

Comparison of Biophysical and Radiological Responses of Bio-test objects to Pulsed and Continuous X- and Neutron-Irradiations

V. Gribkov^{1,2,3}, A. Dubrovsky¹, E. Demina¹, V. Pimenov¹, M. Orlova^{4,5}, O. Kost⁴, S. Ul'yanenko⁶, A. Lychagin⁶, S. Koryakin⁶

¹A.A. Baikov Institute of Metallurgy and Material Science, Russ. Ac. Sci., Moscow, R.F.

gribkovv@yahoo.com

²A.I. Alikhanov Institute of Theoretical and Experimental Physics, Rosatom, Moscow, R.F.

³Institute of Plasma Physics and Laser Microfusion, Warsaw, Poland

⁴M.V. Lomonosov Moscow State University, Moscow, R.F.

⁵Rogachev Federal scientific clinical centre of pediatric hematology, oncology and immunology, Moscow, R.F.

⁶Medical Radiological Research Center, Russ. Ac. Med. Sci., Obninsk, Kaluga region, R.F.

Abstract

A comprehensive investigation of biological effectiveness of irradiations of bio-test objects by neutrons, X- and gamma-rays within a wide range of doses, dose rates (dose powers), durations of action and spectral contents was provided. It was done with the help of well-known and newly elaborated sources of the above radiations based on isotopes, fission reactors, X-ray tubes and Dense Plasma Focus devices. As biological test objects of different contents and complexity we used enzymes of various types, serum, seminal fluid, yeasts, human lymphocytes, mice bone marrow, etc. As it follows from the results of our experiments various biological systems react to neutron radiation of dissimilar dose rates (dose powers) in different ways. However the range of the variations of the coefficient of biological effectiveness as a rule does not go out from the limits of physiological oscillations at the neutron dose power that changes within 9 orders of magnitude. I.e. it is actually the same for pulsed and continuous sources of neutrons till the figure 10^8 Gy/min and it coincides with the "classic" dose rate effect (a quadratic part of dose curves for cell survival or chromosomal aberrations) [1]. This conclusion gives hope of applicability for the potential methods of neutron therapy the neutron generators of the Dense Plasma Focus type instead of dangerous, expensive and cumbersome nuclear reactors. As opposed to the results with neutrons our study in the field of radioenzymology fulfilled in the frame of this set of experiments has confirmed our previous observations [2] that the very powerful X-ray radiation may cardinaly change a response of the enzymes at the upper level of the dose powers. Such an action results in activation or suppression of enzymes at doses differing by 4-5 orders of magnitude (to lesser doses) compared with analogous effects obtained with low power isotope and X-ray tube sources. This effect was observed with X-rays at the dose power from DPF being on its maximal level ($\sim 10^{10}$ Gy/min) provided that their spectrum contains a component in close vicinity to the absorption line of metallic ion belonging to the particular enzyme. Attempts of interpretation of the above results are connected with the threshold-like behavior of the pulsed radiation chemistry in its perfect sense [3].

Introduction

In up-to-date data, circa 70% of oncologic patients have a need in radiation therapy (side by side with surgery, chemotherapy, hormone therapy, immunotherapy or some mixture of the five). X-rays and neutron radiation fluxes here have their own special niches in cancer treatment (together with different ionizing particle beams – protons, muons, etc.), i.e. these fluxes are used for irradiation of different-type tumors positioned at various depths. In particular, neutron therapy is prescribed for about 30% of oncologic patients [4]. This therapy is made in over 20 countries in the world. Under correct patient choice and at the proper quality assurance (QA) procedures for patient dosimetry implemented at neutron therapy (see e.g. [5]) the therapy allows at least 20% enhancement of direct and long-term treatment results [6].

Recently the development of new methods of neutron therapy is under way – combined neutron-capture plus X-ray and neutron-capture plus fast neutron therapy. Together with oncology a series of new applications of neutrons in various fields of clinical practice is developed. One of the most perspective lines in use of neutron radiation is so called boron neutron capture synovectomy, i.e. non-surgery treatment of arthritis (J.C. Yanch, 1996).

Isotopes, nuclear fission reactors, cyclotrons, X-ray tubes, and other-type accelerators are currently used as neutron and X-ray sources for therapeutic purposes. Although they allow large success, some important parameters are desirable to be much better (neutron biological efficiency, dose depth distribution, stability, availability, cost, dimensions, ecological compatibility, possibility of multi-field, multi-beam and rotational irradiation, etc.).

Some problems are badly studied here. These issues relate to dosage for the intermediate layers of organism while deeply located tumor is under irradiation, to neutron scattering and penetration within organism, combined irradiation modes, etc.

Therapeutic efficiency of neutron radiation versus dose rate (or dose power in particular while short-pulse irradiation) and pulse frequency is virtually not studied. In accordance with some theoretical concepts namely pulsed radiation influence upon tumor (of the shock-like nature) may be especially effective because within such a pulse duration reparation and recovery processes have no time enough to take place, and various effects of cumulative influence and synergism are possible [1, 7].

Among fundamental problems of radiation biology a new presently born discipline radioenzymology occupies an important place. It is a comprehensive approach to study molecular mechanisms of ferment injury under effect of radicals produced while tissue's irradiation by different-type ionizing radiation. Radioenzymology is at the same time one of the most sensitive methods to determine conformational changes of molecules. For instance, radioenzymology allows determination of molecular structure and conformational state and also individual amino acid residue effect upon catalysis process while comparable study of ferment mutant forms.

Short powerful neutron and X-rays pulses are necessary for various radiobiological tasks. Between them, for example, the determination of contribution of fast cell metabolism processes (damaged DNA reparation, oxygen effect, etc.) into formation of final cell and organism damages by radiation, effects of different-type radiations, their spectral composition and pulse duration influence upon radiation injury of cells being at various stages of mitosis.

This work is devoted to Comparative Study of Biophysical and Radiological Responses of Bio-test objects to Pulsed and Continuous X- and Neutron-Irradiations.

Apparatus

We used ^{60}Co , medical X-ray tubes and nuclear fission reactor BR-10 as *continuous* sources of X-rays and fast neutrons plus gamma-rays respectively.

Alternatively as *pulsed* sources of neutrons and X/gamma-rays we applied pulsed reactor BARS-6 and Dense Plasma Focus (DPF) devices PF-5M and PF-6.

Dense Plasma Focus device is a type of plasma accelerator that produces:

- directed powerful hot ($T \sim 1$ keV) fast ($v > 10^7$ cm/s) dense ($n_{pl} \sim 10^{16} \dots 10^{18} \text{ cm}^{-3}$) plasma streams,

- high energy ion ($E_i \sim 0.01 - 100$ MeV) and electron ($E_e \sim 0.01 - 1.0$ MeV) beams,

- *soft* ($E_{hv} \sim 0.1 \dots 10$ keV) and *hard* ($E_{hv} \sim 10 \dots 1000$ keV) X-rays and

- *fusion neutrons* ($E_n \sim 2.45$ and 14 MeV).

These radiation types are generated in the DPF due to a number of collective effects taking place inside the plasma because of some turbulent phenomena [8].

Principle of the DPF operation is demonstrated in Fig. 1.

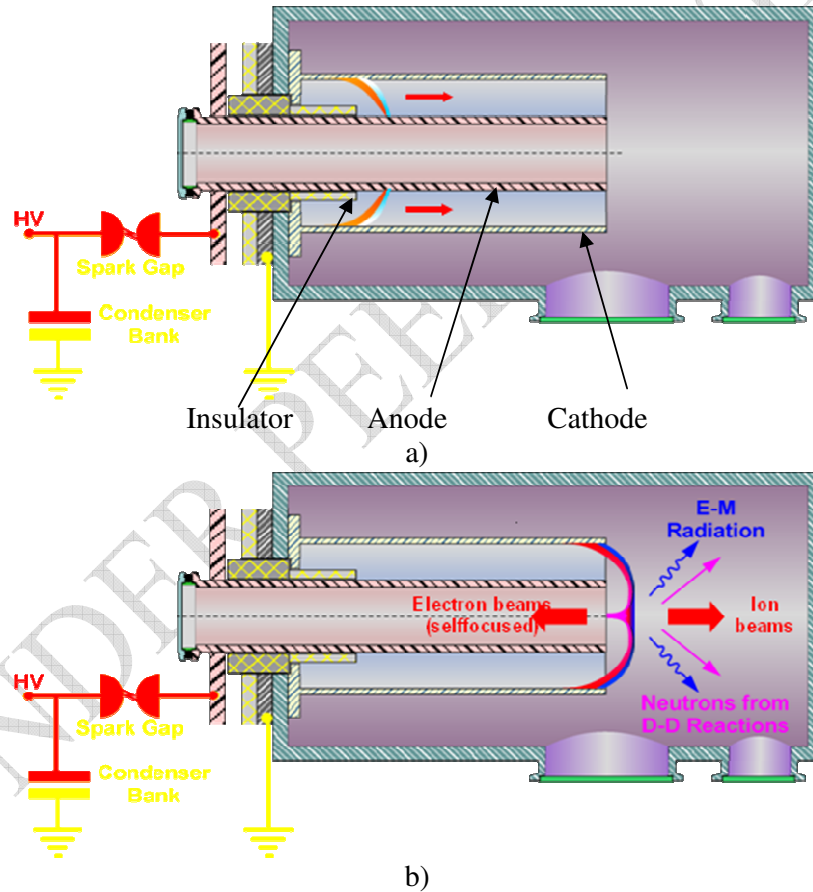


Fig. 1. Principle of operation of a DPF device: the breakdown and accelerating stages (a) and the implosion stage with generation of hard radiations (b)

In the above picture it is shown that after the initial breakdown of gas (being at a pressure of a few mbar) along the insulator a plasma-current sheath is formed. Then it is accelerated by a Lorentz force within the co-axial gap between the tube-like anode and cathode and is imploded subsequently about the axis of the DPF chamber. After the

phenomenon known as the “current abruption” [8] the above-mentioned ionizing radiations are generated.

In the Fig. 2 one may see two of the medium-class (capacitor bank energies are 2 and 6 kJ for PF-5M and PF-6 correspondingly) devices that were used in these experiments.

This type of the sources is more ecologically acceptable compared with isotopic ones, classical high-voltage accelerators and reactor facilities.

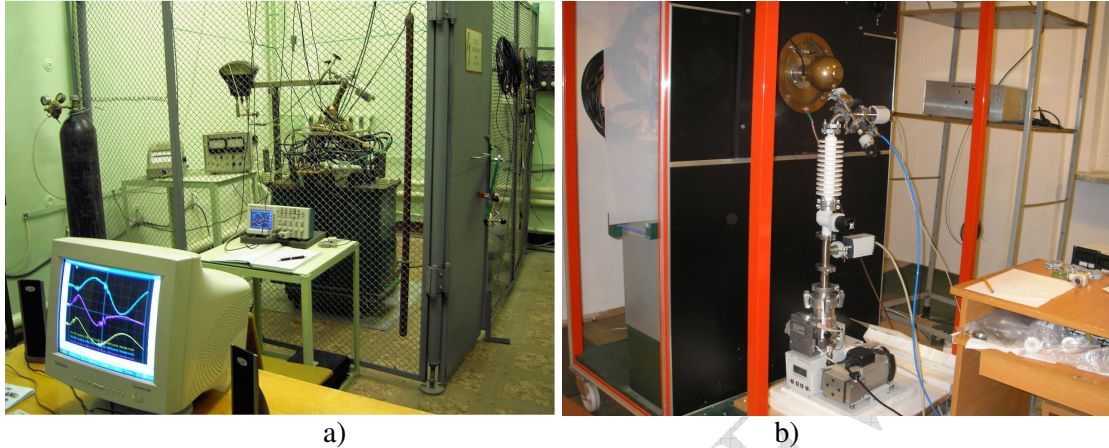


Fig. 2. DPF devices used in these experiments: PF-5M (IMET) (a) and PF-6 (IPPLM) (b)

The reasons are as follows:

- They use relatively low-voltage (10...250 kV) chargers for the capacitor banks, which operate from the mains of 230 V, 6 A, 50-60 Hz
- They become ionizing radiation sources only under electric power application (“on demand”, i.e. they are “push-button” devices)
- In the “OFF” state they are completely safe, and in contrast to isotopes they do not require special containers for storage
- They are accident-free as against nuclear fission reactors (there is no “criticality” parameter in these devices).

In addition these generators have low cost (a few orders of magnitude lower than classic accelerators), compactness and low weight (from 20 to 200 kg), small-sized irradiation chamber (a few cm), and a “record” brightness of emitted ionizing radiations.

In respect to a number of parameters they are more convenient in clinic applications and open therein a series of new opportunities. They may be used in computer tomographic facility for diagnostics with source's rotation around patient, rather than patient's rotation in relation to a beam. Radiation chamber of a miniature generator may be inserted inside a patient's body. In some cases it provides large advantages (for instance, in stomatological panoramic X-ray photography). Portable generators, which can be powered from domestic mains or accumulator, may be indispensable in ambulance, sport medicine and field surgery. On the other hand, the development of a system consisting of a few powerful generators around patient should allow strongly optimize dose field geometry while therapy.

However because they ensure the unprecedented dose powers of X-ray and neutron radiations (due to nanosecond pulse durations) these devices must be examined with various bio-test objects for their safety in comparison with classical sources. From the other side namely this extremely high brightness of them might result in synergetic effects and open perspectives for a low-dose high-power neutron therapy of cancer.

X-ray and gamma spectra of these sources used in our experiments was ranged (and varied) in the limits from a few keV till several MeV with peaks at about 8.979 KeV (DPF), 150 keV (DPF), and 1.3 MeV (^{60}Co).

Neutron energy was ranged from thermal till 16 MeV with peaks at 2.5 (2.7) MeV (DPF), 1.44 MeV (fission reactors) and 14 (16) MeV (PF-6).

The dose rates (for continuous sources) and dose powers (for DPF sources) were varied from 10^{-3} to 10^9 Gy/min for neutron radiation and $10^0 - 10^{11}$ Gy/min for X-rays depending on the devices and on radiosensitivity of biological objects.

Irradiations were produced by X- and gamma-rays and neutrons separately and in combinations. The mixed gamma-neutron character of radiation from the sources based on nuclear fission reactors (the value of $D_\gamma/D_{n+\gamma}$ was varied from 5 to 50%) has been also taken into account.

Materials

As biological test objects of different contents and complexity we used enzymes of various types (angiotensin converting enzyme, different plant peroxidases), serum, seminal fluid, yeasts, human lymphocytes, mice bone marrow (splenic colony-forming units), mice of line Black and F1 (CBA $\times$ C57B1), etc. These materials were obtained from companies of Russia and foreign countries (for instance, "Biozyme", U.K., "Enzymol International Inc.", U.S.A., etc.).

If necessary, all materials were purified using original technique developed at MSU, R.F. Their activity is checked prior to experiment (for example, for ACE – by stoichiometric titration with specific competitive inhibitor – lisinopril). Concentration was determined by spectrophotometry. Thermal stability of peroxidases was studied at incubation of an enzyme (under needed concentration) at 50 and 80°C in the corresponding buffer (for instance, in phosphate-borate one). Operational stability (inactivation during reaction) was calculated on the base of total curve of increased chemiluminescence obtained under optimum conditions (luminometer "Wallac 1251-002"). Preparation's homogeneity was tested by electrophoresis method in poly-acryl-amide gel in presence of potassium dodecylsulphate. In each case albumen content was determined by spectrophotometric method.

Ferment activity prior to and after irradiation was measured using spectrophotometer (Hitachi) within the wavelength range $\Delta\lambda = 300\div 750$ nm according to up-to-date techniques, for instance, for the horseradish peroxidase in relation to the following substrates: ABTS, KI, *o*-phenylendiamine, guayacol, phenol-antipinine probe.

Methodology

Special measures on the following lines were provided during the experiments:

- Cross-correlation of the whole number of diagnostic and dosimetry devices, based on different principles and used on various installations in differing conditions

- Comprehensive (as wide as possible) use of various detectors set in each experiment.

- Measurement of as many parameters as possible in each experiment (dose, pulse duration, spectrum, etc.)

- Support of experimental dosimetry by numerical mathematical modeling of interaction process; special attention therein was paid to difference between stationary (continuous irradiation) and non-stationary (pulsed) modes

- In a course of the experiments same objects was irradiated in different conditions

- Everywhere effects of the non-linear, triggering and hysteresis type were taken into account in dependence on changing of the parameters.

Results and discussions

Objectives/scope of work and technical approach

The ultimate objectives of the work are an elaboration of methods and equipment for diagnostics and therapy of malignant tumors based on pulsed generators of neutron and X-ray radiations. To realize the above aims the following scope of work was provided using the technical approach to obtain expected results described below:

- Elaboration and manufacturing of neutron and X-ray DPF-based generators with parameters: neutron energy – 2.5 and 14.0 MeV, X-ray photon energy – 10...600 keV, duration of neutron and X-ray pulses – 5...15 ns, neutron yield per pulse – $10^8...10^{11}$ neutrons in full solid angle, repetition rate of neutron pulses – 0.1...10 cps, hard X-rays yield – up to 10 J/pulse

- Development of schemes and methods of irradiation of bio-object by the above-mentioned types of radiation, generated by stationary and newly elaborated pulsed DPF-based sources

- Working out of control-measuring and dosimetric equipment, able to operate in pulsed and stationary streams of ionizing radiation and in harsh environment

- Gaining of new fundamental data about physical-chemical and biological influence of powerful flashes of X/gamma-rays and neutron radiations on bio-objects

- Investigation of Relative Biological Effectiveness (RBE) of the influence of X/gamma-rays and neutron radiations upon bio-objects in dependence on parameters of their pulses.

DPF sources of neutrons and X-rays

At the new devices of the type “Dense Plasma Focus” the record characteristics of dose power (P) were achieved. The maximal values of P for X-ray radiation were of the order of 10^{10-11} Gy/min whereas for neutrons these values were about the same as at pulsed fission reactor BARS – around 10^{8-9} Gy/min for the device PF-6. As it was mentioned above these generators of both types of radiations are more convenient for their implementation in clinics for diagnostics and therapy in comparison with sources commonly used at present time (fission nuclear reactors, isotope sources, high-voltage accelerators and X-Ray tubes).

We present here some results on usage of these sources together with classical ones in our irradiation tests.

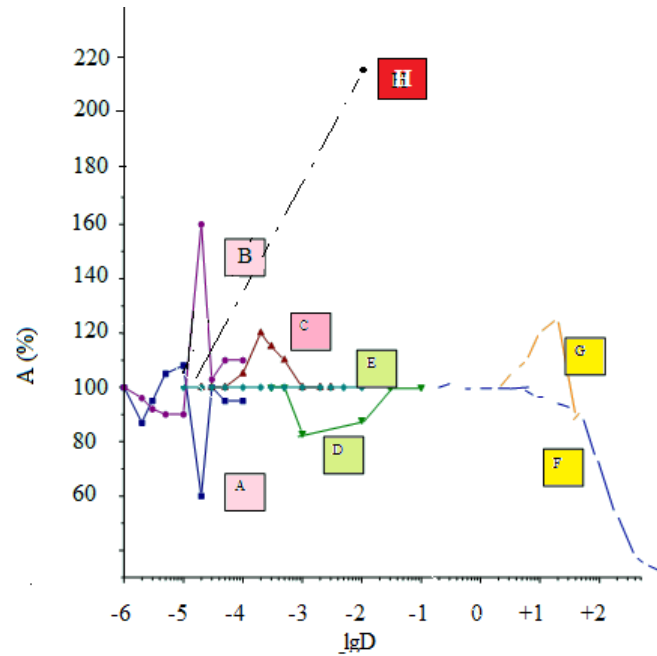
Radioenzymology

Somatic and testicular forms of angiotensin converting enzyme (ACE) as well as some complicated compositions containing dissimilar concentrations of ACE (blood serum and seminal fluid) at variations of conditions of an enzyme existence and on parameters of powerful pulsed and stationary sources of irradiation were investigated. Besides short-pulsed powerful irradiation influence on different types of plant peroxidases were examined.

These experiments in certain cases have shown appearance of deviations in catalytic activity (both activation and inactivation) at super-low doses of X-ray radiation (by 4-5 orders of magnitude lower compared with the case of the isotopes' sources) but at a very high dose power (by -8 – 9 orders of magnitude higher than in the case of irradiations by isotopes). It is supposed that this phenomenon is ruled by a presence of the K_{α} -line of copper with the photon's energy $E_{hv} = 8.98$ keV in the radiation spectrum as well as by the parameter

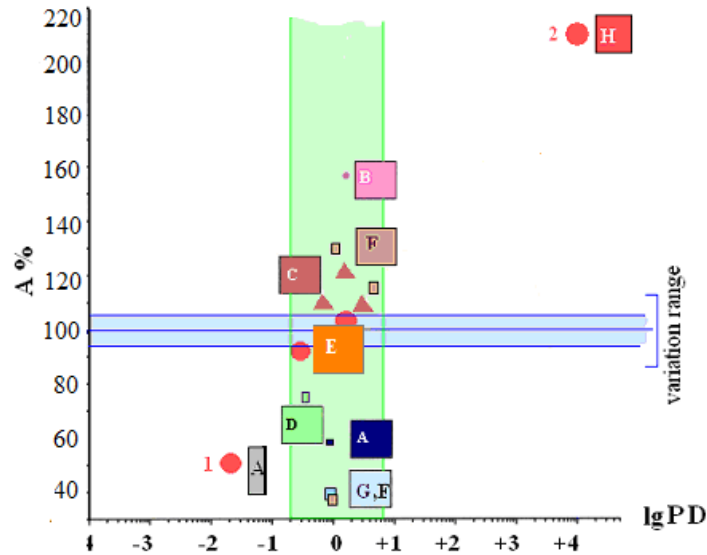
“product of dose and dose power” $D \times P$, in particular within the range of its values $D \times P \approx 0.1 \dots 10 \text{ Gy}^2/\text{s}$ (see Fig. 3).

In these our experiments especially the dose region nearby $D \approx 10^{-4} - 10^{-5} \text{ Gy}$ was marked. One may also see that quite “chaotic” oscillations seen in the picture (a) in the range of doses of about 8 orders of magnitude are collected in the relatively narrow “corridor” in the picture (b) for the product $D \times P$ occupied only 2 orders of magnitudes.



A, B - "PF-0.2" device, Cu filter; C - "PF-5M" device, Cu filter; D - X-ray tube: U - 50 kV, $t = 1 \text{ sec}$, photon energy = 35 keV; E - isotope Sr-Y: photon energy 8 keV...2000 keV, and "PF-0.2" device with Al filter: photon energy - 40 keV...100 keV; F, G - gamma-source Sc-137: photon energy 662 keV; H - "PF-6" device with Cu filter: photon energy is peaked at about 9 keV, $t = 10 \text{ nsec}$

a)



A, B - "PF-0.2" device with Cu filter; D - X-ray tube with U = 50 kV: photon energy maximum = 35 keV; E - isotope beta-source Sr-Y with Al filter: photon energy 40...150 keV; F, G - gamma-source Cs-137 for different enzymes with photon energy 662 keV; H - "PF-6" device with a distance 3 cm, dose D = 0.01 Gy and dose power P = 10¹⁰ Gy

b)

Fig. 3. Dependence of activity of enzymes on the dose (a) and the product of dose and dose power (b) at their irradiation by dissimilar sources of X-rays

It should be noted here that the same effect of a general nature – relative independence of materials damageability on the product of D and P – was observed at the interaction of nanosecond powerful pulses of fast ions generated by DPF with various types of steel and other solid state materials [9] during our radiation tests of elements that are counted to be perspective ones for fusion reactors.

It means that an extrapolation of results obtained with the short strong pulses may be provided for a longer interaction time observed at a lower power. It was declared that such action may be performed using the so-called integral damage factor (IDF) F [9]:

$$F = P \tau^{1/2},$$

where P is power flux density of the irradiation beams and τ is their pulse duration. The method (certainly being very attractive) demands, however, careful investigation of the validity of the above factor for a wide range of parameters and for different materials.

Two exceptions from this basic "accumulation law" – namely points 1 ("A") and 2 ("H") in Fig. 3 b – were obtained correspondingly: first – at the *shortest* pulse duration ($\tau < 2$ ns) and second – at the *highest* product $PD = D^2/\tau = P^2\tau = F^2$ ever achieved before.

Presence of the above effects at so small doses in the conditions of a very high intensity of pulsed radiation (at nanosecond pulses) indicates the existence of peculiar (punctured) dots in the dose curves, which may pose a hazard for biological objects' functioning. Add-on neutron irradiation (at its dose powers $\leq 10^8$ Gy/min) in the conditions of combined X-ray/neutron and γ /neutron fields did not show sound effects in catalytic activity changes of ACE and peroxidases, yet it was reflected in a certain amplification of them.

Studies of chromosomal aberrations in human lymphocytes

In this comparative study the cytogenetic action of neutron fission spectrum, generated by two nuclear reactors BARS-6, BR-10 and of fusion 14-MeV neutrons from PF-6 were studied with one of the test-system that is most widespread in the world – chromosomal aberrations in human lymphocytes [10 -12]. Results obtained with reactors versus ^{60}Co -source are shown in Fig. 4.

With the DPF device 5 plastic tubes with blood samples were placed in a cylinder plastic container near the DPF chamber. Diameter (inner) of the container was 55 mm, diameter of each tube was 10 mm, and wall thickness was 1 mm. Since DPF produced only 1 pulse every 10 minutes, to prevent damage reparation between pulses each container was filled with melting ice. Neutron kerma was calculated using two types of detectors – the fission ^{237}Np detector and the activation silver detector SIVN 61. X-rays doses were measured with a 27012 clinical dosimeter (Dresden). The blood samples were received 16 pulses in total. The maximum 14-MeV neutron tissue kerma was 0.8 Gy, whereas for X-rays it was 0.5 Gy.

Dose curves for the total chromosome aberration yields and for dicentrics yields in human lymphocytes irradiated with unfiltered mixed X-rays and neutron pulsed radiation from dense plasma focus source are presented in Fig. 5 *a)* and *b)* correspondingly. For comparison some cytogenetic results obtained at continuous irradiation regime by the 14-MeV neutron generator known from literature are also shown here.

Experimental data presented in Fig. 5 were fitted to linear regression provided that one excludes from the analysis a data point marked with an arrow. As it follows from the data comparison the DPF neutron source data are closer to the data of Pohl-Ruling et al. [11] if dosimetry is based on the activation silver detector. If dosimetry is based on ^{237}Np fission detector, the experimental data are closer to those of Lloyd et al. [12]. Nevertheless, in both cases the experimental data for the DPF are within the published data for 14-MeV neutrons.

At least two conclusions may be inferred from the Fig. 5. First, the major contribution to the cytogenetic effect of DPF neutron generator radiation is made by neutrons. Second, pulsed neutron radiation of very short, nanoseconds, duration is as effective as it is at continuous mode of neutron radiation, at least, within the mutual experimental uncertainties at its relatively high values ($< 10^8$ Gy/min) for 14-MeV neutrons. It gives hope of applicability of DPF for the potential methods of neutron therapy instead nuclear reactors.

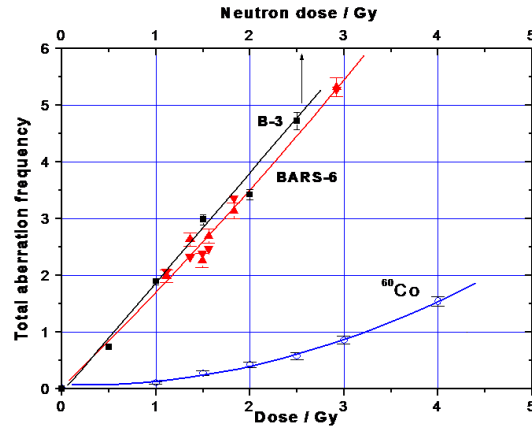


Fig. 4. Dose dependences of the total chromosomal aberration yield in human lymphocytes irradiated with the reactor BARS-6 (pulsed mode); B-3 – effect from the reactor BR-10 (continuous neutron radiation action); and the effect of irradiation by the standard isotope source ^{60}Co of γ -radiation

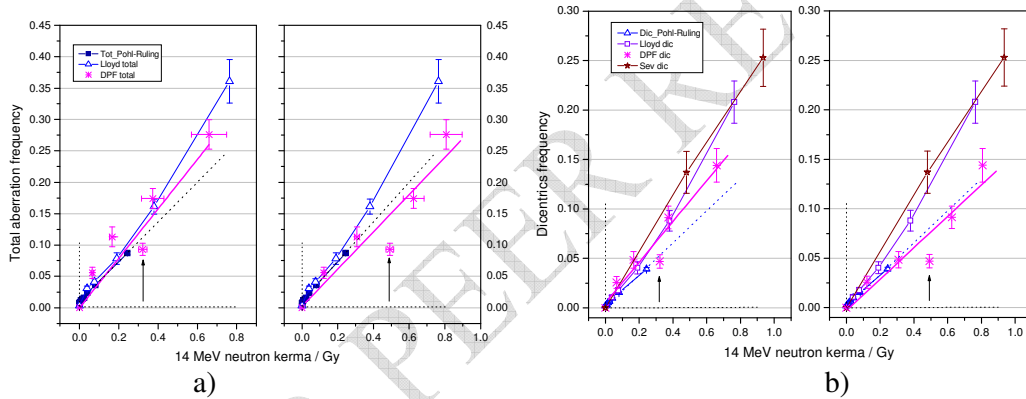


Fig. 5. (a) - Dose curves of the total chromosomal aberration yield in human lymphocytes irradiated with 14 MeV neutrons. Left panel – neutron kerma was calculated using ^{237}Np fission detector; right panel – using SIVN 61 silver detector. Linear regression equation are $Y_{tot}=(0.041\pm0.009) + (0.36\pm0.022)K$ and $Y_{tot}=(0.021\pm0.01) + (0.28\pm0.028)K$, respectively. DPF – dense plasma focus. (b) - Dose curves of the dicentric chromosome yield in human lymphocytes irradiated with 14-MeV neutrons. Left panel – neutron kerma was calculated using ^{237}Np fission detector; right panel – using SIVN 61 silver detector. Linear regression equation are $Y_{dic}=(0.012\pm0.006) + (0.21\pm0.026)K$ and $Y_{dic}=(0.005\pm0.01) + (0.15\pm0.026)K$, respectively

References

- [1] E. Hall, D. Brenner, *Int. J. Radiation Oncology Biol. Phys.* **21** (1991) 1403-1414
- [2] V.A. Gribkov, M.A. Orlova, O.A. Kost et al., *Applied Biochemistry and Biotechnology* **88** (2000) 243-255
- [3] V.A. Gribkov, A.V. Dubrovsky et al., *Research Journal Chem. Envir.* **9**(4) (2005) 11-18
- [4] A.F. Tsyb, S.E. Ulianenکو et al., *Neutrons in the treatment of cancer*, Obninsk: BIST (2003)
- [5] P.M. Munck af Rosenscheold, J. Capala, C.P. Ceberg, V. Giusti, L.G. Salford and B.R.R. Persson, *Quality Assurance of Patient Dosimetry in Boron Neutron Capture Therapy, Acta Oncologica* **43**(4) (2004) 404-411
- [6] A. Wambersie, H.G. Menzel, *Bull. Cancer Radiother.* **83**, Suppl. 1 (1996) 68-77
- [7] V.A. Gribkov, Pulsed radio-biology: possibilities and perspectives, Proc. of the First Workshop on Biological Physics 2000 (BP2K), Editors V. Sa-yakanit, L. Matsson, H. Frauenfelder, World Scientific (2001) 139-171
- [8] V.A. Gribkov, A. Banaszak et al., Plasma dynamics in PF-1000 device under the full-scale energy storage: II. Fast electrons and ions characteristics versus neutron emission parameters, and the gun optimization properties, *J. Phys. D: Appl. Phys.* **40** (2007) 3592-3607
- [9] V.A. Gribkov, A.V. Dubrovsky et al., In-line and following-up tests of perspective fusion-reactor materials in DPF devices, *Czechoslovak Journal of Physics* **56**(12) (2006) 1401-1416
- [10] M. Fujitsuka et al., *J. Nucl. Mater.* **179** (1991) part A, 189-198
- [11] J. Pohl-Ruling, P. Fisher et al., Chromosomal aberrations induced in human lymphocytes by low-dose of D-T neutrons, *Mutat. Res.* **173** (1986) 267-272
- [12] D.C. Lloyd, A.A. Edwards A.A. et al., Chromosome aberrations in human lymphocytes by D-T neutrons, *Radiat. Res.* **98**(3) (1984) 561-573