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RESEARCH PROGRAMME OF THE DEPARTMENT FOR RADIATION AND RADIOBIOLOGICAL RESEARCH: ITS PERFORMANCE IN 2001 AND THE PROGRAMME FOR 2002

Report to the 91st Session
of the JINR Scientific Council
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Dubna 2001

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1. Scientific research programme for 2001

The major lines of the programme in 2001 were concentrated on:

- Simulation of ionizing radiation interaction with matter, shielding calculations and design;
- Study of radiation detector responses;
- Physical support of radiobiological experiments;
- 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;
- The project "Mitra" – investigation of ''methylene blue - ^{211}At'' complex therapy efficiency in melanoma cells.

2. Execution of the 2001 programme

2.1. Radiation researches

Radiation shielding. Radiation from an accelerator installation may extend out to large distances from the source. As well as the radiation coming directly through the shield and in a straight line, radiation may also indirectly reach points at large distances by way of air scatter. This radiation is termed skyshine and is usually due to relatively high levels of neutrons escaping upwards through holes or thin parts of an accelerator shield in areas that are normally inaccessible during operation. These neutrons are then scattered in the air and a proportion arrive back down at ground level. Because the prompt radiation field dominates the radiation environment of high-energy accelerators it is the dominant source of population exposure resulting from accelerator operations. There is no generally accepted method of calculation the population exposure resulting from accelerator operations. *Simulation of the population exposure resulting from accelerator operations was developed by the Monte Carlo method. Neutron and gamma effective doses due to skyshine were calculated at different distances from the accelerators' building depending on ceiling thickness of accelerator's hall.*

In the construction of an accelerator installation some importance must be attached to adequate design of labyrinths through the main biological shield for access of personnel and equipment. These labyrinths act as leakage path for radiation, especially low energy neutrons. They must be carefully designed such that their presence does not impair the efficiency of the main shield. The width of personnel entries may be quite large, particularly if emergency fire and medical services are to be permitted efficient access and egress. *Transmission of radiation down various types of labyrinths in rooms of neutron and proton therapy at cyclotron center in Bratislava was calculated by Monte Carlo technique. The results of these calculations have enabled designing of the labyrinths so that their presence does not impair the efficiency of the main shield.*

Neutron spectrometry. The data of the experiment at the LNP Phasotron on investigation of the differential characteristics of neutrons' yield from the thick lead target simulated the core of the SAD assembly were processed and compared with the calculations by the MCNP4B+LAHET and MCNPX codes. The verification of the neutron transport codes were done for the calculation of: 1) the neutron spectra under several angles; 2) the angular and the spatial distributions of the hadron's yield at different energy thresholds. The good agreement between the calculated and experimental data in wide energy range was obtained.

The neutron detectors responses study. The calculations of neutron detection efficiency of organic scintillators were carried out. The neutron spectrometer with NE213 detector will be used in the next stage of the experiments in frame of the SAD project. The precise calculation of the efficiencies of the gamma spectrometers at various detector-source geometries was carried out too. The responses of solid track detectors on basis of CR-39 (with fission radiators and without its) to the ^{12}C ions with energies 0,5 and 1,0 GeV/ nucleon were investigated.

The physics support of the biological experiment on the blood lymphocytes and mammalian cells in culture irradiation by the ^{12}C ions with energy 0,5 GeV/n was done at LHE Nuclotron. For the biological samples irradiation with the particle beams the near uniform radiation field in area of the samples is formed and the absorbed doses are measured with the calibrated ionizing chamber.

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

2.2. Radiobiological research

The main goal of radiobiological research was connected with investigation of mutagenic effect of heavy charged particles on bacteria, yeast, mammalian and human cells. The study of regularities and mechanisms of point (fepA$^-$, ton B$^-$) and deletion (ton B$^-$trp$^-$) mutation induction in bacteria *E. coli* by radiation with broad region of linear energy transfer (LET) was accomplished. It was shown that the frequency of point mutations as a function of the γ-ray and heavy ion dose (helium and carbon ions with LET 20 - 200 keV/μm) 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 $\approx$ 20 keV/μm. The induction of deletion mutations by helium and carbon ions (LET = 20-200 keV/μm) is described by the linear function. The helium ions with LET=50 keV/μm are more effective in induction of deletion mutations than the carbon ions.

The induction of mutagenic SOS repair in *E. coli* cells after irradiation by deuterons, helium and carbon ions was studied by using the SOS lux test. A genetically controlled luminescent bacterial reporter assay was developed for detection of cell SOS response. The bioassay is based on the recombinant plasmid pPLS-1, which was constructed as a derivative of pBR322, carrying the

promoterless luxCDABFE genes of Photobacterium leiognathi downstream of a truncated cda gene from ColD with a strong SOS promoter. *E. coli* strain containing this construction are inducible to high levels of light production in the presence of agents that cause damage to the DNA of the cells or after irradiation. It was shown that the relationship of SOS induction potency (SOSIP) on LET has a local maximum in region of 50–60 keV/μm. The results that were obtained with bacterial cells indicate on the important role of cluster DNA damages in formation of point mutations. On the other hand, the formation of deletion mutations is connected with induction of direct and enzymatic double strands breaks of DNA.

The important data concerning regulation of cell cycle of *yeast Saccahromyces cerevisiae* and sensitivity to DNA damage agents were obtained in recent years. According to these data, for instance, the RAD9, RAD17, and RAD24 genes are believed to act at the initial steps of damage recognition. The RFC-Rad24 protein complex plays probably a role in loading the Rad17-Mec3-Ddc1-complexes or repair proteins on damaged DNA. The *RAD53* protein kinase is involved in a signal transduction cascade, and the other kinase, CDC28, acts at the final step of cell cycle arrest regulation. Arrest is required for repair of damages. The branched system of regulation of cell cycle arrest due to DNA damage needs studies.

It's known that defects in the mechanisms that regulate cell cycle arrest due to DNA damage are believed to have some definite consequences for cell hereditary apparatus, *viz* to cause genetic instability and increased cell sensitivity to DNA-tropic agents. Indeed, enhances sensitivity to damaging agents has been found in various checkpoint mutants affected in checkpoints. Our and some literature data existed however suggests that *post hoc* may not be completely *propter hoc* here.

Single and double strains were constructed to analyze interactions between the RAD9, RAD17, RAD24, RAD53, and CDC28 genes, and cell sensitivity to γ-radiation was determined. The RAD9, RAD17, and RAD24 genes act in one and the same pathway of radiosensitivity while RAD9 and RAD24 genes have been classified as belong to different groups dealing with DNA damage induced by UV light or MMS.

Protein kinases encoded by the RAD53 and CDC28 genes are epistatic to RAD9 gene but are most probably involved in different pathways of radiation sensitivity control while CDC28 and RAD53 belong to the same branch of the regulation of cell cycle arrest.

We continue to study induction of mutations of different nature on ionizing radiation in particular point mutations used a convenient tester system for 6 possible base-pair substitution diagnostic. We characterized base pair substitution – transversion AT – TA – induced by helium and carbon ions with LET values 80 and 200 keV/μm used the diploid strain YMH53. The mutation induction efficiency of He^{2+}(80 keV/μm) was less than the efficiency of He^{2+}(20 keV/μm). The frequency of AT – TA transversion induced by C^{6+} ions was the lowest.

Frame-shift mutations were tested in the strain RKY2672. This strain has insertions in LYS2 and HOM3 genes, which revert to wild type in results of only frame-shift mutations. Earlier mutation rate induced by ionizing radiation was measured. Now spontaneous reversion rate was monitored. The rate of spontaneous

lys2 reversion was $1x10^{-8}$ and *hom3* reversion – $2.5x10^{-8}$. Our results are consistent with literature data.

An extended time-course study of expression of chromosomal aberrations induced by radiation of wide LET range were finished at GSI (Gesellschaft für Schwerionenforschung), Darmstadt, Germany in collaboration with GSI Biophysics Group. In these experiments the time-dependent expression of particle-induced chromosomal damage has been analyzed in 3 Chinese hamster cell lines: V79, CHO K-1 and xrs5. The cells synchronized by either centrifugal elutriation or mitotic shake off were irradiated in G_1-phase of the cell cycle by X-rays and accelerated heavy ions (Ne, Ar, Kr, Au) obtained at UNILAC and SIS facilities (GSI, Darmstadt) with LET ranging from 360 to 3980 keV/μm. The chromosome damage has been measured at time span ranging from 10 to 34 h after exposure covering the time interval of almost 3 cell generations, so that almost all dividing cells including very delayed were collected. The cells were analyzed at 5-12 subsequent sampling times in 2-4 h intervals preceded by 2 h colcemid treatment. To distinguish between the metaphases of various postirradiation generations the Fluorencense-plus-Giemsa (FPG) technique was applied, and the chromosomal damage was analyzed separately in the first and second generation metaphases.

The irradiation was shown to retard the cell cycle progression and delay the entry of cells into mitosis, while the expression of chromosomal damage was markedly affected by radiation-induced cell cycle delay in dose- and LET-dependent manner. The number of aberrant cells and aberrations have been found to enhance drastically with sampling time after particle exposure and this effect depended on LET: the most pronounced increase was observed for Ar and Kr exposure (LET: 1280 and 3980 keV/μm) reaching 100% of aberrant cells and 10-15 aberrations per cell at later sampling times. The aberration frequency increased in X-ray- and Ne-irradiated samples by a factor of 2-3 while after Ar (4.6 MeV/u, LET 1840 keV/μm) – by a factor of 20.

The observed differences in the cell cycle progression and in time course of aberrant cells after exposure of G_1 cells to densely and sparsely ionizing radiation are obviously related to different spatial energy deposition of both radiation types. The applied dose of X-rays is homogeneously distributed over the cells. Thus, only small fluctuations in the amount of damage within target cell are induced and synchrony of population is at least partially maintained. In contrast, the particle exposure results in high inhomogeneity of energy deposition both in terms of dose distribution inside the track and in the number of particle traversals per cell nucleus. One of the biological consequences of this non-uniformity of energy deposition by particle exposure is the affected time course of the appearance of chromosomal damage: cells with a low number of particle traversals and correspondingly low chromosomal damage enter mitosis earlier than the cells with a high number of hits and severe chromosomal damage. The distribution of particle-induced aberrations among the cells was fitted by Neyman type A distribution that takes into account the stochastic distribution of particle traversals over the cells as well as the stochastic distribution of aberrations per single traversal. The fit parameters clearly reflected

the damage-dependent cell cycle delay: the number of particle traversals per cell appears to increase with sampling time, i.e. highly hit cells are delayed for a longer time and carry correspondingly more aberrations than the cells with low number of particle traversal, less damaged which reach mitosis earlier.

Thus, the RBE (relative biological efficiency) values of high LET particles obtained for single sampling time were shown to be strongly dependent on time: for example, after Ar ion exposure they varied from 0 at 14 h (standard fixation time) to 4.0 at 22 h postirradiation and could thus be misleading.

It became clear that for the correct determination of meaningful RBE values multiple fixation regimes has been used. To account for the time-dependent expression of chromosomal damage a novel mathematical approach has been developed in our group, which allowed to quantify the amount of damage within the whole cell population. Briefly, the number of aberrant cells and aberrations per cell obtained at each sampling time were weighted with the corresponding mitotic index. The data were corrected for cell proliferation to account for the dilution of heavily damaged cells at later sampling times due to the division of undamaged cells. This yielded the corrected frequencies of aberrant cells and aberrations at given time with respect to the entire population. Finally, the total amount of damage was determined by integrating the area under the yield-time curves. The method was shown to be more adequate for the comparison of experimental data obtained for different radiation qualities which caused different cell cycle perturbations compared with the conventional methods based on single sampling time data. It yielded for Ne (LET 460 keV/μm), Ar (1226 and 1840 keV/μm) and Kr (3980 keV/μm) ions values of 3.2, 1.9, 1.4, 1.3, respectively, which were much higher than all previously published RBE values obtained routinely at single fixation regime in this region of very high LET.

The mathematical method was also applied to reconstruct the growth curves and to evaluate the amount of aberrant cells, which reached mitosis during time course of experiment as well as the number of lost cells due to the incomplete sampling or interphase death. We have demonstrated that in case of Chinese hamster cells used here the vast majority of exposed cells were able to reach their first postirradiated mitosis despite of pronounced cell cycle delay.

Finally, LET-dependent alterations in aberration spectrum were demonstrated in line with the general view that the quality of lesions underlying chromosomal aberrations changed with LET. First, it was shown that after high LET radiation the chromosomal breaks were preferentially formed, while after sparsely ionizing radiation the aberration spectrum was dominated by exchange-type aberrations like dicentrics: the fraction of chromosomal breaks increased from 40% after X-ray exposure to 55-65% for particles with LET in the range 1000-4000 keV/μm. A further characteristic feature of densely ionizing radiation was the occurrence of a high number of chromatid-type aberrations despite of cell irradiation in G_1-phase of the cell cycle. While after X-irradiation the chromatid-type aberrations accounted for about 10-15 % of aberration yield, this amount gradually increased with LET being 30% for Ne (LET 460 keV/μm), 35-40% for Ar (1226 and

1840 keV/μm) and 45 % for Kr (3980 keV/μm) ion exposure. This is a consequence of higher complexity and lower reparability of DNA lesions produced by high LET radiation.

As an extension of these studies, the time-course of chromosomal damage induced by radiation of different quality in normal healthy human fibroblast cell lines as well as in human peripheral blood lymphocytes is currently investigating.

The investigation of low dose radiation effects was continued. The induction of cytogenetic damage after irradiation of Chinese hamster cells and human melanoma cells within dose range 1-200 cGy was studied. The anaphase and metaphase analyses of chromosome damage and micronuclei test were applied in asynchronic and synchronic populations. The hypersensitivity (HRS) at doses below 20 cGy and the increased radioresistence at higher doses (IR) were shown for all cytogenetic criteria in both cell lines. The phenomenon of HRS/IR was reproduced in synchronic as well as in asynchronic population of Chinese hamster cells. This reflects that HRS was caused by high radiosensitivity of all cells and can not be explained by changes of radiosensitivity of cells in different phases of the cell cycle. So it was supposed that the increasing radioresistence is determined by the inclusion of the inducible repair processes in all cells. This conclusion consents with the facts, that there was no evidence of HRS on dose-effect curves and that some parts of pre-existent damage was repaired after preliminary irradiation with low doses (1-20 cGy) which induced repair processes. It can be concluded that the same inducible repair processes underlie either HRS/IR phenomenon and adaptive response.

Comparison of radiobiological effective depths in 150-MeV unmodulated proton beams (LNP JINR) was conducted. Cell survival curves were generated with the in vitro colony forming assay. With ^{60}Co gamma-rays as the reference irradiation, the relative biological effectiveness values for a survival fraction level of 0,1 at Bragg peak and plateau are 1,17 and 1,03, respectively. So, to maintain uniformity of radiobiological effectiveness for a target volume, careful attention should be paid to the influence of depth of beam and irradiation dose.

As an extension of our previous studies on the genotoxic effects of low doses of radiation and formation of stable and unstable chromosomal aberrations, the first experiments were performed on mammalian cultured cells and human lymphocytes exposed to ^{12}C ions (480 MeV/n) obtained on the Nuclotron (LHE, JINR). The data are currently analysed.

Studies on targeted therapy of human pigmented melanoma with radiolabeled methylene blue (MTB) were continued. Experiments carried out with MTB labeled with ^{131}I or ^{211}At *in vitro* and *in vivo*. Accumulation of ^{131}I-MTB in human pigmented melanoma cells in vitro is about 4-5 times higher than in non-pigmented cells. The maximum of accumulation occurs in 2 h after injection. The obtained data stay in an accordance with our previous results on ^{211}At-MTB accumulation in *vitro*.

Experiments *in vivo* show rapid excretion of ^{131}I-MTB from all organs of tumor bearing mice during first 24 h. Accumulation of the compound in tumor has maximum (5 %/g of injected activity) in 24 h and remains at a high level at least

first 2 days after injection. Tumour: normal tissues ratios for ^{131}I-MTB accumulation were 36:1 for blood, 47:1 - for muscles, 8:1 - for skin at 24 h after i.v. injection.

Preliminary data on ^{211}At-MTB biodistribution shows high accumulation of the compound in melanoma (6 %/g of injected activity) at 5 h after injection and its' slow excretion from the other organs.

The analysis and comparison different models for describing radiobiological effects to low dose exposure have been done. All models are presented by linear no-threshold term of damage yields, which is and resulting for linear no-threshold model. This fact reflects the regularity, that primary reason of all stochastic radiobiological effects (perhaps DNA breaks) linearity and no-threshold depend from dose. However, linear term of effect is necessary, but insufficiently for adequacy of model, in general case, to the observation result. Linear-quadratic model can be adequate to any data on a cell level to the dose more then 1 Gy, however at the less doses the adequacy often break. Inducible repair model is adequate to the data on cell level. A confidence level of the model of two cell populations electionly adequate to the data on a cell level. The confidence level of the model of two-protection reactions on totality data is the most out of comparison models and suitable for all levels of an organism. On the base of this model the estimation of radiation risk for population of the most contaminated regions of Belarus have been done.

3. Scientific programme for 2002

3.1. Radiation research in 2002 will be concentrated on the following directions:

- *Neutron Spectrometry.* It is planned to prolong the measuring of the neutron spectra generated in the thick lead target by the 650 MeV protons with spectrometer on basis of NE-213 detector. The technique of the neutron spectra unfolding will be developed too.
- *Physical Support of Radiobiological Experiments.* It is planned to ensure the dosimetric support of the radiobiological experiments at the ^{24}Mg beams of the Nuclotron.
- *Shielding Studies.* Application of Monte Carlo and engineering methods for shielding calculation and prognostication of the radiation environment will be continued.
- *Response Detectors Study.* The study of the responses of different type dosimeters and radiation detectors at the JINR basic facilities will be continued.

3.2. Radiobiological research in 2002 will be connected with:

- *Study of regularities of stable chromosomal aberration induction in human lymphocytes* by heavy ions in broad region of LET. The spectrum of induced aberrations in peripheral blood lymphocytes by heavy ions will be analyzed.
- *Investigation of mutagenic action of radiation with different LET on microorganisms* will be continued. The investigations of regularities and mechanisms of induction of precise excision of transposons by densely ionizing radiation will be performed. The study of gene and frame shift mutation

induction in yeast exposed to radiation with broad region of LET will be continued.

- *Biological effects of low-dose exposure* in Chinese hamster cells after γ-irradiation will be continued. The adaptive response of mammalian cells to irradiation under different doses will be studied.
- *The in vitro and in vivo investigations of methods of targeted radiotherapy* with the complex 211-astatin–MTB will be continued. The biological effect of 131-iodine –MTB on melanoma cells will be studied.

4. Conferences and educational activity

The II International Symposium under UNESCO auspices "Problems of Biochemistry, Radiation and Space biology" dedicated to the memory of Academician N. Sissakian and the II N. Sissakian Readings were held in Moscow (A. Bakh Institute of Biochemistry) and in JINR on 29 May-01. June 2001. The Symposium was attended by about 120 participants from Russia and other European countries. The Symposium Scientific Programme included 16 plenary reports and more 80 reports were included in three special sessions on following topics:

- Biochemistry;
- Space biology and medicine;
- General and space radiobiology.

The competition of the reports among the young scientists was organized in the frame of the Symposium. The winners were awarded with the diplomas and premiums.

The education process at the chair "Biophysics" of the International University "Dubna" was continued. 11 new students were admitted in 2001 to the chair on specialty "Radiation protection of people and environment".

5. Administration activity

Personnel. The total personnel of the DRRR (without of the Radiation Protection Division) were 83, including the Directorate staff 5.

Finance. Funding of research in the direction of radiation and radiobiological investigations in 2002 is shown in Table 1.

Table 1. Financing DRRR in 2002.

Area	Financing plan (k$US)
08-9-1015-96/2003 (1-st priority)	280,8
Infrastructure	64,3
Total:	345,1

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