

The Effects of Low Dose Radiation

New aspects of radiobiological research
prompted by the Chernobyl nuclear disaster

Elena B. Burlakova and Valeria I. Naidich

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Introduction

Dear readers!

This Collection is timed to the 20th year of Chernobyl Accident. This sad day - the happening and the catastrophe, requires detailed understanding of the reasons and consequences of it, and the results of investigations must be summed up to know possible consequences, as such accidents may happen in future.

Strange it may seem, but the views of independent scientists from different countries on this problem and conclusions made on the basis of their experimental and clinical investigations are very similar. Let us present the modern results by the leading Russian scientists in this field and some reviews generalizing different opinions, experimental data and empirical suggestions.

The papers in this Collection were written by many scientists mostly from research institutes of Russian Academies of Sciences and Medical Sciences. Their studies focus on the effects of low-dose ionizing radiation on living organisms and involve both experimental works and surveys of cohorts of people living on radionuclide-contaminated territories and liquidators of Chernobyl Accident consequences.

For many years (especially after the Chernobyl Accident), the problem of chronic low-dose irradiation effect is of the prior interest of radiobiologists and radioecologists. There is the common opinion on danger and mechanisms of acute irradiation. Unfortunately, the situation is opposite with interpretation, understating and making conclusions from the investigations concerning low intensive, low dose irradiation.

Investigations of low-dose irradiation enter a new, unknown, and unexplored world of biosphere interactions with the permanent weak man-induced and natural factors, which power and concentration range may be compared with responses of the environment that support the homeostasis in organisms and the entire biosphere. These problems are extremely complicated. Actually, there is not an unanimous point of view regarding favorable or harmful effects of low doses, because the results and conclusions differ depending on the dose value, dose rate, index chosen, level of investigation of the response (e.g. population or an individual organism), and the initial state of the object under study.

What are the specific features outlined by many investigators of the low-dose irradiation? They are:

The effect of low dose radiation

1. Nonlinear shape of the dose dependence and, in some cases, non-monotonic dose-effect dependence. This means that simple extrapolation from high to low doses may not assess the risk of low-dose irradiation-induced damages.
2. Changes in biological object sensitivity to any future impacts after low dose irradiation. This is displayed in the presence of synergic and antagonistic effects.
3. The irradiation effect depends on irradiation intensity, differently in different dose ranges. In some dose ranges, the effect may be higher for lower dose and intensity irradiation.
4. The value and direction of the effect depend upon the initial characteristics of biological object.

First of all, let us discuss the dose – effect dependence features.

As mentioned above, nonlinear shape of the dose - effect dependence makes impossible extrapolation of data from higher irradiation doses to lower doses. Moreover, the question about the presence or absence of a “threshold” in irradiation action is brought up differently. Therefore, the policy of counter measures is also different. One of the fundamental doctrines of classical radiobiology is the statement that the higher the irradiation dose and intensity are, the higher is the damaging effect. An important applied conclusion is made from this doctrine: a linear, linear-quadratic or quadratic-linear effect dependence on the irradiation dose testifies about radiogenic origin of this effect. We have to agree with this statement. However, we have to argue with the inverse statement that “if usual monotonic dependence between the effect and dose is absent, the effect is not caused by irradiation”. All experimental works indicate that low intensive, low dose irradiation may induce more complex dose dependencies.

What are the mechanisms which may stipulate such regularities? Of special attention of many investigators is nonuniformity of the population response to low impacts. They explain a complex, polymodal dose dependence of radiogenic effects suggesting that radiosensitive cells and organisms are responsible for the low dose maximum (minimum) of the effect at low doses, whereas more irradiation resistant cells and organisms are responsible for effects in higher doses.

Another explanation is associated with changes in the relation between damaging and repair processes. In case of low intensive irradiation, repair systems of irradiation-impacted and changed functions and properties of cells

Introduction

and organisms are not activated or activated at lower intensity, or in later time. Therefore, the effect dependence on the dose may be of bimodal type.

Another change of gene activity, different from high irradiation dose, is typical of low irradiation doses. Recently, it was an ambiguity in the literature, if low-intensive irradiation may affect gene expression. However, gene expression was detected already at 0.1 – 0.2 Sv doses and different groups of genes were expressed in different dose ranges. The comparison of gene expressions in animal organs irradiated by low and high doses shows opposite changes in gene activity. This may indicate different mechanisms of the effect at low dose irradiation.

Of special complexity is the question about the effect of low dose irradiation on human health state, impacted by irradiation. The simplest answer may be given using data of Russian epidemiological investigations. Unfortunately, their quality leaves much to be desired. Of the same complexity was found the question about classification of radiogenic diseases. If one suggests a concept that the effects of low dose irradiation may base on mechanisms different from the mechanisms of high doses, the spectrum of diseases developed under the effect of low dose irradiation may be different. Unfortunately, the amount of data of this type is not enough yet.

The data by Japanese scientists show that many somatic diseases in groups of population irradiated by low doses may be of radiogenic origin. These points of view are discussed in the current Collection.

Being the editor of the monograph, I am sure that contents of Russian data will be interesting and useful for professionals and for other people paying attention to problems of interrelations between increased irradiation level and the biosphere state.

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The effect of low dose, low-level radiation on incidence rate and development of spontaneous leucosis in AKR mice

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ABSTRACT

The development of spontaneous leucosis in AKR mice is promoted by low dose (1.2 - 2.4 cGy), low-level irradiation (at 0.6 cGy/day dose rate): the incidence rate increases, deaths of animals - leucosis-carriers occur earlier, which results in shortening the average and maximum life-span of the animals. The dynamics of changes in the mass of the immune system organs (thymus and spleen) has extreme points. The moment of reaching extreme points correlates with time of maximum death rate of the animals.

At present, the effects of low-level irradiation on living organisms became the most urgent in the scope of environmental problems. The investigation of regular trends of low dose irradiation impact is of special importance. According to the studies carried out in Emanuel Institute of Biochemical Physics, Russian Academy of Sciences (Burlakova, 1994), a high-dose irradiation impact on biological objects may be comparable with the effect produced by doses that are several orders of magnitude lower. In this case, the dose-effect dependence of irradiation is non-monotonic and nonlinear. These results are of practical importance, since they may be a factor for choosing models for assessment of radiation risks and working out countermeasures. In the radiation medicine, linear dose dependence is used for evaluating the risks of radiation-induced malignant tumors. This choice of a model is claimed to answer the human purposes: to avoid underestimation of the risk. Nevertheless, some radiobiologists believe that although low dose irradiation below a certain level

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(threshold) produces some effect on biological objects, but the effect is not harmful. However, comprehensive studies of the action of low doses of physical and physicochemical agents indicated this view to be erroneous and alarming (Burlakova *et al.*, 1996).

The objective of this work is to evaluate the effect of low dose γ -irradiation on the occurrence and development of malignant tumors - one of the most harmful long-term biological aftereffects of irradiation. For the tumor growth model we chose leucosis, because it develops spontaneously (it is important) in 65 - 90% AKR line mice aged 6 - 11 months (refer to: *Laboratory Animals Lines for Medicobiological Studies*, 1983). This line of mice with high cancer frequency (instead of the line with low cancer frequency, which is usually the object of most studies) was chosen for comprehensive study of generation (tumor producing transformation of cells) and development of malignant tumors induced by low dose irradiation.

It should be noted that origination, clinical symptoms, and pathological and morphological parameters of spontaneous leucosis in mice are similar to that of leucosis in man (Kassirskii, 1964; Bergoltz and Rumyantsev, 1966). A detailed study of leucosis development kinetics by a number of indices was carried out by Belich and Erokhin (1972). The disease is characterized by leucotransformation of thymus cells, induced by leucosis virus in mice. For a certain age of animals, this transformation is associated with the changes in a number of physicochemical parameters of healthy cells (active oxygen form formation rate, activity of antioxidant enzymes, antiradical and antioxidant activity of lipids, properties of plasmatic membranes in cells - composition, viscosity, etc.), which makes possible the use of these parameters in determining time and dose of irradiation of animals (Burlakova and Molochkina, 1973; Zhizhina *et al.*; Vartanyan *et al.*, 2000). By these parameters the highest sensitivity of animals to irradiation was determined for the age of 3 - 4 months. These data and results of preliminary studies of biochemical parameters of organs and tissues of intact AKR line mice, carried out during spontaneous leucosis generation, allowed the choice of conditions (doses and time) for the most effective irradiation of mice.

MATERIALS AND METHODS

In this work, about 200 AKR female mice were used. The mice were provided by Scientific Research Laboratory of Experimental and Biological Models, Russian Academy of Medical Sciences.

The animals aged 3 to 4 months were γ -irradiated (^{137}Cs) at the State Scientific Center: Institute of Biophysics (1.2 - 2.4 cGy doses, 0.6 cGy/day dose

rate, 2 - 4 days irradiation period). In view of the fact that a stress (similar to irradiation) promotes generation of active oxygen species, control animals were subject to transportation (required to irradiate the experimental animals) together with the experimental group.

Leucosis was diagnosed post mortem in the pathological anatomy studies of thymus and spleen of dead animals. We determined the life-spans, leucoses frequency in the control and experimental groups (the irradiation effect), and changes in thymus and spleen. Before and after the experiments involving irradiation of AKR mice and during leucosis development, a series of biophysical and biochemical studies was performed. The results will be published later. These studies will help to answer the question, what irradiation effect (promoting or inducing) was prevailing in a leucosis process in each particular experiment.

Based on the life-span data, corresponding curves of survival (share of survived animals - age) were plotted. Quantitative analysis of the curves involved a nonlinear approximation of the Hompertz function

$$S(t) = \exp\left[-\frac{h_0}{\gamma}(e^{\gamma t} - 1)\right],$$

where $S(t)$ is the share of survived animals at age t ; γ and h_0 are the function parameters (Konradov and Kutyakin, 1988). The process rate depends on γ parameter.

For a numerical approximation, the Gauss-Newton nonlinear least-square method was used. In the calculations, including statistical ones, the MATLAB mathematical package was used.

RESULTS AND DISCUSSION

Figure 1 shows data on the irradiation effect on AKR female mice; corresponding approximation of these data by the Hompertz function is shown in Figure 2. Curve 1 shows the survival rate (the share of survived animals depending on the age) for mice irradiated by 1.2 - 2.4 cGy doses; curve 2 shows the survival rate for intact animals.

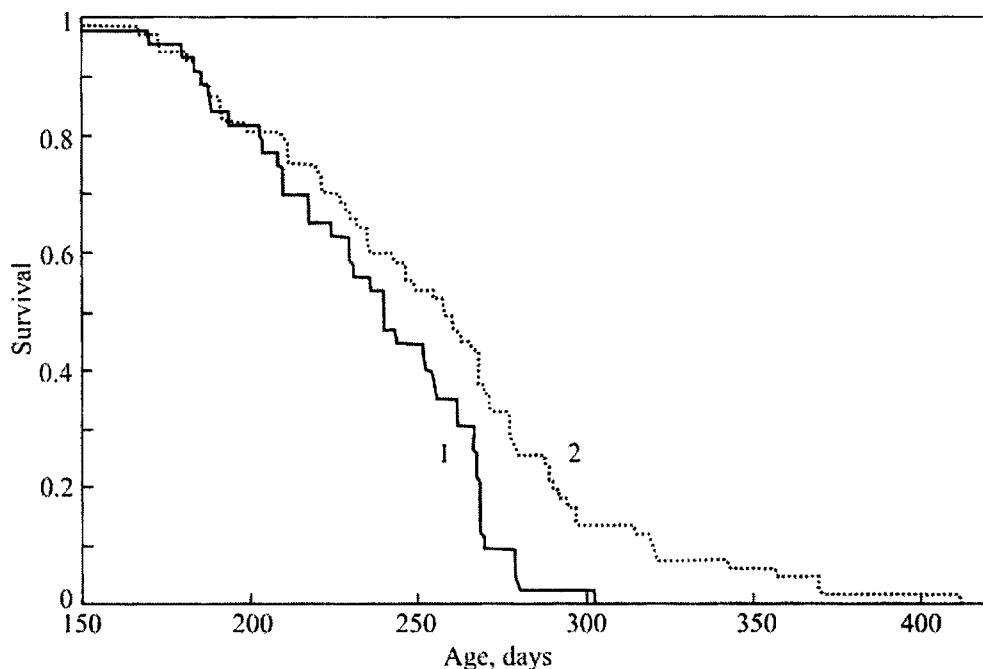


Figure 1. Survival rates of (1) AKR female mice exposed to 1.2 - 2.4 cGy irradiation doses and (2) controls

Figures 1 and 2 show that irradiation of mice with the above doses results in a confident (confidence level $p < 0.05$) 1.3-fold acceleration of the leucosis process (the relationship between the maximum death rate from leucosis in the experiment and control).

The Hompertz function approximation allowed evaluation quantitatively the rate of leucosis process development (Figure 2). The so-called modal age (marked with a dark square in Figure 2) was calculated as follows:

$$\tau_{\text{mod}} = \frac{\ln \gamma - \ln(h_0)}{\gamma}.$$

This is the age at which the function of survival yields a maximum slope (the infliction point of the curve) and the death rate of the population reaches the maximum (mode). The lower the modal age is, the higher the rate of development of the leucosis process occurs; the difference between these values:

$$\Delta \tau_{\text{mod}} = \tau_{\text{mod}}^{\text{contr}} - \tau_{\text{mod}}^{\text{exp}}$$

in the control and experiment may characterize the effect quantitatively. Table 1 shows that irradiation produces a significant promoting effect ($\Delta\tau_{\text{mod}} = 18.7$ days).

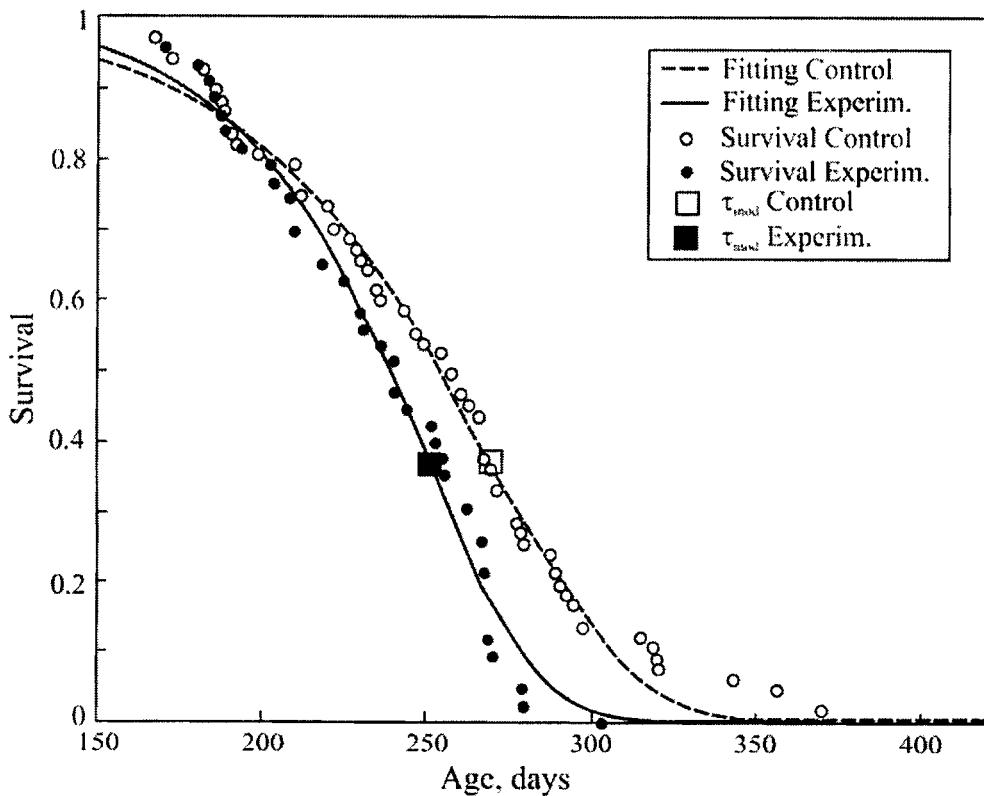


Figure 2. Survival rates approximated using the Hompertz function: in (1) experiment and (2) control

Basing on obtained approximation parameters, the average life-span (LS_{theor}) was calculated by the formula

$$LS_{\text{theor}} = \frac{\ln\gamma - \ln(h_0) - 0.577}{\gamma}$$

(see Table 1). For comparison, the experimental average life-span calculated by the standard formula is also shown in Table 1: obviously, the experimental promoting effect of irradiation on leucosis process resulted in a decrease in the average and maximum life-spans (almost by 20 and 120 days, respectively). In addition, note an increase in the percentage of leucosis cases after single γ -irradiation with 1.1 - 2.4 cGy doses: 80% and 82% in the control and

experiment, respectively ($p < 0.1$; in some experiments, differences were more pronounced). Experimental studies of the irradiation effect on leucosis development in male mice (the data are not presented here) showed that percentage of irradiation-induced leucoses increased in this case (from 64% in the control to 77% in the experiment).

Table 1
Dynamic parameters of spontaneous leucosis development in AKR female mice in control and after exposure to low-level irradiation doses of 1.2 - 2.4 cGy

Survival curve parameters	Experiment	Control
h_0	1.665×10^{-5} , 95%	4.585×10^{-5} , 95%
	confidence interval $[0.77 \pm 2.299] \times 10^{-5}$	confidence interval $[3.551 \pm 5.885] \times 10^{-5}$
γ	0.0296, 95%	0.0229, 95%
	confidence interval $[0.0278 \pm 0.0325]$	confidence interval $[0.0218 \pm 0.0240]$
Modal age, days	251.3	270.0
$\tau_{\text{mod}}^{\text{contr}} - \tau_{\text{mod}}^{\text{exp}}$	18.7	
Average life-span (theor.), days	232.2	244.8
Average life-span (exp.), days	235.4 ± 7.2	254.4 ± 10.7

Apart from the death-rate curves for mice, we analyzed also the curves of changes in the mass of thymus and spleen, i.e. the main organs of the immune system (Figure 3, curves 1 and 2). Curve 1 features 2 distinct maxima: they refer to ages of 120 days (4 months since birth) and 210 days (7 months), respectively. A comparison between curves 1 and 2 (Figure 1) showed that the first peak refers to the end of the latent period on the survival curve. Supposedly, at this time, the strongest response of the immune system to leucosis initiation is induced, which results in increase in the mass of thymus and spleen. The second maximum on the kinetic curve of changes in the mass of thymus refers to the beginning of high death rate of animals from leucosis (about 20%).

In the course of question about the mechanisms of low-level irradiation effect on leucosis development, one should note the following important statement: the mechanisms of low dose chronic irradiation of living objects are significantly different from high dose mechanisms. Changes in biochemical and biophysical characteristics of the cell metabolism, induced by low dose radiation, are of informative, signal, regulatory type. There are many features of low-level irradiation action.

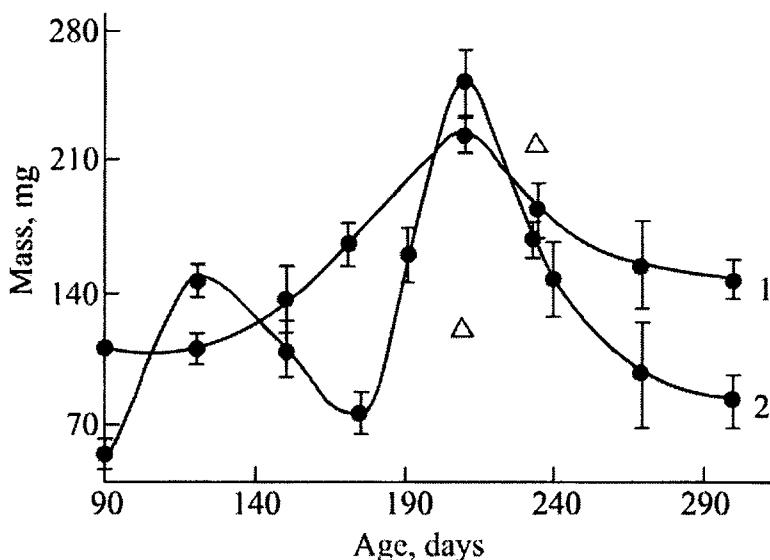


Figure 3. Kinetic curves of changes in the mass of (1) spleen and (2) thymus during leucosis development in intact AKR female mice

One of the main features of the low dose irradiation effect is its nonlinear, sometimes even non-monotonic, dependence on the dose and the dose rate. Some effects manifest themselves within narrow dose and dose rate ranges. This became evident from comparison of data obtained by the authors and Japanese scientists (Ishii *et al.*, 1996). The results were processed with the same statistical methods. Ishii *et al.* (1996) subject AKR mice to fractionated chronic irradiation with 5 and 15 cGy doses three and two times a week, respectively, during 40 weeks (the cumulative doses received by animals were 600 and 1,200 cGy, respectively). In this case, the effect was opposite: an increase was observed in the average and maximum life-spans of animals - leucosis-carriers (by 20 - 30 and 140 days, respectively); the percentage of leucosis incidence decreased. Thus, a comparison between our results and the data obtained by Japanese scientists showed that experimental low-level irradiation with varying irradiation mode may give opposite results.

CONCLUSION

The low-level irradiation effect (1.2 - 2.4 cGy doses at 0.6 cGy/day dose rate) on the incidence rate and development of spontaneous leucoses in AKR mice was studied. Irradiation resulted in the increase in leucosis incidence rate, acceleration of the death rate among animals - leucosis-carriers, and shortening

of the average and maximum life-spans of sick animals. Kinetic curves of changes in the mass of immunocompetent organs (thymus and spleen) were extreme in character. Time of reaching maxima correlated with the maximum death rates of animals.

The authors are grateful to A.A. Konradov and A.V. Krementsova for the help in mathematical processing of the experimental results.

REFERENCES

1. Belich E.I., Erokhin V.N., and Emanuel N.M., *Izvestiya AN SSSR, Ser. Biol.*, pp. 204 - 212 (1972). (Rus)
2. Bergoltz V.M. and Rumyantsev N.V., *Comparative Pathology and Etiology of Human and Animals Leucoses*, 1966, Moscow, Meditsina. (Rus)
3. Burlakova E.B. and Molochkina E.M., *Biofizika*, 1973, vol. **18**(2), pp. 293 - 298. (Rus)
4. Burlakova E.B., Goloshchapov A.N., Gorbunova N.V. et al., *Radiats. Biol. Radioekol.*, 1996, vol. **36**, pp. 610 - 631. (Rus)
5. Burlakova E.B., *Vestnik RAN*, 1994, vol. **64**(5), pp. 425 - 431. (Rus)
6. Ishii K et al., *Radiat Res.*, 1996, vol. **146**(5), pp. 582 - 585.
7. Kassirskii I.A., *Introduction into Clinical Hematology*, 1964, Moscow, Meditsina. (Rus)
8. Konradov A.A. and Kutyakin V.A. *Human Life-Span Distribution and Historical Dynamics* (preprint), 1988, Chernogolovka. (Rus)
9. *Laboratory Animals Lines for Medicobiological Studies*, 1983, Moscow, Nauka, pp. 50 - 53. (Rus)
10. Vartanyan L.S., Gurevich S.M., Kozachenko A.I., et al., *Radiats. Biol. Radioekol.*, 2000, vol. **40**(3), pp. 285 - 291. (Rus)
11. Zhizhina G.P., Skalatskaya S.I., and Burlakova E.B., *Radiats. Biol. Radioekol.*, 1994, vol. **34**(6), pp. 759 - 762. (Rus)

Quality of medical information in the Russian National Medical and Dosimetric Registry (RNMDR) for the Bryansk, Kaluga, Tula and Oryol Oblasts

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Following the Chernobyl accident extensive territories of Russia were exposed to radioactive contamination, the worst affected of them were the Bryansk, Kaluga, Tula and Oryol oblasts. Residents of the contaminated territories have become the largest population group ever exposed to low radiation doses.

In the RNMDR cancer incidence and mortality on the contaminated territories is given special attention. For obtaining convincing results the study population should be fairly large and the study should be thoroughly organized.

We analyzed medical documents on cancer morbidity and mortality (CRF and DCC) for the quality of completion by RCs over five year period (1996-2000) in the Chernobyl contaminated areas.

Figures 1 - 12 show the quality of maintaining CRFs and DCC in different contaminated oblasts from 1996 to 2000 in terms of common and crude errors.

In the contaminated areas, as can be seen from the diagrams, CRFs and DCC are maintained quite competently, as compared to other RCs, and the number of major errors is limited, even though the size of the followed up population is considerable. A relatively high proportion of minor errors in DCCs in 1995-1997 (65% of all submitted documents) was mainly associated with more stringent requirements of the Registry to coding of diseases and death causes relative to the general health care system: mandatory 4 digits in ICD code and two codes for death from external causes. In CRFs the dominant minor error was absence of tumor stage by TNM system. The increasing number of errors observed in the latest years is explained by transition to the ICD-10 coding system.

For comparison of pre- and post-accidental rates individual data for the period 1981-1999 were verified at the state level of the RNMDR. In doing so, the verification procedure was streamlined towards more detailed analysis of

medical information using additional and missing data received from RC on request from the state level RNMDR (abstracts from case history, patient's record). Table 1 shows the number of verified CRFs prepared by RCs of the contaminated oblasts (Kaluga, Tula, Oryol, Bryansk) in the period from 1981 to 1999.

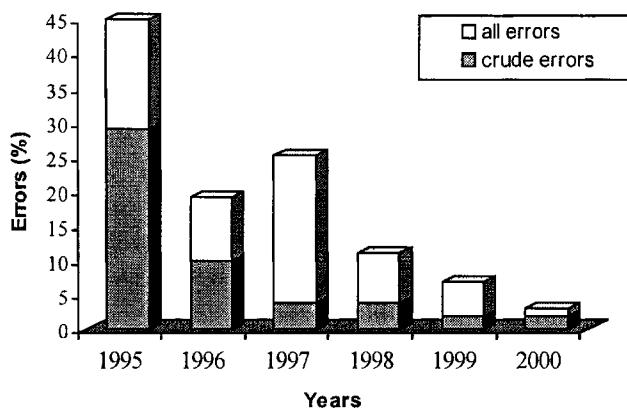


Figure 1. Time changes in the structure of errors detected in DCCs, the Kaluga oblast registry

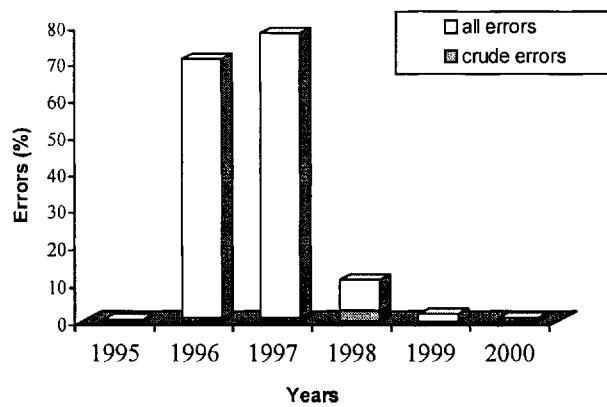


Figure 2. Time changes in the structure of errors detected in CRFs, the Kaluga oblast registry

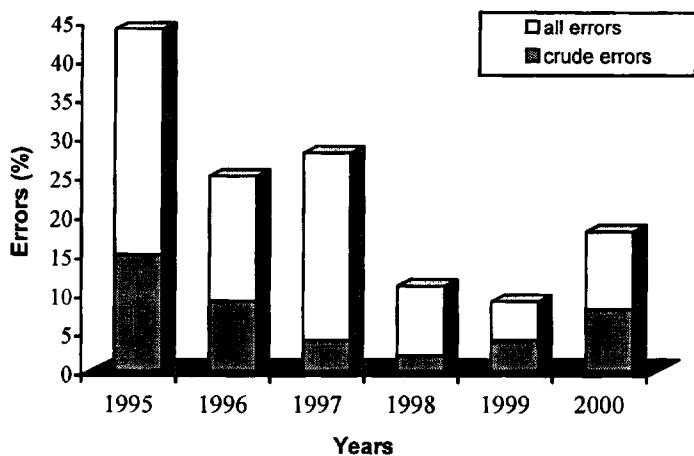


Figure 3. Time changes in the structure of errors detected in DCCs, the Bryansk oblast registry

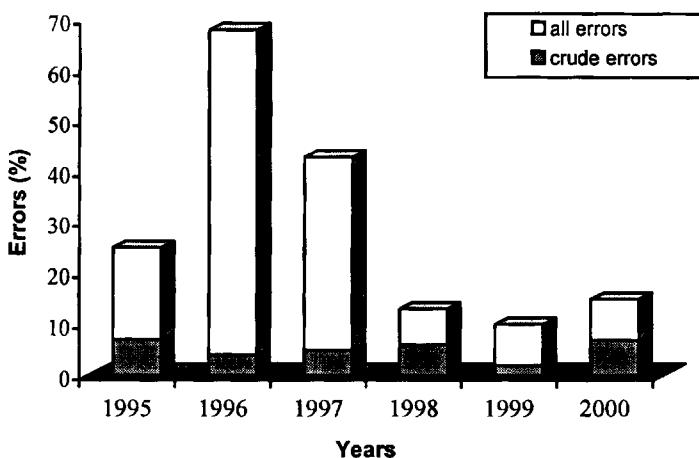


Figure 4. Time changes in the structure of errors detected in DCCs, the Tula oblast registry

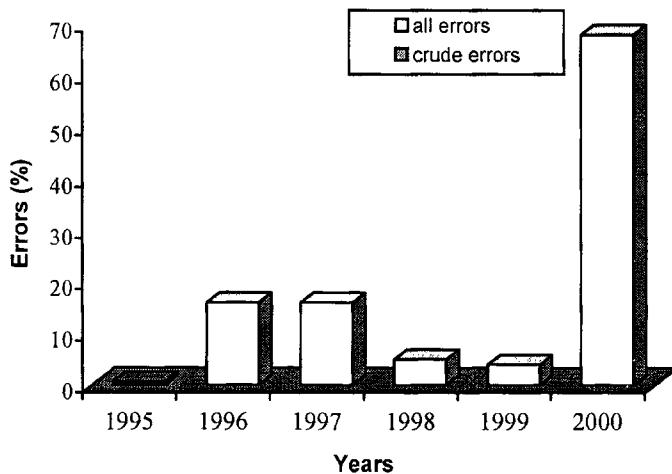


Figure 5. Time changes in the structure of errors detected in CRFs, the Tula oblast registry

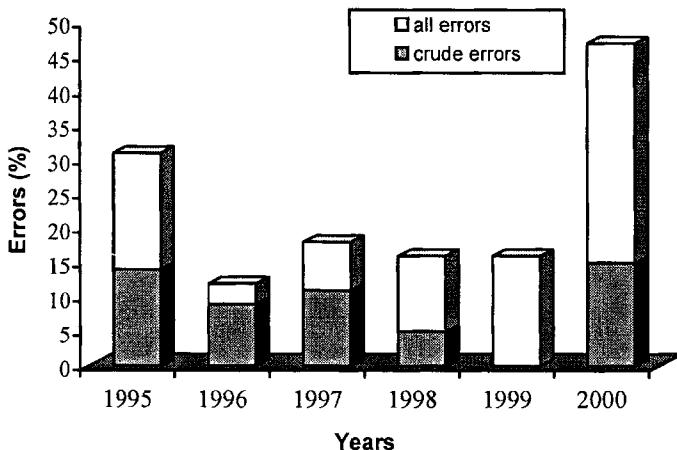


Figure 6. Time changes in the structure of errors detected in DCCs, the Oryol oblast registry.

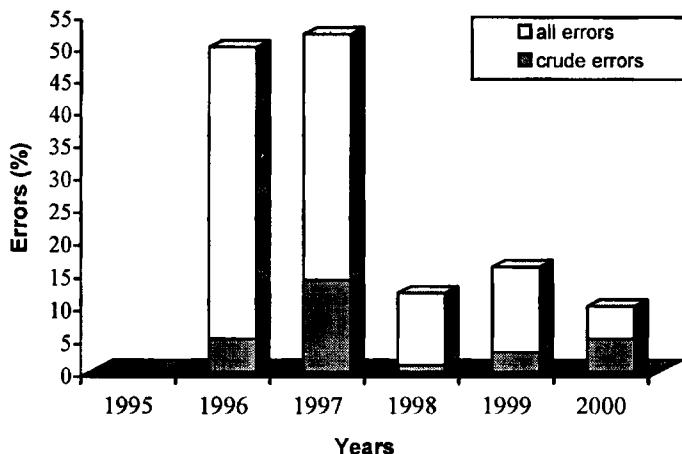


Figure 7. Time changes in the structure of errors detected in CRFs, the Oryol oblast registry

As can be seen from Table 1, a total of 12813 documents were received from the contaminated territories, of them 7540 (58.8%) have been verified. For the Kaluga, Tula and Oryol oblasts 100% of documents have been verified. The documents from the Bryansk oblast were subjected to random checks for 50% of cases. To study the incidence in the pre-accidental period retrospective cases since 1981 were also analyzed and subjected to verification.

Table 2 shows results of the RNMDR quality control of cancer registration in RCs in the contaminated areas.

As can be seen from Table 2, the number of major errors in CRFs decreased from 7.2% to 1.3% as a result of the verification.

The profound checks of registration of cancer cases in the contaminated territories of Russia by the RNMDR population radiation epidemiology laboratory provide a basis for improving the technology of quality control of primary medical documents in the Kaluga, Tula, Oryol and Bryansk oblasts and the Registry itself. To this end, we regularly conduct additional studies of various aspects of quality control.

Table 1
Number of verified cancer cases in the contaminated regions (oblasts) of Russia between 1981 and 1999

	1981-1996	1997	1998	1999	1981-1999	Verified
Kaluga oblast	398	157	151	128	834	834 (100%)
Tula oblast	494	139	112	140	885	885 (100%)
Oryol oblast	173	121	156	98	548	548 (100%)
Bryansk oblast	7187	1127	1245	987	10546	5273 (50%)
Total	8252	1544	1664	1353	12813	7540 (58.8%)

Table 2
Results of verification of malignant neoplasms in the worst contaminated oblasts of Russia

Data quality control results	1981-1996	1997	1998	1999
Number of verified cases	8252	1544	1664	1353
Completed TNM fields (%)	49.8	65.3	67.6	68.3
Available histology statement (%)*	75.9	84.2	89.2	92.8
Errors in 4-th digit (%)	29.1	17.4	11.0	10.1
Crude errors (%)	7.2	3.5	1.6	1.3

* - including negative results.

The medical unit of the RNMDR performed expert checks in the Kaluga and Bryansk oncological dispensaries as part of on-site verification. These efforts were focused on using retrospective data, employing identical methods of data collection and linking collected information with the cancer subregistry data.

Checks for consistency of CRFs and form 030-6/U were performed by the MRRC experts in oncological dispensaries by random sampling of primary documents.

The study objects were 4 cancer sites (stomach, trachea, bronchus, lung, colon, breast) in residents of 5 worst contaminated regions of the Bryansk oblast (about 110,000 persons) and three regions of the Kaluga oblast (about 40,000 persons). The contaminated regions of the Bryansk oblast were the following: Novozybkovsky, Krasnogorsky, Zlynkovsky, Gordeevsky and Klintsovsky. The worst contaminated regions of the Kaluga oblast are Ulyanovsky, Zhizdrinsky and Khvastovichsky. For comparison the regions of the Kaluga oblast with low contamination level were selected as control regions, namely Babyninsky, Baryatinsky and Tarussky with the total population of about 40,000 people.

Kaluga oblast

As mentioned above, the worst contaminated regions in the Kaluga oblast are Ulyanovsky, Zhizdrinsky and Khvastovichsky. In these regions the ^{137}Cs contamination level, on the average, is 18 times higher than in the uncontaminated.

Primary registration of patients is based on referrals from oblast hospitals to the Kaluga OD in case a diagnosis needs to be confirmed. Alternatively, the oncological dispensary receives "Notification of a first time cancer" (form 090/U) from region hospitals and clinics of Kaluga, or other medical facilities. These documents are used for releasing "Patient's record" (form 025-U) with an assigned number by which a patient can be traced later.

The patient's record contains all information about the patient. In the past years, in all oncologist rooms "Control forms of dispensary follow-up" (form 030-6/U) were maintained and all hospital visits and follow-up were documented there. With the system of unified dispensary follow-up abolished and computer database set up in OD, these forms are done away with. Required information can be obtained from patient's record in the registration department

or from the database of the Kaluga OD. Access to the database became possible since 1994 when computer cancer registration was introduced in the Kaluga OD.

Besides the computer database the medical statistics department maintains a catalogue of forms for patients registered in OD, by region of residence, with information identical to form 030-6/U. At the end of the year or beginning of next year, a medical nurse from a region hospital brings a list of deceased patients (according to a region vital statistics office) and their records are withdrawn and transferred to the OD archive where they are to be stored for 25 years. If information on death of the patient is received in OD in the course of the year, his registration form is put aside, but his medical record is not withdrawn until confirmation comes from the region vital statistics office. The database is entered with such information only after full evidence about death is received.

During the checks for data quality and completeness in the Kaluga OD the registry staff studied medical records of the patients currently registered and followed up, as well as deceased patients who were diagnosed cancer in the period 1980 to 1998. Data of the oblast cancer registry since 1994 were also used.

To check for completeness medical records of about 500 patients were selected in the OD registration department by random sampling. Data were abstracted from the medical records of patients followed up in the OD and having the diagnoses of cancer of stomach (ICD-9 code 151), trachea, bronchus and lung (ICD-9 code 162), colon (ICD-9 code 153) and breast (ICD-9 code 174). The abstracts were compared with data in the database (data since 1994). Particular attention was given to transfer of data from patients' records to primary documents of the RNMDR, specifically consistency of diagnosis date, code, diagnosis confirmation method and disease stage.

The checks also included scrutiny of documents for deceased cancer patients with the above cancer localizations. In the Kaluga OD death is documented based on hospital epicrisis, or "Notification" (form N 090/U) with the flag "postmortem registration", or form N 030-6/U, or abstract from the vital statistics office, or death certificate. Data on death are entered in the patient's record or such a record is opened postmortem.

In the Kaluga OD the records of deceased patients are stored in the archive and classified by death date. All available medical records of deceased cancer

patients diagnosed malignancy from 1980 to 1998 were selected from the archive and screened. These patients lived in both the contaminated and control areas.

An alternative source of data on morbidity and mortality among cancer patients are findings of the pathology department of the dispensary where preparations are stored for 25 years. For out-patients the logs contain passport data, birth date, residence place, biopsy date, description of preparation and number of case history for inpatients.

Finally, the following variables were selected and verified for cancer cases in the contaminated and control areas:

- Name,
- Place of residence,
- Sex,
- Age,
- Diagnosis,
- Date of diagnosis,
- Principal method of diagnosis confirmation,
- Site of primary tumor,
- Tumor morphology (histology),
- Information source.

As part of the quality control of information gathered in the three contaminated regions during 1980-1997, records describing methods used for cancer diagnosis verification were scrutinized. In doing so, retrospective archived data were screened and the data of cancer-registry after 1994 were used. Results of the analysis are summarized in Table 3.

As can be seen from Table 3, 57.8% of cases had histological confirmation, the percentage increasing over the years. The role of instrumentation methods (endoscopy, ultrasonic examination) is growing, as hospitals become better equipped and more advanced techniques are used: endoscopy for digestive system and lung cancer, ultrasonic examination for stomach, thyroid and breast cancer. X-ray examination remains to be an important method of malignancy diagnosis, in particular with respect to brain and lung cancer.

Table 3

**Confirmation of cancer diagnosis for three worst contaminated regions of the Kaluga oblast
(Zhizdrinsky, Ulyanovsky, Khvastovichsky)**

Diagnosis confirmation method		Periods			Total
		1980-1985	1986-1991	1992-1997	
	abs.	572	736	930	2238
Histology	%	44.2	60.5	64.0	57.8
Cytology	%	5.1	5.3	2.8	4.2
X-ray examination	%	37.4	22.9	21.4	26.0
Endoscopy	%	1.4	3.2	5.5	3.7
Ultrasonic examination	%	0.9	2.2	1.9	1.7
At surgery	%	2.4	2.8	0.4	1.7
Clinical only	%	10.3	5.8	9.7	8.6

The percentage of cases confirmed only clinically remains to be fairly high, which is mainly explained by lack of capabilities for detailed study of advanced stages of cancer, old age or refusals to be examined or treated, which often happens among the rural population.

Thus, our data show that morphological (histology and cytology) confirmations are available for 66.8% (average 62%) of cases of all malignant neoplasms. About 1% of cases were registered postmortem. By statistics, for patients with first time cancer 70% of diagnoses are morphologically confirmed, the value varying from 60 to 90% in different oblasts of Russia.

Bryansk oblast

As mentioned above, the worst contaminated were the following five regions of the Bryansk oblast: Novozybkovsky, Krasnogorsky, Zlynkovsky, Gordeyevsky, Klintsovsky. It has to be said that the Gordeyevsky region was formed only in 1986 and therefore data were collected starting from 1987. The Zlynkovsky region did not exist earlier and data are available starting from 1989.

Data on cancer incidence for 4 most common localizations (stomach, trachea, bronchus and lung, colon, breast) were gathered and selectively verified. These data cover the period from 1980 to 1998 and were collected in five worst contaminated regions of the Bryansk oblast.

To verify the quality of cancer incidence data supplied from the Bryansk RC experts of the RNMDR were sent on missions to the Bryansk oblast oncological dispensary. Together with OD specialists they studied medical documents relating to cancer cases (individual data).

First, data of forms N 12 were grouped by age and sex for the period 1980-1986 and compared with individual data. The difference between the grouped and individual data for 1980-1986 was insignificant because retrospective information for the indicated period was entered in the latest years.

In the Bryansk oblast OD, like in the Kaluga oblast, cancer patients are registered by the procedure adopted in all hospitals and clinics of the Russian Federation. For each patient "Control form of dispensary follow-up" (form 030-6/U) is completed. These forms contain data on referrals, examinations and tests, disease progression and treatment. Forms 030-6/U are stored in the registration department of the Bryansk oblast OD and after patient's death are handed over to the dispensary archive. We selectively checked forms 030-6/U for alive and deceased patients from five contaminated regions with the diagnoses of stomach, lung, colon and breast cancer.

Random checks were conducted for the quality of individual data on cancer of four localizations (stomach, lung, colon and breast) for the periods from 1980 to 1998 and separately for three other periods (1980-1985, 1986-1991, 1992-1998) in five contaminated regions of the Bryansk oblast. The proportion of random sample with respect to the study group was determined based on prevalence of malignant neoplasms and estimates of sensitivity and specificity of the calculation methods. For this purpose, not less than 25% of cases were selected by random sampling from all primary documents for patients with considered cancer sites diagnosed in a given period. Thus the number of screened cases was the following: for stomach cancer 190 (of 858) primary documents for those who developed the disease in 1980-1985, 184 (of 552) - in 1986-1991 and 284 (of 880) - in 1992-1998; for trachea, bronchus and lung cancer - 111 (of 465) for 1980-1985, 128 (of 416) - in 1986-1991 and 218 (of 701) - in 1992-1998; for colon cancer - 48 (of 123) for 1980-1985, 42 (of 97) - in 1986-1991 and 65 (of 203) - in 1992-1998; for breast cancer - 56 (of 223) for 1981-1985, 106 (of 202) - in 1986-1991 and 110 (of 368) - in 1992-1998.

Techniques used for confirmation of cancer diagnosis were subjected to study. Given several techniques used for diagnosis confirmation, morphological conformation was preferred.

The comparative analysis of confirmations of cancer diagnoses by the morphological method in 1980-1985 was based on using data for the last year of each study period (1985, 1991, 1998) available in the State Statistics Committee for the Bryansk oblast and Russia in general. Table 4 includes results of verifying the records of the Bryansk OD for patients with cancers of stomach, colon, lung and breast in five contaminated regions of the Bryansk oblast.

As can be seen from Table 4, the percentage of morphologically confirmed stomach cancer cases among the population of the contaminated areas of the Bryansk oblast in 1980-1985 is practically the same as in the Bryansk oblast in general (21.5%). In the following period 1986-1990 the proportion of morphologically confirmed stomach cancers was increasing in the contaminated regions as compared with the oblast in general (46.7% against 34.9%). At the same time, the percent of morphologically confirmed stomach cancer in the oblast in general and in the contaminated areas is much lower than in Russia in general (63%). This might be explained by a considerable percentage of older rural residents among the whole population of the Bryansk oblast who delay with attending hospitals and often refuse to be examined and treated. The percentage of morphologically confirmed stomach cancer cases among the population of the Bryansk oblast in 1992-1998 is consistent with data for the RF as a whole (67.1% and 70.9%, respectively) but higher than in the Bryansk oblast as a whole (67.1% against 59.8%). It should be noted that the level of

morphologically confirmed diagnoses of stomach cancer in the contaminated areas is close to the data of 1998 for the RF, with the lag observed at the end of the previous period (1986-1991) decreasing. Over all considered periods the level of morphologically confirmed cases of stomach cancer in the contaminated areas remains higher than in the Bryansk oblast in general.

Figure 8 shows morphologically confirmed cases of stomach cancer in the contaminated areas by study periods, as compared to data for the Bryansk oblast as a whole and Russia in general.

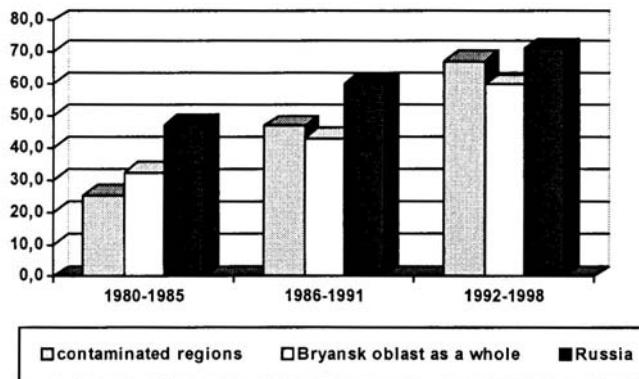


Figure 8. Morphologically confirmed cases of stomach cancer in the contaminated areas by studied periods, as compared to data for the Bryansk oblast as a whole and Russia in general

The study of stomach cancer by periods suggests that the level of morphologically confirmed diagnoses of stomach cancer among residents of the contaminated regions in the third period is close to the level of 1998 for the RF, and the difference observed at the end of the previous period (1986-1991) became smaller. During all the periods considered the level of morphologically confirmed cases of stomach cancer in the contaminated areas was higher than in the Bryansk oblast in general.

Table 4

Confirmation of diagnosis of cancer of stomach, lung, colon and breast in 5 worst contaminated regions of the Bryansk oblast in the periods 1980-1985, 1986-1991, 1992-1998

Diagnosis confirmation method	Period	1985		1986-1991	1991		1992-1998	1998	
		Bryansk	Russia		Bryansk	Russia		Bryansk	Russia
<i>Stomach</i>									
Verified number of cases	abs.	190			184			284	
	%	100			100			100	
Morphological X-ray	%	25.3	21.5	47.2	46.7	34.9	63.0	67.1	59.8
	%		67.8			48.5			29.2
Other (without surgery, clinical only etc.)	%		6.9			4.8			3.7
<i>Trachea, bronchus and lung</i>									
Verified number of cases	abs.	111			128			218	
	%	100			100			100	
Morphological X-ray	%	24.3	10.0	34.6	34.3	21.0	45.6	43.4	44.8
	%		70.3			62.6			55.8
Other (without surgery, clinical only etc.)	%		5.4			3.1			0.8

Table 4 (continued)

<i>Colon</i>									
<i>Verified number of cases</i>	abs.		48		42		65		
	%		100		100		100		
Morphological	%	37.1	38.0	57.3	52.4	32.6	67.5	64.6	71.3
X-ray	%		56.2			42.8			28.7
Other (without surgery, clinical only etc.)	%		6.7			4.8			6.7
<i>Breast</i>									
<i>Verified number of cases</i>	abs.		56		106		110		
	%		100		100		100		
Morphological	%	96.2	83.5	91.4	91.4	80.5	93.0	94.6	92.9
X-ray	%		-			-			1.2
Other (without surgery, clinical only etc.)	%		3.8			8.6			4.2

On the other hand, as can be seen from Figure 8, between 1991 and 1998 the percent of morphologically confirmed cases of stomach cancer increased by more than 20.4% (from 46.7% to 67.1%) similar to the period 1985 to 1991 (from 25.3% to 46.7%). Thus between 1985 and 1998 the level of morphologically confirmed cases of stomach cancer increased by 41.8% (from 25.3 to 67.1%) in the contaminated regions of the Bryansk oblast, by 27.6% (from 32.2% to 59.8%) for the Bryansk oblast as a whole and 23.7% (from 47.2% to 70.9%) for Russia in general. However, this indicator, as mentioned above, both in the contaminated regions and the Bryansk oblast in general remains below a corresponding level in Russia throughout the studied period.

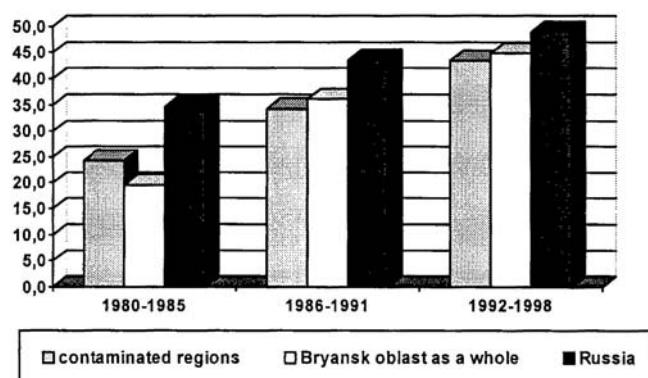


Figure 9. Morphologically confirmed cases of trachea, bronchus and lung cancers in the contaminated areas by studied periods, as compared to data for the Bryansk oblast as a whole and Russia in general

For cancers of trachea, bronchus and lung (Table 4) the percentage of morphologically confirmed cases in the sample from the contaminated regions in 1980-1985 is 24.3%, which is much higher than in the oblast in general in the same period (about 10.0%). During 1986-1991 this gap was reduced (34.3% in the contaminated regions and 21.1% in the oblast as a whole), nevertheless the level of morphologically confirmed cases in the contaminated regions remains higher than in the oblast in general. In Russia in this period (1986-1991) the level of morphologically confirmed cases of lung cancer was higher (45.6%). In the period from 1992 to 1998 the level of morphologically confirmed cases in the contaminated regions of the Bryansk oblast was 43.4%, which is actually

close to the level of the Bryansk oblast as a whole (44.8%), but somewhat lower than in Russia (48.9%).

Figure 9 shows data on morphologically confirmed cases of trachea, bronchus and lung cancers in the contaminated areas by studied periods as compared to data for the Bryansk oblast as a whole and Russia in general.

As can be seen from Figure 9, with respect to cancers of trachea, bronchus, lung and stomach the level of morphologically confirmed cases in the contaminated areas was increasing during all studied periods and this variable tends to become closer to the all-Russia level. In the post-Chernobyl period the percentage of lung cancers with morphological confirmation increased by 19.1% (from 24.3% to 43.4%) in the contaminated areas, by 25.2% (from 19.6% to 44.8%) in the oblast as a whole and 15.3% (from 34.6% to 48.9%) in Russia in general.

Data on the colon cancer incidence (Table 4) in the contaminated areas and the whole oblast in 1980-1985 were actually identical: the percentage of morphologically confirmed diagnoses was 37.1 and 37.6, respectively. At the same time, in the period from 1986 to 1990 the contribution of morphologically confirmed cases in the contaminated regions increased by 52.4%, whereas the value decreased to 32.6% in the oblast in general. In 1991 the indicator for Russia in general for a given localization was 67.5%. The low percentage of this variable in the Bryansk oblast as a whole, as compared to Russia in general could be associated with the lack of endoscopic equipment and trained specialists at that time. In 1992-1998 data on colon cancer in the contaminated areas and the Bryansk oblast as a whole were similar: the percentage of morphological confirmations of this diagnosis was 64.6 and 67.4, respectively, whereas in 1998 in Russia in general it was 71.3% for a given localization. The situation was similar for the above indicated cancer types.

Figure 10 presents data on morphologically confirmed colon cancer cases in the contaminated areas by studied periods, as compared to data for the Bryansk oblast as a whole and Russia in general.

As can be seen from Figure 10, between 1985 and 1998 in the Bryansk oblast the level of morphologically confirmed colon cancer cases increased by 27.5% (from 37.1% to 64.6%) in the contaminated areas and by 29.4% (from 38.0% to 67.4%) in the oblast as a whole, most probably thanks to reinforcement of the health facilities with diagnostic equipment and skilled personnel, while in Russia in general the increase was only 13.9% (from 57.3% to 71.3%).

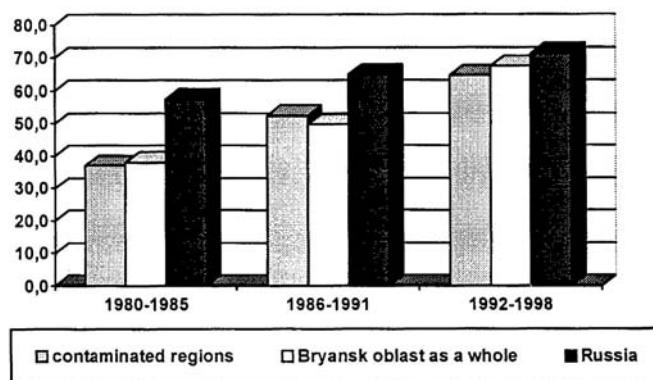


Figure 10. Morphologically confirmed cases of colon cancer in the contaminated areas by studied periods, as compared to data for the Bryansk oblast as a whole and Russia in general.

The study of breast cancer (Table 4) shows that in 1980-1985 the percentage of morphologically confirmed cases was 96.2% in the contaminated regions and 83.5% in the Bryansk oblast in general. During the next six years this level somewhat decreased in the contaminated regions (to 91.4%), but was almost identical to the one for Russia in general (92-95%) and was higher than in the Bryansk oblast as a whole (80.5%). In the period 1992-1998 the level of morphologically confirmed cases of breast cancer was 94.6%. This is a little higher than in Russia (92.1%) and in the Bryansk oblast as a whole (92.9%) in 1998.

Figure 11 presents data on morphologically confirmed breast cancer cases in the contaminated areas by studied periods as compared to data for the Bryansk oblast as a whole and Russia in general.

As can be seen from Figure 11, a relatively high percentage of morphologically confirmed diagnoses of breast cancer cases in the contaminated areas occurring after the Chernobyl accident was also observed prior to 1986 (96.2%) and continues to be close to the all-Russia level. For the Bryansk oblast as a whole the variable is a little lower over the entire studied period, but increased by 9.8% (from 83.1 to 92.9%) during 1985-1998.

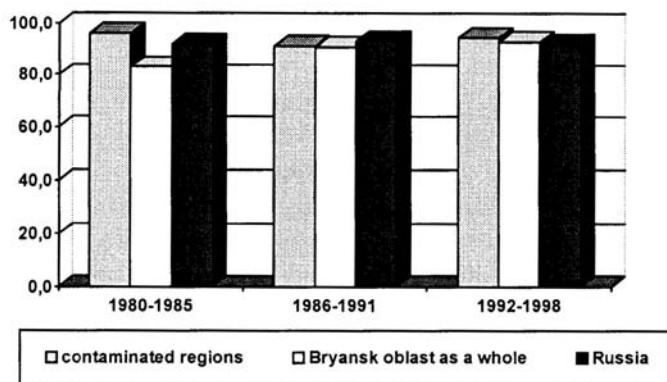


Figure 11. Morphologically confirmed cases of breast cancer in the contaminated areas by studied periods, as compared to data for the Bryansk oblast as a whole and Russia in general

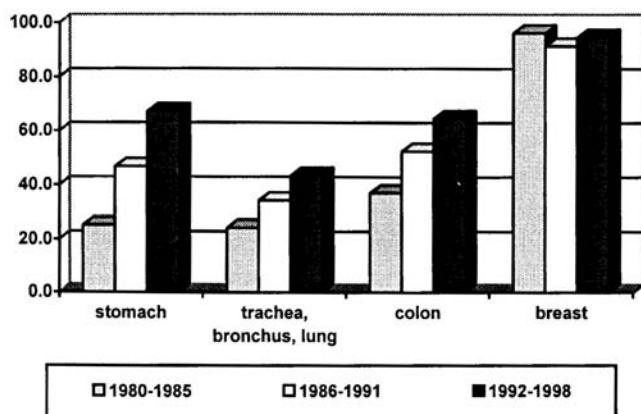


Figure 12. Results of verified morphological confirmations of cancers of stomach, colon, lung and breast in the population of five contaminated regions of the Bryansk oblast in the three periods from 1980 to 1998, as compared to data for the Bryansk oblast as a whole and Russia

Figure 12 shows an overall picture of verified morphological confirmations for cancers of stomach, colon, lung and breast among residents of five contaminated regions of the Bryansk oblast in the three periods from 1980 to 1998 and results of comparative analysis of data for different periods.

As follows from the obtained data (Figure 12), the level of morphologically confirmed cancer cases almost for all studied sites in the contaminated regions of the Bryansk oblast increases steadily with time. For breast cancer the level of morphological confirmations is actually stable during all follow-up periods.

The analysis of other methods used for confirmation of cancer diagnosis shows that the contribution of X-ray examination, as a principal diagnostic method, tends to decline for stomach, lung and colon cancers. This can be explained by advances in diagnostic equipment and increase in the number of morphologically confirmed cases.

Thus, it may be concluded that the information about cancer cases among residents of the Bryansk, Kaluga, Oryol and Tula oblasts available in the RNMDR database can be regarded as satisfying the requirements of epidemiological studies.

Summing up this paper it should be emphasized that the RNMDR medical and organizational provision system was set up and operates on the basis of the recommendations of the WHO and other national and international organizations including those dealing with quality control and quality management. Since 1993 a special medical unit was established within the Registry and works under the guidance of the laboratory of population radiation epidemiology. The staff of the laboratory has developed and introduced quality control criteria for primary medical information and performance of regional centers, developed technology for improving the quality of medical data and lay the groundwork for the quality assurance system as applied to medical information in Russia. The developed medical and organizational framework makes possible operation and development of territorially distributed system of RNMDR which is a reliable information base of radiation protection, health care and social security programs for persons living in Russia exposed to ionizing radiation and other harmful effects of the accident. The activity of the National Radiation and Epidemiological Registry points to the importance of the issue of quality of primary medical information, which forms the documentary base of the RNMDR. To date the country's health care system has no practical experience in medical and organizational provision to automated personalized registration systems of this kind. Therefore, the registry recommendations provide a

documentary base for the activity, define structure and amount of data to be registered and propose methodological approaches to gathering and verification of data, which may be useful for implementation of health care, social security and rehabilitation programs. The medical unit of the Registry places special emphasis on quality control of key epidemiological indicators of exposure to ionizing radiation, namely leukemia and thyroid cancer. Information about malignant neoplasms available in the RNMDR database is well organized and of high quality, it can be used for radiation-epidemiological analysis and is virtually fully confirmed by primary medical documents.

Cytogenetic effects in human beings exposed to irradiation on the Earth and in space

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ABSTRACT

This work represents a review of relative radiosensitivity of different biological markers used for bio-indication and biological dosimetry in radiobiological studies. Cytogenetic effects observed in lymphocytes of spacemen participated in space flights at the MIR station and ISS (37 spacemen) and the effects revealed in human beings exposed to irradiation during radiation situations on the Earth were compared. Expected genetic and carcinogenic risks of space flights determined in accordance with absorbed doses, calculated on the basis of unstable and stable chromosome aberration frequency, are presented.

INTRODUCTION

Natural background of ionizing radiation at the Earth surface consists of radioactivity of mountain rocks and outer space irradiation. The effective irradiation dose of the population from all natural sources equals, on average, 2.4 mSv per year (0.27 μ Sv/h); space irradiation at the sea level gives, on

average, 0.39 mSv per year. For 9 - 12 km altitudes at high latitudes, the effective dose rates fit the range of 5 - 8 $\mu\text{Sv}/\text{h}$, whereas for transatlantic flight from Europe to North America, the route dose would be 30 - 45 μSv . For 18 km altitude, the effective dose rates are 10 - 12 $\mu\text{Sv}/\text{h}$ [1]. According to common assessments, the annual irradiation dose during a space flight may reach 0.7 – 1.0 Sv/year (80 - 110 $\mu\text{Sv}/\text{h}$) [2]. As follows from the data shown, the effective dose intensity during a space flight (for example, to Mars) will be two orders of magnitude higher (by 300 – 400 times) than doses on the Earth surface.

In accordance with these concepts [1], irradiation doses received by human beings in different radiation situations may be arbitrarily classified as small (up to 200 mGy), medium (up to 2 Gy) and high (over 2 Gy) ones. Thus, the doses that may be received by spacemen during the flight should be assigned to classes of small and medium doses. Action of such doses may lead to stochastic (genetic damage, carcinogenesis) and determined radiation effects on the health of spacemen.

A broad variety of sensitive radiobiological methods were developed allowing registration of effects, caused by low dose irradiation. The lower sensitivity boundary of each method is, to a large extent, determined by the size of sensitive target on which observed effect depends (chromosome aberrations, gene mutations, etc.). At the same time, the progress in determining the lower sensitivity limit in the method depends on methodological elaboration of one method or another and the scope of performed experiments. The UNSCEAR report in 2000 [1] gives a review of biological effects of low dose irradiation and the lowest dose irradiation, at which chromosome aberrations and mutations were detected in biological objects exposed to low-LET irradiation. Such doses are 0.1 Gy for cell transformations in the C3H10T1/2 system, 0.01 Gy for pink-eye mutations in mice, and 0.0025 Gy for pink mutations in *Tradescantia*. Sensitive radiobiological methods are widely used in biological dosimetry. However, registration of unstable and stable chromosome aberrations [3, 4] is the method most frequently used in biodosimetry. The analysis of chromosome aberrations detection of increase in their frequency at relatively low irradiation doses: the lower sensitivity limit for unstable chromosome aberrations (dicentrics and centric rings) is 0.02 Gy, for stable aberrations (translocations) – 0.1 – 0.25 Gy [1]. This fact is crucial for determining wide application of cytogenetic methods in biological dosimetry in various emergency irradiation situations.

In connection with long interplanetary space flights, of importance is the study of radiosensitivity of various microorganisms, flora and fauna species that

may accompany human beings in such flights. The study of relative radiosensitivity of species demonstrates its dependence on the nucleus volume (interphase chromosomes). The species are subdivided into several radiotaxons in accordance with the complexity of genome biological organization [5]. During long-term space flights, chronic or prolonged exposure to radiation will usually dominate. The UNSCEAR report in 1996 [6] reviews sensitivity of flora and fauna representatives to chronic irradiation based on the analysis of Kyshtym and Chernobyl accidents and other extreme irradiation situations consequences. According to UNSCEAR assessments, in the most sensitive plant species the effect of chronic irradiation was observed for 1 - 3 mGy/h dose rates. Chronic dose rates below 0.4 mGy/h (10 mGy/day) will have effects, though slight, in sensitive plants but unlikely have significant deleterious effects in the wider range of plants in natural plant communities. For most sensitive species of animals, specifically mammals, there is an indication that 10 mGy/day dose rates for highly exposed individuals might seriously affect mortality of the population. For dose rates up to an order of magnitude less (0.04 – 0.1 mGy/h), the same may be stated with respect to reproductive effects.

Genetic effects in natural populations of animals and plants may be observed at 1 mGy/day (0.3 – 0.4 Gy/year) dose rate or higher [7, 8]. These the lower dose limits, at which radiobiological and genetic effects are observed in human beings, flora and fauna, suggest that for a long interplanetary flight 0.7 – 1.0 Sv/year irradiation doses may be expected. They may produce real radiobiological effects on spacemen as well as on microorganisms, plants and animals forming biocenosis of a spacecraft.

In this study the results of cytogenetic examinations of spacemen participated in space flights at the MIR station and ISS are compared with analysis results of cytogenetic effects in human beings exposed to irradiation in different radiation situations on the Earth. Of importance is to understand what genetic consequences of spacemen irradiation may induce in the following generations.

CYTOGENETIC EFFECTS OBSERVED IN SPACEMEN AFTER SPACE FLIGHTS

Cytogenetic studies of peripheral blood lymphocytes in spacemen before and after space flights were reported in several publications before [9 – 11]. The

main results of these studies are discussed below.

Totally, 133 blood samples from 37 spacemen were examined in the period 1992 – 2003, fifteen of whom participated in short-term flights (up to three weeks) and 22 - in long-term flights (73 - 199 days). Most of the examined spacemen took part in two, three and even four flights. Cytogenetic examinations were performed 1 - 2 months before and 24 hours after the flight. For cytogenetic analysis, blood samples were drawn within February 1992 and March 2003. The standard method [12] with some modifications [13] was used for cultivating blood lymphocytes and analyzing chromosome damages. Slides for conventional analysis were subject to standard fluorescence plus Giemsa (FPG) staining. On average, 900 cells were scored for each spaceman. A mixture of biotin-labelled DNA probes for chromosomes 1, 4 and 12 with digoxigenin-labelled pancentromeric probe was used for analysis of stable chromosomal aberrations (the FISH method). The staining of chromosomes was performed by the Pinkel method [14], modified by Bauchinger *et al.* [15]. Chromosome probes were removed with FITC-labelled streptavidin, and pancentromeric probes - with AMCA-labelled antibodies. Propidium iodide (PI) was applied to counterstaining. From 199 to 3,256 cells (1,500, on average) were analyzed for each spaceman. Complete and incomplete translocations (t_c , t_i) were scored. The results of cytogenetic analysis (conventional and FISH methods) were compared with the measurements of the onboard standard R-16 radiometer, reported by Mitrikas *et al.* [16]. To reveal common tendencies, the average characteristics of cytogenetic effects were estimated in different groups of spacemen formed with respect to flight duration and other criteria. The results obtained were analyzed using standard statistical methods. The Fisher exact test and Student *t*-criterion were used to compare group values.

Table 1 shows average frequencies of chromosome-type aberrations in peripheral blood lymphocytes of spacemen before their first flight and in Moscow region residents, who occupationally did not contact radiation sources. The yield of dicentrics plus centric rings was found 5 times exceeding the background. This may result from more frequent medical X-ray exposures the spacemen underwent during their pre-flight preparation. Thus, pre-flight cytogenetic examinations of spacemen are of great importance for assessment of the irradiation exposure in space.

Table 1

Cytogenetic pools for spacemen before the first flight (conventional method)

Group	Number of persons	Frequency of chromosome aberrations \pm SEM* (per 100 cells)			
		total	dic + R _C	ace	chr
Spacemen	37	1.33 \pm 0.12**	0.07 \pm 0.02**	0.52 \pm 0.04 ^a	0.74 \pm 0.09
Control (Moscow region residents)	115	0.84 \pm 0.09	0.013 \pm 0.005	0.30 \pm 0.05	0.53 \pm 0.07

* standard error of the mean

** statistically differed from the control group, $p < 0.05$ (Student's *t*-test)

dic – dicentrics; R_C - centric rings; ace – acentrics; chr – chromatid aberrations

Space flights result in an increase of chromosomal aberration yields. Table 2 illustrates cytogenetic examination of spacemen after the first space flight. Of special interest are dicentrics and centric rings being the biomarkers of irradiation exposure. Post-flight frequency of dicentrics and centric rings considerably exceeds the pre-flight level.

Moreover, the yield of dicentrics increases with duration of space missions. Thus, short-term space flights resulted in 2.5-fold increase and long-term flights - in more than 6-fold increase of dicentrics plus centric ring frequency compared with the pre-flight level. The results obtained confirm the conclusions that the yield of radiation-dependent chromosomal aberrations (namely, dicentrics and centric rings) statistically increases after the influence of space radiation, as reported in previous works [9 – 11, 17].

The biological dose assessment results for spacemen after first flight are shown in Table 2. Space irradiation doses absorbed by spacemen over their flights were estimated using self calibration curve for dicentrics (acute γ -irradiation at 0.103 Gy/min dose rate). The curve fits the linear-quadratic equation [18]:

$$y = 0.1 + 1.5x + 6.3x^2,$$

where y is the frequency of dicentrics per 100 cells; x is the dose, Gy.

Table 2

Cytogenetic results for spacemen before and after short- and long-term flights, as well as biological dose estimates (conventional method)

Group	Number of persons	Frequency of chromosome aberrations ± SEM (per 100 cells)				Biodosimetry dose estimate, mGy
		total	dic + R _C	ace	chr	
Before the first flight	37	1.38 ± 0.12	0.07 ± 0.02	0.52 ± 0.04	0.74 ± 0.09	-
After short-term flights (1-21 days)	9	1.46 ± 0.24	0.19 ± 0.04 ^a	0.65 ± 0.18	0.65 ± 0.18	60
After long-term flights (73 – 199 days)	17	2.19 ± 0.37	0.41 ± 0.06 ^a	0.96 ± 0.25	0.75 ± 0.16	140

^a - statistically different from the control group, $p < 0.05$ (Student's *t*-test);

dic – dicentrics; R_C - centric rings; ace – acentrics; chr – chromatid aberrations.

The mean doses for spacemen participated in long- and short-term space flights were estimated to be 140 mGy and 60 mGy, respectively. Note that doses were assessed in terms of the acute irradiation calibration curve without taking into account actual space flight environment conditions, such as biological effectiveness and dose rate of the space irradiation.

It should be noted that after space flights spacemen displayed increased frequency of multiaberrant cells. The term "multiaberrant cell" (MAC) is referred by the majority of radiation cytogeneticists to a cell, in which 5 or more exchange chromosome aberrations are detected after standard Giemsa staining. Such cells may contain three or more dicentrics or a corresponding number of polycentrics, centric and acentric rings, atypical monocentrics with corresponding number or an excess of acentric fragments. The history of investigation of this interesting phenomenon is described in the paper by Awa [19]. The picture observed during cytogenetic analysis in this case most closely resembles the situation of very uneven irradiation, when the distribution of aberrations in cells does not agree with Poisson's law. Most of cells have no damage but some contain multiple chromosome aberrations. By the character of aberrations, these cells are identical to those observed at high doses of γ -irradiation. It may be suggested that a cell has undergone a powerful local exposure comparable by its effect with high-dose γ -irradiation. In discussing this phenomenon radiation genesis of rogue cells was questioned by a majority of researchers. Of greatest popularity among cytogeneticists became the theory of viral origin of MAC cells advanced by Neel *et al.*, [20]. An unexpected detection of lymphocytes with multiple chromosome aberrations in the blood of two spacemen after 6 months in [21] and similar results obtained by Obe *et al.* [17], regenerated interest in radiation genesis of MAC. These researchers suggested that multiple chromosome aberrations in lymphocytes of astronauts were induced by high-energy heavy particles of cosmic radiation.

Present results are obtained in the course of 10-year investigation of cytogenetic effects induced by low doses of irradiation. MAC distribution and occurrence frequency as well as the relationship of these parameters to dicentrics and stable aberrations frequency in several groups of irradiated persons were comparatively analyzed. The results obtained are shown in Table 3. MAC were found in most of examined groups and absent in the control persons. These cells are highly abundant in radiochemical plant employees working with Pu-containing compounds. Using conventional staining, MAC were detected in 3 of 8 patients. As shown in Table 3, MAC frequency in spacemen (98,681 metaphases analyzed) is (0.10 ± 0.03) per 100 cells, which is

comparable with MAC frequency detected in the residents of Altai Region, suffered from nuclear tests in Semipalatinsk, and liquidators after Chernobyl Accident.

MAC were found in different categories of examined persons somehow been irradiated before. They are typical of persons being in contact with Pu and other possible sources of α -irradiation and are accompanied by a relative increase in several classical radiation markers (dicentrics and rings). Apparently, occurrence of these cells may be regarded as the indicator of local, exposure of the organism to high-level irradiation. The same is suggested by researchers studying chromosome aberrations induced by α -particles *in vitro*. The increased MAC frequency in spacemen points out their exposure to ionizing radiations with high RBE during the flights.

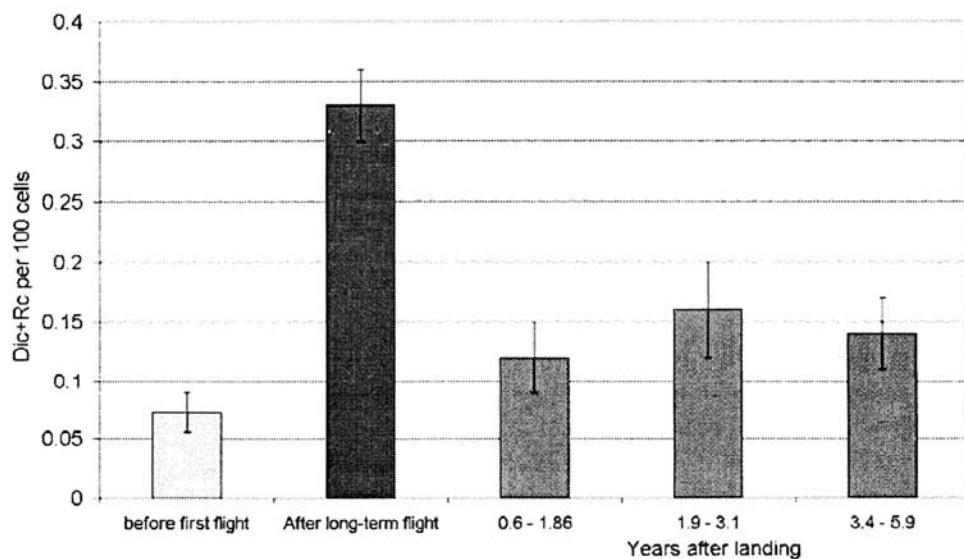


Figure 1. Frequency of dicentrics plus centric rings in lymphocytes from spacemen in various periods after long-term flights

Table 3

**Frequency of multiaberrant cells in human blood lymphocytes in different examined groups
(conventional staining)**

Groups	No. persons (persons with MAC)	No. cells	Multiaberrant cells (5 breaks or more)	
			Number	Frequency per 1,000 cells
Plutonium workers	8 (3)	4,000	11	2.75 ± 0.83
Three Mile Island	29 (2)	15,109	2	0.13 ± 0.09
Spacemen	36 (7)	98,681	10	0.10 ± 0.03
Altai Region	226(6)	49,300	6	0.12 ± 0.05
Chelyabinsk Region (Techa river)	140 (5)	47,160	5	0.11 ± 0.05
Liquidators	1025(23)	380,412	23	0.07 ± 0.01
Nuclear specialists (Sarov)	108 (3)	104,536	3	0.03 ± 0.02
Bryansk Region	80 (0)	21,027	0	-
Controls (Moscow Region)	115 (0)	51,630	0	-

Let us show how dynamics of unstable chromosome aberrations manifest in spacemen after first space flight. As already reported, dicentrics frequency in the post-flight period decreases [10]. Figure 1 shows summarized cytogenetic data on dic+Rc dynamics for spacemen after long flights. The dic+Rc level, increased after long flights, decreases with time, but even 3.4 – 5.9 years after the flight this level remains to be significantly higher than in the pre-flight period. The dic+Rc level increases again after the next flight and after each subsequent flight. It should be noted, however, that the post-flight maximum of the dic+Rc level in this case reduces relative to the maximum observed after the first flight. Therefore, we may indicate a reliable tendency towards slowing down dic+Rc frequency increase during flights with the number of flights ($r = -0.28$, $P = 0.028$). This also indicates induction of adaptive mechanisms in the course of repeated flights.

DYNAMICS OF UNSTABLE CHROMOSOME ABERRATIONS IN PERSONS EXPOSED TO IONIZING RADIATION

The dynamics of unstable chromosome aberrations observed in spacemen are compared with dynamics of chromosome damage in victims of emergency situations. Cytogenetic examinations of Chernobyl Accident liquidators revealed that even over ten years after it they had increased levels of both unstable and stable chromosome aberrations [22, 23]. The highest level of unstable chromosome aberrations (dicentrics and centric rings) observed in 1986 then gradually decreased, but still did not reach the control value in succeeding years (Table 4).

Of interest is the problem, how long unstable chromosome aberrations can be retained in irradiated human beings. The important information was obtained in the studies carried out on Semipalatinsk nuclear test site. Several settlements in Altai Region (7 settlements, 226 persons) were examined 45 years after the first nuclear test in 1949 [24, 25]. These data showed that the residents of these settlements exposed to different doses of irradiation (up to 3 Gy) had an increased level dic+Rc cells in peripheral blood lymphocytes, proportional to irradiation dose. It is believed that the effect observed is caused by irradiation-induced chromosome changes in stem cells of hematopoietic tissues.

Table 4

Cytogenetic results (classical method), pooled data for groups of liquidators

Year of examination	Number of persons	No. cells	Dic+Rc per 100 cells ±SEM
1986	443	41,927	0.33 ± 0.03
1987	280	44,268	0.14 ± 0.02
1990	23	4,268	0.10 ± 0.05
1991	110	20,077	0.09 ± 0.02
1992	136	32,000	0.14 ± 0.02
1993	75	18,581	0.09 ± 0.02
1994	69	20,879	0.16 ± 0.03
1995	110	30,012	0.06 ± 0.01
1996	53	17,960	0.07 ± 0.02
1997	126	63,462	0.09 ± 0.01
1998	77	39,426	0.09 ± 0.01
1999	41	22,524	0.12 ± 0.02
2000-2001	28	14,000	0.09 ± 0.02
Controls	115	51,630	0.013 ± 0.005

Cytogenetic examination of nuclear specialists from Sarov (the city where atomic weapons were created) represents another example of increased level of unstable chromosome aberrations detected 40 - 50 years after irradiation [18]. A majority of examined patients (56 of 108) aged, on average, (61.5 ± 0.7) received 0.10 – 0.19 Gy doses; 7 of them received doses over 1.0 Gy. The rest patients received doses from 0.2 to 1.0 Gy. The frequency of dicentrics in examined patients (0.21 ± 0.01 per 1,200 cells) is 10 times higher compared with controls (Moscow) and 3 times higher compared with controls of similar age from Sarov (Table 5).

Thus, cells with radiation-induced unstable chromosome aberrations (dic+Rc) are gradually eliminated from peripheral blood (apparently by the exponential law). However some part of cells with dic+Rc are retained for several tens of years after irradiation.

Table 5

Cytogenetic examination data on nuclear specialists

Groups	No. persons	No. cells	Frequency of aberrations per 100 cells $\pm m$				
			ab	C_{ab}	dic	ace	chr
Specialists	108	104536	1.82 ± 0.04	1.74 ± 0.04	0.21 ± 0.01	0.61 ± 0.02	0.92 ± 0.03
Control (Sarov)	49	51893	0.92 ± 0.04	0.91 ± 0.04	0.07 ± 0.01	0.32 ± 0.02	0.52 ± 0.03
Moscow Region	115	51630	0.87 ± 0.04	0.85 ± 0.04	0.013 ± 0.005	0.30 ± 0.05	0.53 ± 0.07

ab - total number of aberrations; C_{ab} - the number of cells with aberrations; dic – dicentrics; ace – acentrics;
chr – chromatid aberrations

The above examples (liquidators of the Chernobyl Accident, people living in the neighborhood of the Semipalatinsk nuclear test site, nuclear specialists from Sarov) indicate in favor of long persistence of an elevated (as compared to the control) dic+Rc level 40 - 50 years after irradiation at doses of about 1 Gy. This circumstance should be taken into account in estimation of long-term genetic consequences of ionizing radiation effects on different human cohorts.

ON THE POSSIBILITY OF RECONSTRUCTING ACCUMULATED DOSES IN ANALYSIS OF STABLE CHROMOSOME ABERRATIONS

Stable chromosome aberrations (translocations) do not lead to loss of cellular viability. Therefore cells with translocations can retain through a number of cell cycles and can be detected within many years after the exposure to radiation. In this connection, the analysis of translocation frequencies in lymphocytes of irradiated persons has become one of the most important methods (especially after the development of the FISH method) of biological dosimetry. Several spacemen were examined by the FISH method for the frequency of translocations before and after flights. Leaving aside the individual results for each spaceman, we shall present the general averaged data for groups differing in doses received during space flights according to the measurements of the onboard radiometer. The spacemen were arbitrarily subdivided in 3 groups: before flight (group I), 1 to 100 mGy (group II), 110 to 300 mGy (group III) (Table 6).

It is seen that the pre- and post-flight level of translocations (two groups) statistically significantly exceeds the laboratory control. It may be assumed that the elevated pre-flight level of translocations (as well as of dicentrics and rings as noted above) is due to frequent roentgenologic examinations of the spacemen in the pre-flight period. In the group of spacemen with the highest accumulated doses (group III) a statistically significant ($p < 0.05$) increase (as compared to the pre-flight level) in the translocation frequency is observed. Following the data obtained for the frequency of translocations, the accumulated doses were calculated with the use of the calibration dose-response relationship for the FISH method [18]. The results of the calculations are shown in Table 6.

Table 6

Cytogenetic pooled data for spacemen divided into different dose-range groups (the FISH method)

Group	Number of persons	Mean physical dose over flight, mGy	Cells scored	Translocation frequency $F_p \pm SEM$ (per 100 cells)	Biodosimetry estimate, mGy
I ("before the first flight")	5	0	9710	$0.30 \pm 0.06^*$	80
II (dose range 1-100 mGy)	9	44 ± 4	12140	$0.32 \pm 0.05^*$	110
III (dose range 110-300 mGy)	9	179 ± 6	12651	$0.51 \pm 0.06^{b*}$	260
Control	15	-	21953	0.15 ± 0.03	-

^b - statistically significantly differed from the "before the first flight" group value, $p < 0.05$ (Fisher's exact test)

* - statistically significantly differed from the control group, $p < 0.05$ (Student's *t*-test)

Mean physical doses were measured to be 44 mGy and 179 mGy for groups II and III, respectively. The biological doses for groups II and III, assessed by the translocation frequency (110 and 260 mGy), agree well with the biological dose (140 mGy) estimated by dicentrics scored in the spacemen after the first long-term flight. Since the level of doses accumulated by spacemen during space flights is relatively low, the estimation of individual doses by the FISH method in this investigation is complicated. However, the pooled cytogenetic data for several spacemen make it possible to determine the absorbed dose and thus to obtain additional information on the levels of doses to which spacemen are exposed during space flights.

The results obtained in the analysis of stable chromosome aberrations (the FISH method) in spacemen and persons exposed in various irradiation cases are compared. The level of translocations was studied in liquidators of the Chernobyl accident [26]. The study was performed 8 - 9 years after the accident. If the frequency of translocations significantly exceeded the control level, assessments of individual accumulated doses were based on it. In the group of liquidators, the frequency of translocations was almost 4 times higher compared to controls (0.37 ± 0.03 and 0.10 ± 0.03 per 100 cells, respectively). The accumulated doses, reconstructed by the FISH method (200 mGy), were found equal on average doses determined by physical dosimetry.

Another example of the FISH method use is cytogenetic examination of Altai Region residents, suffered from nuclear test in 1949 [24,27]. In 1994, inhabitants of several settlements were examined, i.e. 45 years after the nuclear test. High frequency of translocations as compared to local control was detected. Accumulated doses, determined from the calibration dose-effect curve, well agree with the doses determined by the physical methods immediately after the test.

The frequency of translocations was also determined in nuclear specialists in Sarov 40 - 50 years after contact with radioactive materials [18]. For the whole group, the average frequency of translocations (0.49 ± 0.06 per 100 cells) is more than 3 times above controls (Table 7). Biological doses assessed in them by the FISH method (from 0 to 1.84 Gy) correlated well with the red bone marrow (RBM) doses accumulated over the period of professional activity (0.07 – 0.86 Sv).

Table 7

**Pooled data on translocations determined by the FISH method
(chromosomes 1, 4 and 12) for the group of Sarov nuclear specialists**

Group	No. persons	No. cells	No. translocations	Frequency of translocations	
				$F_p \pm$ SEM/100 cells	$F_G \pm$ SEM/100 cells
Specialists	17	15,561	77	0.49 ± 0.06	1.56 ± 0.19
Control	15	21,953	33	0.15 ± 0.03	0.48 ± 0.09

The frequency of unstable chromosome aberrations, namely, the frequency of dicentrics and centric rings, in all examined patients is significantly lower than the frequency of translocations. The average frequency of translocations for the group made up 1.56 per 100 cells, the average frequency of dicentrics – 0.23 per 100 cells, i.e. 7 times lower. It is customary to assume that the frequency of dicentrics and translocations immediately after irradiation is practically the same and this is supported in a number of works. In the course of post-exposure time cells carrying dicentrics gradually eliminated from peripheral blood as a result of the complexity of their passage through mitosis. The difference in the frequency of cells with dicentrics and translocations indicates that irradiation might take place several years ago, and the greater is the difference the longer is the time from the moment of exposure till cytogenetic examination.

Thus, the ratio between stable and unstable chromosome aberrations is the important criterion for determining time intervals since exposure. Note that this ratio equals 7 and was obtained by the authors for the inhabitants of Muslyumovo settlement on Techa river. They received highest doses nearly 45 years before cytogenetic examination. The above-mentioned residents of several Altai settlements we also examined 45 years after irradiation caused by nuclear tests [27]. We called this value the correction factor and suggested for assessments of unstable chromosome aberrations elimination rate with time [27].

For spacemen after long flights (17 persons) the average $dic+Rc$ frequency equaled 0.41 ± 0.06 per 100 cells or 0.37 per 100 cells subtracting the pre-flight level. For two groups, the average frequency of translocations after long flights (Table 6) equals 0.42 ± 0.04 per 100 cells or 0.12 per 100 cells without the pre-flight level. In this case the frequency of translocations was calculated for three chromosomes – 1, 4 and 12 (F_p). Calculated for the whole

genome [28], the frequency of translocations induced during flights makes up 0.37 per 100 cells (F_G). The ratio between stable (0.37 per 100 cells) and unstable (0.34 per 100 cells) chromosome aberrations after long flights (160 days, on average) is about 1.0. This indicates that changes of the ratio between stable and unstable chromosome aberrations during long-term flights are insignificant. Therefore, calculation of accumulated doses after long space flights based on the frequency of dic+Rc does not require introduction of any correction factor, taking into account elimination of cells with such aberrations.

How dangerous are doses received by spacemen and other groups of irradiated persons in relation to possible genetic and carcinogenic effects? Let us observe expected genetic and carcinogenic risks from conventional dose of 100 mSv basing on materials presented in UNSCEAR reports of 2000, 2002 [1, 29, 30].

The risks of exposure-induced death from all solid cancers combined with the acute dose of 0.1 Sv during the life is assessed as 0.9% for men, 1.3% for women and 1.1% for children [1]. Assessments may be reduced by 50% for chronic exposures (0.55%). The death probability from irradiation-induced leukemia after received 0.1 Sv acute dose equals 0.1%. The expected genetic risks for children (100 mSv dose) equal 0.03 – 0.05% of acute genetic diseases (compare this value with the spontaneous mutagenesis level in persons – 73.8% Mendelian hereditary diseases, chromosomal diseases, congenital abnormalities and multifactor diseases manifested throughout the human life). Thus, irradiation by 0.1 Sv dose will promote the expected increase of genetic and carcinogenic effects in irradiated persons.

CONCLUSIONS

1. Modern radiobiology has radiation-sensitive biomarkers that enable biodosimetry and biomonitoring to be performed during long-term space flights.
2. Cytogenetic analysis of lymphocytes from MIR and ISS spacemen showed a significant increase in frequency of stable and unstable chromosome aberrations after space flights;
3. For the case of several space flights, the level of unstable chromosome aberrations decreases in the first post-flight period then increases after the

- second and each subsequent flight while the value of post-flight maximum reduces with respect to the maximum observed after the first flight;
4. The use of biological dosimetry methods (analysis of stable chromosome aberrations, the FISH method) allows assessment of accumulated doses for a group of spacemen exposed to high-dose radiation during flights;
 5. The comparison of cytogenetic effects observed after space flights, Chernobyl and other radiation accidents promotes for the analysis of unstable chromosome aberrations dynamics over decades after ionizing radiation impacts.
 6. An assessment of possible genetic and carcinogenic irradiation-induced consequences may be made for various groups of irradiated persons basing on doses accumulated in them and calculated with the help of biological dosimetry methods.

REFERENCES

1. *United Nations. Sources and Effects of Ionizing Radiation. UNSCEAR 2000 Report to the General Assembly with Scientific Annexes*, United Nations sales publication, No.E.00.IX.4, 2000, United Nations, New York.
2. Dudkin V.E., Kovalyov E.E., Kuznetsov V.G., and Smirennny L.N., *Biological Effects of High-Energy Protons*, Ed. Yu.G. Grigoryev, 1967, Moscow, Atomizdat, pp. 8 – 21. (Rus)
3. Bender MA, Awa AA, Brooks AL. et al., *Mutat. Res.*, 1988, vol. 196, pp. 103 - 159.
4. *IAEA: Cytogenetic analysis for radiation dose assessment. IAEA Technical report N. 405*, International Atomic Energy Agency, Vienna, 2001.
5. Sparrow A.N., Underbrink A.G., and Sparrow R.C., *Radiat. Res.*, 1967, vol. 32, pp. 915 - 945.
6. *United Nations. Hereditary effects of radiation. UNSCEAR 1996 Report to the General Assembly with Scientific Annex*, United Nations sales publication. No.E.96.IX.3, 1996, United Nations, New York.
7. Shevchenko V.A., Proc. Seminar on Comparative Assessment of the Environmental Releases during Three Major Accidents, Luxem., 1991, pp. 821 - 866.

8. Shevchenko V.A., Kalchenko V.A., Abramov V.I. et al., *Low Doses of Radiation: Are They Dangerous?*, 2000, Nova Science Publishers, New York, 378 p.
9. Fedorenko B., Druzhinin S., Yudaeva L., et al., *Adv. Space Res.*, 2001, vol. **27**, pp. 355 - 359.
10. Fedorenko B.S., Druzhinin S.V., Snigiryova G.P. et al., *Microgravity and Space Station Utilization*, 2001, vol. **3**(2), pp. 5 - 9.
11. Durante M., Snigiryova G., Akaeva E. et al., *Cytogenet. Genome Res.*, 2003, vol. **103**, pp. 40 - 46.
12. Moorhead, P.S., Nowell P.S., and Mellman W.J., *Exp. Cell Res.*, 1960, vol. **20**, pp. 613 - 616.
13. Bauchinger M., Salassidis K., Braselmann H. et al., *Int. J. Rad. Biol.*, 1998, vol. **73**, pp. 605 - 612.
14. Pinkel D., Straume T., and Gray J.W., *Proc. Nat. Acad. Sci. US*, 1986, A, vol. **83**, pp. 29 - 34.
15. Bauchinger M., Schmid E., Zitzelsberger H. et al., *Int. J. Radiat. Biol.*, 1993, vol. **64**, p. 179.
16. Mitrikas V.G., Tsetlin V.V., Teltssov M.V., and Shumshurov V.I., *Radiat. Meas.*, 2002, vol. **35**, pp. 515 - 525.
17. Obe G., Johannes I., Johannes C., *Int. J. Radiat. Biol.*, 1997, vol. **72**(6), pp. 727 - 734.
18. Snigiryova G.P., Bogomazova A.N., and Novitskaya N.N., *Proc. Intern. Conference "Genetic Consequences of Emergency Radiation Situations"*, Moscow, Russia, June 10-13, 2002, RUDN Publishing, pp. 313 - 328.
19. Awa A., *Mutat. Res.*, 2003, vol. **543**, pp. 1 - 15.
20. Neel J.V., Major E.O., Awa A.A. et al, *Proc. Nat. Acad. Sci. USA*, 1996, vol. **93**, pp. 2690 - 2695.
21. Testard I., Ricoul M., and Hoffschir F., *Int. J. Radiat. Biol.*, 1996, vol. **70**, pp. 403 - 411.
22. Shevchenko V.A., Akaeva E.A., Yeliseyeva I.M. et al., *Mutat. Res.*, 1996, vol. **361**.
23. Snigiryova G.P. and Shevchenko V.A., *In Recent Research Activities about the Chernobyl NPP Accident in Belarus, Ukraine and Russia*, Research Reactor Institute, Kyoto University, July 2001, pp. 256 – 267.
24. Shevchenko V. and Snigiryova G., *Radiation Exposure by Nuclear Facilities*, Berlin, 1998, pp. 216 - 226.

25. Shevchenko V.A., Shoikhet J.N., Algasin A.I. et al., *Estimation of Long-Term Consequences of Nuclear Tests at the Semipalatinsk Test Site for the Altai Population. NATO ASI Series*, 1998, vol. 36(2), pp. 157 - 185.
26. Snigiryova G., Braselmann H., and Salassidis K., *Int. J. Radiat. Biol.*, 1997, vol. 71(2), pp. 119 - 127.
27. Shevchenko V. and Snigiryova G., *Radiation Exposure by Nuclear Facilities*, 1998, Berlin, pp. 329 - 331.
28. Lucas J.N., Awa A., Straume T. et al., *Int. J. Radiat. Biol.*, 1992, vol. 62, pp. 53 - 63.
29. United Nations. *Hereditary effects of radiation. UNSCEAR. 2001 Report to the General Assembly with Scientific Annexes*, United Nations sales publication. No.E.01.IX.2, 2001, United Nations, New York.
30. Shevchenko V.A., *On The Genetic Risks From Exposure Of Human Population To Radiation*, Nova Science Publishers, New York, 2000, pp. 65 - 81.

The effect of epithelial thymus cells on post-irradiation apoptosis display of thymocytes in vitro

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ABSTRACT

Human thymocyte apoptosis was estimated after irradiation by γ -quanta from ^{137}Cs *in vitro*. The apoptosis was estimated using techniques based on the flowing cytofluorometry – the expression of phosphatidylserin binding annexin V and hypodiploid cell accumulation 4 and 24 hours after irradiation, respectively. The annexin technique helped in recording the amount of apoptotic cells in the early post-irradiation period even at 0.1 Gy irradiation dose. Injection of non-irradiated thymic epithelial cells (TEC) in the culture of irradiated thymocytes eliminated the increase of apoptotic cell content (in %). On the contrary, the thymocyte apoptosis increase at joint irradiation of thymocytes and TEC. Injection of TEC, irradiated by 1 and 5 Gy doses, to the culture of non-irradiated thymocytes increased their apoptosis comparative to the effect of thymocyte irradiation. TEC irradiation induced their activation, which was displayed in intensification of CD54, HLA-DR and Fas-ligand (CD178) molecules expression and IL-7 secretion. Hence, TEC apoptosis was not induced. It is suggested that thymocyte apoptosis induction by irradiated TEC is stipulated by the Fas-dependent mechanism.

Keywords: thymus, thymocytes, apoptosis, thymic epithelial cells, annexin V technique, hypodiploid cells, TEC monoculture supernatant, cytokine

Thymocytes are one of the most radiosensitive cells in the organism [1], whereas death of a significant part of these cells is the natural display of thymocyte clone selection, performed in the thymus. Thymus epithelial cells (TEC) represent the main component of intra-thymus micro-environment, which determines the selection process: they are the main factor of positive selection and may also (besides dendrite cells) implement negative selection of thymocytes. At positive selection, TEC are the source of signals providing survival of thymocytes capable of distinguishing (with the intermediate affinity degree) autogenic complexes of MNS and peptide molecules. At the negative selection, these molecules are sources of signals stipulating death of thymocyte clones, which recognize the mentioned complexes with high affinity [2]. It is also found that TEC in the thymocyte co-culture (both syngenic and allogenic) may induce their apoptosis [3, 4]. This, apparently, reflects TEC ability to kill thymocytes, which are not selection supported.

Basing on the fact that TEC are capable of alternate influence on thymocyte survivability (with respect to the type of signals from T-cell receptor and other membrane molecules, received by the cell). Of interest also is the study of TEC modifying effect on apoptosis of thymocytes, induced by ionizing radiation. This work studies manifestations of irradiated thymocyte under conditions of co-cultivation with intact and irradiated TEC, and some manifestations of radiation-induced TEC activation.

MATERIALS AND METHODS

Cells and their sources

Thymocytes were derived from thymus in mice or fragments of human thymus [4]. The fragments were obtained from children, aged 1 month to 1 year, undergoing the cardiac surgery in accordance with the present surgical practice. The fragments were cut by scissors and thymocytes were suspended by repeated suction through a syringe needle. Only freshly obtained cells were used for cultivation.

Cultures of TEC were obtained by the following method. Thymic fragments were placed to a plastic Petri dish for 7 days in the presence of 20 μ M epidermal growth factor ("Sigma", USA). TEC migrated from the fragments were detached from the plastic surface by 0.1% Versene solution with 0.05% trypsin ("Sigma"). Then the cells were washed and transferred to a fresh

medium with 20 µM epidermal growth factor. The cells were passed 3-4 times prior to the use in experiments. About 90% of cells in cultures bound monoclonal antibodies against cytokeratin 8/18 (CAM5.2; "Becton Dickinson", USA). The cultivation techniques for human and mouse TEC were identical.

Culture conditions and radiation exposure

Thymic cells were cultivated in 96- or 24-well plastic plates ("Costar") and plastic Petri dishes ("Linbro"). Cells were grown at 37°C in 5% CO₂ in CO₂-incubator ("Jouan", France), in 10% FCS-supplemented RPMI 1640 medium with adding 300 µg/ml L-glutamine ("Flow", Scotland) and 0.02 M HEPES-buffer ("Sigma"). Initial concentration of thymocytes in cultures was 5·10⁶ cells per ml. The ratio of thymocytes and TEC in co-cultures was 10:1 [4].

The cell cultures were exposed to γ-rays of ¹³⁷Cs on "Stebel" apparatus. The control cultures were transported together with experimental cultures, but were not irradiated.

Flow cytometric analysis of the cell phenotype

Monoclonal antibodies of the following specificities were used for immunofluorescent staining – fluoresceine isothiocyanate (FITC)-labeled: anti-CD54, anti-CD80, anti-CD178, anti-HLA-DR; phycoerythrine-labeled anti-human CD95 (all - "Caltag", USA). Stained samples were analyzed using cytofluorometer FACSCalibur ("Becton Dickinson") and CellQuest 3.1 Software (Becton Dickinson, Mountain View, CA) or WINMDI software (JoeTrotter, The Scripps Research Institute) for 10,000 cells per sample.

Measurement of cell apoptosis

Two techniques were used for estimating the apoptosis. The first technique is based on detection of cells expressing phosphatidyl serin [5]. Cells were treated with 3 µg/ml FITC-labeled Annexin V ("Sigma") and 50 µg/ml of propidium iodide (PI; "Sigma") in HEPES- buffer, pH 7.2. After 15 minutes of incubation, the cells were analyzed by two-color flow cytometry. Apoptotic cells bind Annexin V but not PI.

The second technique was based on the estimation of hypodiploid cell amount [6]. Thymocytes were fixed in 70% ethanol for 1 hour, washed and stained by 0.15% PI solution in phosphate buffer contained 0.1% Triton X-100 (“Sigma”) and 0.1% sodium citrate. Cells were analyzed by flow cytometry. Apoptotic cells are located in hypodiploid region of histogram.

Cytokine assay

Cytokine concentration was determined in the supernatants of the 1-day monocultures of TEC by solid-phase immunoenzyme assay with the use of kits for detecting IL-1 β and IL-7 (“Cytimmune”, USA).

Statistical Analysis

The significance between the results for different groups was estimated using two-tailed Student’s test. The results are present as means \pm SE.

RESULTS

Irradiation of human thymocytes *in vitro* in the absence TEC (monoculture) causes the dose-dependent increase of the ratio of apoptotic cells. Similar results were obtained at the estimation of apoptosis on binding annexin V, performed 4 hours after irradiation, and accumulation of hypodiploid cells, detected by cytofluorometric technique a day post-irradiation coloring by propidium iodide (Figure 1). The apoptosis level dependence on the irradiation dose is clearer at its earlier estimation by the annexin technique. In this case, apoptosis intensification is recorded even at 0.1 Gy dose. At 5 Gy a half of the cells are subject to apoptosis, which is 4 times greater than in non-irradiated

Table 1

TEC modifying effect on the level of thymocyte apoptosis (the content of apoptotic cells), induced by γ -radiation

Cultivated cells	Irradiation dose, Gy			
	0	0.1	1.0	5.0
Apoptosis determination by the number of cells binding annexin V ($n = 3$)				
Thymocytes	10.5 \pm 1.8	20.9 \pm 2.0**	34.8 \pm 2.9**	35.5 \pm 4.2**
Irradiated thymocytes and non-irradiated TEC	10.7 \pm 2.1	19.4 \pm 2.3*	10.4 \pm 2.5	10.6 \pm 3.6
Non-irradiated thymocytes and irradiated TEC	10.7 \pm 2.0	10.8 \pm 2.3	33.0 \pm 2.6**	35.2 \pm 3.0**
Determination of apoptosis by the number of hypodiploid cells ($n = 8$)				
Thymocytes	17.1 \pm 4.5	18.8 \pm 6.5	32.7 \pm 5.7*	34.7 \pm 5.5*
Irradiated thymocytes and non-irradiated TEC	17.0 \pm 6.7	30.9 \pm 4.7	20.7 \pm 7.1	13.7 \pm 6.2
Non-irradiated thymocytes and irradiated TEC	16.8 \pm 2.2	34.9 \pm 6.0*	40.7 \pm 6.2*	30.3 \pm 4.5*

Note: * $p < 0.05$;

** $p < 0.01$.

culture. Further intensification of apoptosis with irradiation dose duplication is not observed. As the apoptosis was estimated a day after irradiation by the number of hypodiploid cells, the effect close to the threshold one is observed: apoptosis is not intensified by 0.1 Gy dose irradiation, whereas 1, 5 and 10 Gy doses give approximately equal percentage of apoptotic cells, which is duplicated compared with the control and does not exceed the one-third part of cultivated thymocytes.

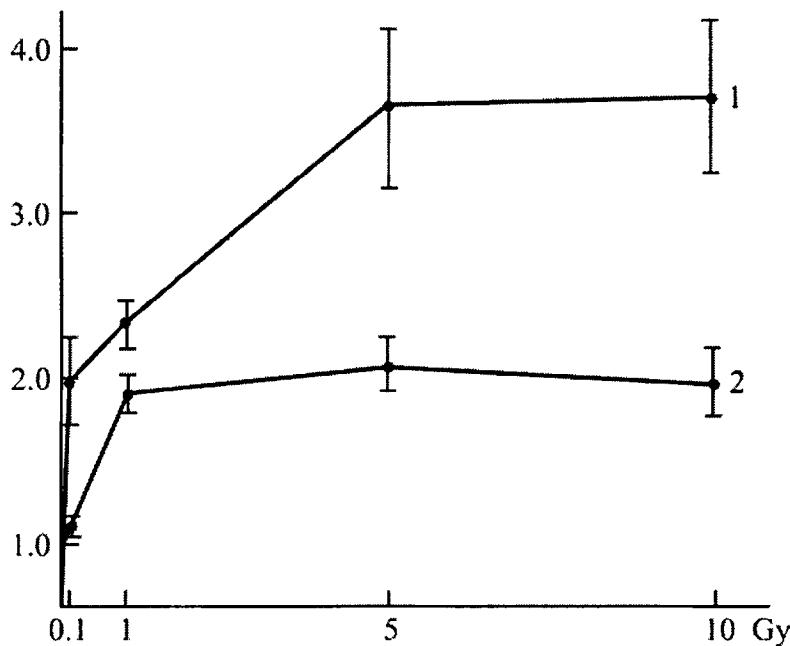


Figure 1. The dependence of thymocyte apoptosis level in monoculture on γ -irradiation dose

Data on thymic apoptosis are given in relative units; for 1.0, the value of non-irradiated control is accepted (hereinafter, data are shown in the $M \pm SE$ shape). The *Annexin* curve gives the content of cells binding annexing V ($n = 3$); curve *PI* gives the content of hypodiploid cells ($n = 8$)

Abscissa axis: irradiation dose, Gy;

Ordinate axis: the content (%) of apoptotic cells relative to control

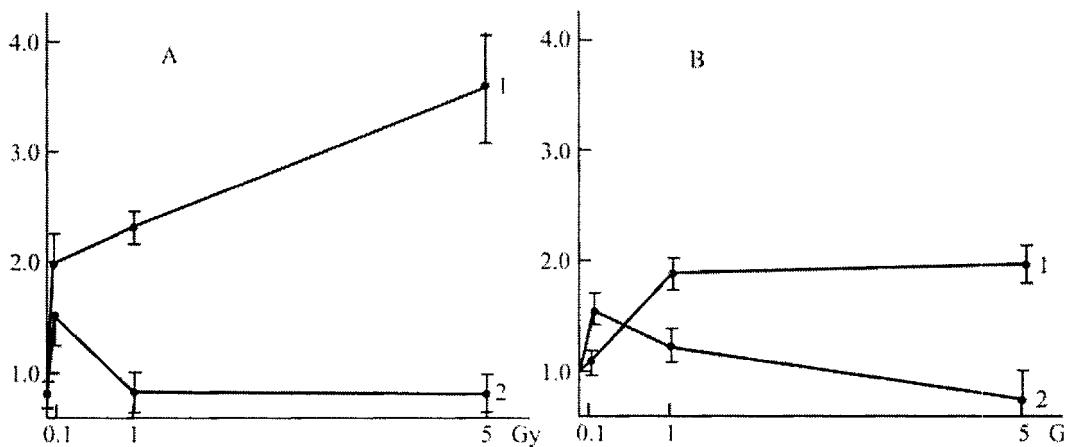


Figure 2. The effect of co-cultivation with non-irradiated TEC on irradiated thymocyte apoptosis expression

A – apoptosis was determined 4 hours after irradiation by FITC-labeled ($n = 3$) annexin V binding;

B – apoptosis was determined 24 hours after irradiation by the number of hypodiploid cells ($n = 8$).

Th-Ir is the monoculture of irradiated thymocytes;

Th-Ir + TEC is the co-culture of irradiated thymocytes and non-irradiated TEC.

Abscissa axis: irradiation dose, Gy;

Ordinate axis: the content (%) of apoptotic cells relative to control

Injection of non-irradiated TEC into the culture of irradiated thymocytes (at the ratio thymocyte:TEC = 10:1) strongly modifies the dose dependence of irradiated thymocyte apoptosis (Table 1, Figure 2). TEC themselves had no effect on the apoptosis of thymocytes, but almost completely eliminated intensification of thymocyte apoptosis at irradiation doses of 1 and 5 Gy. This was determined at both the early apoptosis by the annexin technique and the late apoptosis by increase of hypodiploid cell part. Hence, the apoptosis level remained enhanced at 0.1 Gy dose, and at determination of the late apoptosis such increase was induced by TEC addition.

Meanwhile, as thymocytes are irradiated in the presence of TEC, the apoptosis level of thymocytes was higher than at irradiation of isolated thymocytes (Figure 3). Hence, the plateau of thymocyte death was observed already at 1 Gy dose.

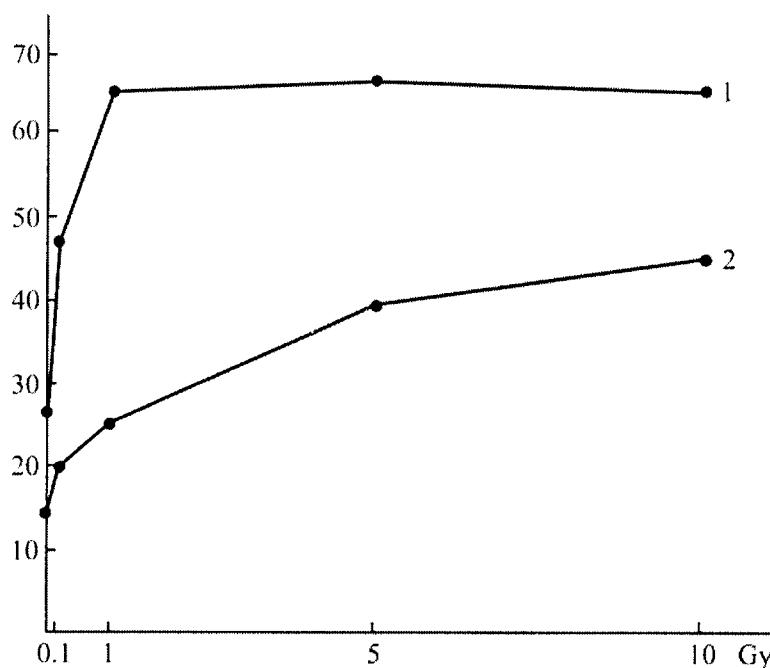


Figure 3. The level of thymic radiation apoptosis at monoculture (Th) and co-culture with TEC (Th + TEC) irradiation. Data on typical experiment are shown
Abscissa and ordinate axes – see figures above

Addition of irradiated TEC to non-irradiated thymocytes induced an action similar to irradiation of thymocytes themselves: apoptosis increased to 30 – 35% both at 1 and 5 Gy doses (Table 1, Figure 4). The highest effect on the late apoptosis of thymocytes was caused by irradiated TEC, whence the apoptosis increase was recorded even after TEC irradiation by 0.1 Gy dose.

Table 2

Irradiation-induced (5Gy dose) expression of TEC activation markers (the part of cells carrying the molecule, $M \pm SE$)

Irradiation	Activation molecules			
	HLA-DR (n = 5)	CD54 (n = 5)	CD80 (n = 5)	CD178 (n = 3)
-	46.3 ± 6.8	55.5 ± 5.3	51.4 ± 6.8	1.8 ± 0.6
+ (5 Gy)	86.6 ± 12.0*	88.9 ± 10.4*	60.9 ± 10.2	5.5 ± 0.7*

Note: * $p < 0.05$.

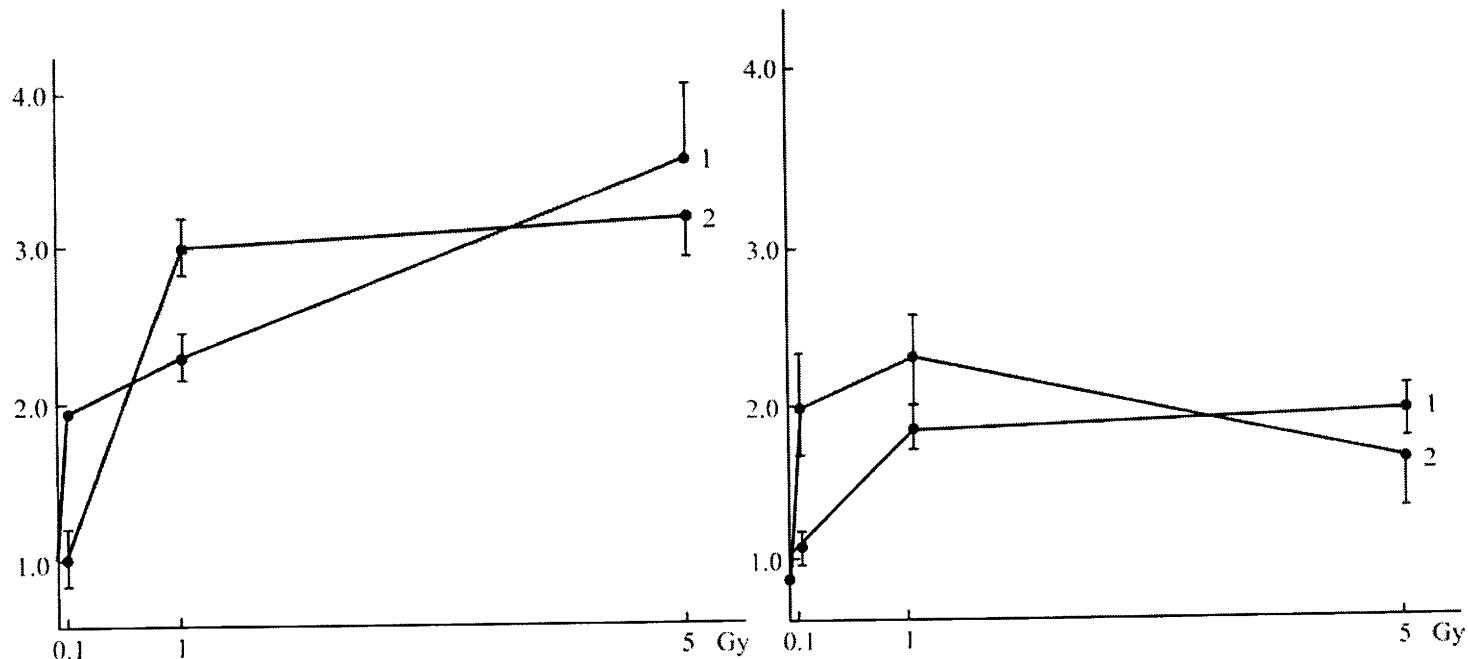


Figure 4. The induction of thymic, non-irradiated apoptosis at co-cultivation with irradiated TEC.
 A – apoptosis was determined 4 hours after irradiation by FITC-labeled ($n = 3$) annexin V binding;
 B – apoptosis was determined 24 hours after irradiation by the number of hypodiploid cells ($n = 8$).
 Th-Ir is the monoculture of irradiated thymocytes;
 Th-Ir + TEC is the co-culture of irradiated thymocytes and non-irradiated TEC.
 Abscissa and ordinate axes – see figures above

TEC irradiation in monoculture was found insignificant for their apoptosis which, primarily, was quite high (15 – 30%). On the TEC surface, radiation increases expression of activators of these molecules, which are class II MHC (HLA-DR) and β_2 -integrin receptor ICAM-1 (CD54); a tendency to increase expression of costimulatory CD80 molecule is displayed. Fas-ligand (CD178) was expressed on the surface of some part of TEC (Table 2). The parallel increase of expression on Fas-receptor (CD95) thymocytes was not observed: the percentage of CD95 $^{+}$ thymocytes in non-irradiated and differently irradiated populations varied in narrow range from 35 to 55%.

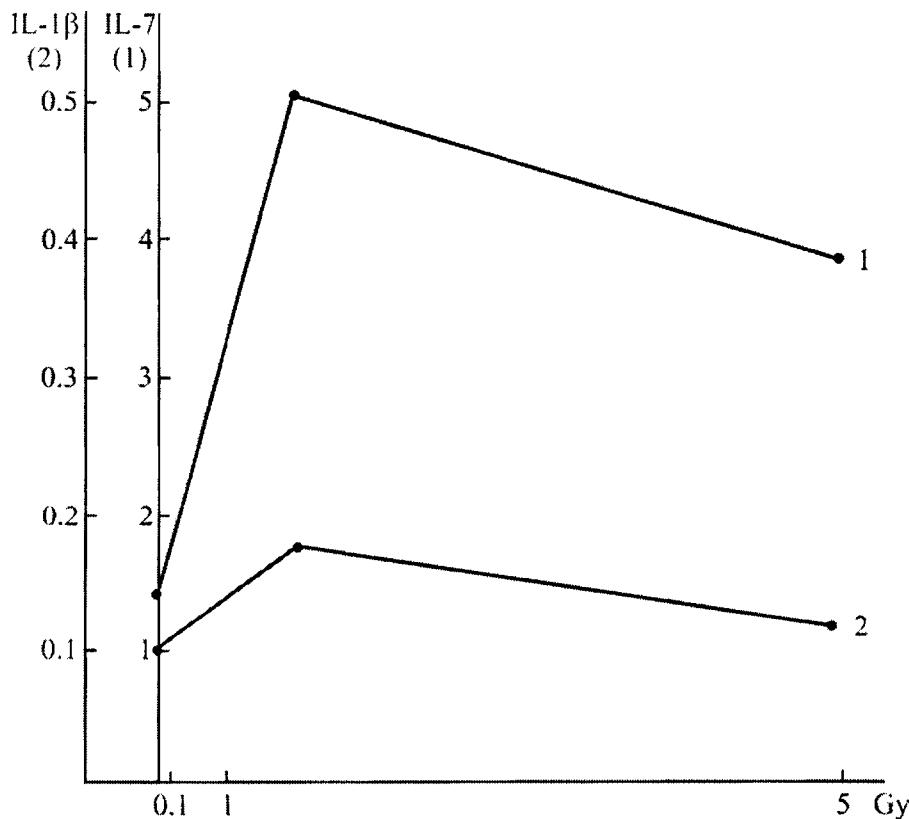


Figure 5. The effect of TEC irradiation on IL-7 (A) and IL-1 β (B) secretion. Data present the averaged of two experiments.
 Abscissa axis: see above figures;
 Ordinate axis: cytokine concentration in the 1-day supernatant of TEC monocultures, ng/ml

Irradiation intensifies secretion of epithelial cells IL-7. The concentration of these cells in supernatant of TEC monoculture 3 – 4-fold increased at irradiation doses of 1 and 5 Gy. Irradiation had practically no effect on IL-1 β secretion: just a slight increase in the concentration of this cytokine is observed in supernatants at 1 Gy dose, and at 5 Gy the effect was not observed at all (Figure 5).

DISCUSSION

The data obtained are summed up as follows. Induction of human thymocyte apoptosis is registered early after irradiation *in vitro* already at 0.1 Gy irradiation dose. Maximal level of apoptosis is reached at 5 Gy dose and embraces about a half of cells in the studied population of thymocytes. In the presence of non-irradiated TEC, radiation-induced apoptosis of thymocytes was not detected. In the studied dose range (0.1 – 10 Gy) radiation does not intensify apoptosis of TEC themselves. Meanwhile, irradiation manifests some signs of activation of these cells which is displayed by activation marker expression and intensification of IL-7 secretion. Irradiation does not affect the amount of thymocytes expressing Fas-receptor, but increases the number of TEC expressing Fas-ligand.

Clearer information on the radiation-induced apoptosis is obtained for the early post-irradiation period, using the annexin test which allows detection of phosphatidylserin expression already at early stages of the apoptosis development. Basing on the data on thymocyte apoptosis level, one is unable to calculate classical parameters of their sensitivity to radiation, because there is no information about total amount of survived cells. Nevertheless, the data obtained correlate with the results of radiobiological studies indicating extremely high thymocyte sensitivity to radiation ($D_0 \approx 0.5$ Gy) [1].

These data correlate with the ideas on TEC resistance to radiation [7, 8]. The data on activating action of radiation on TEC are comparable with similar results for macrophages. Usually, irradiation stimulates phagocytic activity of macrophages and other myeloid cells [1], induces occurrence of these cells and endothelial cells of integrine receptor ICAM-1 [9], CD80 [10] on the surface, as well as gene expression of cytokines, most likely TNF α [11]. Epithelial cells (TEC, in particular) respond to activation similar to endothelial cells and, therefore, take on some properties of macrophages (phagocytic activity, the ability to release cytokines, express adhesion molecules typical of

macrophages). Ionizing radiation is one of the activators of the above-enumerated cells. The expression increase for ICAM-1, HLA-DR and to some extent CD-80 on the TEC surface induced by irradiation is also shown and the possibility of radiation-induced intensification of IL-7 secretion by these cells is determined. These data conform to the results published in the literature [12] (though there is also information about radiation-induced death of cells - producers of IL-7 [13]).

The most unexpected are data on the effect of non-irradiated and irradiated TEC on the thymocyte apoptosis. In the presence of TEC, elimination of the radiation apoptosis of thymocytes may, at least, be dually explained – by secretion of radioprotective substances (mostly cytokines) and elimination of apoptotic cells by means of phagocytosis. It is known that among cytokines produced in the thymus, IL-7 is the most important factor for thymocyte survival and plays this role at different stages of this cell development [14]. It is found that TEC is one of the sources of IL-7 in the thymus [15]. The mechanisms of radiation-induced apoptosis and apoptosis “by default” (for thymocytes, mostly because the indication for IL-7 via receptor is absent) are principally analogous [16], which assumes possible the protective action of IL-7. So far as concerns the probability of phagocytizing of dead thymocytes by thymus epithelial cells, it is known that apoptotic cells may be eliminated by both specific phagocytes and other cells [17]. These suggestions may be easily confirmed in experiments.

It is much more difficult to explain induction of thymocyte apoptosis by irradiated TEC. As mentioned above, irradiation increases phagocytic activity of the cells (apparently, this also concerns TEC) and stimulates IL-7 secretion, i.e. irradiation would decrease concentration of apoptotic cells. However, irradiation may induce some new property of TEC, which promotes realization of their apoptotic (apoptogenic) action. Apparently, it consists of intensification of Fas-ligand expression. This work shows preliminary data in favor of such radiation effect. According to data reported in the literature, intensification of the Fas-ligand expression at the cell stress, which accompanies irradiation, is stipulated by ceramide synthesis [18]. Therefore, injection of TEC expressing CD178 (Fas-ligand) leads to the receptor mechanism of CD95⁺ thymocyte apoptosis realization.

It is obvious that the modifying action which TEC cause in the culture of irradiated thymocytes is somehow realized *in vivo*. In this case, it may be suggested that thymocytes in the whole organ possess much higher resistance to radiation than in the monoculture *in vitro*, because TEC must additionally contribute to the apoptosis induction. However, this is not obvious, because

processes proceeding in the thymus frequently depend upon the three-dimensional (spatial) organization of the organ and not much any effects, reproduced in the two-dimensional culture, are realized in the organ culture or the whole organ [19]. Thus the ultimate assertion in relation to TEC contribution to the radiation-induced death and post-apoptotic elimination of thymocytes can be made only on the basis of their study in the organ culture of the thymus.

REFERENCES

1. Anderson R.E. and Warner N.L., 'Ionizing radiation and the immune response', *Adv. Immunol.*, 1976, vol. **24**, pp. 216 - 336.
2. Starr T.K., Jameson S.C., and Hogquist K.A., 'Positive and negative selection of T-cells', *Ann. Rev. Immunol.*, 2003, vol. **21**, pp. 139 - 176.
3. Schreiber L., Sharabi Y., Schwartz D., Goldfinger N., Brodie C., Rotter, and V.J. Shoham, 'Induction of apoptosis and p53 expression in immature thymocytes by direct interaction with thymic epithelial cells', *Scand. J. Immunol.*, 1996, vol. **44**, pp. 314 - 322.
4. Sharova N.I., Dzutsev A.K., Linvina M.M., Pleskovskaya G.N., Kharchenko T.Y., and Yarilin A.A., 'Thymic epithelial cells induce Fas-independent activation apoptosis of thymocytes', *Immunol. Lett.*, 2001, vol. **78**, pp. 201 - 207.
5. Van Engeland M., Nieland L.J.W., Ramaekers F.C.S., Schutte B., and Reuelingsperger C.P.M., 'Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine exposure', *Cytometry*, 1998, vol. **31**, pp. 1 - 9.
6. Mac Closkey T.W., Oyaizi N., Coronezi M., and Pahwa S., 'Use of flow cytometric assay to quantitate apoptosis in human lymphocytes', *Clin. Immunol. Immunopathol.*, 1994, vol. **71**, pp. 14 - 18.
7. Sharp J.G. and Watkins E.B., *Cellular and immunological consequences of thymic irradiation. Immunopharmacologic effects of radiation therapy*, Ed. by J.B. Dubois, B. Serrou, and C. Rosenfeld, New York: Raven Press, 1981, pp. 137 - 179.
8. Yarilin A.A., 'Radiation-induced damage to thymocytes and thymic stromal cells, manifestation and after-effects', *Physiol. Gen. Biol. Rev.*, 1995, vol. **10**, pp. 1 - 58.

9. Molla M., Gironella M., Miquel R., Tovar V., Engel P., Biete A., Pique J.M., and Panes J., 'Relative roles of ICAM-1 and VCAM-1 in the pathogenesis of experimental radiation-induced intestinal inflammation', *Int. J. Radiat. Oncol. Biol. Phys.*, 2003, vol. **57**, pp. 264 - 273.
10. Morel A., Fernandez N., de La Cote A., Haddada H., Viguier M., Polla B.S., Antoine B., and Kahn A., 'Gamma-ray irradiation induces B7.1 costimulatory molecule expression in various murine tumor cells', *Cancer Immunol. Immunother.*, 1998, vol. **46**, pp. 277 - 282.
11. Chang C.M., Limanni A., Baker W.H., Dobson M.E., Kalinich J.F., and Patchen M.L., 'Sublethal gamma irradiation increases IL-1alpha, IL-6, and TNF-alpha mRNA levels in murine hematopoietic tissues', *J. Interferon Cytokine Res.*, 1997, vol. **17**, pp. 567 - 572.
12. Toki J., Adachi Y., Jin T., Fan T., Takase K., Lian Z., Hayashi H., Gershwin M.E., and Ikehara S., 'Enhancement of IL-7 following irradiation of fetal thymus', *Immunobiology*, 2003, vol. **207**, pp. 247 - 258.
13. Chung B., Barbara-Burnham L., Barsky L., and Weinberg K., 'Radiosensitivity of thymic interleukin-7 production and thymopoiesis after bone marrow transplantation', *Blood*, 2001, vol. **98**, pp. 1601 - 1606.
14. Von Freeden- Jeffry U., Solvason N., Howard M., and Murray R., 'The earliest T lineage-committed cells depend on IL-7 for Bcl-2 expression and normal cell cycle progression', *Immunity*, 1997, vol. **7**, pp. 147 - 154.
15. Wolf S.S. and Cohen A., 'Expression of cytokines and their receptors by human thymocytes and thymic stromal cells', *Immunology*, 1992, vol. **77**, pp. 362 - 368.
16. Wu X. and Deng Y., 'Bax and BH3-domain-only proteins in p53-mediated apoptosis', *Front. Biosci.*, 2002, vol. **7**, pp. 151 - 156.
17. McConkey D.J., Zhivotovsky B., and Orrenius S., 'Apoptosis – molecular mechanisms and biomedical implications', *Molec. Aspects Med.*, 1996, vol. **17**, pp. 1 – 110.
18. Herr I., Wilhelm D., Bohler T., Angel P., and Debatin K.M., 'Activation of CD95 (APO-1/Fas) signaling by ceramide mediates cancer therapy-induced apoptosis', *EMBO J.*, 1997, vol. **16**, pp. 6200 - 6208.
19. Van Ewijk W., Wang B.P., Hollander G., Kawamoto H., Spanapoulou E., Itoi M. et al., 'Thymic microenvironments, 3D versus 2-D', *Sem. Immunol.*, 1999, vol. **11**, pp. 57 - 64.

Radiation-induced genome instability: phenomenon, molecular mechanisms, pathogenic significance

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ABSTRACT

The data were considered regarding radiation-induced genome instability, which is a specific state of in vitro irradiated cells descendants and progeny of cells after whole body ionizing irradiation, which makes them substantially different from the normal, not irradiated cells. This state is characterized by citogenetic, molecular-biological, cytological and biochemical manifestations, which are not usual for normal cells, is controlled by mechanisms of cell cycle checkpoints regulation and by process of apoptosis, which are in turn controlled by gene *p53*. There is an evidence that this state is transferred to survived progeny of irradiated cells by through epigenetic mechanisms and will continue to exist until the cells they return to basal (prior to irradiation) state of response to DNA damage. From the point of view of genome inconstancy concept which considers mechanisms of chromatin rearrangement as those, which provide for adaptive-evolutionary process directed to adaptation of a species to changing conditions of environment this radiation-induced genome instability may be interpreted as a transition of a progeny of irradiated cells into a state of readiness to adaptive changes with two alternative results: 1) adaptation to the given conditions and gradual normalization of cellular functions and phenotype (transition into a normal, steady state); 2) transition into a transformed state with retention of genome instability and increase of probability for tumor transformation.

Keywords: DNA, repair, genome, ionizing radiation, low doses, apoptosis, *p53*, epigenetic inheritance

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Not so long ago we have considered some recent data regarding molecular basis for reproductive cell death, apoptosis and necrosis directly after irradiation, which are main causes of radiation sickness and death of mammals and humans [1]. According to the analysis results these deterministic effects of ionizing radiation are of common molecular nature, which become best evident after studying apoptosis process and consist of complicated genetic and biochemical phenomenon which accompany DNA repair.

Continuing the analysis regarding stochastic radiation effects it became necessary to consider, first of all, the problem of radiation-induced genome instability (RIGIS) in an analogical manner. This issue is directly connected to mechanisms of such important radiobiological phenomenon as radiation mutagenesis, carcinogenesis and ageing, which are main consequences of ionizing radiation exposure. In the last decade there have been accumulated interesting results, which break common radiobiological concepts; also, an attempt has been made to directly extrapolate these results to the practical sphere of evaluation of radiation risk and radiation regulation [2].

Recently, the main demonstrations of the broader phenomenon of genome instability were chromosomal rebuilding and in particular chromosomal aberration, and its basic mechanism was fixation of cellular progeny damages in initial DNA structure, which has not been eliminated by repair systems due to their temporary failure or their natural inclination to errors (error prone repair). Structural changes in several genes are the basis of pathological processes during such heritable diseases as ataxia teleangiectasia, Fanconi's anemia, Blume syndrome, etc., for which irregular response to DNA damages, disorder of control of programmed cell death (apoptosis) and increased possibility of tumor transformation are common [3].

Alongside with permanent, genetically inheritable genome instability, specific radiation-induced genome instability (RIGIS), which appears due to ionizing radiation influence, has been recognized within a few last years. This develops in the form of cell late reproductive death (late lethal mutations), chromosome destabilization, somatic mutations and gene amplification, changes of radiosensitivity. Ideas on RIGIS are based on results of investigations of cells in tissue culture.

Two features of RIGIS are distinguished [4] as common features of proliferative cells. One of these features characterizes a phenomenon in general as a permanent decrease of possibility of growing and replication of irradiated cells without further mutations in genetic material (in other words genome instability is an increased possibility of mutation changes). The other feature

emphasizes on the fact that those cells, which possess genome instability after being irradiated are generated with high frequency though they do not form a uniform clone and gene damages detected in these cells are accidental, unpredictable in terms of their frequency, time of development and value.

RIGIS is inherited to many generations of cells, which are generated by means of replication; herewith genetic changes detected in cells of daughter generations are different from those generated in "parent" ones, i.e. in an irradiated cell itself. Naturally, radiation increases frequency of spontaneous genetic changes in survived irradiated cells or, to be more precise, in cellular populations formed by these cells at their normal functioning [5].

LATE REPRODUCTIVE CELL DEATH

It was noted already in 1986 that late lethal mutations provide for reduction of ability of irradiated cells progeny to form colonies *in vitro* [6]. This data were confirmed with cells of hybrid line GGL1 (HeLa X skin fibroblasts) exposed to γ -radiation of 7 Gy. Decreasing of clonogenic activity of progeny of irradiated cells formed through reseeding with the similar with control by the density of plating and conditions of cultivation were explained by progeny late expression of lethal mutations [7]. This phenomenon was called late reproduction cell death.

It was displayed that after irradiation this phenomenon develops in many generations of cells of mammals [8] and is accompanied by progeny phenotype, which is different from a *parental* phenotype. Thus, progeny of the 12th-14th cycles of doubling of survived cells of Chinese hamster had different anomalies including decreased ability of adherence to substrate and retarded passage through a cell cycle. An increased number of aborted colonies and colonies with giant cells were detected after plating of single cells. This made it possible to make a conclusion that "traces" of damages are transferred to survived progeny of irradiated cells through many mitotic cycles and that the damages may appear *de novo*.

Thus, a stable phenotype with late death of irradiated cells CHO and SQ-20B of human cancer retained after irradiation in more than 50 generations and was accompanied by aneuploidy and formation of colonies with a great number of giant cells. By somatic hybridization these cells with intact cells this type of inheritance turned out to be dominant due to the fact that effectiveness of

cloning hybrids was substantially lower than that of hybrids of cells that were not irradiated [9].

When HeLa cells were γ -irradiated of 10 to 50 cGy it was noted that survival was reduced at the dose of 50 cGy both in generation of irradiated cells and their progeny, and no changes appeared after irradiation these cells in lower doses. However, extra treatment with mixture of cytosine arabinoside (inhibitor of DNA repair synthesis) and hydroxyurea (inhibitor of replicative synthesis) reduced viability of cell descendants, which, in authors' these publications opinion, indicates genome instability [10, 11].

Phenotype of late reproductive cell death can be induced in CHO cells under other DNA-damaging factors, such as ethylmethanesulfonate and Hinfl endonuclease, which provokes single stranded DNA breakages. UV-radiation did not lead to generation of cells with phenotype of late death. At the same time CHO xrs-5 cells, which were mutant by repair of double-stranded DNA breakages after exposure to ionizing radiation did not generate any progeny with late reproductive cell death possibly due to quicker elimination [12]. This indicates that DNA breakage and mechanisms of double-stranded breakages are connected to formation of RIGIS.

Prolonged effect of ionizing radiation in increasing doses may provoke late reproductive death and formation of giant cells as RIGIS manifestation [13].

In order to study the dynamics and dependency upon irradiation dose of late reproductive death induction in progeny of irradiated cells, human keratinocytes of HPV-G line were exposed to γ - and α -radiation, which was maintained in the culture up to 72 duplications [14]. Progeny of cells survived after γ -irradiation in doses 1 and 3, but not 0.5 Gy, displayed decreased ability to form colonies. It was also observed that 15 - 20% of clonogenic cells were lost at every duplication up to the 72nd. Apoptosis death was observed in all colonies at doses 1 and 3 Gy after the 30th and 72nd duplication but not after irradiation at 0.5 Gy. The authors' opinion is that this process indicates RIGIS formation threshold by the effect of rarely ionizing γ -irradiation referenced to index of late reproductive death.

Irradiation by a beam of α -particles of 0.5 Gy led to approximately 20% reduction of ability of irradiated cells to form colonies with respect to control value.

CHROMOSOMAL DESTABILIZATION

Chromosomal destabilization has already long been considered as the first and direct feature of common genome instability. Radiation-induced chromosomal instability is distinguished in different cells of mammals [15, 16]. While studying different populations of metaphase cells in colonies developed from the same hybrid cell, which had been X-irradiated at doses 5 and 10 Gy, maintaining chromosomal instability was also detected [17]. This instability developed in multiple rearrangements of human chromosome 4 in a set of hamster chromosomes in 29 and 60% of cells colonies survived after irradiation at 5 and 10 Gy respectively. Correlation of maintaining instability of chromosomes with late reproductive death (decreased clonogenic activity) was detected. After making a correction to the length of chromosome all chromosomes in the survived V79 cells take part in generating dicentric aberrations with relatively equal probability [18].

Due to different reasons the total number of cells with aberrations of chromosomes decreases after every mitosis. At the same time clones with chromosomal instability can regain stability in the next cellular population, maintain the same level of instability or become even more unstable [19]. According to the authors' data [20] part of Chinese hamster cells, which did not have chromosomal aberrations in the 1st post-irradiation mitosis but contained free fragments in the 2nd and 3rd mitosis is about 5-10%. Chromosomal instability turns out to be a stable feature in those cells that underwent malignant transformation [21].

It is known that development of cells with chromosomal aberrations and micronuclei has common material basis. While examining cytogenetical, lethal (induction of micronuclei) and non-lethal (sister chromatid exchanges) damages as manifestation of genomic instability in Chinese hamster and HeLa cells culture, the authors studied dependency of RIGIS expression by these indicators on radiation dose in more than 30 generations of progeny of irradiated cells [22]. Induction of sister chromatid exchanges, increasing number of cells with sister chromatid exchanges and micronuclei, increasing of radiosensitivity and absence of adaptive response from progeny of irradiated cells was also observed. Recovery from potentially lethal damages was accompanied by decreasing number of cells with micronuclei (i.e. evidence of lethal damages) was observed in descendants of irradiated cells in more than 20 generations. RIGIS expression degree by these factors depended on irradiation dose and was

observed after exposure to radiation at the dose of 0.5 Gy. Thus, even minor DNA damages may induce RIGIS.

Part of cells with chromosomal aberration among cells progeny effected by γ - and α -radiation with doses of 0.5, 1 and 3 Gy after 30 duplications was significant higher than in reference control. Chromosomal aberration type (chromatid rupture, fragments and small chromosomes) indicated the inheritance of chromosomal instability in generations. Expression of instability by this index reduced between the 30th and the 72nd duplication of the population after irradiation by 0.5 and 3 Gy doses and maintained until the 72nd duplication after irradiation with a dose 1 Gy. During the effect of α -particles the expression of chromosomal instability in cells progeny was more evident and remained almost unchanged by the time. According to the authors' opinion [14] unstable chromosomal aberrations typical to RIGIS may lead to apoptosis and contribute to formation of late reproductive death/lethal mutations in HPV-G cells. The authors think that there are no any direct correlation and simple ratios between such two displays of RIGIS, as late reproductive death and chromosomal aberration, especially after exposure to radiation at low doses.

However, correlation was detected between late death and increase of the number of cells with micronuclei during RIGIS. Thus, after X-irradiation V79 cells by doses 1 to 9 Gy an increased number of cells with micronuclei formed by the 7th day, maintained at the same level within 4 weeks being independent from the irradiation dose within the range of 3 to 9 Gy [23, 24]. After high LET irradiation, dose-dependent increase of cell frequency with micronuclei was detected in the population of V79 cells of Chinese hamster after a week. This raise of cell number was accompanied by a maintained increase of the number of dicentric chromosomal aberrations [25].

LATE MUTATIONS

In 1990, it was shown [26] that dozens of cell generations had an increased number of mutations after irradiation. Mutation frequency in locus of guanine-hypoxanthine-phosphoribosiletransferase (*hprt*) of generation of irradiated cells after 95 - 100 divisions was 2 degrees higher than its spontaneous level and achieved 10^{-3} and demonstrated possibility to initiate a mutant phenotype related to reproductive cell death [27]. It is important to note that mutation mechanisms under RIGIS are different from those that appear in

directly irradiated cells. While more than 70% of mutations under direct irradiation are related to deletions [28, 29], the mutations appearing as RIGIS demonstration are described as point mutations effecting many genes (specifically *hprt*, ouabain resistance, thymidilate kinase (*tk*) and tumor suppressor *p53*) [30 - 32]. In the beginning of the 1990's and later it was demonstrated that mutation process which is specific for genome instability draws not only encoding but also minisatellite genome areas [33 - 35].

MINISATELLITES

Minisatellites is a class of tandem-type repetitions actively moving in genome and influence the expression of genes by modifying encoding sequences or by forming "fragile" areas. Instability in GC-enriched minisatellites joins the mutation processes in somatic cells and gametes. Complex of changes takes place in human gametes during meiosis when repetitions probably control the intensity of recombinant DNA activity aligning into flanking areas and localizing meiotic recombination. On the contrary, AT-enriched minisatellites probably start influencing the development of intraallelic processes during replication [36].

Experiments on TK6 line of lymphoblastic human cells showed that ionizing radiation increases mutation frequency in minisatellite locuses [35]. It was determined later that TK6 clones selected by mutation in *tk* gene localized in chromosome 17q displayed higher spontaneous minisatellite mutability in different chromosomes than mutability detected in cells of a wild type and that this mutability is induced by ionizing radiation [32]. These tests prove the fact that general genome instability plays a great role in mutagenesis but to the authors' point of view the tests do not actually prove the fact that the changes observed in minisatellites are specifically induced by the effects of ionizing radiation and are not spontaneous.

The other phenomenon, which is regarded as genome instability demonstration, is *genes amplification* [37]. It was determined that ionizing radiation causes cell death of EMT-6 mice cells, which depends on the radiation dose. At the same time radiation induces a significant amount of survived cells methotrexate (MTX) resistance by amplification of dihydropopholatereductase (dhfr). So, the maximum output of mutants (8 times higher than that of

spontaneous level) is observed during irradiation in dose D₃₇ against high level of *dhfr* gene amplification in MTX-resistant cells [38].

APOPTOSIS

In the early 1990's, it was discovered that every cell has a supervision function, which prevents from transferring such structural genome damages from parental cell to daughter cell that were acquired by a parental cell and were not eliminated by repair systems. This system is controlled by tumor suppressing gene *p53*, which does not only produce conditions for DNA repair through G₁- and G₂-block of the cell cycle, but also starts one of the cell death programs, which is apoptosis (in case when genome has changes that are unacceptable for further vital functions) [39].

Later on, 48 hours after exposing to γ -radiation at doses of 1 to 12 Gy the detection frequency of V79 cells that die of apoptosis increased to the maximum of 35% and decreased to 10% by the 3rd day depending on the dose of irradiation. Even 2 weeks after irradiation the level of apoptosis was 10 times higher than in the control, which had not been irradiated [40]. Similar data were received in research [4]. At the same time, it turned out that in cells with RIGIS apoptosis developments depend greatly upon metabolic factors and may be completely eliminated for example under influence of cyclosporine A.

Activation of apoptosis process under RIGIS is probably connected with the fact that the total amount of changes maintained in DNA in every cells generation with unstable genome periodically causes part of cells to start the cell death program.

IN VIVO MANIFESTATION OF RIGIS

Mutagenesis observed in cells of the organism under real conditions e.g. intense background of man-induced effects is determined by the factors, which diversity and effects may hardly be recorded. Though radiation induced changes in the progeny of in vitro irradiated cells can be definitely determined as causally related to initial radiation effect and inheritance under epigenetic mechanisms due to standard controlled conditions, in vivo RIGIS may develop

from different genotoxic factors; thus, the relation with radiation does not appear to be completely evident and requires a specific proof.

Already in 1986 it was assumed that the effects observed *in vitro* regarding late lethal mutations existed also after irradiation *in vivo* [6]. In opinion of the authors of the cited work a well-known fact points to that: irradiated but looking healthy tissue displays unusual sensitivity to the further ionizing irradiation. Furthermore, quantitative evaluations of late cell death based on *in vitro* data may significantly diminish actual evidence of the effect *in vivo*.

These assumptions were conformed experimentally. In accordance with data from the authors' work [41] genome instability induced *in vitro* affects 10-12% of the total number of progeny of cells exposed to ionizing radiation. Special experiments carried out in order to solve this problem showed that in DNA of progeny of mouse cells C3H/10T1/2 that were *in vitro* exposed to X-rays, grown *in vitro* or in bodies of syngeneic mice C3H (after equal number of divisions, 25 passages during approximately 6 months) frequency of rearrangements in cells *in vivo* was considerably higher than under *in vitro* conditions [42]. DNA rearrangements affect 50 - 100% of cell subclones.

Moreover, when 10% of cells were exposed to α -radiation ^{238}Pu during cultivation of hematopoietic stem cells, karyotypic anomalies were observed in 40 - 60% of cells [15]. This result makes it possible to conclude that progeny maintains signals that are transferred from irradiated to unirradiated cells inducing the formation of genomic instability. The assumption regarding the fact that genomic instability is modulated by factors inherent in an animal body was generally proved [5, 43].

In recent years, earlier experimental data and results of the latest investigations of the ionizing radiation impact consequences on human beings especially after the Chernobyl Accident were associated with development of genome radiation instability *in vivo*. Distinctions, which sometimes can hardly be made, between inherited aftereffects and aftereffects of continuing radiation or other genotoxic factor exposure often are not made. Perhaps it is one of the main difficulties to prove the RIGIS manifestation in actual conditions *in vivo*.

Taking into account the fact that research of minisatellite locuses of tandem-type repeats might be useful for monitoring radiation induced mutations of mammalian cells [44] frequency of development of such mutations was studied in children that were born in the families residing in Mogilyov Area, Byelorussia, influenced with Chernobyl radioactive contamination. It turned out that this frequency is twice higher than in controls from Great Britain [45, 46].

There question is brought up still: is this observation sufficient to regard such developments as RIGIS ones or should they be considered as manifestations of genome instability caused by other reasons?

The exposure of cultures of HeLa cells and hybrid mice (CBA×C57B1)F₁ in the area polluted with γ -radionuclides (5 - 40 Cu/km²) located in a 10-kilometer zone of the Chernobyl accident during certain periods (1 to 12 days) with the dose rate of radiation of 2.4 cGy/day and further exposure to radiation of 1 to 4 Gy for the purpose to evaluate the adaptive response by a number of tests made it possible to determine that the adaptive response develops neither in cells culture nor under *in vivo* conditions. On the contrary, high sensitivity to subsequent irradiation was registered. The number of citogenetic damages (such as chromosomal and micronuclei aberrations) increased both in HeLa cells and in bone marrow cells of mice; aberrations spectrum varied to chromosomal type at that. Cells with multiple chromosomal aberrations and phenomena of apoptosis activations were observed [46].

Similar data were received from experiments with blood lymphocytes of both adults and children residing in the area polluted with γ -radionuclides (5 - 40 Cu/km²) after Chernobyl accident [47, 48]. In comparison with the Moscow residents those people displayed reduced mitotic lymphocyte index stimulated *in vitro* with phytohemagglutinin [47]; frequency of lymphocytes with micronuclei in blood of children was 2.3 times higher than that of control group from Moscow [48].

The observations on people showed that despite a long-term exposure to radionuclides in the area of Chernobyl accident initially induced unstable chromosomal aberrations were gradually eliminated [49].

The observations on clean-up workers of the Chernobyl accident exposed to low dose radiation (below 0.25 Gy) showed that after 10 years the frequency of mutations in somatic cells was increased (glycophorine test). This phenomenon the authors refer to strengthening of spontaneous mutagenesis and development of genome instability [50, 51].

Frequency of cytogenetic changes in somatic cells of the first generation progeny born from field voles [52], mice [53], rats [54], monkeys [55] and people [56 - 58] who were exposed to radiation before conception was high, which indicates on the rise of molecular-genetic changes in the cells of progeny characterized at present by the "genome instability" term.

This instability registered through effects of inductions after blood irradiation *in vitro* of chromosomal aberrations in blood lymphocytes of children borne from patient who underwent radiotherapy and cytostatic

antitumor therapy. Differences in types of chromosomal aberrations of examined children were detected in comparison with control group. The essence of this difference includes the fact that in chromosomal aberrations the frequency of mutations of exchange type (dicentrics and rings) increased, which was typical for radiation effect and effects which quantitatively depend upon the radiation dose [57]. The conclusion was made that children who suffered genotoxic actions on the stage of parental gametes are related to a group of high mutation and carcinogenic risk.

Examination of 3036 persons living in the western part of the Altai Region bordering the Semipalatinsk Nuclear Test Site resulted in genome instability developments (high frequency of lymphocytes with micronuclei in blood) with those who were born during intensive nuclear tests (1949 to 1962) [58].

The question is brought up: how long shall genome instability induced in vivo remain in progeny? Mutant karyotypes of rodents inhabiting the area of East Urals radioactive zones of Kyshtym accident are observed after 30 - 40 [61] and even after 70 - 80 generations since the radiation influence began [52].

Investigations of laboratory colony of field-voles parents caught not far from East Urals radioactive zones showed that the first generation maintained high frequency of chromosomal aberrations typical to their parents, which decreased to reference value in the next generations (the 3rd generation) [62]. This phenomenon denotes the fact that cytogenetic effects are connected with plutonium pollution of rodents' environment, which causes mutations in germ cells leading to genome instability in progeny. Manifestations of this instability are gradually eliminated in the next generations of animals in case they live outside of the polluted area. Probably, this is the reason why high frequency of chromosomal mutations was not found in the children whose parents suffered nuclear attacks of Hiroshima and Nagasaki [63].

GENOME INSTABILITY FORMATION MECHANISMS

As noted, RIGIS is a form of general genome instability, which may be developed in cellular systems in response to effects of genotoxic factors of

certain power [4], genetic susceptibility and even spontaneously under the reasons that have not been found out yet. Mechanisms of its formation have not been ascertained though intensive research on molecular mechanism of cell cycle regulation, apoptosis and malignant transformation carried out within the last few years directly point out that genome instability is connected with the above.

Recently cytologic explanation of RIGIS mechanism was presented [64]. Animal and human organisms are supposed to contain a small sub-population of cells with unstable genome, which are called evolutionary and/or ontogenetic cell reserve. Genome instability is induced in these cells by epigenetic program. This program causes DNA damages mostly in “hot points” of chromosomes. The result is rise of new genetic variants.

However, there is a great number of molecular and genetic ways that may cause different RIGIS manifestations in the majority of cells of any type. The experiments on hybridome GM10115 line of cells, which contained chromosome 4 surrounded by 20-24 hamster chromosomes did not show significant correlation between chromosomal instability and sister chromatid exchanges, late mutations and mispair DNA repair. At the same time demonstrations of late gene amplification remained on the significance border of correlation ($p < 0.1$) and cell death delay significantly correlated with chromosomal instability ($p < 0.05$) [65].

Three interrelated systems providing for maintaining genome stability are known at cellular level:

- 1) homeostasis redox system, which produces different cytotoxic factors including reactive oxygen species (hydrogen peroxide, superoxide anion-radical, OH[•] radical) that take part in elimination of genetically foreign material;
- 2) cell cycle control system at check-points, which provides emergency elimination of cells with pathologically modified DNA that may lead to disturb genome stability; the main component of this system is tumor suppressor – protein p53;
- 3) DNA repair mechanisms.

REACTIVE OXYGEN FORMS AND GENOME INSTABILITY

In recent years, the concept development of redox homeostasis role in radiobiological effects at low doses of ionizing irradiation was observed [66]. The main point of this thesis means the provision of balance between production of reducing substances and oxidants because of which reactive oxygen species (including superoxide) act as substances having both protective and cytotoxic functions. Similar ideas are developed in terms of nitric oxide ion and radical, and peroxynitroxide – the products of nitrogen oxide, discovered and studied in the recent decades [67].

There was assumed that the main source of RIGIS in progeny of irradiated cells is the expression of production of high quantities of reactive oxygen species (ROS) in these cells. As a result cellular components and firstly DNA are permanently damaged, which results in increase of frequency of cell death, mutations and chromosomal aberrations.

Really during the comparison of frequency of chromosomal aberrations by hybrid F₁ mice of radiosensitive CBA/H and DBA/2 line and radiation-resistant C57BL/6 line after exposure to α -particles animals of radiosensitive line displayed dominant inheritance for both high rise of chromosomal aberrations and ability of tissues to generate high amounts of superoxide anion-radical, which accompanied the process [68]. These results display the importance of genotype for RIGIS formation.

It is also important that the observed instability effects may result from displacement in redox homeostasis system towards high production of ROS [69].

Thus, cultivation of progeny of human keratinocytes of HaCaT line with low-molecular antioxidants changed the strength of RIGIS manifestation after exposure to γ -radiation with dose 5 Gy and led to decrease of apoptotic cells number accordingly to the dose and recovered clonogenic activity [4]. This phenomenon points out to low ability of irradiated cells progeny to maintain redox homeostasis due to disturbance in mechanism of adaptive production of endogenous antioxidants synthesis regulators.

Actually, in response to oxidative stress the contents in normal cells of anti-apoptosis protein bcl-2 and thermal shock protein Hsp 25/27 that cause raise of anti-oxidant glutathione contents increases [70]. This mechanism does not work in progeny of irradiated cells with RIGIS manifestations cultivated without anti-oxidants; as a result of this, percentage of bcl-2 positive cells is

low. On the contrary, cultivation of cells in anti-oxidant environment recovers the percentage of bcl-2-positive cells to normal level [4].

Analogical data were received from the experiments with culture of bone marrow cells which differed twofold in their ability to produce endogenetically superoxide anion-radical: initiation of "respiratory burst" led to development of a great number of chromatid breaks in cells of the line which are characterized by 2 times higher capacity of superoxide anion-radical production [71].

The given data directly display changes of cellular system of redox homeostasis during RIGIS and agree with the fact of increasing the frequency late apoptosis process. However, in general the role and mechanisms of ROS participation in rise of RIGIS needs to be studied further.

CELLULAR CYCLE CONTROL AS MECHANISM FOR MAINTAINING GENOME STABILITY AND RIGIS FORMATION

As it was mentioned earlier features of RIGIS manifestations are chromosomal aberrations, low ability of irradiated progeny cells to form colonies and high susceptibility to apoptosis. All these features are closely connected to genetically controlled cell cycle process and are caused by its disorders. It is known that main role in integration of damaging signals that influence a cell (first of all, its DNA) is performed by tumor suppressor protein p53, which is controlled by a respective gene. Disturbances in functioning of tumor suppressors (p53, pRb) and activation of proto-oncogenes (Myc, Ras and maybe some other) leads to dysfunction of checkpoints of the cell cycle and genome instability [72].

Thus, the influence of ionizing radiation on L mouse cells that had wild type gene p53 led to activation of mutant protein p53(175(Arg>His)) that in its turn led to disability of protein to block cells in phase G₁ under the γ -radiation exposure. Mutant phenotype was characterized by 5-20 times higher level of spontaneous homologous recombination between intrachromosomal direct sequences. This data indicate on the defect in the gene and protein p53, which is a reason for genetic instability rise [73]. Appearance of multiple mutations in gene p53 are noticed in progeny of normal epithelial human cells exposed to γ -radiation of comparatively low (0.5 Gy) and high (5 Gy) doses. In both cases multiple "focuses" of morphologically normal dividing cells with high expression of p53 were developed in the cellular culture after 5-10 cycle of

cellular division. Analysis of gene *p53* structure in such cells that was carried out by methods of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) showed that the progeny of cells irradiated even under 0.5 Gy developed point mutations in the given gene. As a result mutant protein p53 loses its ability to fully cooperate with a chromosome: maintaining its own high expression it loses the ability to control fully other genes, specifically *p21*, *bcl-2*, *bax* participating in monitoring the passage of cells through the cycle and elimination of damaged part of the cellular contingents by apoptosis. As a result, some mutated cells remain in progeny and become one of the reasons for maintaining genome instability in generations [74]. These results agree with the previously noticed fact of genome destabilization in progeny of irradiated cells and after irradiation with low doses.

Actual existence of this mechanism is proved by information on the fact that loss of control over checkpoints of replication and DNA damages in the cellular cycle, e.g. during inactivation of protein Hus 1 at mice (analogue of gene *hus 1* product – sensitivity product to urea *Saccharomyces pombe*) brings to accumulation of genome damages. If during this process gene *p21/WAF1*, that product is inhibitor of cyclin-dependent kinases, which is responsible for arrest of cells in phase 'G₁' in response to hyperexpression of p53 and DNA damages, inactivated the proliferation of cells continues, but these cells become significantly more sensitive to hydroxyurea (inhibitor for DNA replication) and UV-radiation and become insignificantly more sensitive to the exposure of ionizing radiation [75].

It is known that penetration of oncogenes by means of viruses or their activation in genome cause genome instability. One of the possible mechanisms of its development may be mutation in gene *p53* due to activation of oncogenes or protogenes under the influence of radiation. At that, when contents of tumor suppressor protein p53 in normal cells raised dozens of times, the cells transformed by oncogenes such phenomenon did not display [76].

Loss function of full value by tumor suppressor, which is controlled by gene localized in chromosome 11 led to increased demonstration of genome instability (late death) in progeny of hybridome CON104(-11)/CGL1 human cells in response to exposure of ionizing radiation [77].

Usually, RIGIS was considered as a stage prior to tumor transformation. However, if the loss of gene of tumor suppressor of chromosome 11 is sufficient to form RIGIS, radiation-induced tumor transformation requires inactivation of suppressing genes both in chromosome 11 and chromosome 14. At the same

time tumor suppressor gene of chromosome 14 does not play any role in RIGIS development [78].

DNA REPAIR MECHANISMS AND GENOME INSTABILITY

Temporarily, whole genome mutations which were called adaptive are recombination-dependent and appear in response to stress exposures. In prokaryotes they are regulated by the SOS-response – a complex of following reactions, which include induction of gene products that block cell division and assist mutation, DNA recombinations and repairs. SOS-response, which is well studied with *Escherichia coli* is global response to DNA damages and a prototype of control mechanism of cell cycle in checkpoints and of DNA repair system. Central part of SOS-response is derepression of more than 20 genes, which are under direct and indirect transcriptional control of repressor LexA. In the absence of functionally valuable SOS-response cells are sensitive to DNA-damaging agents [79].

Analogous response system to DNA damages may be found in cells of mammals [80]. Similarly, to provide for normal functioning of cells of some types, e.g. those of the immune system, mammal organisms undergo mutations, which are analogical to those adaptive mutations of prokaryotes, with their further selection.

Thus, immunoglobulin gene mutates in hypermutation processes, which take place in blastogenic centers of lymph nodes, spleen and Peyer's plaques. It was concluded from the experiments on transgenic mice that such mutations result from transcription process, where “hot points” for these mutations in immunoglobulin genes are short repeated sequences.

There are replication and recombination models, which describe this mutagenesis.

The attempts to associate such mutations with changes by transcription repair have not led to decoding of mechanisms of this mutagenesis. It turned out that with those mice that had defects in nucleotide excisional repair associated with transcription, somatic mutations in immunoglobulin occur with the same frequency, which indicates the fact that mutagenesis does not depend on presence of such repair.

Since “hot points” in DNA of immunoglobulin genes are presented as short repeated sequences, it was assumed that mismatch repair system (MMR)

was associated with somatic mutagenesis. Investigation of MMR showed that repair proteins of this system are not actually required for somatic hypermutability in germ center B-cells of mice [81]. This why induced or increased somatic mutagenesis in this case has different molecular basis. The phenomena connected with V(D)J-recombination can be regarded to as such basis.

It was displayed that double strand DNA breaks (DSB) in cells of mammals may result from normal metabolic processes, activity of DNA-damaging agents, such as e.g. ionizing radiation, or specific enzymes, which initiate V(D)J-recombination in developing lymphocyte. Unrepaired DSB may lead to such consequences as cell death or neoplastic cell transformation. Repair of DSB in mammals is carried out by means of either homologous recombination or nonhomologous DNA ends joining (NHEJ).

Five components of NHEJ system are known. Three of them are subunits of DNA-dependent protein kinase (DNA-PK), proteins Ku70 and Ku86, which form DNA end binding complex and catalytic subunit. Two other complexes are respectively DNA ligase IV and XRCC4 protein, which act in reaction as a catalytic ligase complex.

It was determined that the path of homologous recombination plays an important role in maintaining chromosome stability. However, main part in preventing instability is performed by NHEJ including such feature as its translocation. Repair of DSB by means of NHEJ is a critical guardian of genome of mammals, which influences both spontaneous and exogenously induced chromosome damages [82].

At studying influence of mutation p53, appearing during RIGIS in part of cells, on spontaneous homologous recombination between intrachromosomal direct repeated sequences in mice L cells it was proved that during hyperexpression of mutant protein p53 (175(Arg>His)) one can observe absence of cell arrest in G_1 -phase of the cycle after γ -radiation exposure and 5-20-fold increase of spontaneous recombinations compared to cells of wild type (p53 $^{+/+}$) [83].

Spontaneous DSB in cells of TK-E6 line, which was transfected by human papilloma virus 16, started a cycle of break-fusion-bridge that led to multiple chromosome aberrations and loss of function of p53, which was accompanied by increase of frequency of spontaneous mutations at heterozygous locus of thymidine kinase gene. In cells wild type p53 $^{+/+}$ of spontaneous DSB restored completely by means of recombination between homologous chromosomes [84].

To maintain genome stability, of importance is to obtain valuable and functional NHEJ components due to the fact that defects with Ku70, Ku86, Lig4 and XRCC4 lead to premature aging, acute disorder of V(D)J-recombination and increase of cell sensitivity to DNA-damaging agents inducing DSB. At mice the inactivation of any of the four genes, which code the enumerated components, leads to multiple disorders, including growth disorder, different immunodeficiencies and massive increase of apoptosis frequency among newly formed postmitotic neurons. Insufficiency of catalytic DNA-PK subunit also leads to increase of sensitivity to DNA-damaging agents and V(D)J-recombination disorder though its absence does not lead to such evident insufficiency manifestations as it can be observed with deficiency of the other four known NHEJ components.

In general, insufficiency in NHEJ may lead to high frequency of neoplastic transformation. However, the extent to which the discussed disorders in components of NHEJ system during recombinational repair are connected to formation of RIGIS still remains unknown.

Apart from one of DNA-PK subunits, which belongs to the class of phosphatidyl-inositol-3-kinase, in the cells of mammals there were identified at least two more specimens of the same kinase class: ATM and ATR. ATM takes part in DNA break discerning and transmission of a signal to the lower targets, including protein p53 (by serine-15 phosphorylation in it), which influences cell cycle, transcription and/or apoptotic mechanisms [85].

The existence of two classes of ATM mutations was determined: null-mutation, which leads to ataxia-teleangiectasia and dominant negative missense mutations, predisposing to cancer in heterozygous condition. ATM plays the main role in maintaining genome stability by providing high accuracy of processes that occur to chromosomes. Defects by ATM gene at humans cause exceptionally high radiosensitivity [86].

Participation of ATM gene in formation of RIGIS through gene p53 product is very probable but has not yet been proved by anybody and needs further study. Moreover, there is no information about state of ATM with already formed RIGIS.

In some cases, DNA repair requires gene expression. In its turn in order to activate many of inducing genes it is necessary to change chromatin conformation – movement or “rearranging” of nucleosome, in particular. This makes it possible transcriptional factor binding, which actually start the process of gene expression. As for other inducible genes, particularly for *GADD45*, such change is not required – nucleosomes are already aligned around area of binding

transcriptional factors prior to DNA damaging in such a way that regulator areas are maintained in conformation, which is free for binding with transcriptional factors at any moment [87].

A high mobility group (HMG) of chromosomal proteins, which form three subfamilies has been known. These are HMG-1/-2, HMG-I/-Y and HMG-14/-17. These proteins facilitate through local changing of chromatin conformation such DNA-dependent processes as transcription, replication and recombination; in particular proteins HMG-1/-2 take part in stimulation of V(D)J-recombination [88]. One of the proteins - HMG-1 – facilitates activation of gene p53. Disorder in higher levels of DNA laying and its organization in chromatin threads after radiation exposure with changes of conditions of HMG-proteins functioning may effect the efficiency of V(D)J-recombination and thus lead to reduction of genome stability.

The analysis of information from the literature demonstrated importance of Gadd45 in maintaining normal genome stability and, consequently, it may participate in forming RIGIS. *GADD45* gene is connected to processes of cell cycle arrest (in the S-phase) and DNA repair. Under UV-radiation, methylmethanesulfonate or stress gene *GADD45* activation depends upon cooperation of transcriptional factors with promotor. In case of ionizing radiation it may include DNA-protein interactions both in promotor and gene *GADD45* intron 3 area, and potential interaction between complexes in both areas. As demonstrated, quick regulation of *GADD45* expression after exposure to ionizing radiation does not require synthesis of new transcriptional factors, changes in chromatin structure or new radiation-induced protein interactions. This means that gene *GADD45* relates to a type of genes, which may be activated by transcriptional factors without preliminary changes of chromatin structure [89]. However, data on this point are yet insufficient.

Phenotypes common for p53-defective mice such as high radiation carcinogenesis and genome instability were observed in Gadd45a-zero line mice. Genome instability in them developed in the form of aneuploidy, chromosomal aberrations, genes and centrosome amplifications, and was accompanied by anomalies in mitosis, cytokinesis and growth control. Unequal segregation of chromosomes due to large number of spindle poles during mitosis was determined in cells of several Gadd45a(-/-) lines. Thus, Gadd45 is one of p53 path components, which takes part in maintaining genome stability [89].

Expression of *p21* and *Gadd45* genes was observed after irradiation in doses < 50 cGy, and levels of corresponding mRNA content in cells linear

depended on irradiation doses from 2 to 50 cGy. While irradiation by doses from 2 to 50 cGy did not decrease the efficiency of cell cloning or increased death ratio by apoptosis, transitory delay of cell cycle was observed. Induction of *p21* and *GADD45* genes under irradiation in doses that do not provoke cytotoxic effect showed that survived cells contribute significantly to the observed stress reactions, which may be related to epigenetic changes [90].

Since during RIGIS loses valuable p53 control over processes, which take part in maintaining genome stability, reduction of Gadd45 participation efficiency in providing condition for DNA repair is possible. This is proved by the data justifying the fact that activation of oncogenes – human papilloma virus in transformed HOK-16B keratinocytes of human mouth mucous tunic under UV-radiation is accompanied with disability of mechanisms of nucleotide excision repair to eliminate pyrimidine dimers from gene *p53*. This leads to synthesis of functionally defective protein p53, which concentration in such cells remains low as well as contents of protein *p21/WAF1/CIP1*, while in normal cells its concentration increases 30 times. These data display the fact that activation of oncogenes induces genetic instability through disturbance of nucleotide excision repair in cells, which is controlled by gene *p53* [77].

EPIGENETIC MECHANISMS OF RIGIS

Genetic inheritance is understood as transfer of information recorded in DNA from parents to their progeny, which is necessary for reproduction of certain biological structures. *Epigenetic* inheritance is understood as transfer of information about *functional* condition of genetic programs for the named structures, i.e. inherited changes of *activity* of a gene or a group of genes after exposure on the cells by internal or external modifying factor.

According to [91], this phenomenon is of high importance for RIGIS: changes in the gene expression model peculiar for an unirradiated cell, transfer of a changed model to daughter cells and its reproduction in the following generations on epigenetic level.

It is assumed [92, 93] that this process is associated with conformational rearrangements of chromatin. These changes in chromatin structure may appear after irradiation, e.g. during formation of radiation-induced exchange aberrations of chromosomal type in AT-enriched recombinogenic chromatin sites, connected to nuclear matrix [94], remain in cells and be transferred to

their progeny, despite the completion of DNA repair processes [95]. Changed gene expression may be inherited together with that. Its consequences in regard to V(D)J-recombination have been discussed above.

Another possible event of epigenetic expression inheritance is the gene “sleeping” state transfer after gene methylation. Though DNA methylation is not the main item in controlling the expression regulation of tissue specific genes in differentiating cells, changing products of its reactions in regulatory important areas of DNA may have important consequences as well as during RIGIS formation.

It is known that melanoma antigen-encoding gene (MAGE) family localized at humans and animals in X-chromosome, cells of all tissues (except testicles) remains in inherited “sleeping” (“silent”) condition [96] because of methylation of CpG dinucleotides in promotor area [97]. Influence of ionizing radiation may provoke changes in methylation of promoter area of genes both as a result of errors in DNA repair (including 5-methyl dCTP) and during fermentative methylation, which is catalyzed by DNA-methyltransferase [98] and because of possible demethylation. As a result genes that were “silent” before irradiation become active and their state is inherited by their progeny. Epigenetic inheritance of active condition of genes in mitosis is, by the [99] authors’ opinion, carried out through of nucleus matrix structures, which are associated with active genes and necessary enzymes. It is assumed that relaxed (active) monoreplicon chromatin loops are transferred in such state from parental to daughter cells and remain in this state in daughter cells. Mechanism of epigenetic inheritance is provided in those authors’ opinion also due to the fact that during division of a parental cell its progeny receives informosomes, which are ready structures of protein synthesis taking part in reproduction of epigenotype being inherited.

“BYSTANDER” EFFECT

Recently, there were attempts to explain RIGIS initiation mechanism by clarification of reasons for the so-called “bystander” effect. For the first time, this phenomenon was detected in experiments when cultural medium of irradiated cells was seeded with intact cells, which did not undergo the ionizing radiation exposure. Soon the cells and their progeny start to exhibit all or many features typical for RIGIS as if they appeared from an irradiated cell [100].

The “bystander” effect defined as the induction of genetic changes in nuclei of non-irradiated cells may reflect the development of at least two different mechanisms [101].

The first mechanism assumes that bystander effect is performed through intercellular contacts, which turn on p53-mediated path for signal of damage [102, 103]. According to the second mechanism irradiated cells secret cytokines and other factors, which in unirradiated cells increase intercellular level of reactive oxygen species [100, 104, 105].

Evidences for p53-mediated signal path in bystander effect were presented for the first time in the research work [106] while studying influence of low doses of radiation on lung epithelial cells of male rats of F344 line. Flow cytometry made it possible to determine a fraction of cells with high content of protein p53 and with that high expression of this protein was observed in a greater part of cells than in those that were directly exposed to α -particles. It is important to note that higher contents of protein p53 was observed even after irradiation by α -particles in doses as low as 0.6 cGy, while during sparsely ionizing X-ray exposure the dose used was 10 cGy.

Investigation of reaction of initial diploid human fibroblasts culture at low-energy α -radiations in low doses noted that though tracks of particles went only through 5% of nuclei, 3-4 fold increase of protein p53 and p21^{waf} contents was observed. High expression of these proteins in cells culture was abolished by preincubation with lindane – inhibitor of intercelular contact interactions, which points to the important role of signal transfer from directly radiation-damaged cells through intermolecular contacts.

Realization of “bystander” effect expression of gene, connected to cellular cycle, - p53, p21/Waf, p34^{edc2}, cyclin B1 (new names TP53, CDKN1A, CDC2, CCNB1 respectively) and rad51 was noted. Thus during irradiation of cell culture by low-energy α -particles, which provides intersection of approximately 2% of nuclei high gene expression was noted in limited areas. At that several groups of cells, which were not exposed to radiation, displayed high level of respective proteins, while in other cells of the same medium their contents remained at initial level. “Bystander” effect was substantially suppressed by lindane and also by low density of cellular population.

This result shows that direct cellular contacts are important for spreading damages also because in the presence of secreted diffusing factor we should have expected more homogeneous increase of *p53* and *p21^{waf}* gene expression [107].

The second mechanism of the bystander effect is secretion of factors to the culture medium. Thus, the culture medium from cells that were irradiated by a beam of low-energy α -particles, which was used for incubation of unirradiated test-cells, caused increase of frequency of sister chromatid exchanges [108]. Due to the fact that the used medium not exposed to direct radiation but only after cultivation of irradiated cells, the observed effect could only be a result of secretion of certain factors from irradiated cells. With that it is important to note that irradiated cells remained in the culture medium for at least within 24 hours after radiation exposure, which makes it possible to assume a possibility for long-term development and secretion by these cells of the above-mentioned factors to the extent of recovery of the damaged cellular DNA to the basal level. The “bystander” effect did not develop in case the medium was not exposed to thermal treatment or in case irradiated cells were treated with inhibitors of protein synthesis before the medium was collected. This proves that the secreted factors are in fact proteins [109, 110]. These factors of this medium also induce increase of intracellular level of reactive oxygen species including superoxide and hydrogen peroxide, which are critical intermediaries in damage expansion.

For realization of “bystander” effect in accordance with this mechanism it is extremely important state of systems that control redox homeostasis, which is proved by research with mutant cell lines. It studied clonogenic ability of human keratinocytes or mutant cells of Chinese hamster CHO-K1 after their exposure to γ -radiation or during cultivation in culture medium from the cell lines that had defects in lactate dehydrogenase (LDH $^{-/-}$) or glucose 6-phosphatehydrogenase (G6PD $^{-/-}$). While using apoptosis inhibitors or medium from (LDH $^{-/-}$) or (G6PD $^{-/-}$) cells it was noticed that “bystander” effect was reduced or abolished. Transfection of cells by G6PD gene led to recovery of the “bystander” effect. Treatment by antioxidants – L-lactate and L-deprenyl – prevented the influence of the “bystander” effect connected to cell death, which points to the participation of energy/redox metabolism in expression of radiation-induced “bystander” effect [111]. The “bystander” effect does not have linear dependence upon radiation dose. It is maximal at low doses.

Induction of mutation during irradiation of cytoplasm by α -particles is directly connected to “bystander” effect due to the fact that it leads to genetic changes in unirradiated nuclei.

It is obvious that both mechanisms of the “bystander” effect are important for the RIGIS initiation: both damage signals transferred through cultural medium and signal transferred through cellular contacts from progeny of irradiated cells to intact ones. The former may be carried out by means of

cytokine-type factors and the transfer process for RIGIS formation both with progeny of irradiated cells and with non-irradiated cells is independent of p53 [107].

At the same time, p53-dependent secretion of stress-dependent inhibitors of growth factors was described that determines the “bystander” effect [112]. These factors were discovered in cultural medium of transplantable and primary cellular cultures after γ -radiation exposure and also in urine of irradiated mice [113], which means the reality of observed mechanisms from the point of view of in vivo irradiation. Presently the research is performed to study the role of transforming growth factor [TGF- β 1], which is able to increase the contents of reactive oxygen species in unirradiated cells as one of possible substances, which are responsible for “bystander” effect in vivo [114].

p53-Dependent “bystander” effect is also obvious [74] when, besides usual growth, the growth of multiple isolated “focuses” with significant hyperexpression of p53 was observed in epithelocytes of human urinary tracts were observed under 0.5 Gy γ -irradiation exposure after 7 - 10 division cycles. The frequency of p53 gene changes in cells with normal morphology, developed after irradiation, was significantly higher.

It is believed that genetic instability derives from the cell cycle state checkpoints regulation and apoptosis mechanisms, which is controlled by gene p53 [115]. However, actual interaction and interrelation of these processes under RIGIS when the condition of this gene is already changed requires special investigations.

In the [101] authors' opinion still there are no evidences saying that “bystander” effect must exist in many generations; however, it was proved that it exists until the irradiated cells come back to initial reaction levels to DNA damage.

Thus, maintained genome instability may be induced through “bystander” mechanism. The initial profile of damage is amplified by “bystander” effect and the cells, which were affected by mechanism of “bystander”, may remain in the area of high risk of genetic changes for many generations.

PATHOGENETIC SIGNIFICANCE

From the analysis performed one may note that, phenotypically, RIGIS is a specific state of irradiated cell progeny, which makes them considerably

different from normal, unirradiated cells. At present this state is characterized by citogenetical, molecular-biological, cytologic and biochemical manifestations, which are not typical for normal cells. At citogenetic level this is a transfer of disposition to formation of chromosomal aberrations *de novo* with significantly low than normal-conditions levels of genotoxic exposures. This is the affinity to form point mutations, especially affecting regulatory genes and making them ineffective particularly in terms of maintaining genome stability, rate of cell renovation and elimination of foreign cells that facilitates tumor transformation. At molecular level this is the existence and transfer to progeny of epigenetic, conformational changes in chromatin structure, the activity typical for condition of irradiated maternal or neighboring cell, including expression and production of proteins, which take part in performing functions of checkpoints in the cellular cycle at first of all in p53. It maintains changed biochemical phenotype of cells and facilitates mutagenesis. Changes in location of mini- and microsatellite DNA sequences, genes amplification also facilitates phenotypic developments of mutagenesis. At cytological level it develops as a reduction of viability of cells in conditions considered normal for them, and reduction, at least *in vitro*, of their clonogenic ability. Biochemical features of such condition and mechanisms that cause late lethal mutations/reproduction death have been studied episodically, fragmentary and do not allow making some kind of integral pattern.

Biochemical development of RIGIS phenotype known at present in the progeny of irradiated cells is a mutation that depends on gene bcl-2 in the system of redox homeostasis in direction of reduction of ability of irradiated cells progeny to produce reduction metabolites of glutathione type leading to balance disorder in direction of dominant generation with cytotoxic influence of reactive oxygen species, including nitroxide derivatives (nitroxide and peroxide ions and radicals).

As for radiobiological characteristics of RIGIS, one can note that this phenomenon develops after irradiation in the wide range of doses, including low ones (< 20 cGy), especially during influence of dense ionizing radiation; it depends on radiation dose in probably a limited range; it is maintained in progeny of cells and probably has a tendency to be reduced in cells exposed to radiation for long periods during dozens of generations.

Phenotypic developments similar to RIGIS *in vitro* are also observed *in vivo*, but convincing proof of complete analogy of the observed effects are not yet available due to the fact that under real conditions *in vivo* there are

unavoidable additional genotoxic influences upon progeny of irradiated cells, which are able to simulate RIGIS effects.

Nevertheless it seems important to estimate pathogenetic significance of RIGIS. From the position of genome inconstancy concept [37] considering various mechanisms of chromatin rearrangement as ensuring adaptive-evolution process directed to adaptation of the species to environment changing conditions, RIGIS phenomenology can be interpreted as transition of irradiated cells progeny into the status of readiness to adaptation changes. Such status can have two ways-out: adaptation to arisen conditions with gradual normalization of cell functions and phenotype through repair and elimination of defected cells, transition to normal stable status or on the contrary to status with maintaining genome instability and acquisition of malignant characteristics.

Duality of RIGIS possible way-out, potential danger of such status of cells can be exciting for specialists in the field of radiation safety and standardization. For optimists it carries reassuring information that there are compensatory mechanisms in the nature that are able to bring disturbance caused in cells and even in the organism by irradiation in low doses and characterized with increase of mutation changes possibility, to initial status, to well-being without any harmful consequences.

For pessimists it serves a signal for stating the question on immediate reconsidering of the existing radiation safety standards towards their toughening.

The current analysis of data in the literature points out, on the one hand, the importance and urgency of the RIGIS problem and, on the other hand, its expressed incompleteness, insufficiency, fragmentariness in many things and antipathy of modern scientific information on the RIGIS phenomenon both for its exhaustive description in terms of molecular biology and biochemistry, and for adequate estimation of pathogenetic significance for radiobiology and radiation medicine. Thus, special experiments on animals which would eliminate any ambiguity seem urgent.

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REFERENCES

1. Mazurik V.K. and Mikhailov V.F., 'On some molecular mechanisms of the main radiobiological consequences of ionization effect to mammalian organism', *Radiats. Biol. Radioekol.*, 1999, vol. **39**(1), pp. 91 - 98. (Rus)
2. Baverstock K., 'Radiation-induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer', *Mutat. Res.*, 2000, vol. **454**(1-2), pp. 89 - 109.
3. Meyn S.M., 'Chromosome instability syndromes: lessons for carcinogenesis', *Curr. Topics Microbiol. Immunol.*, 1997, vol. **221**, pp. 71 - 148.
4. Mothersill C., Crean M., Lyons M. et al., 'Expression of delayed toxicity and lethal mutations in the progeny of human cells surviving exposure to radiation and other environmental mutagens', *Int. J. Radiat. Biol.*, 1998, vol. **74**(6), pp. 673 - 680.
5. Little J.B., 'Radiation-induced genomic instability', *Int. J. Radiat. Biol.*, 1998, vol. **74**(6), pp. 663 - 671.
6. Seymour C.B., Mothersill C., and Alper T., 'High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation', *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.*, 1986, vol. **50**(1), pp. 167 - 179.
7. Mendonca M.S., Kurohara W., and Antoniono R., 'Red-path J.L. Plating efficiency as a function of time postirradiation: evidence for the delayed expression of lethal mutations', *Radiat. Res.*, 1989, vol. **119**(2), pp. 387 - 393.
8. Chang W.P. and Little J.B., 'Delayed reproductive death in X-irradiated Chinese hamster ovary cells', *Int. J. Radial. Biol.*, 1991, vol. **60**(3), pp. 483 - 496.
9. Chang W.P. and Little J.B., 'Delayed reproductive death as a dominant phenotype in cell clones surviving X-irradiation', *Carcinogenesis*, 1992, vol. **13**(6), pp. 923 - 928.
10. Alferovich A.A., Gotlib V.Ja., Konradov A.A. et al., 'Gamma-irradiation low doses effect to mammalian cells', *Izv. RAN, Ser. Biol.*, 1992, No. 1, pp. 127 - 130. (Rus)
11. Alferovich A.A., Gotlib V.Ja., and Pelevina I.I., 'Changing of proliferative activity of cells under irradiation in low doses', *Izv. RAN, Ser. Biol.*, 1995, No. 1, pp. 15 - 18. (Rus)

12. Chang W.P. and Little J.B., 'Evidence that DNA double-strand breaks initiate the phenotype of delayed reproductive death in Chinese hamster ovary cells', *Radiat. Res.*, 1992, vol. 131(1), pp. 53 - 59.
13. Alferovich A.A., Gotlib V.Ja., and Pelevina I.I., 'Influence of irradiation in low doses to survival of cells and their progeny', *Izv. RAN, Ser. Biol.*, 1995, No. 2, pp. 137 - 141. (Rus)
14. Mothersill C., Kadhim M.A., O'Reilly S. et al., 'Dose-and time-response relationships for lethal mutations and chromosomal instability induced by ionizing radiation in an immortalized human keratinocyte cell line', *Int. J. Radiat. Biol.*, 2000, vol. 76, No. 6, pp. 799 - 806.
15. Kadhim M.A., MacDonald D.A., Goodhead D.T. et al., 'Transmission of chromosomal instability after plutonium α -particle radiation', *Nature*, 1992, vol. 355(6362), pp. 738 - 740.
16. Sabatier L., Dutrillaux B., and Martins M.B., 'Specific radiation-induced chromosomal instability', *Nature*, 1992, vol. 357(6379), p. 548.
17. Marder B.A. and Morgan W.F., 'Delayed chromosomal instability induced by DNA damage', *Mol. Cell. Biol.*, 1993, vol. 13(11), pp. 6667 - 6677.
18. Trott K.R. and Teibe A., 'Lack of specificity of chromosome breaks resulting from radiation-induced genomic instability in Chinese hamster cells', *Radiat. Environ. Biophys.*, 1998, vol. 37(3), pp. 173 - 176.
19. Ponnaija B., Limoli C.L., Corcoran J. et al., 'The evolution of chromosomal instability in Chinese hamster cells: a changing picture?', *Int. J. Radiat. Biol.*, 1998, vol. 74(6), pp. 765 - 770.
20. Zhloba A.A. and Sevan'kaev A.V., 'Identification of chromosome aberrations demonstrating irradiated cells progeny genome instability', *Dokl. RAN*, 1991, vol. 316(5), pp. 1239 - 1244. (Rus)
21. Rabbits T.H., 'Chromosomal translocation in human cancer', *Nature*, 1994, vol. 372(6502), pp. 143 - 149.
22. Pelevina I.I., Gotlib V.Ja., Kudryashova O.V. et al., 'Irradiated cells progeny characteristics', *Cytology*, 1998, vol. 40(5), pp. 467 - 477. (Rus)
23. Jamali M. and Trott K.R., 'Persistent micronucleus frequency in the progeny of irradiated Chinese hamster cells', *Int. J. Radiat. Biol.*, 1996, vol. 69(3), pp. 301 - 307.
24. Trott K.R., Jamali M., Manti L., and Teibe A., 'Manifestations and mechanisms of radiation-induced genomic instability in V-70 Chinese hamster cells', *Int. J. Radiat. Biol.*, 1998, vol. 74(6), pp. 787 - 791.

25. Manti L., Jamali M., Prise KM. *et al.*, 'Genomic instability in Chinese hamster cells after exposure to X rays or alpha particles of different mean linear energy transfer', *Radiat. Res.*, 1997, vol. 147(1), pp. 22 - 28.
26. Little J.B., Gorgojo L., and Vetrovs H., 'Delayed appearance of lethal and specific gene mutations in irradiated mammalian cells', *Int. J. Radiat. Oncol. Biol. Phys.*, 1990, vol. 19(6), pp. 1425 - 1429.
27. Chang W.P. and Little J.B., 'Persistently elevated frequency of spontaneous mutations in progeny of CHO clones surviving X-irradiation: association with delayed reproductive death phenotype', *Mutat. Res.*, 1992, vol. 270(2), pp. 191 - 199.
28. Fuscoe J.C., Zimmerman L.J., Fekete A. *et al.*, 'Analysis of X-ray-induced HPRT mutations in CHO cells: insertion and deletions', *Mutat. Res.*, 1992, vol. 269(2), pp. 171 - 183.
29. Nelson S.L., Giver C.R., and Grosovsky A.J., 'Spectrum of X-ray-induced mutations in the human hprt gene', *Carcinogenesis*, 1994, vol. 15(3), pp. 495 - 502.
30. Little J.B., Nagasawa H., Pfenning T., and Vetrovs H., 'Radiation-induced genomic instability: delayed mutagenic and citogenetic effects of X-rays and α -particles', *Radiat. Res.*, 1997, vol. 148(3), pp. 299 - 307.
31. Selvanayagam C.S., Davis CM., Cornforth M.N., and Ulrich R.L., 'Latent expression of p53 mutations and radiation-induced mammary cancer', *Cancer Res.*, 1995, vol. 55(15), pp. 3310 - 3317.
32. Little J.B., 'Radiation-induced genomic instability', *Int. J. Radiat. Biol.*, 1998, vol. 74(6), pp. 663 - 671.
33. Jeffreys A.J., Bois P., Buard J. *et al.*, 'Spontaneous and induced minisatellite instability', *Electrophoresis*, 1997, vol. 18(9), pp. 1501 - 1511.
34. Paquette B. and Little J.B., 'Genomic rearrangement in mouse C3H/10T1/2 cells transformed by X-rays, UV-C and 3-methylcholanthrene, detected by a DNA fingerprint assay', *Cancer Res.*, 1992., vol. 52(20), pp. 5788 - 5793.
35. Li S.-Y., Yandell D.W., and Little J.B., 'Evidence for confident mutations in human lymphoblast clones selected for functional loss of a thymidinkinase gene', *Mol. Carcinogen*, 1992, vol. 5(4), pp. 270 - 277.
36. Bois P. and Jeffreys A.J., 'Minisatellite instability and germ-line mutation', *Cell. Mol. Life Sci.*, 1999, vol. 55(1), pp. 1636 - 1648.
37. Khesin R.B., *Genome Inconstancy*, Moscow, Nauka, 1985, 472 p. (Rus)

38. Hahn P., Nevaldine B., and Morgan W.F., 'X-ray induction of metholrexate resistance due to ghfr gene amplification', *Somat. Cell. Mol. Genet.*, 1990, vol. 16(5), pp. 413 - 423.
39. Lane D.P., 'p53, guardian of the genome', *Nature*, 1992, vol. 358(6381), pp. 15 - 16.
40. Jamali M. and Trott K.R., 'Persistent increase in the roles of apoptosis and dicentric chromosomes in surviving V79 cells after X-irradiation', *Int. J. Radial. Biol.*, 1996, vol. 70(6), pp. 705 - 709.
41. Little J.B., 'Induction of genetic instability by ionising radiation', *C. R. Acad. Sci. III*, 1999, vol. 322(2-3), pp. 127 - 134.
42. Paquette B. and Little J.B., 'In vivo enhancement of genomic instability in minisatellite sequences f mouse C3H/10T1/2 cells transformed *in vitro* by X-rays', *Cancer Res.*, 1994, vol. 54(12), pp. 3173 - 3178.
43. Little J.B., 'Induction of genetic instability by ionising radiation', *C. R. Acad. Sci. III*, 1999, vol. 322(2-3), pp. 127 - 134.
44. Dubrova Y.E., Jeffreys A.J., and Malashenko A.M., 'Mouse minisatellite mutations induced by ionizing radiation', *Nature Genet.*, 1993, vol. 5(1), pp. 92 - 94.
45. Dubrova Y.E., Nesterov V.N., Krouchinsky N.G. *et al.*, 'Human minisatellite mutation rate after the Chernobyl accident', *Nature*, 1996, vol. 380(6576), pp. 683 - 686.
46. Pelevina I.I., Afanasjev G.G., Gotlib. V.Ja. *et al.*, 'Exposition of cells in tissue culture and animals (mice) in 10-km zone of Chernobyl accident. Influence on sensitivity to further irradiation', *Radiats. Biol. Radioekol.*, 1993, vol. 33(1(4)), pp. 508 - 520. (Rus)
47. Pelevina I.I., Nikolaev V.A., Gotlib. V.Ja. *et al.*, 'Adaptive reaction of human blood lymphocytes exposed to chronic exposure of radiation in low doses', *Radiats. Biol. Radioekol.*, 1994, vol. 34(6), pp. 805 - 817. (Rus)
48. Pelevina I.I., Gotlib. V.Ja., Kudryashova O.V. *et al.*, 'Genome instability after low dose radiation effect (in 10-km zone of Chernobyl accident and in laboratory)', *Radiats. Biol. Radioekol.*, 1996, vol. 36(4), pp. 546 - 560. (Rus)
49. Sevanjkaev A.V., 'Some results of citogenetic research regarding the estimation of Chernobyl accident consequences', *Radiats. Biol. Radioekol.*, 2000, vol. 40(5), pp. 590 - 597. (Rus)
50. Saenko A.S., Zamulaeva I.A., Smirnova S.G. *et al.*, 'Determination of mutations frequency on glycophorine A and T-cellular receptor loci:

- Chernobyl accident liquidators examination', *Radiats. Biol. Radioekol.*, 1998, vol. **38**(2), pp. 181 - 185. (Rus)
51. Saenko A.S. and Zamulaeva I.A., 'Results and prospective of using the methods of mutant cells frequency determination on glycophorine A and T-cellular receptor loci for estimation of genotoxic effect of ionizing irradiation in remote terms after influence', *Radiats. Biol. Radioekol.*, 2000, vol. **40**(5), pp. 549 - 553. (Rus)
 52. Gileva E.A., Ljubashevsky N.M., Starichenko V.I. *et al.*, 'Inheritable chromosome instability in common vole (*Microtus arvalis*) of Kyshtym nuclear accident region – fact or hypothesis?', *Genetika*, 1996, vol. **32**(1), pp. 114 - 119. (Rus)
 53. Bezlepkin V.G., Vasiljeva G.V., Lomaeva M.G. *et al.*, 'Research of genome instability through method of analysis of fingerprints of progeny DNA of male mice exposed to chronic low dose γ -irradiation', *Radiats. Biol. Radioekol.*, 2000, vol. **40**(5), pp. 506 - 512. (Rus)
 54. Vorobtsova I.E., 'Mutability of irradiated male rats progeny liver cells', *Radiobiologia*, 1987, vol. **27**(3), pp. 377 - 383. (Rus)
 55. Kosichenko L.P. and Alexjan A.A., 'Citogenetic research of bone marrow cells of young monkeys in the first generation of irradiated ones', *Cytology*, 1991, vol. **33**(7), pp. 117 - 121. (Rus)
 56. Stepanova E.I., Vanjurykhina E.A., Kondrashova V.G., and Tschepelakina L.A., 'Clinic and citogenetic features of children born from fathers – participants of Chernobyl accident liquidation, having acute radiation sickness', *Pediatria*, 1996, No. 1, pp. 63 - 64. (Rus)
 57. Vorobtsova I.E., Vorobjeva M.V., Korytova L.I., and Shust V.F., 'Research of citogenetic reaction of lymphocytes to in vitro irradiation of children born as patients after antitumour radiation and chemical therapy', *Tsitologiya*, 1995, vol. **37**(5/6), pp. 449 - 457. (Rus)
 58. Ilyinskikh N.N., Isaeva T.M., Ivanchuk I.I. *et al.*, 'Frequencies of micronucleated lymphocytes and Epstein-Barr virus contamination in Altai region residents living near the Semipalatinsk atomic testing ground', *Environ. Mol. Mutagen.*, 1998, vol. **31**(1), pp. 11 - 17.
 59. Nikiforov Y.E., Nikiforova M., and Fagin J.A., 'Prevalence of minisatellite and microsatellite instability in radiation-induced post-Chernobyl paediatric thyroid carcinomas', *Oncogene*, 1998, vol. **17**(15), pp. 1983 - 1988.
 60. Richter H.E., Lohrer H.D., Hieber L. *et al.*, 'Microsatellite instability and loss of heterozygosity in radiation-associated thyroid carcinomas of

- Belarus children and adults, *Carcinogenesis*, 1999, vol. 20(12), pp. 2247 - 2252.
61. Shevchenko V.A., Abramov V.I., and Pechkurenkov V.L., *Genetic research in East Ural radiation track. South Ural radiation pollution ecological consequences*, Moscow, Nauka, 1993, pp. 258 - 302. (Rus)
 62. Gileva E.A., Nokhrin D.Ju., and Starichenko V.I., 'Chromosome instability in progeny of common voles from radiation unfavorable zone', *Genetika*, 2000, vol. 36(5), pp. 714 - 717. (Rus)
 63. Awa A.A., Honda T., Neriishi S. *et al.*, 'Citogenetic study of the offspring of atomic bomb survivors Hiroshima and Nagasaki', *Citogenetics*, Berlin, Springer-Verlag, 1987, pp. 166 - 183.
 64. Spitkovsky D.M., 'New biophysical and biochemical aspects of ionizing radiation low doses influence mechanisms', *Radiats. Biol. Radioekol.*, 1999, vol. 39(1), pp. 145 - 155. (Rus)
 65. Limoli C.L., Kaplan M.I., Corcoran J. *et al.*, 'Chromosomal instability and its relationship to other end points of genomic instability', *Cancer Res.*, 1997, vol. 57(24), pp. 5557 - 5563.
 66. Vartanyan L.S., Gurevich S.N., Kozachenko A.I. *et al.*, 'Changes in superoxide radicals generation rate and activity of superoxide dismutase and glutathione peroxidase in subcellular microbodies of mouse liver in low dose low-level irradiation', *Biokhimia*, 2000, vol. 65(4), pp. 522 - 527. (Rus)
 67. Menjtschikova E.B., Zenkov N.K., and Reutov V.P., 'Nitrogen oxide and NO-synthases in mammalian organism under different functional statuses', *Biokhimia*, 2000, vol. 65(4), pp. 485 - 503. (Rus)
 68. Watson G.E., Lorimore S.A., and Wright E.G., 'Genetic factors influencing alpha-particle-induced chromosomal instability', *Int. J. Radiat. Biol.*, 1997, vol. 71(5), pp. 497 - 503.
 69. Aslund P. and Beckwith J., 'Bridge over troubled waters: sensing stress by disulfide bond formation', *Cell*, 1999, vol. 96(6), pp. 751 - 753.
 70. Benjamin I.J. and McMillan D.R., 'Stress (heat shock) proteins. Molecular shaperones in cardiovascular biology and disease', *Circulat. Res.*, 1998, vol. 83(2), pp. 117 - 132.
 71. Glutton S.M., Townsend K.M.S., Walker C. *et al.* 'Radiation-induced genome instability and persisting oxidative stress in primary bone marrow cultures', *Carcinogenesis*, 1996, vol. 17(8), pp. 1633 - 1639.

72. Kopnin B.P., 'Oncogens and tumor suppressor effect target: the key to understanding of carcinogenesis basic mechanisms', *Biokhimia*, 2000, vol. **65**(1), pp. 5 - 33. (Rus)
73. Bertrand P., Rouillard D., Boulet A. *et al.*, 'Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein', *Oncogene*, 1997, vol. **14**(9), pp. 1117 - 1122.
74. Colucci S., Mothersill C., Harney J. *et al.*, 'Induction of multiple PCR-SSCPE mobility shifts in p53 exons in cultures of normal human urothelium exposed to low dose gamma radiation', *Int. J. Radiat. Biol.*, 1997, vol. **72**(1), pp. 21 - 31.
75. Weiss R.S., Enoch T., and Leder P., 'Inactivation of mouse Hus1 results in genomic instability and impaired responses to genotoxic stress', *Genes Develop.*, 2000, vol. **14**(15), pp. 1886 - 1898.
76. Rey O., Lee S., and Park N.H., 'Impaired nucleotide excision repair in UV-irradiated human oral keratinocytes immortalized with type 16 human papillomavirus genome', *Oncogene*, 1999, vol. **18**(50), pp. 6997 - 7001.
77. Mendonca M.S., Howard K., Desmond L.A., and Derrow C.W., 'Previous loss of chromosome 11 containing a suppressor locus increases radiosensitivity, neoplastic transformation frequency and delayed death in HeLa x fibroblast human hybrid cells', *Mutagenesis*, 1999, vol. **14**(5), pp. 483 - 490.
78. Mendonca M.S., Desmond L.A., Temples T.M. *et al.*, 'Loss of chromosome 14 increases the radiosensitivity of CGL1 human hybrid cells but lowers their susceptibility to radiation-induced neoplastic transformation', *Mutagenesis*, 2000, vol. **15**(3), pp. 187 - 193.
79. McKenzie G.J., Harris R.S., Lee P.L., and Rosenberg S.M., 'The SOS response regulates adaptive mutation', *Proc. Natl. Acad. Sci. USA*, 2000, vol. **97**(12), pp. 6646 - 6651.
80. Jin S., Antinore M.J., Lung F.D. *et al.*, 'The GADD45 inhibition of Cde2 kinase correlates with GADD45-mediated growth suppression', *J. Biol. Chem.*, 2000, vol. **275**(22), pp. 16602 - 16608.
81. Kim N., Bozek G., Lo J.C., and Storb U., 'Different mismatch repair deficiencies all have the same effects of somatic hypermutation: intact primary mechanism accompanied by secondary modifications', *J. Exper. Med.*, 1999, vol. **190**(1), pp. 21 - 30.
82. Ferguson D.O., Sekiguchi J.A.M., Chang S. *et al.*, 'The non-homologous end-joining pathway of DNA repair is required for genomic stability and

- the suppression of translocations', *Proc. Natl. Acad. Sci. USA*, 2000, vol. **97**(12), pp. 6630 - 6633.
- 83. Bertrand P., Rouillard D., Boulet A. *et al.*, 'Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein', *Oncogene*, 1997, vol. **14**(9), pp. 1117 - 1122.
 - 84. Honma M., Momose M., Tanabe H. *et al.*, 'Requirement of wild-type p53 protein for maintenance of chromosomal integrity', *Mol. Carcin.*, 2000, vol. **28**(4), pp. 203 - 214.
 - 85. Smith G.C.M., Gary R.B., Lakin N.D. *et al.*, 'Purification and DNA binding properties of the ataxia-teleangiectasia gene product ATM', *Proc. Natl. Acad. Sci. USA*, 1999, vol. **96**(20), pp. 11134 - 11139.
 - 86. Khanna K.K., 'Cancer risk and the ATM gene: a continuing debate', *J. Natl. Cancer Inst.*, 2000, vol. **92**(10), pp. 795 - 802.
 - 87. Graunke D.M., Fornace A.J. Jr., and Pieper R.O., 'Presetting of chromatin structure and transcription factor binding poised the human GADD45 gene for rapid transcriptional upregulation', *Nucl. Acid. Res.*, 1999, vol. **27**(19), pp. 3881 - 3890.
 - 88. Bustin M., 'Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins', *Mol. Cell. Biol.*, 1999, vol. **19**(8), pp. 5237 - 5246.
 - 89. Hollander M.C., Sheikh M.S., Bulavin D.V. *et al.*, 'Genomic instability in Gadd45a-deficient mice', *Nat. Genet.*, 1999, vol. **23**(2), pp. 176 - 184.
 - 90. Amundson S.A., Do K.T., and Fornace A.J. Jr., 'Induction of stress genes by low doses of gamma rays', *Radiat. Res.*, 1999, vol. **152**(3), pp. 225 - 231.
 - 91. Gerasjkin S.A. and Srapuljzev B.I., 'Stochastic model of induced instability of genome', *Radiats. Biol. Radioekol.*, 1995, vol. **35**(4), pp. 451 - 462. (Rus)
 - 92. Spitskovsky D.M., 'The concept of ionizing irradiation low doses effect to cells and its possible application to medical-biological consequences interpretation', *Radiobiologia*, 1992, vol. **32**(3), pp. 382 - 400. (Rus)
 - 93. Talyzina T.A. and Spitskovsky D.M., 'Structural changes of human lymphocytes nuclei under influence of ionizing irradiation in the range of doses causing adaptive response', *Radiobiologia*, 1991, vol. **31**(4), pp. 606 - 611. (Rus)
 - 94. Akif'ev A.P., Khudoly G.A., Yakimenko A.V. *et al.*, 'C-process in human lymphocytes cultivated with PHA and generation of radiation-induced

- aberrations of chromosomes', *Genetika*, 1995, vol. **31**(4), pp. 485 - 491. (Rus)
- 95. Pelevina I.I., Saenko A.S., Gotlib V.Ja., Synzynys B.I., *Survival of irradiated mammalian cells and DNA repair*, Moscow, Energoatomizdat, 1985, 120 p. (Rus)
 - 96. Jungbluth A.A., Busam K.J., Kolb D. et al., 'Expression of MAGE-antigens in normal tissues and cancer', *Int. J. Cancer*, 2000, vol. **85**(4), pp. 460 - 465.
 - 97. De Smet C., Lurguin C., Lethe B. et al., 'DNA methylation is the primary silencing mechanism for a set of germ line- and tumour-specific genes with a CpG-rich promoter', *Mol. Cell. Biol.*, 1999, vol. **19**(11), pp. 7327 - 7335.
 - 98. Holliday R. and Ho T., 'Evidence for gene silencing by endogenous DNA methylation', *Proc. Natl. Acad. Sci.*, 1998, vol. **95**(15), pp. 8727 - 8732.
 - 99. Mikheev A.N., Gutscha N.I., and Malinovsky Ju.Ju., 'Epigenetic reactions of cells to ionizing radiation effect', *Radiats. Biol. Radioekol.* 1999, vol. **39**(5), pp. 548 - 556. (Rus)
 - 100. Seymour C.B. and Mothersill C., 'Delayed expression of lethal mutations and genomic instability in the progeny of human epithelial cells that survived in a bystander-killing environment', *Radiat. Oncol. Investig.*, 1997, vol. **5**(3), pp. 106 - 110.
 - 101. Grosovsky A.J., 'Radiation-induced mutations in non-irradiated DNA', *Proc. Natl. Acad. Sci.*, 1999, vol. **96**(10), pp. 5346 - 5347.
 - 102. Hickman A.W., Jaramillo R.J., Lechner J.F., and Johnson N.F., 'Alpha-particle-induced p53 protein expression in a rat lung epithelial cell strain', *Cancer Res.*, 1994, vol. **54**(22), pp. 5797 - 5800.
 - 103. Azzam E.I., de Toledo S.M., Goading T., and Little J.B., 'Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low influences of alpha particles // Radiat. Res.', 1998, vol. **150**(5), pp. 497 - 504.
 - 104. Mothersill C. and Seymour C.B., 'Genomic instability, bystander effects and radiation risks: implications for development of protection strategies for man and the environment', *Radiats. Biol. Radioekol.*, 2000, vol. **40**(5), pp. 617 - 622. (Rus)
 - 105. Lehnert B.E. and Goodwin E.H., 'Extracellular factor(s) following exposure to alpha particles can cause sister chromatid exchanges in normal human cells', *Cancer Res.*, 1997, vol. **57**(11), pp. 2164 - 2171.

106. Hickman A.W., Jaramillo RJ., Lechner J.F., and Johnson N.F., 'Alpha-particle-induced p53 protein expression in a rat lung epithelial cell strain', *Cancer Res.*, 1994, vol. 54(22), pp. 5797 - 5800.
107. Azzam E.I., de Toledo S.M., Goading T., and Little J.B., 'Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low influences of alpha particles', *Radiat. Res.*, 1998, vol. 150(5), pp. 497 - 504.
108. Lehnert B.E. and Goodwin E.H., 'Extracellular factor(s) following exposure to alpha particles can cause sister chromatid exchanges in normal human cells', *Cancer Res.*, 1997, vol. 57(11), pp. 2164 - 2171.
109. Lehnert B.E. and Goodwin E.H., 'A new mechanism for DNA alterations induced by alpha particles such as those emitted by radon and radon progeny', *Environ. Health Perspect.*, 1997, vol. 105, Suppl. 5, pp. 1095 - 1101.
110. Narayanan P.K., Goodwin E.H., and Lehnert B.E., 'Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells', *Cancer Res.*, 1997, vol. 57(18), pp. 3963 - 3971.
111. Mothersill C., Stamato T.O., Perez M.I. *et al.* 'Involvement of energy metabolism in the production of "bystander effects" by radiation', *Brit. J. Cancer*, 2000, vol. 82(10), pp. 1740 - 1746.
112. Qaslibash M.H., Xiao X., Seth P. *et al.*, 'Cancer gene therapy using a novel adeno-associated virus vector expressing human wild-type p53', *Gene Ther.*, 1997, vol. 4(7), pp. 675 - 682.
113. Komarova E.A., Diatchenko L., Rokhlin O.W. *et al.*, 'Stress-induced secretion of growth inhibitors: a novel tumour suppresser function of p53', *Oncogene*, 1998, vol. 17(9), pp. 1089 - 1096.
114. Iyer R. and Lehnert B.E., 'Factors underlying the cell growth-related bystander responses to alpha particles', *Cancer Res.*, 2000, vol. 60(5), pp. 1290 - 1298.
115. Turovez N.A., Chumakov N.A., and Kopnin B.P., 'Influence of activation of different components of signal paths of tumor suppresser p53 to genome instability', *Genetika*, 1999, vol. 35(12), pp. 1651 - 1658. (Rus)

Induced Mini- and Microsatellite Instability in the Genome of Animal Germline Cells

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ABSTRACT

The data from the literature on the phenomenon of ionizing radiation-induced genomic instability (GI) in mini- and/or microsatellite sequences in germline cells are reviewed. The natural hypervariability of minisatellites (MNS) and microsatellites (MCS) widely found in the eukaryotic genome makes them convenient markers for assaying the frequency of germline mutations induced by the influence of genotoxic factors in low doses. These markers provide highly sensitive direct measurements with no use of extrapolation approaches. The non-target mechanisms of fixation of radiation injuries and the recombination events of meiosis or early embryogenesis are suggested to play a role in the induction of GI in germline cells. A quantitative estimation of the level of induced GI in germline cells is assumed to be adequate to assess genetic risks and the probability of arising of malignant and other pathologies in the progeny of radiation-exposed parents.

Keywords: eukaryotes, germline cells, genome, instability, minisatellites, microsatellites, ionizing radiation

In the radiobiology of the recent decade, the phenomenon of induced GI has been widely investigated in connection with elaboration of approaches to assess the genetic risk of radiation [1 - 7]. As a result of these studies, some problems arose which cannot be satisfactorily solved within the existing paradigm of low-dose effects but can be treated in terms of the conception of genomic instability [5]. Experimental studies that demonstrated the phenomenon of mini- and/or microsatellite GI were of great importance in the development of modern views of possible genetic consequences of low-dose exposures. The present work gives a brief review of studies that, using MNS and MCS as genetic markers with no phenotypic manifestation, demonstrate induction of GI in progeny generations from parents exposed to ionizing radiation or other genotoxic factors.

DEFINITIONS OF CONCEPTS

When the genome in cultured cells or cells within the organism shows an increased level of spontaneous lesions, a high frequency and rate of mutations, an elevated level of gene amplification and alterations in gene expression – this situation has been generally called “genomic (sometimes “genetic”) instability”. For cultured cells, this spectrum of changes is supplemented by an increase in clone heterogeneity and frequency of malignant transformations of cells, as well as by a delay in reproductive death and changes in some biochemical parameters. At the organism level, a characteristic feature of genomic instability is the increased risk of malignancies and other pathologies. It should be noted that the term “genomic instability” is equally used to describe changes occurring in the genome of both somatic and germline cells, although there is a substantial difference between the phenomena taking place in successive generations of somatic cells and in successive generations of animals or humans. In order to emphasize that some GI state can be transmitted from one generation to the next at the cell and/or organism level, the term “transmissible genomic instability” is frequently used [3, 8]. To accent the transmission of GI from parents to their progeny, i.e. from the genome of parental gametes to the somatic cells of a progeny organism, the term “germline genomic instability” is used [9]. General mechanisms for realization of “hereditary effects” in mammals in the progeny of one or both irradiated parents have been recently reviewed in [10]. The subject of our consideration is the transmissible germline GI induced by the action of a

genotoxic factor on parental gametes and manifested by the polymorphism of mini- and microsatellite loci.

MNS AND MCS AS HYPERVARIABLE GENETIC MARKERS

MNS and MCS are simple tandem-repeating nucleotide sequences uniformly distributed over the genome of eukaryotic cells and forming its substantial part. MCS are usually defined as relatively short sequences in DNA (100-300 bp). They are among most widely spread types of intergene repeats. In the human genome, they comprise about 25%. According to [11, 12], one block of trimeric or tetrameric repeats is present, on average, in every 20 thousands of base pairs. The minimal repeating unit, "core", in a MCS contains, as a rule, from 1 to 6 bp [11 - 15]. For comparison MNS repeats can be several thousand base pairs in length, the core part being 15-70 bp long. A variety of types of MCS repeats are represented to varying degrees in the mammalian genome. For instance, 76% of MCS in man fall within five types of repeats of above 20 nucleotides in length: $(A)_n$, $(AC)_n$, $(AAAN)_n$, $(AAN)_n$, and $(AG)_n$ (listed in order of decreasing occurrence; N is any of the three nucleotides C, G or T) [11]. Only 12% of MCS are above 40 nucleotides long. In rats, $(AC)_n$, $(AG)_n$, $(A)_n$, $(AAAN)_n$, and $(AAGG)_n$ are most frequently occurring types. On the average, they are longer than human MCS. The fraction of rat MCS repeats of more than 40 nucleotides is 43% [11]. The number of "core" units in definite MNS and MCS types varies, thus providing the basis for a high level of their natural polymorphism in length in the genome of animals and humans [12, 16]. In this connection, the following abbreviations are used in the literature to denote simple tandem repeat blocks: VNTP (variable number of tandem repeats), STR (short tandem repeats), SSR (simple sequence repeats) or SSTR (simple sequence tandem repeats). Recently it has been proposed to denote unstable minisatellite loci as ESTR (expanded simple tandem repeats) to distinguish them from more stable "true MNS" in the mouse genome [17, 18].

The high level of natural variability characteristic of MNS and MCS makes them rather attractive genetic markers in assaying linkage in genome mapping, in studying genetic variability, in certifying animal and plant breeds, in determining relationship and in identifying personality, in prediction of possible heterosis effect on hybridization etc. [19 - 23]. The high level of variability of simple repeats in mammal populations was proposed to be due to the increased

mutation frequency in these sequences compared to other genome regions. It was also noted that the MCS spectrum remains stable in the genome of every organism throughout its lifetime, provided that there have been no pathologies and external genotoxic influences [6].

The progress in the field of genomic fingerprinting of a variety of organisms on the basis of MNS and MCS polymorphism coupled with the Southern hybridization was largely determined by the use of high-efficiency hybridization probes (for instance, DNA from M13 phage) capable of detecting various types of hypervariable minisatellite loci in the genome [24 - 26]. Development of methods to produce microsatellite hybridization probes extended substantially the spectrum of assayed polymorphism types of simple repeats and the application of genomic fingerprinting [26 - 27]. The application of microsatellite markers to assay the size variability of MCS alleles became especially popular in the last decade due to the progress in using the polymerase chain reaction (PCR) and the computer-aided analysis of fingerprints of amplification products [6, 13, 28 - 33]. This method of molecular cloning enables studies with very small amounts of genetic material, even if its purification degree is not very high, and the results are more easily interpreted than in the case of assaying VNTR polymorphism coupled to the Southern hybridization.

BACKGROUND FOR THE DEVELOPMENT OF CONCEPTS OF INDUCED GERMLINE GENOME INSTABILITY

In the early 1990's, the results of several studies were published demonstrating an instability of dinucleotide MCS in somatic cells of persons with some spontaneous forms of cancer [32, 34], as well as with heritable non-polypoid colorectal cancer (HNPCC) [35]. These data served as the basis for the development of concepts of microsatellite instability as a characteristic sign ("mutatory phenotype") of increased risk of malignant pathology [35 - 37]. Later on, similar conclusions were made about the correlation of instability of triplet repeats with the high probability of neuromuscular and neurodegenerative disorders in humans (see reviews [38, 39]).

For families with a high risk of HNPCC it was convincingly demonstrated that the "mutatory phenotype" correlates with mutations in the genes that control the repair of mispairs arising in replication and genetic

recombination [40, 41]. In this connection it was proposed that the defects of this repair pathway play a significant role in the formation of transmissible microsatellite GI state, as well as in the processes of cell malignization and carcinogenesis [33, 40, 42 - 51]. Attempts were made to develop models to explain the mechanisms of arising and inheritance of GI with regard to the contributions from various types of disturbances in germline DNA, the role of different pathways of their repair in gametogenesis, as well as hereditary mutational defects of DNA repair [43, 46, 47]. A natural result of this more extended look at the problem is the suggestion of an induced GI form relating to both somatic and germline cells [3, 5, 7, 48, 49, 50, 95].

EXPERIMENTAL EVIDENCE FOR THE EXISTENCE OF INDUCED GI PHENOMENON IN GERMLINE CELLS

The phenomenon of radiation-induced GI persisting in many generations of cultured somatic cells was convincingly documented in experimental studies [1, 3, 7, 8, 44, 50]. Induced somatic GI is primarily characterized by an increase in the frequency of chromosomal and gene mutations, in the levels of micro- and minisatellite variability and malignant transformation, as well as by a pronounced growth of all parameters of spontaneous somatic GI intrinsic in unexposed cell cultures. The manifestation of induced GI in simple repeat sequences in the somatic cells of a radiation-exposed organism frequently correlates with the development of tumor pathologies [51 - 58].

Estimation of germline mutation frequency seems to be a natural approach to assess induced transmissible GI of germline cells (Table 1). At first glance, the most substantiated factual evidence for such quantitative estimates, at least for humans, must be epidemiological data. However, because of difficulties in collecting and interpreting data of this kind, the results of epidemiological surveys are taken by specialists too ambiguous to be considered as a basis for definitive conclusions. Therefore, various experimental approaches to assaying the frequency of induced germline mutations in animal and human populations are still of current interest. In experimental studies of transmissible GI, consideration is given only to situations with relatively low doses of radiation when lethal lesions in parental gametes are absent or low in number and the reproductive function persists. This circumstance naturally raises the requirements for adequacy and sensitivity of methods of GI registration.

Traditional studies in this field are most frequently based on estimation of mutations at loci with distinct phenotypic or biochemical manifestations, but in the genome of higher animals, such loci, as a rule, either have only few copies or are unique. Hence, experimental estimation of genetic risks of low-dose ionizing radiation exposures from the frequency of mutations induced in one-copy genes requires many thousands of animals [54]. In this connection, approaches using as genetic markers repeating sequences such as hypervariable MCS and MNS presented in the genome by a great number of loci look promising in assessing genetic risks [9, 28, 29, 48, 49, 56]. Table 2 summarizes the results of most interesting studies using this technology to assay GI.

In a recent review [6], procedures for technical realization of microsatellite GI assays are discussed in detail. Here we only emphasize that variants with direct registration of MNS and MCS alterations on the bases of genomic DNA fingerprinting by means of hybridization with locus-specific probes or by PCR (Tables 1 and 2) proved to be most widely used. An alternative for this technical approach is detection of reversion or inactivation of chosen marker sequences in the genome as well as artificial sequences. However, because of some limitations, the latter version can successfully be realized mostly with cultured animal cells and yeast [6].

In the early 1990's, two groups of scientists ([55] and [48, 56]) presented experimental evidence for induction of GI at minisatellite loci in the progeny of male mice exposed to acute radiation at doses from 0.5 to 3 Gy (Table 2). Both of these groups revealed minisatellite mutations by the Southern blot assay of total DNA preparations from tail tissues of mice using a locus-specific hybridization probe Ms6hm. The results were however different with respect to what spermatogenesis stage in male parent organism was most sensitive to induction of GI by radiation. According to [55], a substantial increase (3.7 times) in the mutation frequency of progeny cell MNS was observed when male parents that subsequently fertilized the egg cells of intact females were exposed to whole-body irradiation at doses 0.5 and 1.0 Gy in the premeiotic period of gamete maturation. In [48, 56], an increased mutation frequency in MNS of progeny cells was detected when male parents were irradiated (^{60}Co , 1, 2 and 3 Gy) at the stage of postmeiotic spermatides; at the same time, no significant difference with the control was found in the case of irradiation at the premeiotic stage. A similar feature in the results of both groups was the absence of a linear dependence of minisatellite mutation frequency on radiation dose. In subsequent studies, the conclusion about the higher sensitivity of premeiotic spermatogonia and stem cells to radiation, as compared to postmeiotic spermatides, was

confirmed [9, 57]. Also, a linear dose dependence (the slope angle for the linear regression curve of induced mutation frequency was evaluated at $0.3379 \pm 0.0867 \text{ Gy}^{-1}$) was found for the yield of paternal mutations, whereas the frequency of maternal mutations did not grow with dose when irradiated males were mated to unexposed females [57]. The latter fact was subjected to revision in a recent study [58] with reciprocal mating of females from the F1 progeny hybrids of inbred lines C57BL/6N×C3H/HeN to gamma-irradiated (6 Gy) males of F0 generation. It was possible to demonstrate in the somatic cells of the progeny a 20% increase in the mutation frequency of maternal alleles (C3H/HeN) of the minisatellite locus Ms6hm, the frequency of spontaneous germline mutations being 9.8%. In these experiments, males were irradiated at the spermatozoid stage of spermatogenesis. The frequencies of minisatellite mutations of paternal alleles, according to the authors' estimates, reached 22% and 19% when males were irradiated at the stage of spermatozoids or spermatogonia, respectively, the level of spontaneous germline mutations being 8.4%. The results were interpreted as an evidence for the non-target mechanism of germline GI induction. Besides, the potential genetic risk of damage to the combined genome of zygote (including its maternal part) due to the presence of lesions in its paternal part was stressed [58].

The conclusion about the increased sensitivity of the premeiotic spermatogonia of male mice to mutagenic influence is consistent with the results of independent studies with lacI transgenic mice [59], which demonstrated the maximum frequency of spontaneous mutations for the A type spermatogonia and a decrease in the frequency at later stages of spermatogenesis. It should be noted that transgenic animal models are also used directly to detect induced germline GI. Experiments with transgenic mice have been described according to which the transmissible GI is determined from an increase in mutation frequency in the haemopoietic cells of the F1 progeny of mice exposed to acute gamma-radiation at a dose of 4 Gy [60]. However, at doses 0.1 – 1.0 Gy, the mutation frequency characteristic of GI corresponded in these experiments to the values obtained for control animals. Probably, the relatively low sensitivity of assays was due to that here coding loci, and not minisatellite loci, were used in mutation frequency estimates. Also, it is not improbable that the problem consists in the specificity of meiotic control of inheritance of transgenes. For instance, in the germline cells of transgenic mice with introduced human locus MHCMS32(DIS8) it was impossible to detect GI at this locus, although the somatic GI was successfully registered. At the same time, with human genome DNA preparations, the

minisatellite locus MS32(DIS8) enabled detection of both somatic and germline GI. [61].

Most studies of the phenomenon of induced mini- and microsatellite GI in germline cells have been performed in experiments with acute exposures of male mice to X-ray- or gamma-radiation. Recently, data have been published which demonstrate similar quantitative patterns of mutation rate increase in MNS in the F1 progeny of CBA/H mice both due to acute irradiation of male parents and as a result of their chronic exposure to sparsely ionizing radiation, at least for the dose range from 0.5 to 1.0 Gy. The linear regression curves of mutation yield dose dependence are characterized by the slope angles (0.338 ± 0.087) and (0.373 ± 0.082) for the X-radiation (0.5 Gy/min) and gamma radiation (1.66×10^{-4} Gy/min), respectively. These results enable the authors to believe that the increased level of mutations due to the premeiotic irradiation of males does not directly depend on the time within which the organism cells are able to repair DNA lesions (two minutes or 100 hours) [62].

Chronic irradiation of male mice with fission neutrons at a dose of 0.5 Gy (^{252}Cf ; $E_n = 2$ MeV; 0.003 Gy/min) proved to be more efficient with respect to induction of minisatellite (at Ms6hm and Hm-2 loci) germline mutations. The value of RBE for this type of high LET radiation (the contributions of neutrons and gamma radiation to the absorbed dose are 67% and 33%, respectively) is 3.36 [62]. Interestingly, in the studies of *D. melanogaster* with acute neutron exposure ($E_n = 0.85$ MeV), the coefficient of RBE was evaluated at more than 2. This value was calculated from the efficiency of induction of recessive mutations in the X-chromosome in the F1 progeny of irradiated males (stage of mature spermatozoa) and non-irradiated females. At the same time, neutrons appeared less effective with respect to point mutations: the RBE was 0.44 [63].

In our own experiments [64], male BALB/c mice, 15 days after chronic irradiation at doses 0.1 to 0.5 Gy (^{137}Cs ; 1 cGy/day), were mated to non-irradiated females. To assess induced germline GI, we used an “arbitrarily” primed PCR (AP-PCR) [28, 29] with individual DNA preparations from tail tip tissues of parent animals and their F1 progeny as a template with subsequent assays of fingerprints of amplification products [28, 29]. A 20-mer oligonucleotide, TGGTGTTCCTGCCACAGAAA, from a sequence flanking the microsatellite locus Atp1b2 on murine chromosome 11 was chosen as a primer [66]. AP-PCR was earlier successfully applied to study the induction of transmissible GI in fish [65]. Comparative analysis of the spectrum of AP-PCR products seen as bands on individual fingerprints of DNA from the progeny of exposed and unexposed males reveals an increase in variability of microsatellite-

associated sequences in the genome of the progeny of males irradiation at 0.25 – 0.5 Gy. The total number of bands on fingerprints increases by 30 to 50% and the number of “non-parental” bands (absent in both parents) by 37% per descendant. These results suggest that DNA changes contributing to GI may be transmitted from male parents exposed to chronic low-dose irradiation to the somatic cells of the progeny [64]. The results of our experiments on mice do not contradict the data on the increased level of minisatellite mutations in the blood cells of children born in families living in the Mogilev Region of Belarus (the equivalent dose for their parents has been evaluated at about 27 mSv) [9, 67, 68].

PERSISTENCE OF GI IN CONSECUTIVE GENERATIONS OF ANIMALS EXPOSED TO GENOTOXIC INFLUENCE

The persistence of GI in subsequent generations of animals and humans is an essential issue of induced GI as a whole. The data on the germline GI revealed by cytogenetic methods are rather conflicting. Mutant caryotypes were detected in rodents inhabiting the zone of the Eastern Urals trace of Kyshtym accident radioactive deposits, after 30 - 40 [69] and even 70 - 80 generations [70] in the population of field mice living in the contaminated territory since the time of the accident in 1957. Under the conditions of laboratory breeding of field mice on the bases of parental pairs caught in the zone of the Eastern Urals trace in 1994, a persistence of the increased aberration frequency in the cells of the “laboratory” F1 progeny and its decrease to the control level in the F3 generation were shown [71]. The authors of [72] used the activity level of signal proteinkinases as a marker of inheritable GI, which permitted them to demonstrate the persistence of increased GI level in the F3 progeny of male CD1 mice exposed to acute gamma radiation at a dose of 1 Gy 6 weeks before mating to intact females.

As regards the possibility of persistence (or transmission) of induced minisatellite GI in subsequent generations obtained from irradiated parents, the publications on this subject are not numerous [73, 109]. In the experiments described in [73], male CBA/H mice were irradiated for almost three hours with fission neutrons at a dose of 0.5 Gy (^{252}Cf ; $E_n = 2\text{MeV}$; 0.003 Gy/min). In the F1 progeny of irradiated males mated (10 weeks after treatment) to non-irradiated females, a nearly 6-fold increase in mutation rate of paternal alleles of the minisatellite loci Ms6hm and Hm-2 was revealed. The F2 generation obtained

from non-irradiated animals of the F1 generation retained the high mutation rate (6-fold and 3.5-fold increase for paternal and maternal alleles, respectively) as compared to non-irradiated families. The authors suggest that this transgenerational GI at MNS is due to the increased level of mutation mosaicism in the germ cells of the F1 generation [73].

Among other experimental models to reveal and assess induced transmissible GI in the sequences of simple tandem repeats, studies with fish, insects and plants [33, 65, 74] (see Table 2) are of significant interest both in using these systems in ecological monitoring and in elucidating GI induction mechanisms. A sensitive system using an inbred fish line, *Oryzias latipes* (Japanese medaka), was developed to reveal radiation-induced microsatellite mutations in male germline cells [65]. Owing to some peculiarities of reproduction biology in fish, that model enabled registration of changes in the genome of progeny both in viable embryos and in embryos with dominant lethal mutations before the elimination of the latter in the course of development.

The experimental approach used in [65] is of interest, besides the object, also because it was the first one in which the suitability of AP-PCR in conjunction with fingerprinting of PCR products as a technique to reveal and assess induced transmissible GI in germline cells was demonstrated. In their experiments, the authors obtained the offspring of Japanese medaka males in such a way that the latter were exposed to gamma radiation (4.75 or 9.50 Gy) at the stage of spermatozoa or spermatides and their semen was subsequently used to fertilize the spawn of non-irradiated females. It was shown that both in embryos with lethal mutations and in viable specimens the level of MCS polymorphism increased with dose, which was judged from the frequency of band loss on the DNA-fingerprints of amplification products. Also, they managed to register the appearance of a new band, a microsatellite-like repeat (ATGT)_n with a Mendelian type of inheritance. This type of induced MCS variability did not influence the survival or the phenotype of the progeny [65].

The studies [33] with *D. melanogaster* are of interest because the authors have tried to correlate the quantitative estimates (obtained by means of PCR with nine locus-specific primers) of microsatellite GI induced by a mutagenic compound, 2-acetylaminofluorene (2-AAF), with the total genome damage (revealed as specific changes in AP-PCR fingerprints), all estimates being performed with the same preparations of DNA extracted from individual flies. Analysis of genomic DNA of F1 generation descendants obtained from wild-type males treated with 2-AAF and females of the mutagen-sensitive line mus-201 (deficient in DNA repair) revealed the induction of transmissible

microsatellite GI when the mutagen-treatment of male parents was performed at the spermatozoid (not spermatide) stage of spermatogenesis. Along with the pronounced changes in MCS, some features of general genome damage were observed although no quantitative correlations could be revealed. The authors emphasize that the extent of genome damage depends on the repair capacity of progeny organism and suggest that the *mus-201* gene product essential in excision repair is not involved in the process of mismatch repair [33].

Experiments to reveal induced GI in wheat [74] attract attention by both the chronic type of radiation exposure and by an unexpectedly high mutation rate. Winter wheat was grown on a heavily contaminated plot (900 Ci/km^2) near the Chernobyl NPP and for the period of vegetation (10 months) received a total dose of 0.3 Gy (the external and internal components were 0.2 Gy and 0.1 Gy, respectively). The control population was seeded on a field similar in the agrochemical characteristics and with a pollution level below 1 Ci/km^2 . Assays of PCR-fingerprints of parental and F1 generation seeds at 13 single-copy monomorphous MCS enabled the mutation rate to be evaluated at 6.63×10^{-3} per locus for the population from the contaminated region, whereas for the control population the value of mutation rate was 1.03×10^{-3} per locus. The authors emphasize that this more than 6-fold increase in MCS mutation rate per generation caused by low-dose exposure can hardly be explained in terms of modern conceptions of genetic effects of low-level irradiation [74].

To conclude, some experimental studies with rodents and other biological models provided evidence for the transmission of radiation-induced GI at MNS and MCS from parental lines exposed to non-lethal doses of radiation to the somatic cells of their offspring. This genomic instability transmitted to the somatic cells of progeny is revealed both as an increased mutation frequency in the cells and as an increased risk of tumor pathologies in the progeny [49, 65, 75, 76].

EPIDEMIOLOGICAL EVIDENCE FOR GERMLINE GI INDUCTION

Epidemiological data on induced germline GI in humans are presently not abundant. The results of surveying a great cohort of children born from parents survived from A-bomb attacks in Hiroshima and Nagasaki still remain the subject of contradictory propositions in respect to the genetic consequences of radiation influence [4, 7]. On the one hand, a large part of researchers believe

that the genetic consequences of the low-dose exposure of parents have been not recognized in children because of difficulties in interpreting observation results and imperfect research techniques used [67, 79, 111]. On the other hand, the absence of reliable results, allow some authors to suggest that the genetic consequences in the progeny of irradiated parents are actually absent [77] or the risk of adverse hereditary effects is small [106]. The Russian National Medical and Dosimetric Registry (RNMDR) of 1996 has data on 18816 children born from Chernobyl liquidators who worked in the accident zone in 1986 - 1987 [78]. The available literature for this group of persons (4.3% of the total quantity of those registered in the RNMDR) has not given any estimates for genetic consequences of exposure of their parents to radiation. It cannot be ruled out that this is connected with relatively low dose loads (about 25 cSv on the average) for most liquidators. Besides, when used at remote times, the traditional cytogenetic method to reveal radiation effects (from the frequency of dicentrics in peripheral blood lymphocytes) gives no pronounced correlation between effect and accumulated dose even for liquidators [79].

An unexpectedly high level of minisatellite mutations was detected in children whose parents had been exposed to chronic radiation at doses about 25 mSv during their residence in radiation-contaminated areas in the Mogilev Region after the Chernobyl accident [9, 67, 68]. The level of these mutations in the progeny of irradiated parents (127 families) assessed by fingerprinting of peripheral blood DNA with two multi-locus and six hypervariable single-locus minisatellite probes (33.15, 33.6, B6.7, CEB1, CEB15, CEB25, CEB36, and MS32) significantly exceeded the expected values of point mutations revealed at coding loci. The mutation rates for two groups of families with total dose received by one parent above and below 25 mSv, respectively (median dose distribution) differed 1.35 times on the average. A two-time excess in mutation rate was found for these 127 families compared to non-irradiated families from Great Britain. The results of studies on the Mogilev group as calculated per radiation dose unit differ substantially from the data obtained for the Japanese cohort [4, 9, 67, 68] and are taken ambiguously by specialists [4, 7].

POSSIBLE ROLE OF RECOMBINATION EVENTS IN THE INDUCTION OF GI IN GERMLINE CELLS

Experimental studies suggest that spontaneous mutations in MNS of germline cells are produced with participation of a complex of recombination and conversion events [80-83]. Analogously, the transmissible germline GI induced by genotoxic agents may, in particular, be caused by an increased level of recombination-like events in diploid germline cells of males. These assumptions are consistent with the experimental results of [84] which demonstrate the induction of transmissible minisatellite GI in germline cells of mice exposed to a Diesel engine exhaust (by inhalation) and/or polychlorinated biphenyl (Aroclor 1254; intraperitoneally). As is shown in [85], Aroclor 1254 possesses a recombinogenous activity in animal and insect cells. And it is just the combined action of this compound and Diesel engine exhaust, according to [84], that provides a maximum yield of mutations, as revealed by hybridization with a PC-1 probe (analog of Ms6hm), in the genome of liver cells obtained from the progeny of treated males and intact C57B1/6 females.

Detection of allele variability in a telomere-like sequence (TTAGGG), associated with a fragile site on chromosome 2 of CBA/H mice was interpreted as an evidence for the involvement of recombination in the radiation induction of the GI state [86]. The restriction fragment length polymorphism (RFLP) assay was used to compare spleen DNA preparations from male parents exposed to a single dose of 3 Gy and from 14-day embryos of their F1 progeny. As the authors of [86] suggest, the polymorphous alleles registered in the somatic cells of embryos may arise both in the germline cells of exposed male parents and in the early embryogenesis due to recombinant losses or duplication of initial parental alleles. Interestingly, the polymorphism of only one of the four RFLP-variants ("alleles") of the above sequence typical for the inbred line of CBA/H mice appeared to be associated with their predisposition to radiation-induced pathology (acute myeloid leukemia) [86].

If radiation-induced recombination events leading to an increase in GI take place in the meiotic crossingover, it would be reasonable to expect a direct correlation between the level of induced germline GI and the frequency of crossingover. A special experimental examination of this assumption was made [17] by assessing crossingover frequencies in male mice gametes at 25 polymorphic microsatellite loci localized in 6 chromosomes and spaced nearly at 20 cM intervals (thus covering 421 cM or 28% of the genome). It was

recognized that whole-body irradiation of male mice with X-rays (1 Gy) 4, 5 or 6 weeks (premeiotic stage of spermatogenesis) before mating with unexposed females did not influence the frequency of crossingover events at the meiotic stage of spermatogenesis [17], although as it was shown earlier in the same laboratory, it is just under such conditions of radiation influence that germline GI was induced [9, 57].

According to [87], intraperitoneal injection of the antitumor preparation *cis*-platinum to male mice (10 mg/kg) produced an increase in the level of meiotic crossingover in the genomic DNA. However, an attempt to register the induction of transmissible germline GI failed: not only no increase in minisatellite mutation frequency in the genome of tail tissue cells in the F1 progeny was found but also no support for the earlier evidence for the increase in crossingover frequency was obtained [17]. Thus the suggestion of recombination events in murine germline cells as a major mechanism for induction of the induced transmissible mini- and microsatellite instability state, which has been repeatedly discussed by many authors, has so far not been justified experimentally as to the involvement of meiotic crossingover in this process. Taking into account this circumstance as well as other relating findings, it has been suggested that mutations at minisatellite loci occur either in premeiotic spermatogonia of any type but before the meiotic crossingover, or at the early stages of zygote cleavage [17, 86].

NON-TARGET MECHANISMS FOR GI STATE FORMATION

According to the conclusions made by a number of authors, MNS or MCS *per se* in parental gametes do not present a direct and obligatory target for radiation. Primary lesions occur in them with the same probability as over the genome as a whole and it is far from being always that minisatellite mutations can be detected in the genome of germline cells after the whole-body irradiation of animals [73, 88]. The cause of GI formation in the somatic cells of progeny is believed [9, 48, 56, 67, 73] to be the primary-lesions-induced non-target fixation of damage in the genome. These events are likely to take place in the meiosis and/or at the early stages of embryogenesis over the whole genome, in particular (and possibly at a higher probability) in the region of simple tandem repeats.

It should be noted that, as some authors believe, the non-target principle of arising of "germline" mutations not only determines the mutagenesis of

paternal alleles (in irradiated males) but also explains the increased genetic risk for the maternal genome by the presence itself of the damaged genome of paternal gametes in the zygote [58]. Among the disturbances (both spontaneous and radiation- or chemical mutagen-induced ones) registered in MNS and MCS in the genome of the progeny obtained from irradiated parents, deletions, insertions, and translocations are rather frequent. They are registered as allele length changes in simple tandem repeats [58] and also as heterozygosity loss or, less commonly, as arising of new alleles [65, 89] as a result of occurrence of various processes in which the DNA of germline cells carrying non-lethal lesions is implicated. It is assumed that MNS and MCS may contain “hot spots” or sites at which recombinational and conversional events are realized [80 - 83].

PROBLEMS OF TERMINOLOGY AND TECHNICAL APPROACHES IN DEFINING THE STATE OF INDUCED GENOMIC INSTABILITY (GI)

The term “genomic instability” has been used by researchers engaged in a variety of topics in biology and in studies with different objects and with the use of various approaches and techniques.

This universality of the term may however be the source of misunderstanding and erroneous conclusions when general models of induced GI are discussed with no regard for the specific features of particular studies. For instance, though there is a formal analogy of phenomena observed in dividing cell cultures (“somatic” GI) and in a series of successive generations in animal and human populations (“germline” GI), these two phenomena differ essentially from each other [81]. As for the choice of approaches to assess GI, a dramatic illustration of difficulties in this way was given in [90] whose authors studied genome polymorphism by the method of cytogenetics and the method of molecular genetics. The discrepancy between the results was so high that the authors assumed the existence of different states of GI or different manifestations of this phenomenon [90]. Within the cytogenetic approach, it was impossible to recognize any significant correlation between chromosomal instability and sister chromatid exchanges as well as between delayed mutations and mismatch repair in DNA in assessing GI, the chromosomal instability significantly correlating with reproductive cell death delay [91].

In the recent decade, progress has been made in studying GI by newly developed techniques based on the polymerase chain reaction (PCR) though its application sometimes gives ambiguous results. Thus, the PCR assay of variability of the human minisatellite locus MS32 (D1S8) inserted to the genome of transgenic mice [61] is suitable to detect "somatic", not "germline" GI. The application of two different PCR variants enabled estimation of induced microsatellite GI and the total genome damage in *D. melanogaster*. However, though the spectra of MCS changes revealed by these two versions of the method were qualitatively similar, no quantitative correlations were found [33].

The mutation rate assessed by identical methods in the same animals or insects, but at different mini- or microsatellite loci, may differ quantitatively for both spontaneous and induced germline mutations [33, 84]. The results obtained recently enabled the authors to believe that the level of spontaneous GI in mice in expanded simple tandem repeats (without interruptions by variant units) positively correlated with the length of the latter [93]. Spontaneous mutation frequencies at the minisatellite loci PC-1 and PC-2 were evaluated at 8.8% and 3.3%, respectively, per gamete per generation in hybrids of BALB/c and SM/J mice [92]. However, this parameter for C57B1/6 mice was evaluated at 2.5% for both loci [18]. Recently it has been shown that the levels of spontaneous mutation rates and radiation-induced transgenerational instability vary between strains (BALB/c > CBA/H > C57BL/6) [109]. Moreover, we succeeded to show that the GI level in different types of tissue cells in the progeny of irradiated males varied depending on differentiation and specialization degree and proliferative activity of these cells [110].

Thus, the correctness and adequacy of one or another assay for induced GI in assessing genetic risks is primarily determined by proper allowance for the peculiarities of biological models and approaches used as well as by the presence of a correlation between DNA polymorphism level and expected biological effects.

MINI- AND MICROSATELLITE GI OR GERMLINE CELLS AS A MARKER OF RISKS OF MALIGNANT AND OTHER PATHOLOGIES

The totality of data discussed here are, in our opinion, sufficient to prove the existence of the phenomenon of induced GI at MNS and/or MCS in germline cells and permit one to characterize it to some extent. The idea of the germline

GI induced by low-dose radiation is consistent with the propositions of the conception of genome variability ("instability") [94 - 96, 107, 108] and points to the urgency of designing new, more sensitive test-systems to assay genetic risks [5, 97, 106, 112]. The experimental and epidemiological results published recently demonstrate an adequacy and a high accuracy of quantitative estimation of mini- and microsatellite GI in the progeny of parents exposed to radiation and chemical mutagens. For example, the frequency of induced mutations estimated by assays of MNS and MCS sequences as genetic markers was about ($10^{-3} - 10^{-2}$)/Gy/locus (Table 2) [9, 48, 56, 62, 73, 74], whereas the frequency of radiation-induced germline mutations in mice assayed by testing at 7 specific single-copy loci ("Russel 7-locus test") was evaluated at about 10^{-5} /Gy/locus [104].

Also, it is essential to note that the gonadal doubling dose in mice (parameter used to calculate the genetic risk of radiation), 0.33 Gy, estimated from the assay of minisatellite GI in paternal alleles [9] is in a good agreement with the doubling dose (0.17 – 0.56 Gy) obtained earlier with other methods, including the "Russel 7-locus test". It should be noted that the statistically significant evidence for the occurrence of mutations in germline cells was obtained on a rather small sample (252 specimens) of animals: the descendants of male parents exposed to doses of 1 Gy, whereas the other methods for estimation of doubling dose require hundreds and even thousands of experimental animals (see Table 2) and application of radiation doses substantially exceeding the doubling dose [9, 55].

There are sufficient experimental and epidemiological data that enable us to suggest that the influence of ionizing radiation on parents is a factor of risk of tumors and other pathologies in their offspring [98 - 101, 105]. According to some findings [105], the frequency of induced developmental anomalies and malignant pathologies in the F1 progeny of irradiated parents may be as high as 10^{-1} /Gy. The radiation-induced germline GI registered in the experiments as an increased level of MNS and MCS mutations (about 10^{-2} /Gy/locus) in the genome of somatic cells in the offspring of exposed parents is in a better agreement with the high probability of transgenerationally induced tumor pathologies than with the mutation rate values determined at single-copy specific loci.

It is not improbable that the increased GI level in progeny cells plays a role in the etiology of frequent leukemia and other pathologies in children whose parents were exposed to low-doses of low-intensity radiation occupationally or as a result of nuclear accidents [98, 100]. The competence of these propositions is supported by the well known data on the correlation of the high level of GI and mutations in the genes that are involved in mismatch repair with the

increased risk of some malignant and other pathologies [32, 36, 37, 45, 102]. The induced allele variability of MNS and MCS registered as deletions and insertions of core sequences as well as in the form of a heterozygosity loss is likely to reflect complex rearrangements in the genome. Possible mechanisms for the involvement of MNS and MCS in the processes of non-target fixation of primary lesions as a stage in the complex of events leading to cell malignization and carcinogenesis have been discussed [26, 32, 89, 103].

Further accumulation of data on the significant correlation between the level of induced germline GI in mini- and/or microsatellite sequences and the probability of tumor development and other pathologies in the offspring of parents exposed to radiation and other genotoxic agents in low doses will promote the development of direct sensitive tests to assess the genetic consequences of radiation and the influence of other environmental genotoxic factors in animal and human generations.

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Table 1

Frequency of spontaneous germline mutations assessed with the use of MNS and MCS as genetic markers

Object	Method	Mutation frequency	Refs.
Mice	"Russell 7-locus test"	$0,75 \cdot 10^{-5}$ /locus	[104]
Mice C3H × C57BL, F1	SBA with MNS-probe Pc-1	$9,6 \cdot 10^{-2}$ /gamete/locus	[48]
Mice C57BL × C3H, F1	— " —	$9,7 \cdot 10^{-2}$ /gamete/locus	[48]
Mice C3H × C57BL, F1	SBA with MNS-probe Pc-1	$10 \cdot 10^{-2}$ /gamete/locus	[56]
Mice CBA/H, F0	SBA with MNS-probe Pc-1 coupled with HZA	$4 \cdot 10^{-2}$ /gamete/locus	[51]
— " —	Assay of 20 MCS loci by PCR coupled with HZA	$3 \cdot 10^{-3}$ /gamete/locus	[51]
Mice CBXD, F0	SBA with MNS-probe 33.6 coupled with HZA	$2,4 \cdot 10^{-2}$ /gamete/locus	[18]
Mice CBA/H, F1	SBA with MNS-probe MMS10	$1,3 \cdot 10^{-2}$ /gamete/locus	[9]
	SBA with MNS-probe 33.15	$1,9 \cdot 10^{-2}$ /gamete/locus	[9]
Mice BALB/c, F1	SBA with MNS-probe Pc-1	$8,8 \cdot 10^{-2}$ /gamete/locus	[92]
	SBA with MNS-probe Pc-2	$3,3 \cdot 10^{-2}$ /gamete/locus	[92]
Winter wheat, F1	Assay of 13 MCS loci by PCR method	$1,03 \cdot 10^{-3}$ /locus ^A	[74]
Fish <i>Oryzias latipes</i> <i>NHI</i> , F1	AP-PCR of genomic DNA at MNS loci	$4,8 \cdot 10^{-2}$ /zygote ^B	[65]
Man (105 families from Great Britain), F1	SAGD with MNS-probe 33.15	$1 \cdot 10^{-2}$ /fingerprint band	[68]
— " —	SAGD with 6 locus specific probes	$1 \cdot 10^{-2}$ /fingerprint band	[68]
Man (Asian-Indian family), F1	SAGD with MNS probes 33.15 и 33.6	$4 \cdot 10^{-3}$ /gamete/locus	[24]

Footnote:

A, Mutation frequency as a proportion of microsatellite variants in the F1 progeny per MCS locus.

B, Mutation frequency as a proportion of variants with a loss of a part of amplified products in the heterozygous F1 progeny.

SBA, Southern blot analysis of genomic DNA; HZA, heterozygosity analysis.

Table 2

Estimates of induced GI in germline cells for different organisms

Object ^A	Inducing factor	GI induction conditions in the F0-generation		Estimation conditions in somatic cells of the progeny		Registered parameter		Induction coefficient ^B	Ref.
		Dose and type of influence	Spermatogenesis stage	Method	Progeny ^A	Designation	Value		
Mice	γ-radiation, ¹³⁷ Cs	600 P (Ac)	SPG	"Russell 7-locus test"	F1 (1.2 10 ⁵)	MF in specific single-copy loci	13.29 10 ⁻⁵ /locus	17.7	[104]
— " —	— " —	861 P (Ch)	SPG	"Russell 7-locus test"	F1 (2.4 10 ⁴)	— " —	7.06 10 ⁻⁵ /locus	9.4	[104]
Mice C3H/ HeN (10)	γ-radiation, ⁶⁰ Co	3 Gy (Ac)	SPZ	SBA with MNS probe Pc-1	C3H×C57BL. F1 (77)	MF in MNS <i>Ms6hm</i>	14 10 ⁻² /gamete/ locus	1.44	[48]
Mice C3H/ HeN (11)	— " —	— " —	SPT	— " —	C3H×C57BL. F1 (54)	— " —	19 10 ⁻² /gamete/ locus	1.96	[48]
Mice C3H/ HeN (7)	— " —	— " —	SPG	— " —	C3H×C57BL. F1 (53)	— " —	11 10 ⁻² /gamete/ locus	1.13	[48]
Mice C3H/ HeN (9)	— " —	— " —	SPZ	— " —	C57BL×C3H. F1 (56)	— " —	13 10 ⁻² /gamete/ locus	1.35	[48]
Mice C3H (28)	— " —	— " —	SPZ	— " —	C3H×C57BL. F1 (182)	— " —	15 10 ⁻² /gamete /locus	1.5	[56]
Mice C3H (25)	— " —	— " —	SPT	— " —	C3H×C57BL. F1 (104)	— " —	20 10 ⁻² /gamete /locus	2	[56]
Mice C3H (12)	— " —	— " —	SPG	— " —	C3H×C57BL. F1 (95)	— " —	14 10 ⁻² /gamete /locus	1.4	[56]

The effect of low dose radiation

Table 2 (Continued)

1	2	3		4		5		6	7
Mice CBA/H	X-radiation	3 Gy (Ac)		SBA with probe Pc-1, coupled with HZA	CBA/H F0. (15)	MR in MNS <i>Ms6hm</i>	27 10 ⁻² / locus	6.75	[51]
Mice CBA/H	X-radiation	1 Gy (Ac)	SPT	SBA with MNS-probes 33.15 n MMS10	F1. (62)	MF in MNS <i>Ms6hm</i>	(0 - 11.3) 10 ⁻² /gamete /locus (2.7 - 24.7) 10 ⁻² /gamete /locus	1	[9]
— " —	— " —	— " —	SPG	— " —	F1. (73)	— " —	— " —	1.9	[9]
— " —	— " —	— " —	SGLC	— " —	F1. (25)	— " —	— " —	1.85	[9]
Mice CBA/H (4)	Fission neutrons, ²⁵² Cf	0.5 Gy (Ch)	SGLC	SBA with MNS-probe MMS10	F1. (7)	MF in MNS <i>Ms6hm</i> and <i>Hm-2</i>	32 10 ⁻² /gamete /locus 30 10 ⁻² /gamete /locus	6	[73]
— " —	— " —	— " —	SGLC	— " —	F2. (11)	— " —	— " —	5.6	[73]
Mice CBA/H	γ-radiation, ⁶⁰ Co	0.5 Gy (Ch)	SGLC	SBA with MNS probe MMS10	F1. (53)	MR in MNS- loci	1.79 10 ⁻² / fingerprint band 2.33 10 ⁻² / fingerprint band	1.5	[62]
— " —	Fission neutrons, ²⁵² Cf	0.5 Gy (Ch)	SGLC	— " —	F1. (54)	— " —	— " —	2.95	[62]

Table 2 (Continued)

1	2	3		4		5		6	7
Man (127 families from Belarus) — " —	Residence in radiation-contaminated areas — " —	27.6 mSv (Ch) ^c — " —	SPZ — " —	SBA with MNS probe 33.15 SBA with 6 locus-specific probes	F1, (127) — " —	MR in MNS-loci — " —	$1.98 \cdot 10^{-2}$ /fingerprint band $4.27 \cdot 10^{-2}$ /fingerprint band	1.98 1.69	[68] [68]
<i>D.melanogaster CS</i> — " —	2-AAF — " —	24 h maintenance on a medium with 2-AAF — " —	SPZ — " —	AP-PCR of genomic DNA — " —	<i>CS × CS</i> , F1 <i>CS × mus-201</i> , F1	PDF ^E MF	0.01 (M) 0.21 (N)	— N/M = 21	[33] [33]
Fish <i>Oryzias latipes NHI</i> — " —	γ -radiation — " —	4.75 Гп (O) — " —	SPZ — " —	AP-PCR of genomic DNA — " —	F1 F1	PCR product loss frequency _F Frequency of dominant lethal mutations	$1.7 \cdot 10^{-2}$ /generation $14.3 \cdot 10^{-2}$ /zygote	— 2.98	[65] [65]

Footnotes:

- A - in brackets are numbers of exposed F0-generation males or number of examined offsprings in the F1 generation.
- B - ratio between the values of registered parameter for exposed and control objects.
- C - average dose estimate using ^{137}Cs radiation as an index of overall exposure to radionuclides.
- D - mutation frequency as a proportion of microsatellite variants in the F1-progeny per 1 MCS locus.
- E - PDF, progeny damage fraction, the fraction of altered amplification products in the set of products registered in DNA fingerprints for the F1 progeny from exposed male flies.
- F - mutation frequency as a fraction of variants with a loss of a part of amplified products in the heterozygous F1 progeny.
- (Ac) or (Ch) - acute or chronic exposure;
- SPZ - spermatozoids;
- SPT - spermatides;
- SPG - spermatogonia;
- SGLC - stem germline cells;
- SBA - Southern blot analysis;
- HZA - heterozygosity analysis;
- MF - mutation frequency;
- MR - mutation rate;
- 2-AAF - 2-acetylaminofluorene.

REFERENCES

1. Morgan W.F., Day J.P., and Kaplan M.I., *Radiat. Res.*, 1996, vol. **146**, pp. 247 - 258.
2. Shevchenko V.A., *Consequences of the Chernobyl Accident: Human Health*, Ed. E.B. Burlakova, Moscow, Russian Center of Ecological Policy, 1996, pp. 50 - 67. (Rus)
3. Little J.B., *Int. J. Radiat. Biol.*, 1998, vol. **74**, pp. 663 - 671.
4. Neel J.V., *Proc. Nat. Acad. Sci. USA*, 1998, vol. **95**, pp. 5432 - 5436.
5. Baverstock K., *Mutat. Res.*, 2000, vol. **454**, pp. 89 - 109.
6. Machara Y., Oda S., and Sugimachi K., *Mutat. Res.*, 2001, vol. **461**, pp. 249 - 263.
7. Mazurik V.K. and Mikhailov V.F., *Radiats. Biol. Radioekol.*, 2001, vol. **41**, pp. 272 - 289. (Rus)
8. Harms-Ringdahl M., *Mutat. Res.*, 1998, vol. **404**, pp. 27 - 33.
9. Dubrova Y.E., Plumb M., Brown J., and Jeffreys A.J., *Int. J. Radiat. Biol.*, 1998(a), vol. **74**, pp. 689 - 696.
10. Nefedov I.Yu., Nefedova I.Yu., and Palyga G.F., *Radiats. Biol. Radioecol.*, 2000, vol. **40**, pp. 358 - 372. (Rus)
11. Beckman J.S. and Weber J.L., *Genomics*, 1992, vol. **12**, pp. 627 - 631.
12. Edwards A., Hammond H.A., Jin L. et al., *Genomics*, 1992, vol. **12**, pp. 241 - 253.
13. Weber J.L. and May P.E., *Am. J. Hum. Genet.*, 1989, vol. **44**, pp. 388 - 396.
14. Litt M. and Luty J.A., *Am. J. Hum. Genet.*, 1989, vol. **44**, pp. 397 - 401.
15. Dib C., Faure S., Fizames C. et al., *Nature*, 1996, vol. **380**, pp. 152 - 154.
16. Tautz D., *Nucleic Acids Res.*, 1989, vol. **17**, pp. 6463 - 6471.
17. Barber R., Plumb M., Smith A.G. et al., *Mutat. Res.*, 2000, vol. **457**, pp. 79 - 91.
18. Kelly R., Bulfield G., Collick A. et al., *Genomics*, 1989, vol. **5**, pp. 844 - 856.
19. Jeffreys A.J., Wilson V., and Thein S.L., *Nature*, 1985, vol. **316**, pp. 76 - 79.
20. Kuhnlein U., Sabour M., Gavora J.S. et al., *Poul. Sci.*, 1989, vol. **68**, pp. 1161 - 1167.
21. Hearne C.M., Ghosh S., Todd J.A. et al., *Trans. Genet.*, 1992, vol. **8**, pp. 288 - 294.
22. Ryskov A.P., *Molekulyarnaya Biologiya*, 1999, vol. **33**, pp. 997 - 1011. (Rus)

23. Fregeau C.J. and Fourney R.M., *BioTegniques*, 1993, vol. **15**, pp. 101 - 118.
24. Jeffreys A.J., Wilson V., and Thein S.L., *Nature*, 1985, vol. **314**, pp. 67 - 73.
25. Vassart G., Georges M., Monsieur R. et al., *Science*, 1987, vol. **235**, pp. 683 - 684.
26. Nakamura Y., Leppert M., O'Connel P. et al., *Science*, 1987, vol. **235**, pp. 1616 - 1632.
27. Cifarelli R.A., Gallitelli M., and Cellini F., *Nucleic Acids Res.*, 1995, vol. **23**, pp. 3802 - 3803.
28. Welsh J. and McClelland M., *Nucleic Acids Res.*, 1990, vol. **18**, pp. 7213 - 7218.
29. Williams J.G.K., Kubelik A.R., Livak K.J. et al., *Nucleic Acids Res.*, 1990, vol. **18**, pp. 6531 - 6535.
30. Litt M., *PCR: A practical approach*, M.C. McPherson, P. Quirke, G. Taylor, Eds., Oxford, Oxford Univ. Press, 1991, pp. 85 - 89.
31. Weber J.L., *Curr. Opin. Biotechnol.*, 1990, vol. **1**, pp. 166 - 171.
32. Ionov Y., Piendano M., Malkhosyan S. et al., *Nature*, 1993, vol. **363**, pp. 558 - 561.
33. Lopez A., Xamena N., Cabre O. et al., *Mutat. Res.*, 1999, vol. **435**, pp. 63 - 75.
34. Thibodeau S.N., Bren G., and Shaid D., *Science*, 1993, vol. **260**, pp. 816 - 819.
35. Peltomaki P., LotheR.A., Aaltonen L.A. et al., *Cancer Res.*, 1993, vol. **53**, pp. 5853 - 5855.
36. Wada C., Shionoya S., Fujino Y. et al., *Blood*, 1994, vol. **83**, pp. 3449 - 3456.
37. Loeb L.A., *Cancer Res.*, 1993, vol. **54**, pp. 5059 - 5063.
38. Wells R.D., *J. Biol. Chem.*, 1996, vol. **271**, pp. 2875 - 2878.
39. Djian P., *Cell*, 1998, vol. **94**, pp. 155 - 160.
40. Parsons R., Li G.M., Longley M.J. et al., *Cell*, 1993, vol. **75**, pp. 1227 - 1236.
41. Umar A., Boyer J.C., Thomas D.C. et al., *J. Biol. Chem.*, 1994, vol. **269**, pp. 14367 - 14370.
42. Fang W.H., Li G.M., Longley M. et al., *Cold Spring Harbor Symp. Quant. Biol.*, 1993, vol. **58**, pp. 597 - 603.
43. Modrich P. and Lahue R., *Ann. Rev. Biochem.*, 1996, vol. **65**, pp. 101 - 133.
44. Morgan W.F., Corcoran J., Hartmann A. et al., *Mutat. Res.*, 1998, vol. **404**, pp. 125 - 128.
45. Janin N., *Adv. Cancer Res.*, 2000, vol. **77**, pp. 189 - 221.

46. Bohr V.A., *Carcinogenesis*, 1995, vol. **16**, pp. 2885 - 2892.
47. Baarends W.M., van Der Laan R., and Grootegoed J.A., *Reproduction*, 2001, vol. **121**, pp. 31 - 39.
48. Sadamoto S., Suzuki S., Kamiya K. *et al.*, *Int. J. Radiat. Biol.*, 1994, vol. **65**, pp. 549 - 557.
49. Jeffreys A.J., Bois P., Buard J. *et al.*, *Electrophoresis*, 1997, vol. **18**, pp. 1501 – 1511.
50. Mothersill C., Crean M., Lyons M. *et al.*, *Int. J. Radiat. Biol.*, 1998, vol. **74**, p. 673 - 680.
51. Fennelly J., Wright E., and Plumb M., *Leukemia*, 1997, vol. **11**, pp. 807 - 810.
52. Nikiforov Y.E., Nikiforova M., and Fagin J.A., *Oncogene*, 1998, vol. **17**, pp. 1983 - 1988.
53. Richter H.E., Lohrer H.D., Hieber L. *et al.*, *Carcinogenesis*, 1999, vol. **20**, pp. 2247 - 2252.
54. Russell L.B. and Russell W.L., *Proc. Nat. Acad. Sci. USA*, 1996, vol. **93**, pp. 13072 - 13077.
55. Dubrova Y.E., Jeffreys A.J., Malashenko A.M., *Nat. Genet.*, 1993, vol. **5**, pp. 92 - 94.
56. Fan Y.J., Wang Z., Sadamoto S. *et al.*, *Int. J. Radiat. Biol.*, 1995, vol. **68**, pp. 177 - 183.
57. Dubrova Y.E., Plumb M., Brown J. *et al.*, *Proc. Nat. Acad. Sci. USA*, 1998, vol. **95**, pp. 6251 - 6255.
58. Niva O. and Kominami R., *Proc. Nat. Acad. Sci. USA*, 2001, vol. **98**, pp. 1705 - 1710.
59. Walter C.A., Intano G.W., McCarrey J.R. *et al.*, *Proc. Nat. Acad. Sci. USA*, 1998, vol. **95**, pp. 10015 - 10019.
60. Luke G.A., Riches A.C., and Bryant P.E., *Mutagenesis*, 1997, vol. **12**, pp. 147 - 152.
61. Bois P., Collick A., Brown J., and Jeffreys A.J., *Hum. Mol. Genet.*, 1997, vol. **6**, pp. 1565 – 1571.
62. Dubrova Y.E., Plumb M., Brown J. *et al.*, *Mutat Res.*, 2000, vol. **453**, pp. 17 - 24.
63. Aleksandrov I.D., Aleksandrova M.V., Lapidus I.L., and Korablinova S.V., *Radiats. Biol. Radioekol.*, 2001, vol. **41**, pp. 245 - 248. (Rus)
64. Vasil'eva G.V., Bezlepkin V.G., Lomaeva M.G. *et al.*, *Mutat. Res.*, 2001, vol. **485**, pp. 133 - 141.

65. Kubota Y., Shimada A., and Shima A., *Proc. Natl. Acad. Sci. USA*, 1995, vol. **92**, pp. 330 - 334.
66. Santos J., Perez de Castro I., Herranz M., and Fernandez-Piqueras J., 1995, vol. **71**, pp. 223 - 224.
67. Dubrova Y.E., Nesterov V.N., Krouchinsky N.G. et al., *Nature*, 1996, vol. **380**, pp. 683 - 686.
68. Dubrova Y.E., Nesterov V.N., Krouchinsky N.G. et al., *Mutat. Res.*, 1997, vol. **381**, pp. 267 - 278.
69. Shevchenko V.A., Abramov V.I., and Pechkurenkov V.L., *Ecological Consequences of Radioactive Contamination in the Southern Urals*. Moscow, Nauka, 1993, pp. 258 - 302. (Rus).
70. Gileva E.A., Lubashevsky N.M., Starichenko V.I. et al., *Genetika*, 1996, vol. **32**, pp. 114 - 119. (Rus)
71. Gileva E.A., Nokhrin D.Yu., Starichenko V.I. et al., *Genetika*, 2000, vol. **36**, pp. 714 - 717. (Rus)
72. Baulch J.E., Raabe O.E., and Wiley L.M., *Mutagenesis*, 2001, vol. **16**, pp. 17 - 23.
73. Dubrova Y.E., Plumb M., Gutierrez B. et al., *Nature*, 2000, vol. **405**, p. 37.
74. Kovalchuk O., Dubrova Y.E., Arkhipov A. et al., *Nature*, 2000, vol. **407**, pp. 583 - 584.
75. Vorobtsova I.E., *Radiobiologia*, 1987, vol. 23, pp. 377 - 381. (Rus)
76. Mohr U., Dasenbrock C., and Tillmann T., *Carcinogenesis*, 1999, vol. **20**, pp. 325 - 332.
77. Awa A.A. and Neel J.V., *Proc. Natl. Acad. Sci. U S A*, 1986, vol. **83**, pp. 1021 - 1025.
78. Ivanov V.K. and Tsyb A.F., *Consequences of the Chernobyl Accident: Human Health*, Ed. E.B. Burlakova, Moscow, Russian Center of Ecological Policy, 1996, pp. 10 - 23. (Rus)
79. Sevan'kaev A.V., *Radiats. Biol. Radioekol.*, 2000, vol. **40**, pp. 589 - 595. (Rus)
80. Jeffreys A.J., Tamaki K., MacLeod A. et al., *Nat. Genet.*, 1994, vol. **6**, pp. 136 - 145.
81. Jeffreys A.J., Barber R., Bois P. et al., *Electrophoresis*, 1999, vol. **20**, pp. 1665 - 1675.
82. Buard J. and Vergnaud G., *EMBO J.*, 1994, vol. **13**, pp. 3203 - 3210.
83. Buard J., Shone A.C., and Jeffreys A.J., *Am. J. Hum. Genet.*, 2000, vol. **67**, pp. 333 - 344.

84. Hedenskog M., Sjogren M., Cederberg H., and Rannug U., *Environ. Mol. Mutagen.*, 1997, vol. **30**, pp. 254 - 249.
85. Butterworth F.M., Pandey P., McGowen R.M. *et al.*, *Mutat. Res.*, 1995, vol. **342**, pp. 61 - 69.
86. Silver A. and Cox R., *Proc. Natl. Acad. Sci. USA*, 1993, vol. **90**, pp. 1407 - 1410.
87. Hanneman W.H., Legare M.E., Sweeney S., and Schimenti J.C., *Proc. Natl. Acad. Sci. USA*, 1997, vol. **94**, pp. 8681 - 8685.
88. May C.A., Tamaki K., Neumann R. *et al.*, *Mutat. Res.*, 2000, vol. **453**, pp. 67 - 75.
89. Malkhosyan S., Yasuda J., Soto J.L. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1998, vol. **95**, pp. 10170 - 10175.
90. Foucault F., Buard J., Praz F. *et al.*, *Mutat. Res.*, 1996, vol. **362**, pp. 227 - 236.
91. Limoli C.L., Kaplan M.I., Corcoran J. *et al.*, *Cancer Res.*, 1997, vol. **57**, pp. 5557 - 5563.
92. Mitani K., Takahashi Y., Kominami R., *J. Biol. Chem.*, 1990, vol. **265**, pp. 15203 - 15210.
93. Bois P.R., Southgate L., and Jeffreys A.J., *Mamm. Genome*, 2001, vol. **12**, pp. 104 - 111.
94. Georgiev G.P., *Eur. J. Biochem.*, 1984, vol. **145**, pp. 203 - 220.
95. Khesin R.B., *Instability of the Genome*, Moscow, Nauka, 1985. 472 p. (Rus)
96. Gvozdev V.A. and Kaidanov L.Z., *Zhurnal Obshchey Biologii*, 1986, vol. **47**, pp. 51 - 63.
97. Mothersill C. and Seymour C., *Radiats. Biol. Radioecol.*, 2000, vol. **40**, pp. 615 - 620. (Rus)
98. Gardner M.J., Snee M.P., and Hall A.J., *Br. Med. J.*, 1990, vol. **300**, pp. 423 - 429.
99. Vorobtsova I.E., Vorob'eva M.V., Korytova L.I., and Shust V.F., *Tsitologia*, 1995, vol. **37**, pp. 449 - 457. (Rus)
100. Draper G.J., Little M.P., Sorahan T. *et al.*, *Br. Med. J.*, 1997, vol. **315**, pp. 1181 - 1188.
101. Lord B.I., Woolford L.B., Wang L. *et al.*, *Int. J. Radiat. Biol.*, 1998, vol. **74**, pp. 721 - 728.
102. *Genetic Instability and Tumorigenesis*, Ed. M.B. Kastan, Berlin, Springer, 1997, 210 p.
103. Bouffler S., Silver A., and Cox R., *Bioassays*, 1993, vol. **15**, pp. 409 - 412.

104. Russell W.L. and Kelly E.M., *Proc. Natl. Acad. Sci. USA*, 1982, vol. **79**, pp. 542 - 544.
105. Nomura T., *Nature*, 1982, vol. **296**, pp. 575 - 577.
106. Sankaranarayanan K., *Radiats. Biol. Radioekol.*, 2000, vol. **40**, pp. 621 - 626. (Rus)
107. Barton NH., *Genetics*, 1990, vol. **124**, pp. 773 - 782.
108. Gillespie J.H., *Genetics*, 1984, vol. **107**, pp. 321 - 330.
109. Barber R., Plumb M.A., Boulton E. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2002, vol. **99**, pp. 6877 - 6882.
110. Bezlepkin V.G., Lomaeva M.G., Vasil'eva G.V., *et al.*, *Radiats. Biol. Radioekol.*, 2004, vol. **44**, pp. 133 - 137. (Rus)
111. Suzuki K., Ojima M., Kodama S., and Watanabe M., *Oncogene*, 2003, vol. **22**, pp. 6988 - 6993.
112. Chenal C., Legue F., Nourgalieva K. *et al.*, *Radiats. Biol. Radioekol.*, 2000, vol. **40**, pp. 627 - 629. (Rus)

The Redox Homeostasis System in Radiation-Induced Genome Instability

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ABSTRACT

The participation of the redox homeostasis system in the rise of the radiation-induced genome instability and new data in literature that give direct evidence of the presence of this instability *in vivo* are considered. The O_2^\bullet , H_2O_2 and NO^\bullet role as signal molecules, that triggered the cascade of active responses to change the redox status of cells are discussed. These compounds are mediators, which triggered the expression of specific genes. The exposure of reactive oxygen species (ROS) reorganizes the membrane physical-chemical system of cell metabolism regulation. The composition, structure and function of cell membranes are changed and the membrane-bound proteins activity is modified. The data on changes in ROS generation system, including NO, that lead to genome instability after ionizing irradiation even in low doses, are analyzed. It is noted, that radiation induced genome instability and ROS production increase may be observed both in direct irradiated cells and their progeny and in the cells that are not in ionization tracks, and their progeny. There are some evidences that genome instability of irradiated cell progeny is maintained by the increased ROS production. One of the mechanisms of genome instability transduction is realized through “bystander effect”, triggered by ROS.

Keywords: ionizing radiation, low doses, DNA repair, genome, instability, reactive oxygen species (ROS).

Until recently it was self-evident in the radiobiology that such biological consequences of ionizing radiation effect to human organism as cell loss, mutations, chromosomal aberrations and malignant transformation are the result of only irreversible primary structure DNA damage that appears in cells during irradiation. But the experimental biology development during the last decade shook greatly the notion that this is the only basis for the mentioned biological events. The convincing data was received, that some cells-survivors after irradiation can *give* functionally modified progeny, in which de novo chromosome aberrations and genome mutations appear with high frequency during the several generations that in same cases lead to increased cell mortality through apoptosis. These delayed manifestations of radiation effect have no clonal character and were named radiation-induced genome instability. Though the mechanisms of transduction and maintenance of this state in cellular progeny are not clear, manifestations of this phenomenon, ascertained mainly in experiments *in vitro*, are found with high frequency; they are very depending upon the type of influencing radiation, type and "genetic predisposition" of the irradiated cells [1].

Unlike the permanent genome instability inherent to some heritable diseases (ataxia teleangiectasia, Fanconi's anemia, Blum syndrome, etc.) radiation-induced genome instability is not determined by copying in the progeny of radiation DNA damages, that initial arise in their structure in the parents' cells, has no clone character and can appear in cells not exposed to irradiation, but having received damage signals from the irradiated ones. Apparently it is caused by steady acquired change in functioning of the cell as a whole, transferred to the progeny by the epigenetic mechanisms [2].

There are data in literature evidencing the existence in long period after the irradiation of effects typical for radiation-induced genome instability not only *in vitro* but also *in vivo* [3 - 8]. However, direct evidences for the benefit of this phenomenon *in vivo* were just received. It is determined that in progeny of mouse C3H/10T1/2 cells exposed to X-irradiation *in vitro*, the frequency of genome rearrangements *in vivo* is substantially higher than *in vitro* under the *in vivo* upgrowth in syngeneic C3H mouse organism, as well as *in vitro* (after the equal amount of divisions, 25 passages in about 6 months). The rearrangements affect 50 - 100% of cellular sub-clones [9]. Under transplantation of bone marrow cells of male CBA/H mice exposed to 2.2 MeV neutrons in 0.5 Gy dose (^{252}Cf , 0.04 Gy/min) to females-recipients X-irradiated in the dose of 10 Gy, radiation-induced genome instability occurred in the course of the whole term of research (13 months). Transplantation of syngeneic irradiated (40XY) and non-

irradiated (40XYT6T6) bone marrow cells differing from the first ones by stable T6-reciprocal translocation between chromosomes 14 and 15 showed the existence in progeny of non-irradiated 40XYT6T6 cells of chromatid and chromosome breakages typical for the induced genome instability, which amount was indeed somewhat lower than in terms of cultivation in vitro [10].

Some authors suppose that initial DNA damage and ROS increased production is the necessary conditions for appearance of radiation-induced genome instability [11].

A hypothesis was set up that the mechanism of induced genome instability lies in the fact that the stable increase of ROS formation appears in the progeny of the irradiated cells that brings to the DNA oxidative damage and, as a result, increases the frequency of cellular mortality, somatic mutations and chromosome aberrations [12].

However the mechanisms of change of the redox homeostasis in the trend of possible ROS production predominance in the progeny of the irradiated cells, as well as their participation in the formation of genome instability remain still not elucidated, though recently many works appeared allowing to get closer to understanding of the place of the redox metabolism part connected to ROS generation in the system of events leading to the appearance and maintenance of the induced genome instability.

Analysis of the problem current state is the main goal of this study.

ROS METABOLIC GENERATION. NO SYSTEM

Redox reactions are known to be the normal foundation for the organism vital functions. Owing to them the transformation of energy occurs that was accumulated in the so called "fuel" molecules (carbohydrates, lipids), through the system of electron carriers to the energy of energy-rich compounds consumed in biomolecule synthesis reactions, vital functions products detoxication, in active transport of molecules and ions, for maintenance of the membrane potential. Aerobes have O_2 as the only acceptor of electrons, from which as result of the normal vital activity of an organism occurs formation of highly reactive compounds – endogenous ROS. The most important of them are superoxide anion radical (O_2^\bullet), singlet oxygen (O^1_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^\bullet).

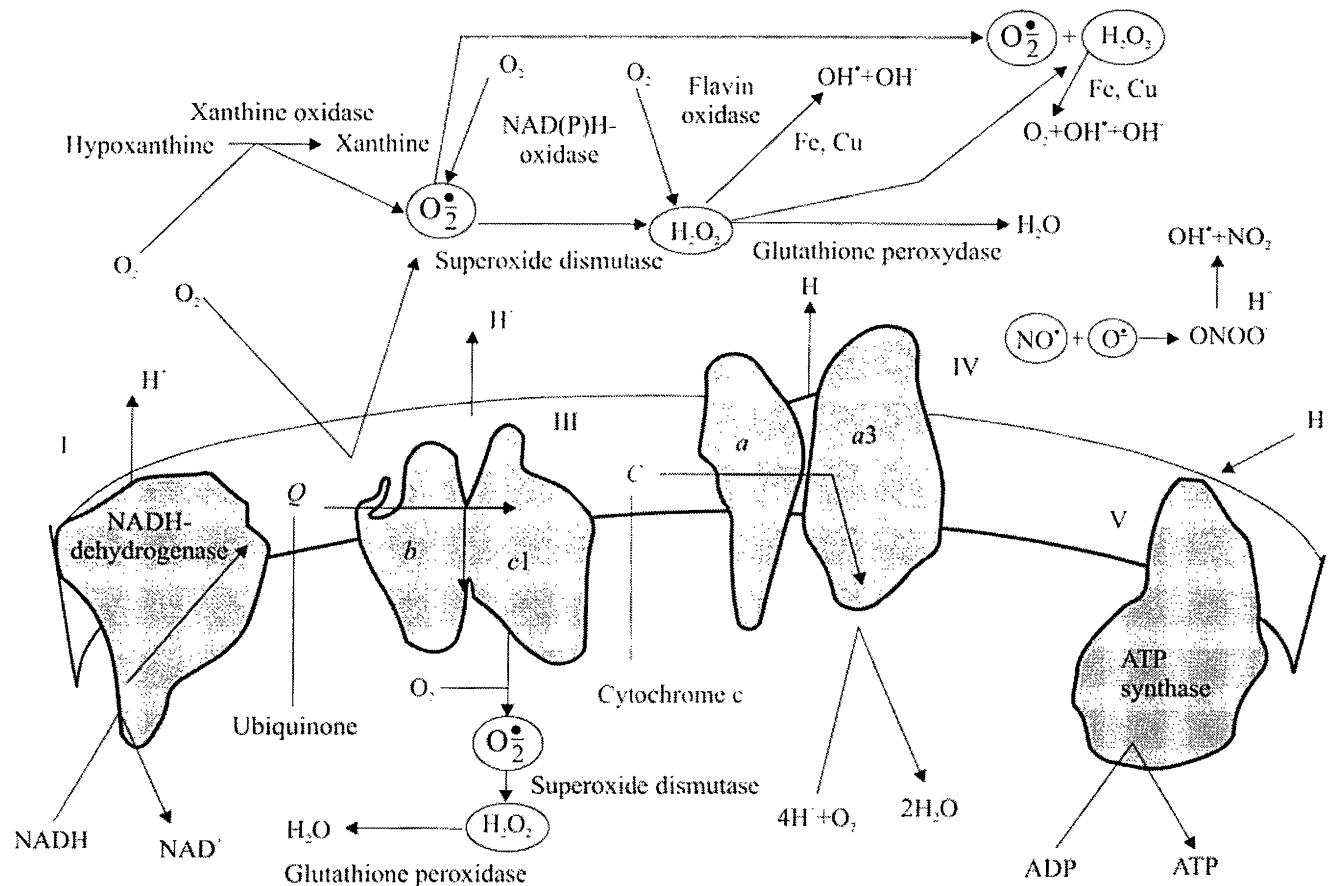


Figure. Metabolic generation of reactive oxygen species in cell. Sensor molecules are in ovals

Figure shows metabolic ways for ROS formation. ROS occur in mitochondrial and microsomal oxidation, NADP.H oxidase action, for which reduced nicotinamide adenine dinucleotide phosphate (NADP.H) is the substrate, etc. It was determined that the main places of their formation are mitochondria, endoplasmic reticulum and nuclear membrane.

Free radicals attack DNA molecule and cause radical formation both in bases and sugar-phosphate fragment in the molecule. Beyond chemical modification of DNA nucleotide radical formation may also change the highest levels of its structure organization [13]. Being the main damaging DNA oxyradical, hydroxyl radical is generated in Fenton reaction from hydrogen peroxide under the influence of Fe^{2+} (Cu^+) ions. Another product of this reaction is hydroxyl anion. If superoxide radical O_2^\bullet interacts with hydrogen peroxide in the presence of Fe^{2+} (Cu^+) ions O_2 , OH^- and hydroxyl radical OH^\bullet are generated (Haber-Weiss reaction).

An important influence on proceeding of redox reactions, products of which are ROS, has the recently discovered nitric oxide system as a significant regulator of many biochemical and physiological processes [14].

Nitric oxide (NO) is a highly reactive radical molecule with unpaired electron on the outer π -orbital. It is formed oxidation of L-arginine through an intermediate product N-hydroxy-L-arginine in the reaction catalyzed by NO-synthases. Another way of NO generation can be transformation of nitro-, nitroso- and other nitrogen-containing compounds to nitrites (NO_2^-) which are reduced owing to nitrite reductase activity of haem-containing proteins (haemoglobin, cytochrome oxidase, cytochrome P₄₅₀) [15,16]. Nitric oxide can directly interact with haem, SH-groups, metals of variable valence included into protein prosthetic groups rousing inhibition of some enzymes of oxidative homeostasis system (NAD.H:ubiquinone oxidoreductase, succinate:ubiquinone oxidoreductase, NADP.H oxidase, superoxide dismutase, glutathione peroxidase), as well as enzymes of biosynthesis and reparation of DNA-ribonucleotide reductase, DNA ligase, formamide pyrimidine DNA glycosylase, etc. [17]. NO is capable to activate some important regulatory enzymes: ADP ribosiltransferase, cyclooxygenase, plasminogen activator [16].

Nitric oxide and oxygen as low-polarity molecules are collected inside the hydrophobic globules of proteins molecules converting their SH groups into nitrosothiols [18]. In aqueous phase superoxide radical O_2^\bullet interacting with NO generates peroxynitrite (ONOO^-), a highcytotoxic compound capable to trigger processes of peroxidation of lipids in membranes and lipoproteins, oxidize NH_3

and SH-groups of proteins. Unlike NO, this compound generates irreversible inhibition of complexes I and II of mitochondria. Interacting with hydrogen ion peroxy nitrite generates OH[•] radical without participation of variable valence metals (see the Figure). It is believed [19] that this reaction is one of the basic cellular reactions leading to generation of hydroxyl radical.

The ROS effect to the membrane lipids causes activation of peroxide oxidation together with forming of high-reactive radical compounds. The latter interact with DNA molecules forming such compounds as malonedialdehyde DNA adducts with deoxinucleosides of adenine, guanine, cytosine and hydroxypropane-deoxiguanosine appearing in DNA reaction with other products of lipid peroxidation – acrolein and crotone aldehyde [20].

Taking into account high ROS reactivity and their products in reactions with NO, participation of these molecules in different vital activity processes the maintenance of the necessary level of their content is controlled not only by generation rate of the mentioned compounds but also by the state of antioxidant protection system. Important components of this system are superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, catalase as well as compounds containing SH-groups (glutathione, cysteine). Bioantioxidants as free radical scavenger traps play an important role in the system of antioxidant protection. Biological activity of such compounds as ubiquinone, tocopherol, vitamin K, vitamin A, etc. correlate with their anti-radical properties [21].

Oncoprotein bcl-2 localized in the same cell microstructures where O₂[•] generates, influences greatly the cellular antioxidant protection. Activation of *bcl-2* gene prevents oxidative damage of cellular components, hinders in the apoptosis development.

In general, ROS and antioxidants form a single prooxidant – antioxidant system. Until recently this system was connected only with protection functions: of some cell types – against alloantigens (pro-oxidant activity), and by exposure to ionizing radiation, which effects are significantly caused by formation of free radicals – with inactivation of the latter (antioxidant activity). At present owing to the success of modern biochemistry the prooxidant- antioxidant system, which prooxidant part is determined as ROS, should be considered as a part of broader system of cell oxidative homeostasis joining all processes going under participation of molecular oxygen. Important for adaptation processes is the existing in the organism physical-chemical system of regulation of cellular metabolism by membranes based on the correlation of lipid peroxidation rate on the one hand and membrane lipid content changing on the other hand. Activation of peroxidation leads to acceleration of liberation of the most easily oxidized

lipids and enrichment of membrane lipids with fractions resistant to oxidation [22]. This in its turn decreases lipid peroxidation rate and returns it to the norm. Thus physical-chemical regulation system connected to cellular membranes exercises the function of homeostasis regulation and adaptation to the changing conditions. The change of peroxidation rate, lipid content and thus membrane structural characteristics influences on the activity of membrane-connected proteins: enzymes, channel building proteins, receptors, etc. It means that ROS concentration change influences on the membranes and leads to the change of cell functional activity.

Owing to existence in the cell oxidative homeostasis system of sensory-regulatory and realizing components adequate adaptation of cell for the environment is ensured by way of adaptation responses to its signals including damaging effects including ionizing irradiation.

Sensory components are presented in the oxidative homeostasis system by molecules perceiving biologically significant signals about the status of intercellular medium oxygenation – O₂ tension [23, 24].

The data from the literature summarized in the present work for the role of sensory molecules in the oxidative homeostasis system at present claim such ROS as superoxide radical (O₂[•]), H₂O₂, as well as nitric oxide (NO) that has also endocrine and paracrine effect. Exceptionally important for the cell fate hydroxyl radical (OH[•]) and peroxinitrite (ONOO⁻) can not play the role of signal compounds as they are high reaction capable and actually enter into biochemical reactions at the place of generation.

Radical O₂[•] and H₂O₂ sensor function regarding O₂ tension and the status of metabolic processes in cell is realized in its different parts in the following way. Under hypoxia xanthine dehydrogenase converts into xanthine oxidase that leads to ROS generation. Activity of another enzyme – microsomal NAD.H oxidase also depends on the amount of O₂ and NAD.H reaching it; cyclooxygenase activity – on O₂ concentration and NADP.H contents. In mitochondria under hypoxia reduction of O₂ up to H₂O catalyzed by cytochrome oxidase c (complex IV) is inhibited, as a result of electrons transferred in complex III go to generating of O₂[•] that increases the content of peroxide radical [21] (see the Figure). The change in value of ROS signal molecules content triggers the cascade of mitogen-activated protein kinase (MAPK), etc., activates transcription factors initiating gene expression and rearrangement of metabolic processes in cell. H₂O₂ and O₂[•] content increase is the signal of

hypoxia and trigger in mitochondria processes necessary for adaptation of cells to these conditions. In conditions of inhibition of complex I the system response changes - O_2^\bullet generation decrease occurs. It is characteristic for endoplasmatic reticulum that O_2^\bullet content decrease leads to decrease in O_2^\bullet and H_2O_2 generation in reaction catalyzed by NADP.H oxidase.

In case of other stress effects, production of ROS and, consequently, signal molecules also increases. In reviews [25, 26] the data are summarized that clearly prove the influence of the oxidative stress on gene activity. The authors suppose that discovery of specific genes and paths, affected by ROS, allows consideration ROS to be subcellular mediators in regulation of gene expression and signal transduction. Besides antioxidants also when changing redox-potential can cause activation of genes and metabolic paths, particularly modulate processes of collagen transformation, post-translation control of ferritin metabolism, transcription factors such as Myb, Egr-1. Some pro-oncogens become transcription active owing to oxidative processes intensification. For example, influence of ROS signal molecules – superoxide radical, hydrogen peroxide stimulates activation of expression of jun-B, jun-D, c-fos and foc-B.

Thus ROS are molecules able not only to bring damaging effect promoting function of protection from allogen aggression but also to play an important role in processes assisting adaptation of cells to changes in environment conditions.

ROS SYSTEM UNDER IONIZING RADIATION EXPOSURE

Under ionizing radiation exposure as a result of direct and indirect radiation effects (due to generation of radicals of water, which output is increased in presence of O_2) genome DNA damage in part of a second occurs. At the same time radiation radicals become a signal for activation of processes triggered from membrane structures usually under other stress effects ensuring adaptation of cells to changes in environment conditions. In reaction of irradiated cells to occurred damage they use constitutive, pre-existing mechanisms including two types of adaptation: immediate (urgent) and long-term. The former is ensured by activation of early regulatory genes coding protooncogenes and stress proteins, the latter – by activation of late structure

genes including genes of Ca^{2+} -ATPase, antioxidant enzymes, nitric oxide cycle, etc. [27 - 29].

For the initial stage of immediate (urgent) adaptation, ROS production increase is characteristic in cells in response to irradiation leading to increase by them of radiation DNA damage. Thus in research of ROS production in human lungs fibroblasts exposed to of α -particles in dose of 0.4 - 19 cGy in the whole used dose range the significant and prolonged (for 24 hours) increase in intracellular generation of O_2^\bullet and H_2O_2 in comparison with sham-irradiated cells observed. Membrane-connected NADP.H oxidase was responsible for these ROS generation increase. O_2^\bullet and H_2O_2 hyperproduction effect was observed also in the event when cell nucleus and even the cell itself were not subject to the direct hit of α -particle. Non-irradiated cells intensified ROS production in incubation with the medium containing serum subjected to influence of α -particles or with supernatant of irradiated cells culture medium [30]. O_2^\bullet and H_2O_2 generation activity by mitochondria after ionizing irradiation depends on the status of electron transduction chain, as increase of ROS production by complex III (cytochrome c reductase) is observed under condition of actively functioning complexes I and II [31].

Thus it is obvious that at least some DNA damage effects observed after ionizing radiation exposure are mediated of ROS-dependent mechanisms.

Note also a possibility of nitric oxide radical participation in realization of the radiation effect that was not considered before. It can promote fixation of radiation damages under influence on DNA in its structure [17]. NO interaction with superoxide anion-radical leads to generation of peroxynitrite with significantly higher reaction capability than NO and O_2^\bullet , a strong DNA destructive agent [32], that leads to DNA damage as well as mitochondria oxidation increase. Thus it was determined that under ionizing radiation exposure on precursors of haematopoietic cells with increased content of nitric oxide in them suppression of mitochondrion function connected with inhibition of respiratory complex I (NAD.H dehydrogenase) and III (cytochrome c reductase) because of aggravated generation of peroxynitrite observed [33].

The principal difference between reactions of the cell to non-irradiation influences and ionizing radiation exposure is that in the latter case cytoplasmatic signals arising in cell go to damaged genome, if it was affected by ionization. These signals as it was previously mentioned appear as a result of changes in concentration of ROS, part of which being signal molecules triggers activation

of preceding transcription factors by means of their phosphorylation with the help of proteinkinase - MAPK activated by mitogens. The latter interact with chromatin causing transcription activation and preferable translation of stress proteins accompanied by simultaneous cutting-off of translation of other proteins [34, 35]. Increase of synthesis of stress proteins and proto-oncogens due to their genes activation is the link of non-specific adaptation to exposure of different factors. And though in both cases mechanisms of expression of the same genes begin to work (including genes of oxidative metabolism/production of ROS as the prompt response system) the response of the system of lasting adaptation under ionizing radiation exposure will differ as reaction of damaged chromatin to transcription signals can be faulty till DNA reparation is carried out.

There are two possible outcomes of radiation exposure in connection with damages existing in genome: 1) cell death including through apoptosis, and 2) their survival with: a) rise of mutations in cellular progeny; b) adaptation and return of the cells to initial state, and c) changes of functional state caused by adaptation processes making cells differ from the existed before the irradiation. The latter as well as the fully restored and the mutant ones are able to give progeny with the increased probability of accidental display of radiation-induced genome instability in it.

The choice of alternative of possible way-out of radiation damage depends significantly on the state of the key links of redox homeostasis. Thus under irradiation of mice lymphoma B cells of two lines – Lyas line - sensitive for apoptosis (similar in radiosensitivity to ataxia teleangiectasia cells) and Lyar line - resistant (similar in radiosensitivity to Chinese hamster cells), differing before irradiation in content of glutathione and protein bcl-2 and expression of several genes participating in the maintenance of redox potential (in particular, of genes of tetrospanin CD53 and fructose-1,6-diphosphatase), in cells of radiosensitive Lyas line fast expression was observed of cluster of genes, which protein were involved into mitochondrial electron transport and promote uncoupling of respiration with phosphorylation and loss of membrane potential. Accordingly in radioresistant Lyar cells the suppression of these biochemical paths and activation of the path promoting the retention of high redox potential through the increase of glutathione level observed [36].

In the cells of radioresistant human glioblastoma T98G line in time from 1 hour up to 24 hours after the ionizing radiation exposure increase of expression of genes coding the subunits 1 and 2 of cytochrome c oxidase and subunit 4 of NAD.H dehydrogenase was observed. Mitochondrial genes expression and the synthesis of corresponding proteins lead to increase of production of ATP and

mitochondrial potential without changing of mass of mitochondria and mitochondrial DNA content [37].

The described processes appear under ionizing radiation exposure though not in all cells subject to irradiation but with increasing of radiation dose cover their more and more number. Thus in experiments on cells of five lines it is determined that already in several minutes after of γ -irradiation increase in production of ROS and reactive nitrogen species appear in culture. Quantitatively generation of these compounds in each cell did not depend on the dose of irradiation in the range 1-10 Gy; their content in cell remained permanent. Though the observed effect of activation of production of ROS and reactive nitrogen species was conditioned by the increase in the amount of cells in which it took place: starting from 20% under the dose of 1 Gy up to 80% under the dose of 10 Gy [35]. The analogous data was received in [30].

Thus it is obvious that after irradiation in part of cells which increases with increasing of absorbed radiation dose oxidative metabolism and gene expression profile are changed that is expressed in increased ROS production. Moreover irradiated cells secretes cytokines and other factors of protein nature that provoke increase content of reactive oxygen species in non-irradiated cells [38-40], in frequency of sister chromatid exchange [40], etc. As a result in such cells not directly affected by radiation exposure changes are generated that are characteristic for irradiated cells, phenomenon appears that is called "bystander effect". For its realization the state of systems that control oxidative homeostasis is extremely important that is confirmed by results of research in mutant cell lines.

Thus under growth of human keratinocytes or Chinese hamster CHO-K1 cells in the cultural medium from irradiated cells bystander effect was observed that was displayed in decrease in clonogenic activity of non-irradiated cells. Bystander effect decrease or abolition was observed when inhibitors of apoptosis or culture environment from irradiated cells of mutant lines defective in lactate dehydrogenase (LDH^{-/-}) or glucose-6-phosphate dehydrogenase (G6PD^{-/-}) were used. Under transfection of non-irradiated LDH- or G6PD-defective cells with gene *G6PD* the growing medium after cultivation such cells in it newly had the ability to challenge bystander effect. Incubation of irradiated cells of wild type or cells with eliminated defect in G6PD with antioxidant compounds – L-lactate and L-deprenyl prevented from generation of factor of bystander effect connected to cell death that indicates to participation of redox metabolism in expression of radiation-induced bystander effect [41].

This effect can originate also as a result of direct contact between irradiated and non-irradiated cells. To the non-irradiated cells the state is transferred that is characteristic for the irradiated ones of elevated ROS production, increased rate of p53 protein, activation of expression of genes connected to the cell cycle arrest in checkpoints, participating in DNA repair and under its incapacity – triggering apoptosis. Apparently physical-chemical system of membrane regulation of cellular metabolism participates in transduction of signal inducing instability. Depending on type of cell the bystander effect can be geared through either this or that mechanism. Thus it was shown using hybrid culture of cells with include into hamster K-1 cell nucleus of the single copy of human chromosome 11 that after α -particles exposure radiation-induced genome instability arises in neighbor cells through mechanism of intercellular contact but not due to excretion to the intercellular medium of factor(s) connected to ROS production [42].

Bystander effect is considered during last years as one of important mechanisms of originating radiation-induced genome instability for which delayed reproductive cell death (delayed lethal mutations) is characteristic with increased frequency of appearance of mortality through apoptosis mechanism, chromosome destabilization, somatic mutations and amplification of genes, radiosensitivity changing.

The key role in arising and maintenance of radiation-induced genome instability is played intensive ROS production as manifestation of the changed redox metabolism inherited epigenetically from parent cells going to this status after irradiation [11, 12, 29, 30, 34].

Till recently all manifestations of radiation-induced genome instability investigated in particular in experiments using α -particles beam irradiation could be characterized besides genetic stability also by increased frequency of cell death through apoptosis. These data were well adjusted to the fact of increase in p53 protein contents that as we know bring not only to cell cycle arrest but also start the apoptosis processes in cells where DNA repair did not complete by radiation defect elimination [43, 44].

But such response of cells to irradiation can be not the only one. The evidence of unstable phenotype can vary from significant to zero including intermediate variants depending also on genotype and genome functional state at the moment of exposure. Thus it was determined that in CBA/H mice with expressed genome instability after irradiation is shown a lower level of delayed cell mortality and apoptosis frequency in comparison with C57/BL6 mice characterized by lower inclination to generation radiation-induced genome

instability [45]. Exposure of short-living α -particles of osteotrophic radionucleotide ^{224}Ra to the cells of CBA/H mice bone marrow challenge no manifestation of genome instability both in vitro and in vivo during 100 days after irradiation though in one experiment bystander effect on chromosome aberration was registered [46]. Irradiation of normal human embryo fibroblasts in the dose of 6 Gy led to rising of genome instability in progeny shown in decrease of clonogenic capability, appearance of giant cells in colonies and chromosomal bridge in anaphase. As a result of preliminary irradiation in the dose of 2 cGy for 5 hours before X-radiation exposure in the dose of 6 Gy genome instability in progeny was manifested not so unambiguously: clonogenic activity somewhat increased though not significant, and appearance frequency of giant cells and chromosomal bridges was not so expressed [47]. In another work describing experiments on human normal fibroblasts increase of proliferative activity was found after α -particles exposure accompanied by decrease in p53 protein contents [29]. The investigation authors suppose that order of events leading to growth of cell proliferative activity is the following. After ionizing radiation exposure ROS production increase takes place that leads to appearance in intercellular fluid of free transformation factor $\beta 1$ (TGF- $\beta 1$). TGF- $\beta 1$ contents increase observed also by other authors after irradiation even in low doses can take place due to fast secretion as a result of its liberation from beta-glycane receptors as well as from non-cellular matrix. Interacting with cells TGF- $\beta 1$ activates membrane-bound NADP.H oxidase contained in fibroblasts that in turn leads to increase of intracellular O_2^\bullet and H_2O_2 production. Besides TGF- $\beta 1$ additionally activates membrane-bound NAD.H oxidase at the cell surface that supports H_2O_2 production for a long period at least of 24 hours after irradiation. Changing of TGF- $\beta 1$ interaction with cells leads to increase of their proliferative activity.

In another work they observed significant increase of interleukin-8 with simultaneous increase of its mRNA contents in normal human lungs fibroblasts at 30 minutes after α -particles exposure in the dose of 3.6-19.0 cGy. In cells irradiated in the presence of antioxidants – superoxide dismutase or dimethyl sulfoxide – the interleukin-8 contents was significantly lower. Such influence was caused by transcription inhibitor dexamethasone. Simultaneous intensification of production of ROS and interleukin-8 with promitogen effect can promote the hyperplastic orientation of the irradiated cells and their neighbors [28].

As the carried out earlier analysis shows [48] the status of radiation-induced genome instability in progeny of the irradiated cells can be considered

as the manifestation of being prepared to adaptation changes. Two outcomes are possible for it: a) adaptation to the arisen conditions with gradual normalization of cellular functions and phenotype through repair and elimination of defective cells, returning into the normal stable state, or b) growing of pathological states first of all of the most probable – premature ageing and malignant transformation with genome instability.

Probability of the second version of the outcome of radiation-induced genome instability allows thinking that prolonged preservation of cells in this state increases the risk of rise of pathology. Hence the question arises apparently on necessity and search of possible ways to modify radiation-induced genome instability on the basis of the considered here role of redox homeostasis in its rise and maintenance. As it is supposed that radiation-induced genome instability can go on until the cells return to their initial reaction levels to DNA damage [49], some possible ways to solving this problem can be found in assistance to normalization of control mechanisms in cell cycle checkpoints, DNA repair processes and redox homeostasis. It is appropriate to give here some data received regarding the considered topic.

Experiments on progeny of human NaCaT keratinecyte line with expressed manifestation of genome instability as a result of influence on parent cells of γ -radiation in the dose of 5 Gy show that growing in the presence of low molecular antioxidants decreases the amount of apoptotic cells and restores clonogenic activity in comparison to cells grown without antioxidants. If the part of bcl 2-positive cells is lowered in the culture of progeny of the irradiated cells supported in absence of antioxidants, the addition of antioxidants to the medium restored the percentage of bcl 2-positive cells up to normal level [11].

However the problem of radiation-induce genome instability modification with the help of antioxidants is not that simple. The data was already given above showing that in some cell types rise and maintenance of genome instability due to bystander effect goes without excretion to the medium of factors connected to ROS production [42].

In cases if instability of irradiated cellular progeny is supported by increased ROS production, cell reaction to antioxidants depends a lot on the fact how far the process of their being in the state of genome instability has gone. Thus on two lines of transformed cells it is shown that effective antioxidant glutathione is a toxic agent increasing H_2O_2 production for one of them, with low level of antioxidant genes expression [50].

However these data should be evaluated very carefully as they were received in experiments *in vitro* and there is no data regarding the fact how the

considered effects can be applied to significantly more complicated situation of radiation-induced instability on organism level.

CONCLUSION

The carried out analysis shows that redox reactions system existing in the cells of mammalian has regulatory mechanisms ensuring their adaptation to the changing environment conditions. An important role in this system belongs to signal molecules – superoxide anion-radical O_2^\bullet , hydrogen peroxide, and NO radical influencing the physical-chemical system of membrane regulation of cellular metabolism. The changes in content, structure and functioning of membranes occur, membrane-connected proteins activity is modified. The same signal molecules switch on the cascade of biochemical reactions leading to activation of genome and adaptive synthesis of some proteins providing metabolism compensatory changes.

ROS generation increases severely under ionizing radiation exposure both due to appearing of radiation-induced radicals including ROS and as a result of their metabolic generation increase. ROS increased production leads to generation in the irradiated cells of signals transferred to the part of cells that was not directly subject to irradiation exposure. These cells react to signals from directly irradiated cells through ROS generation and originating of damages in DNA under their influence. The so called “bystander effect” arises contributing greatly into proportions of cell contingents affected by genome instability. DNA repair mechanism activation in cells with high level of antioxidant mechanisms effectiveness leads to elimination of structural damages in genome and metabolic processes normalization. Some cells having restored damages are eliminated though apoptosis mechanisms. Though cells remain that damages overcame barriers of DNA repair and apoptosis and became mutant. Finally experimental data received in experiments in vitro and in vivo testify that one more part of cells survive after the irradiation – those in which adaptive mechanisms were activated but didn't come into norm at the moment of division. There are no DNA damages in such cells that prevent from overcoming of barrier of cell cycle checkpoints but ROS generation in it is elevated and their DNA is sensitive to effect of oxyradicals and other genotoxic agents. Radiation-induced genome instability with all its phenotype manifestations is observed in these cells. It is important to notice that rising of this instability is

experimentally established in some cases after ionizing radiation exposure even in low doses. Prolonged retention of progeny of the irradiated cells in this state increases the risk of malignant transformation and thus research of this problem and finding the remedies and ways to overcome the unfavourable consequences of radiation-induces genome instability become the actual task of the modern radiobiology.

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REFERENCES

1. Wright E.G. ‘Inducible genomic instability: new insights into the biological effects of ionizing radiation’, *Med. Confl. Surviv.*, 2000, vol. **16**(1), pp. 117 - 130.
2. Simons J.W., ‘Coming of age: “dysgenetics” – a theory connecting induction of persistent delayed genomic instability with disturbed cellular aging’, *Int. J. Radiat. Biol.*, 2000, vol. **76**(11), pp. 1533 - 1543.
3. Seymour C.B., Mothersill C., and Alper T., ‘High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation’, *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.*, 1986, vol. **50**(1), pp. 167 - 179.
4. Kadhim M.A., MacDonad D.A., Goodhead D.T. *et al.*, ‘Transmission of chromosomal instability after plutonium α -particle radiation’, *Nature*, 1992, vol. **355**(6362), pp. 738 - 740.
5. Dubrova Y.E., Jeffreys A.J., and Malashenko A.M., ‘Mouse minisatellite mutations induced by ionizing radiation’, *Nature Genet.*, 1993, vol. **5**(1), pp. 92 - 94.
6. Pelevina I.I., Afanasjev G.G., Gotlib V.Ja. *et al.*, ‘Cell exposition in tissue culture and animals (mice) in 10-kilometer zone of Chernobyl APP catastrophe. Influence on sensitivity to further irradiation’, *Radiats. Biol. Radioekol.*, 1993, vol. **33**(1(4)), pp. 508 - 520. (Rus)
7. Dubrova Y.E., Nesterov V.N., Krouchinsky N.G. *et al.*, ‘Human minisatellite mutation rate after the Chernobyl accident’, *Nature*, 1996, vol. **380**(6576), pp. 683 - 686.
8. Little J.B., ‘Radiation-induced genomic instability’, *Int. J. Radiat. Biol.*, 1998, vol. **74**(6), pp. 663 - 671.

9. Paquette B. and Little J.B., 'In vivo enhancement of genomic instability in minisatellite sequences of mouse C3H/10T1/2 cells transformed in vitro by X-rays', *Cancer Res.*, 1994, vol. 54(12), pp. 3173 - 3178.
10. Watson G.E., Lorimore S.A., Macdonald D.A. *et al.*, 'Chromosomal instability in non-irradiated cells induced in vivo by a bystander effect of ionizing radiation', *Cancer Res.*, 2000, vol. 60(20), pp. 5608 - 5611.
11. Mothersill C., Crean M., Lyons M. *et al.*, 'Expression of delayed toxicity and lethatl mutations in the progeny of human cells surviving exposure to radiation and other environmental mutagens', *Int. J. Radiat. Biol.*, 1998, vol. 74(6), pp. 673 - 680.
12. Clutton S.M., Townsend K.M.S., Walker C. *et al.*, 'Radiation-induced genomic instability and persisting oxidative stress in primary bone marrow cultures', *Carcinogenesis*, 1996, vol. 17(8), pp. 1633 - 1639.
13. Sharpaty V.A., 'Radiation modification of DNA sugar enzyme: generation of breaches, polymer conformation changing; transduction of damage to basis', *Radiobiologia*, 1992, vol. 32(2), pp. 180 - 193. (Rus)
14. Vanin A.F., 'Nitric oxide in biology: history, status and prospective of research', *Biokhimia*, 1998, vol. 63(7), pp. 867 - 869. (Rus)
15. Gorren A.K.F. and Mayer B., 'Universal and complex enzymology of nitric oxide synthase', *Biokhimia*, 1998, vol. 63(7), pp. 870 - 880. (Rus)
16. Menjtshikova E.B., Zenkov N.K., and Reutov V.P., 'Nitric oxide and NO-synthase in mammal organism under different functional status', *Biokhimia*, 2000, vol. 65(4), pp. 485 - 503. (Rus)
17. Vink D.A., Vodovoz Y., Cook G.A. *et al.*, 'Nitric oxide chemical characteristics significance for curing of oncogenic diseases', *Biokhimia*, 1998, vol. 63(7), pp. 948 - 957. (Rus)
18. Nedospasov A.A., 'Biogenic NO in competitive relations', *Biokhimia*, 1998, vol. 63(7), pp. 881 - 904. (Rus)
19. Rubbo H., Darley-Usmar V., and Freeman B.A., 'Nitric oxide regulation of tissue free radical injury', *Chem. Res. Toxicol.*, 1996, vol. 9(5), pp. 809 - 820.
20. Marnett L.J., 'Oxyradicals and DNA damage', *Carcinogenesis*, 2000, vol. 21(3), pp. 361 - 370.
21. Burlakova E.B., Krashov S.A., and Khrapova N.G., 'The role of tocopherols in biomembrane lipid peroxidation', *Membr. Cell. Biol.*, 1998, vol. 12, pp. 173 - 211.

22. Burlakova E.B. and Khrapova N.G., 'Gerpoxide oxidation of membrane lipids and natural antioxidants', *Uspekhi Khimii*, 1985, vol. **54**(9), pp. 1540 - 1558. (Rus)
23. Volin M.S., Davidson K.A., Kaminsky P.M. *et al.*, 'Transduction mechanisms of nitric oxidant-oxide in vascular tissue', *Biokhimia*, 1998, vol. **63**(7), pp. 958 - 965. (Rus)
24. Semenza G.L., 'Perspectives on oxygen sensing', *Cell*, 1999, vol. **98**(3), pp. 281 - 284.
25. Allen R.G. and Tresini M., 'Oxidative stress and gene regulation', *Free Radical Biol. Med.*, 2000, vol. **28**(3), pp. 463 - 499.
26. Somasundaram K. and El-Deiry W.S., 'Tumor suppressor p53: regulation and function', *Frontiers in Biosci.*, 2000, vol. **5**, d424 - d437.
27. Malyshev I.Ju. and Manukhina E.B., 'Stress, adaptation and nitric oxide', *Biokhimia*, 1998, vol. **63**(7), pp. 992 - 1006. (Rus)
28. Narayanan P.K., LaRue K.E., Goodwin E.H. *et al.*, 'Alpha particles induce the production of interleucin-8 by human cells', *Radiat. Res.*, 1999, vol. **152**(1), pp. 57 - 63.
29. Iyer R. and Lehnert B.E., 'Factors underlying the cell growth-related bystander responses to alpha particles', *Cancer Res.*, 2000, vol. **60**(5), pp. 1290 - 1298.
30. Narayanan P.K., LaRue K.E., Goodwin E.H. *et al.*, 'Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells', *Cancer Res.*, 2000, vol. **60**(5), pp. 3963 - 3971.
31. Pham N.A. and Hedley D.W., 'Respiratory chain-generated oxidative stress following treatment of leukemic blasts with DNA-damaging agents', *Exp. Cell. Res.*, 2001, vol. **264**(2), pp. 345 - 352.
32. Maeda X. and Akaike T., 'Nitric oxide and oxygen radicals in infection, inflammation and cancer', *Biokhimia*, 1998, vol. **63**(7), pp. 1007 - 1019. (Rus)
33. Pearce L.L., Epperly M.W., Greenberger J.S. *et al.*, 'Identification of respiratory complexes I and III as mitochondrial sites of damage following exposure to ionizing irradiation and nitric oxide', *Nitric oxide*, 2001, vol. **5**(2), pp. 128 - 136.
34. Benjamin I.L. and McMillan D.R., 'Stress (heat shock) proteins. Molecular shaperones in cardiovascular biology and disease', *Circulat. Res.*, 1998, vol. **83**(2), pp. 117 - 132.

35. Leach J.K., Van Tuyle G., Lin P.-S. *et al.*, ‘Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen’, *Cancer Res.*, 2001, vol. 61(10), pp. 3894 - 3901.
36. Voehringer D.W., Hireshberg D.L., Xiao J. *et al.*, ‘Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis’, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97(6), pp. 2680 - 2685.
37. Gong B., Chen Q., and Almasan A., ‘Ionizing radiation stimulates mitochondrial gene expression and activity’, *Radiat. Res.*, 1998, vol. 150(5), pp. 505 - 512.
38. Seymour C.B. and Mothersill C., ‘Delayed expression of lethal mutation and genomic instability in the progeny of human epithelial cells that survived in a bystander-killing environment’, *Radiat. Oncol. Investig.*, 1997, vol. 5(3), pp. 106 - 110.
39. Mothersill C. and Seymour C.B., ‘Genomic instability and radiation risks: implications for development of protection strategies for man in the environment’, *Radiats. Biol. Radioekol.*, 2000, vol. 40(5), pp. 615 - 620.
40. Lehnert B.E. and Goodwin E.H., ‘Extracellular factor(s) following exposure to alpha particles can cause sister chromatid changes in normal human cells’, *Cancer Res.*, 1997, vol. 57(11), pp. 2164 - 2171.
41. Mothersill C., Stamato T.D., Perez M.L. *et al.*, ‘Involvement of energy metabolism in the production of bystander effects by irradiation’, *Br. J. Cancer*, 2000, vol. 82(10), pp. 1740 - 1746.
42. Zhou H., Randers-Pehrson G., Waldren C.A. *et al.*, ‘Induction of a bystander mutagenic effect of alpha particles in mammalian cells’, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97(5), pp. 2099 - 2104.
43. Hickman A.W., Jaramillo R.J., Lechner J.F. *et al.*, ‘ α -Particle-induced p53 protein expression in a rat lung epithelial cell strain’, *Cancer Res.*, 1994, vol. 54(22), pp. 5797 - 5800.
44. Azzam E. I., de Toledo S.M., Gooding T. *et al.*, ‘Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low influences of α -particles’, *Radiat. Res.*, 1998, vol. 150(6), pp. 497 - 504.
45. Mothersill C., O’Malley K.J., Murphy D.M. *et al.*, ‘Identification and characterization of three subtypes of radiation response in normal human urothelial cultures exposed to ionizing radiation”, *Carcinogenesis*, 1999, vol. 20(12), pp. 2273 - 2278.

46. Bouffler S.D., Haines J.W., Edwards A.A. *et al.*, 'Lack of detectable transmissible chromosomal instability after in vivo or in vitro exposure of mouse bone marrow cells to ^{224}Ra alpha particles', *Radiat. Res.*, 2001, vol. **155**(2), pp. 345 - 352.
47. Suzuki K., Kodama S., and Watanabe M., 'Suppressive effect of low dose pre-irradiation on genetic instability induced by X-rays in normal human embryonic cells', *Radiat. Res.*, 1998, vol. **150**(6), pp. 656 - 662.
48. Mazurik V.K. and Mikhailov V.F., 'Radiation-induced genomic instability: phenomenon, molecular mechanisms, pathogenetic significance', *Radiats. Biol. Radioekol.*, 2001, vol. **41**(3), pp. 272 - 289. (Rus)
49. Grosovsky A.J., 'Radiation-induced mutations in non-irradiated DNA', *Proc. Natl. Acad. Sci.*, 1999, vol. **96**(10), pp. 4346 - 5347.
50. Perego P., Gatti L., Carenini N. *et al.*, 'Apoptosis induced by extracellular glutathione is mediated by H_2O_2 production and DNA damage', *Int. J. Cancer*, 2000, vol. **87**(3), pp. 343 - 348.

Detection of genome instability in descendants of male mice exposed to chronic low-level γ -irradiation using the test “adaptive response”

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ABSTRACT

Genomic instability (GI) in somatic cells of the progeny (F_1 generation) of male mice chronically exposed to low-dose γ -radiation was studied by comparative analysis of chromosome damage. BALB/C male mice exposed to 0.1 Gy (0.01 Gy/day) and 0.5 Gy (0.01 and 0.05 Gy/day) were mated with unirradiated females 15 days after irradiation.

For comparison of radiosensitivity, two-month-old males, the descendants of irradiated and unirradiated animals, were subjected to irradiation with a dose of 1.5 Gy (0.47 Gy/min) from a ^{60}Co source. GI was revealed by the standard scheme of adaptive response.

The experiments indicated that, using the “adaptive response” test, it is possible to detect the transition of γ -radiation-induced genomic instability in germ cells of male parents into somatic cells of mice (F_1 generation) judging either by changes in radiosensitivity or by the absence of the adaptive response induced by a standard scheme.

Keywords: genomic instability, adaptive response, mice, micronucleus test

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INTRODUCTION

In recent years, along with mutation processes induced by various physical and chemical factors much attention is given to the problems of genomic instability, which makes itself evident in many generations. GI was mainly studied on *in vitro* cultured cells using various criteria: frequency of chromosome aberrations [1, 2], point and minisatellite mutations [3, 4], transformations [5, 6], and reproductive cell death [7]. The transition of GI in human and animal parent germ cells, induced by sublethal doses of ionizing radiation, into somatic cells of descendants was also reported in the literature [8, 9]. The data on GI in descendants of parents irradiated with low radiation doses were obtained mainly by extrapolating the effects of high doses, and these data are contradictory and ambiguous. On the one hand, there is evidence indicating an increase in the frequency of chromosome aberrations in hepatocytes of descendants of irradiated rats [10]; on the other hand, in children of parents who survived atomic bombardments, no increase in the level of chromosomal mutations was noted compared to other people. In order to estimate the genetic risk of the effect of low-radiation doses using conventional genetic methods of screening, great quantities of experimental animals and large cohorts of humans are needed [11, 12].

In our previous studies concerned with the effect of low radiation doses on bone marrow cells of mice, we found that, in animals preliminarily irradiated with a dose of 0.1Gy, the yield of chromosome aberrations after subsequent irradiation with a high dose was lower than in animals that were not subjected to preliminary irradiation; i. e., a radiation-induced adaptive response (RAR) occurred. It was also found that in mice exposed to the adapting dose, RAR persisted over a long period of time (up to 12 months) comparable with the lifetime of the animal [13]. It should be emphasized that, after irradiation with adapting doses (0.1 Gy) only, the level of cytogenetic damage in early terms increased, whereas in remote terms, it was even lower than the level of spontaneous cytogenetic damage in unirradiated animals [14]. Despite the absence of increased level of chromosomal lesions in remote terms after irradiation, we revealed also in this case a change in cell radiosensitivity using the “adaptive response” test, which suggested a relationship between the adaptive response of the organism and instability of the genome. Thus, low radiation doses transfer the organism to a new state, which is characterized by an increased resistance of the genome to damaging influences throughout the life of

the animal. In view of this, the goal of our work was to study the relationship between the adaptive state of the parent induced by low doses of γ -radiation *in vivo* and the genomic instability of descendants (F_1 generation) by measuring the radiosensitivity and using the “adaptive response” test.

MATERIALS AND METHODS

Two-month-old male BALB/C mice were exposed to chronic radiation in the γ -field (^{137}Cs) with a dose of 0.1 Gy (0.01 Gy/day) and a dose of 0.5 Gy (0.01 and 0.05 Gy/day) with an interval of 30 min every day. Control animals were maintained under similar conditions but without irradiation. Fifteen days after the irradiation, males from the irradiated and control groups were mated in separate cages with unirradiated females for two weeks. The term 15 days was chosen because this period involves the stage of spermatides, which then take part in the impregnation of the ovule. Male descendants of irradiated and unirradiated parent at an age of two months were subjected to irradiation with a dose of 1.5 Gy (0.47 Gy/min) from a ^{60}Co source. Another group of male descendants of irradiated and unirradiated parent was exposed to acute irradiation by the scheme of adaptive response: with an adapting dose (D_1) of 0.1 Gy (0.125 Gy/min) followed a day later by a challenging dose (D_2) of 1.5 Gy. After 28 h, the animals of all groups were killed by the method of cervical dislocation. Bone marrow specimens for calculating micronuclei (MN) in polychromatic erythrocytes (PCE) were prepared by a conventional method [15] with minor modifications. For each experimental point five animals were taken, and approximately 10000 cells were counted. The experimental data were subjected to statistical processing. The variations in mean values were estimated from the standard error value, and the statistical significance of differences between groups was evaluated by the Student *t*-test.

RESULTS AND DISCUSSION

It follows from the results in Table 1 that, in descendants of unirradiated parent and parent irradiated with a dose of 0.1 Gy, a statistically insignificant increase in the yield of PCE with aberrations occurs. In descendants of irradiated

parent with a dose of 0.5 Gy, the yield of PCE with MN increases. At this radiation dose, the yield of aberrations depends on dose rate. Similar data were obtained in other studies on rodents by the criterion of frequency of chromosomal aberrations and tumor induction [1, 5] and variability of minisatellite tandem repeats [8].

Table 1
Yield of PCEs with MN in bone marrow cells of the descendants born to male mice chronically exposed to gamma-rays

Dose	Number of mice	Number of PCEs	Number of PCEs with MN	Percent of PCEs with MN \pm SE
0 (non-irradiated)	5	12836	96	0.75 \pm 0.09
0.1 Gy (0.01 Gy/day)	5	11200	100	0.89 \pm 0.14
0.5 Gy (0.01 Gy/day)	5	10384	115	1.10 \pm 0.17
0.5 Gy (0.05 Gy/day)	5	11276	175	1.40 \pm 0.11

Table 2
Yield of PCEs with MN in bone marrow cells of the descendants gamma-irradiated *in vivo* with the dose 1.5 Gy

Dose	Number of mice	Number of PCEs	Number of PCEs with MN	Percent of PCEs with MN \pm SE
0 + 1.5 Gy (F1)	5	10954	1186	10.9 \pm 1.05
0.1 Gy(0.01 Gy/day) + 1.5 Gy (F1)	5	10698	278	2.6 \pm 0.18
0.5 Gy (0.01 Gy/day) + 1.5 Gy (F1)	5	10268	369	3.6 \pm 0.91
0.5 Gy (0.05 Gy/day) + 1.5 Gy (F1)	5	8257	270	3.3 \pm 0.28

Table 2 presents the data on radiosensitivity of descendants of irradiated and unirradiated parent. It was found that in the progeny of irradiated parent, the yield of PCE with MN decreases three-to-four times, depending on the dose of irradiation of a parent. As is evident from Table 3, no RAR was observed in descendants of irradiated parent that were irradiated by the scheme of adaptive response: acute D₁ (0.1 Gy) followed by D₂ of 1.5 Gy. This indicates that the adapting irradiation does not decrease the yield of PCE with MN (with D₂ of 1.5

Signal function of reactive oxygen species in regulatory networks of the cell response to damaging exposures: participation in radiosensitivity realization and genome instability

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ABSTRACT

Reactive oxygen species (ROS) have a few possible effects such as metabolic (participation in regulation of protein functions), damaging (oxidative damage to proteins, lipids and nucleic acids) and signal; the latter is reviewed in the article. Superoxide anion-radical O_2^\bullet , hydroperoxide (H_2O_2) and nitroxide (NO) are capable to act as signal substances in the cell regulatory network, which determines a model of cell response to indignating exposure: proliferation rate, a direction of differentiation or a start of the apoptosis program. A role of ROS in this network is considered: significance of ROS contents in a cell; signal pathways triggered the programs of cell response on exposures; links of regulatory networks conditioned of ROS contents in the cell; ROS reactions with network components which influences its functioning. A significance of the ROS-bound segment of network that realizes regulatory signals of the damage in development of radiobiological effect is estimated. The data obtained by the authors are submitted; the prospects of studying substances

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REFERENCES

1. Kadhim M.A., MacDonald D.A., and Goodhead D.T., *Nature*, 1992, vol. **355**, pp. 738 - 740.
2. Sabatier L., Dutrillaux B., and Martins M.B., *Nature*, 1992, vol. **357**, p. 548.
3. Little J.B., Gorgojo L., and Vetrovs H., *Int. J. Radiat. Oncol. Biol. Phys.*, 1990, vol. **19**, pp. 1425 - 1429.
4. Dubrova Y.E., Plumb M., Brown J., Fennelly J., Bois P., Goodhead D., and Jeffreys A.J., *Proc. Natl. Acad. Sci. USA*, 1998, vol. **95**, pp. 6251 - 6255.
5. Mohr U., Dasenbrock C., Tillmann T., Kohler M., Khamo K., Hagemann G., Morawietz G., Campo E., Cazorla M., Fernandez P., Hernandez L., Cardesa A., and Tomatis L., *Carcinogenesis*, 1999, vol. **20**, pp. 325 - 332.
6. Nomura T., *Nature*, 1982, vol. **296**, pp. 575 - 577.
7. Seymour C.B. and Mothersill C., *Mutat. Res.*, 1992, vol. **267**, pp. 19 - 30.
8. Luke G.A., Riches A.C., and Bryant P.E., *Mutagenesis*, 1997, vol. **12**, pp. 147 - 152.
9. Dubrova Y.E., Nesterov V.N., Krouchinsky N.G., Ostapenko V.A., Neumann R., Neil D.L., and Jeffreys A.J., *Nature*, vol. **380**, pp. 683 - 686.
10. Vorobtsova I.E., *Radiobiologia*, 1987, vol. **23**, pp. 377 - 381. (Rus)
11. Russel L.B. and Russel W.L., *Proc. Natl. Acad. Sci. USA*, 1996, vol. **93**, pp. 13072 - 13077.
12. Neel J.V., *Proc. Natl. Acad. Sci. USA*, 1998, vol. **95**, pp. 5432 - 5436.
13. Klokov D.Yu., Zaichkina S.I., Rozanova O.M., Aptikaeva G.F., Akhmadieva A.Kh., Smirnova E.N., and Balakin V.Y., *Biological Effects of Low Dose Radiation*, Ed. Yamada T., Mothersill C., Michael B.D., Potton C.S., Elsevier, Amsterdam, 2000, pp. 87 - 91.
14. Balakin V.Ye., Zaichkina S.I., Rozanova O.M., Klokov D.Yu., Aptikaeva G.F., Akhmadieva A.Kh., and Smirnova E.N., *Dokl. Akad. Nauk*, 2000, vol. **374**, pp. 271 - 273.
15. Schmid W., *The micronucleus test for cytogenetic analysis, Chemical mutagens: Principles and Methods For Their Detection*, Ed. Hollaender A., New York, Plenum, 1976, pp. 31 - 53.
16. Pelevina I.I., Afanasev G.G., Gotlieb V.I., Alferovich A.A., Antoshchina M.M., Riabchenko N.I., Saenko A.S., Riabtsev I.A., and Riabov I.N., *Radiats. Biol. Radioekol.*, 1993, vol. **33**, pp. 508 - 520.

Signal function of reactive oxygen species in regulatory networks of the cell response to damaging exposures: participation in radiosensitivity realization and genome instability

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ABSTRACT

Reactive oxygen species (ROS) have a few possible effects such as metabolic (participation in regulation of protein functions), damaging (oxidative damage to proteins, lipids and nucleic acids) and signal; the latter is reviewed in the article. Superoxide anion-radical O_2^\bullet , hydroperoxide (H_2O_2) and nitroxide (NO) are capable to act as signal substances in the cell regulatory network, which determines a model of cell response to indignating exposure: proliferation rate, a direction of differentiation or a start of the apoptosis program. A role of ROS in this network is considered: significance of ROS contents in a cell; signal pathways triggered the programs of cell response on exposures; links of regulatory networks conditioned of ROS contents in the cell; ROS reactions with network components which influences its functioning. A significance of the ROS-bound segment of network that realizes regulatory signals of the damage in development of radiobiological effect is estimated. The data obtained by the authors are submitted; the prospects of studying substances

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(such as phenosane, etc) which can actively influence redox processes, as means of modification of radiation-induced genome instability and prevention of malignant transformation are considered.

Keywords: Reactive oxygen species, signal function, cell, regulatory network, ionizing radiation, radiosensitivity, radiation-induced genome instability, phenosane

Transit of cell through its vital cycle, choice of direction for development and response to exposure of different medium agents are realized by means of selective trigger of sequences of biochemical reactions, which were developed evolutionary, are genetically controlled and forming peculiar biological modules [1]. Choice, incorporation and realization of the function of the above-mentioned module programs is carried out by means of complicated cellular network of regulatory signal transmission, which change the profile of gene expressions and provide for activation of synthesis of respective proteins.

In the last few years substantial progress was achieved in understanding the role of reactive oxygen species in normal vital functions and their participation in inducing pathology. It was only recently that reactive oxygen species (ROS) – superoxide anion-radical O_2^{\bullet} , H_2O_2 , HO^{\bullet} were considered as only molecules, which induce oxidative damages in proteins, lipids and nucleic acids. Analysis of last published data shows that intracellular formation and elimination of ROS are connected not only with development of destructive processes but also have a role of important unit in mechanisms of homeostasis through redox regulation of a number of biological processes [2, 3].

The purpose of this work is to understand molecular mechanisms of ROS participation in functioning of intracellular network of regulatory signals transmission and their role in radiosensitivity/radioresistance and genome instability as a stage of forming late radiation effects and also sensitivity to modifying action of antioxidant.

INTRACELLULAR NETWORK FOR SIGNAL TRANSMISSION UNDER DAMAGING FACTORS EXPOSURE

Different signals (hypoxia, DNA damage, thermal shock, metabolic changes, cytokines, viral infection, activation of oncogenes, etc.) induce biochemical programs in response to the above-mentioned exposures. Particularly, DNA damage cause stabilization and functional activation of protein p53 which acts as an integrator of damaging signals and transcription factor triggering expression genes takes part in checkpoints of cell cycle regulation which provides for conditions and intensifying of DNA repair and under certain conditions – activation of cell differentiation or apoptosis [4 - 6].

Figure 1 displays part of network connected to activity of damaging factors, which transmits signals in executive unit of cell response system. It consists of receptor unit; cascade of mitogen-activated protein kinases (MAP-kinase); transcription factors, which activate certain genome domains; products of genes expression; inhibitors and activators of biochemical reactions and executes three types of responses: alteration of proliferation rate, entry into differentiation or initiation of apoptosis program. Direction of cellular growth/differentiation is carried out mostly with such MAPK as extracellular regulated kinase signals (ERK 1/2). Cascades with c-Jun of N-terminal kinase (JNK) and p38 MAPK respond to stress influences by inflammation and apoptosis development [7].

Apoptosis is one of the main cell responses to damaging influences, which is initiated at the moment when cell is unable to eliminate completely the existing defect, which creates the threat to maintain genome stability.

Apoptosis initiation is performed in two pathways: external – through the so called superficial death receptors (DR) and internal – through mitochondrion in a cell.

The first (external) pathway starts from five death receptors: CD95 (former name was receptor for Fas-ligand or Apo-1L); tumor necrosis factor α receptor – TNFR1 (DR2); DR3; DR4 (receptor for TRAIL - TNF-related apoptosis-inducing ligand (former name was receptor for Apo-2L)) и DR5 (KILLER). Latter two are typical only for human tumor cells.

Outbreak of free ligands of corresponding receptors results in their connecting to the last ones and in induction of a cascade of apoptosis reactions, which end with activation of 11 caspases - cysteine with aspartate specificity proteinases [8]. Majority of protein components of a cell are disintegrated by

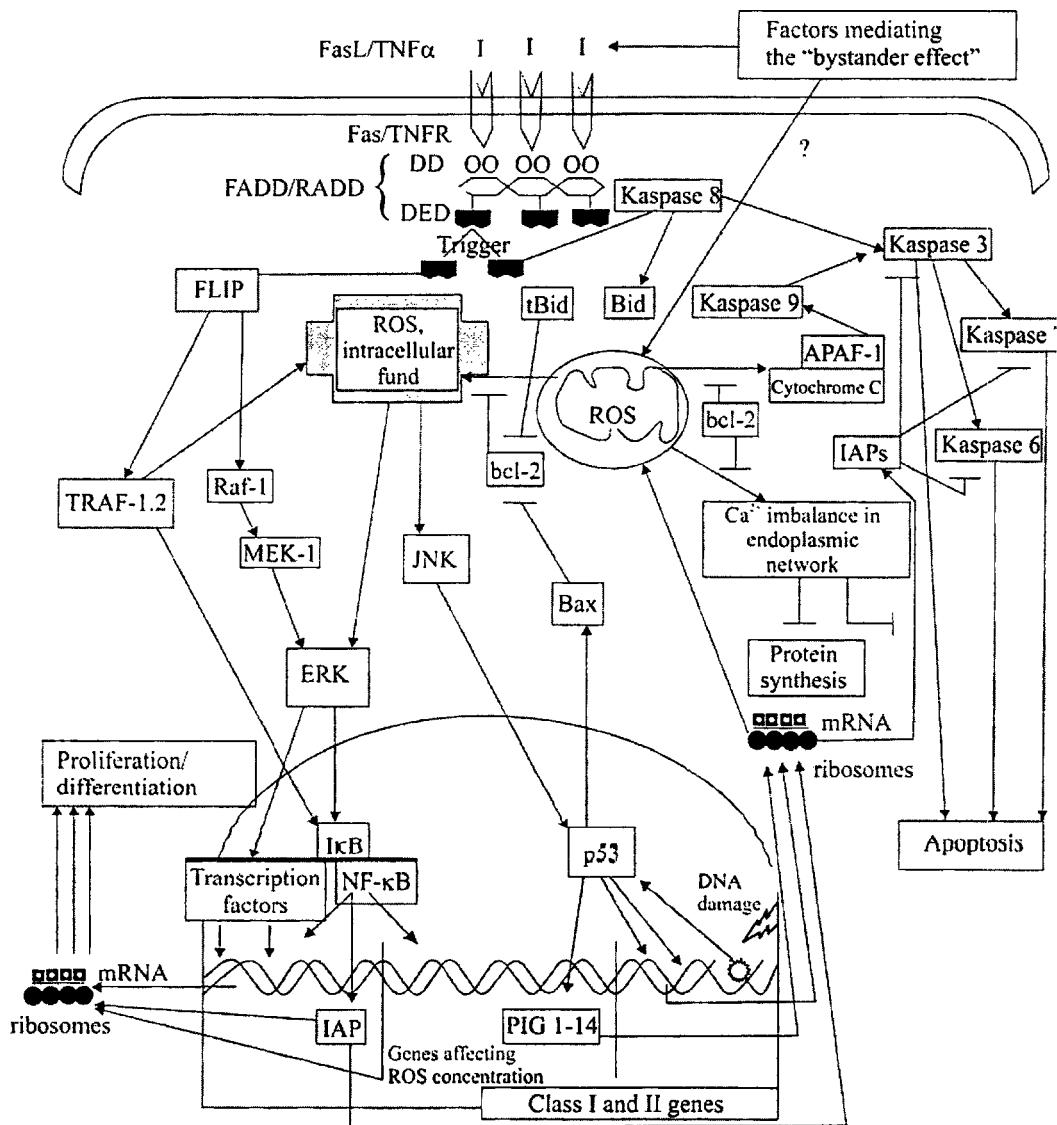


Figure 1. Scheme of cell response regulatory pathways to damaging exposures with ROS participation.

Abbreviations: FasL – ligand for Fas cellular death receptor;
TNF α - tumor necrosis factor α ;
TNFR - tumor necrosis factor α receptor;
DD – death domain;
DED - death effector domain;
FADD - Fas-adapting death domain protein;
RFADD – adaptive protein of death domain receptor;
FLIP – Fas-inhibitor of inhibitor protein of FADD type interleukin-1 β -converting enzyme;
TRAF-1,2 – factors binding TNFR;
Rafl – factor under which binding a signal is transferred from FLIP to Erk;
bcl 2 – antiapoptosis protein bcl 2;
Bid – bcl 2 homologue;
tBid – extended Bid active enzyme;
Apaf1 – activation protease apoptosis factor;
IAPs – inhibitors of apoptosis proteins;
MEK-1 – mitogen activated protein kinase;
JNK – c-Jun N-end kinase;
Bax – protein member of bcl family;
Erk – extracellular signals regulated kinases;
NF- κ B – nuclear of κ B factor;
IkB – inhibitor of nuclear of κ B factor;
p53 – protein suppressor of tumors;
PIG1-14 – p53-induced genes connected to ROS production.

caspases 3 and 7, lamin is selectively destroyed by caspase 6. One of caspases disintegrates cytoplasmic inhibitor of DNA fragmentation factor – caspase-activated specific for apoptosis endonuclease (DNA-ase). This makes it possible for DNA-ase to move from cytoplasm into the cell nucleus and initiate internucleosome fragmentation of genome DNA and form products, which build “staircase” of DNA fragments of different molecular mass, which are typical for apoptosis during these fragments electrophoresis [9, 10].

Mitochondrial (external) pathway of apoptosis process activation is connected with cytochrome c exiting from intermembranous mitochondrial space to cytoplasm due to their damages, that causes activation of caspase 3 and apoptosis initiation. Also, there is another variant of mitochondrial pathway, when as a result of opening of permeability pores and loss of proton and electric gradient located on internal mitochondrial membrane protein, which is called as apoptosis-inducing-factor (AIF), leaves mitochondrion and directly activates caspase 3 [10].

It must be noted that activation of above-noted signal pathways under the influence of damaging factors does not always cause apoptosis. It becomes evident from Figure 1 that apoptotic signals are confronted with protein families with anti-apoptotic functions and particularly some proteins from bcl family, apoptosis inhibitors proteins (IAPS), bifunctional apoptosis regulator (BAR), which is able to suppress transmission of signals both through external and internal ways [11].

Thus, apoptosis regulation represents an example of balanced mechanism with multiple duplication of counterbalances, which must provide for reliable control of maintaining cellular homeostasis under stress conditions and exposure of damaging environmental factors.

Lymphocyte apoptosis, at least of T-cells is initiated by two signals. After antigenic activation these cells acquire cytokine receptors and become dependent from their for growth and survival. Cytokine deficiency may be regarded as a signal for inflammation development.

The second signal, which initiates death of T-lymphocytes, may be repeated antigen receptors stimulation, which results in induction of pro-apoptotic Fas-ligand and tumor necrosis factor α (TNF α).

It was determined that activation of cell death receptors by apoptotic mechanism may initiate an apoptosis alternative – cellular proliferation and differentiation [12, 13]. So, when signal passes through Fas-receptor, which is the only for Fas-ligand, growth signal is initiated in fibroblasts, solid tumors, hepatocytes; amplification of dendrite differentiation is initiated in neurons;

physiological and morphological changes during myocardium are initiated in cardiac hystiocytes.

Transmission of signal is performed by means of trimerization or, which is more probable, by higher factor of Fas polymerization, which causes bounding of Fas-adapting protein (FADD) with death domains (through their common areas) and formation of death-inducing signal complex. The other end of FADD contains two domains of death effector (DED), which cooperate with kaspase 8 or FLIP – inhibitor protein of FADD-type interleukin-1 β -converting enzyme [12]. Interaction of DED with FLIP but not with kaspase 8 switches response from apoptosis to proliferation or differentiation (Figure 1).

ROS ROLE IN REGULATORY NETWORK OF CELL RESPONSE TO DAMAGING EXPOSURES

ROS content in cell as a regulatory factor

Fluctuations of ROS content in cells during apoptosis initiation or cellular growth/differentiation may be the factors that determine regulatory functions of ROS, their participation in activation of programs of response to damaging exposures.

Actually, metabolic production of ROS occurs due to exposure of damaging factors. With that ROS has the role of signal molecules in regulatory network of cellular response to the above-mentioned exposures.

Most often fluctuation of ROS production is elevation. Thus, it was determined that apoptosis initiation may occur not as a result of cytochrome c exiting from mitochondria to cytoplasm, but due to protein p53 participation through increase of generation of reactive oxygen species in mitochondria due to hyper-polarization of membranes during persisting alteration of their membrane delta- ψ potential ($\Delta\delta\cdot\psi$) [14]. With that p53-induced increase of ROS production occurs in the early stage of response to damaging exposure precedes kaspase activation and has metabolic character.

For example, cells of myeloid leukemia with high initial level of peroxide production have high sensitivity to apoptosis induction. Apoptosis induction by protein p53 occurs after increase of peroxide production and protective action of cytokines is not connected to reduction of ROS contents. Thus, initial level of ROS may regulate susceptibility of cells to p53-dependent and p53-independent

apoptosis [15]. Increased ROS production, which occurs due to suppression of superoxide dismutase activity, also causes apoptosis activation in such cells [16].

In a number of cases signal function of ROS is realized through reduction of their content. So, reduction of O_2^\bullet concentration due to hyper-expression of Cu,Zn-superoxide dismutase with transgene mice caused in norm reduction of thymocyte number due to apoptosis and increased sensitivity of these cells to introduction of bacterial lipopolysaccharide. Susceptibility of bone marrow cells and thymus to lipopolysaccharide-induced apoptosis with these animals could be connected to increasing formation of hydrogen peroxide and amplification of lipids peroxidation [17]. On the contrary increase of both superoxide radical O_2^\bullet and nitric oxide prevented death of cells infected with virus SV (Sinbid) and reduced mice death rate infected with this virus [18].

Many investigations determined that level of ROS production plays a substantially important role not only in realization of mechanism for genome stability maintenance by means of apoptosis of irreversibly altered cells, but also in providing optimal cellular response to damaging exposure directed to maintaining their vital activity under such conditions.

Amplified production of superoxide anion-radical may be regarded to as a signal for cells to enter the proliferation pathway when exogenic mitogenic stimuli are absent [19]. There is indication saying that for many types of tumor cells elevation of production of intracellular O_2^\bullet causes apoptosis inhibition caused by Fas-receptor activation. On the contrary, reduction of O_2^\bullet concentration sensibilizes cells, which are initially resistant to Fas-induced apoptosis [20].

Transcriptional activation of a certain number of stress-responding genes occurs under the influence of H_2O_2 . As a result anti-apoptosis effect is provided [21].

NO is able to perform both pro- and anti-apoptotic function [22].

Finally, one can note that changes of contents of such ROS as superoxide anion-radical, H_2O_2 and NO may determine the direction and strength of responses to damaging influences through activation of that or either program: apoptosis or growth/differentiation.

Apoptosis that occurs due to dexamethasone exposure to thymocytes, precedes reduction of ROS production by mitochondria of these cells. On the contrary, during stimulation of lymphoid cells with such mitogens as

phytohemagglutinin, concanavalin A and etc., production of ROS increases. Consequently, two effects – apoptosis and stimulation of cell division and differentiation – correspond to two opposite levels of ROS content in cells. This directly displays the important regulator role of ROS in determination of direction of cell responses to damaging stimulus [23] depending on its concentration in cells, type of cells and cooperation with other ROS.

Signal pathways connected to ROS

Signal role of ROS is realized by means of their participation in activation of two different pathways of intracellular regulation network: ERK-directed cascade and JNK-cascade (Fig. 1). In the first case this causes changes the activity of transcriptional factors, including nuclear factor κ B (NF κ B), which is directed to maintaining the condition of growth/differentiation in cells. In the second case JNK-cascade causes stabilization and development of functional activity of tumor suppressor protein p53, which results in possible triggering of apoptosis program.

JNK-kinases may be activated by such stimulus as ionizing and UV-radiation, inhibitors of protein synthesis, ceramides, DNA-damaging agents, tumor necrosis factor α , interleukin 1 and also during activity of such mitogenic signals as growth factor, oncogenic Ras, activation signals of T-cells.

One of JNK functions is apoptosis initiation. It was displayed that hyperexpression of MEKK kinase of JNK kinase is lethal for fibroblasts. Tumor suppressor p53, which is necessary for radiation-inducing apoptosis, acts as substrate for JNK1 *in vivo* [21]. Realization of apoptosis, developed in fibroblasts of mice by means of H_2O_2 , is carried out by means of activation of JNK-signal way and p53 [24]. The importance of p53 participation in realization of JNK-dependent pathway of apoptosis is proved by experiments, when injection of protein p53 and Δ MEKK1, which mediates signal from JNK, into p53-knockout cells recovered their ability to p53-dependent apoptosis [25].

The important role in determination of the cell destiny during stress is played by nuclear factor NF- κ B. Under the influence of H_2O_2 this factor turns into active state and causes transcriptional activation of a family of stress responding genes. As a result anti-apoptotic effect is provide despite the fact that cells of certain type and under certain stress conditions stress activation of nuclear factor κ B may cause apoptosis. Activation of nuclear factor κ B

involves both ERK- and JNK/SAPK signaling ways through phosphorylation of this factor inhibitor (IkB) [21].

Balance between activities of JNK/SAPK- and ERK-cascades is a key factor for apoptosis regulation. Activation of ERK-cascade by such oxidants as H₂O₂ is important for long-term cell survival [21].

Links of regulation network, which determine ROS contents in cells

By the present time there have been discovered and studied at least two of such links: one of them means the participation of death receptor, the other means the participation of tumor suppressor of p53. In the first case activation of signal way of tumor necrosis factor α receptor (TNF α R) initiates metabolic generation of ROS in a cell, which turns nuclear factor κ B and ERK-cascade into active state (Fig. 1). The key role in this process is played by TRAF2 – a factor, which is associated with receptor TNF α turning over the signal to a way of surviving or apoptosis by changing electronic transport in mitochondria and redox status of cells [26].

The other link of regulatory network, which determines ROS contents in a cell, is connected to protein p53 of DNA reparation and apoptosis activation, acting as “genome guardian”, which arrests cellular cycle. Participation of ROS in p53-mediated apoptosis is performed through three stages: 1) transcriptional induction of expression of redox metabolism genes (PIG1-PIG14); 2) ROS formation; 3) oxidative degradation of mitochondrial components, which concludes cell death [27].

Two gene classes, which are responsible for p53-dependent transcription activation, are distinguished: class I – genes that expression is observed in all cell types, class II – genes, which are expressed only in a certain amount of cell types [28].

Mitochondrial protein ferredoxin [29], cytoplasmic protein PIG3 - homolog of oxidoreductases [30], NADH quinone oxidoreductases 1 [31], protein PIG8, which modifies p53-dependent apoptosis in tumorous cells [32], are regarded as those controlled by genes PIG1-PIG14 (proteins of p53-induced genes 1-14) and providing maintenance of redox homeostasis.

Cooperation of ROS with components of cellular network, generating response to disturbing exposures

Till recently the attention of researchers was concentrated on studying ROS damage influence on biological objects of different levels. One of important manifestations of this influence is initiation of free radical reactions of peroxidation and production of toxic products in lipid components of cells by different damaging agents [33, 34]. Investigation of biological role of biomembranes gave proofs for at least two more functions of ROS in the vital functions of cells.

By modulation of activity of phospholipases and enzymes of arachidonic acid metabolism they take part in normal metabolism, i.e. perform metabolic function. According to [35] ROS (by influencing lipoxygenases) may render signal action by stimulation inflammatory reaction through leukotrienes. Investigation of complex networks of intracellular regulation directed to realization of cell response to moving aside exposures brought to a conclusion that ROS in these networks act as signal molecules, which take part in initiation and conduction of signals through regulatory pathways. Along with this increase of ROS concentration may cause direct chemical interactions with components of these pathways and cause damaging changes directly or through intermediary products. So, in case when oxidative stress induces regulatory pathways, which lead to apoptosis, there takes place the caspase activation; on the contrary, chemical action of ROS over caspase, which causes their oxidization, is accompanied with their inactivation. The same statement is true for nuclear factor κ B, which acts in important role in transcriptional activation [36].

Potential points were located of transmission of signals by ROS, including respective nitrogen compounds, to components of regulatory network (see Table). It can be seen that superoxide anion-radical may interact with nitric oxide. Superoxide interacts with NO of low nanomolar concentrations and reduces it concentration, which causes changes of NO influence over guanylate cyclase [37]. Reaction of superoxide with NO amplifies potential O_2^\bullet , which acts as inhibitor of mitochondrial respiration [38]. It considerably depends on availability superoxide dismutase (SOD) in compartment and on concentration of interacting compounds. NO of high nanomolar concentrations is able to effectively compete with SOD, which makes formation of peroxynitrite – a high-toxic compound, which damages DNA and other macromolecules, preferable [39].

Table

Signals and damaging effects of reactive oxygen species in cells

ROS species	Reaction substrates	Effects	
		Signal	Damaging
O_2^\bullet	NO	As result of reaction with NO and decrease of letter contents inhibition of NO-dependent stimulation of soluble guanylate cyclase	Damage of biological important macromolecules as result $ONOO^-$ arise
	Fe-S complexes	Inhibition of aconitase and mitochondrial respiration by Fe loss	Damage of biological important macromolecules as result OH^\bullet arise
H_2O_2	Catalase	Stimulation of soluble guanylate cyclase	-
	Redox cycle of glutathione	Exhaustion of reduced glutathione and NADP.H contents by high H_2O_2 concentration and inhibition of NADP.H dependent reactions, increase H_2O_2 contents, modification regulator proteins by thiolation, transnitrosoylation and disulfides formation as the sequence	Decrease of antioxidant defense of cell structures: modification of structure proteins by thiolation, transnitrosoylation and disulfides formation
	Cyclooxygenase	Activation by small H_2O_2 levels	-
	Prostaglandine-I ₂ – syntase	Inactivation by high ROS levels	-

Highly concentrated superoxide anion-radical effectively interacts with Fe-S-complexes causing inhibition of aconitase (Krebs cycle enzyme, which is a critical target of toxic action of ROS over cells of mammals) and mitochondrial respiration [40, 41].

Hydrogen peroxide acts as a regulatory molecule by generating intermediary catalase form (compound-1) during enzymatic formation of neutral oxygen molecules and water from active H₂O₂. About 40% of catalase may remain in such state in case contents of hydrogen peroxide is high. Catalase is able to activate dissolvable guanyl cyclase while in the form of compound-1 [43].

Increase of hydrogen peroxide content causes a shift in redox glutathione cycle in the direction to increase of contents of oxidated glutathione and its reduction of NADP.H. This causes increase of H₂O₂ contents, reduction of antioxidant protection of cellular structures and inhibition of reactions, which depend on NADP.H.

As a result there is modification of structural and regulatory proteins by means of their thiolation, transnitrosylation and disulphides formation. Inhibition of tyrosine phosphatases by ROS influence causes autoactivation of tyrosine kinases and increase phosphorylation of tyrosine with subsequent activation of MAPK pathways [44 - 46]. Low concentrations of H₂O₂ is able to activate cyclooxygenase and in case of high concentration it may inhibit prostaglandin-I₂-synthase [47, 48].

Changes of ROS production, which are caused by mitochondrial respiration, is a necessary event in the process of adaptive changes in a cell as a response to moving aside stimulus. It is closely connected to reversible depolarization of mitochondrial membranes and changing permeability of calcium channels, which participate in Ca-homeostasis control in endoplasmatic reticulum. Disruption of this homeostasis causes suppression of synthesis of majority of proteins [49].

Loss of transmembrane mitochondrial potential $\Delta\Psi_m$ induces opening of big conducting channels, which are known as pores of mitochondrial permeability. Opening of these nonselective channels causes uncoupling of respiration chain, disorder in mitochondrial volume regulation, membrane disruption and outflow of proteins, which activates caspases, into cytosol. Inhibitors of pore opening and protein bcl 2 may prevent this process. Oxidants and pathologic increase of Ca²⁺ concentration cause pore opening and may directly initiate disruption of mitochondrial membrane. Outflow of mitochondrial proteins, which causes apoptosis realization, may also occur prior

to loss of transmembrane mitochondrial potential $\Delta\Psi_m$ due to pore disruption under the influence of caspases in case they are activated by other ways [10, 50].

In general role of ROS in terms of signal molecules is developed especially evidently under such factors exposure as stress, bacterial lipopolysacharides, transition metals, tumor necrosis factor α , interleukin-1, ionizing radiation, etc. At that, manner in which cells respond to damages depends on volume of damage, type of cells and their state and may remain as changed for long period of time; but in case ROS and reactive nitric oxide species production is high, may act as a basis for many clinical pathologies.

FEATURES OF IONIZING RADIATION INFLUENCE ON REGULATORY NETWORK OF SIGNAL TRANSMISSION IN CELL

Ionizing radiation exposure imposes specific feature to the character of analyzed system of cell response to damaging factors.

First of all radiation causes dose-dependent non-metabolic formation of ROS along ionization tracks in cells. At that ROS concentration in separate cell compartments typical for its initial state may be significantly modified causing changes in both conduction of signals through regulatory network and structural damages.

DNA damages occur as a result of direct and indirect effects of ionizing radiation. Activation of signal pathways occurs as a result of this action. Identification of radiation DNA damage is performed by proteins of poly(ADP-ribose)polymerase, DNA-dependent protein kinase, proteins p53 and ATM (ataxia-telangiectasia mutated). Signal paths whose initial link is located on membrane level are also activated along with that. As a result, realization of above mentioned module-programs of cell response to damages is activated and is accompanied with activation of tyrosine kinases (such as stress activated proteinkinase (SAPK)/c Jun N-terminal kinase (JNK), protein kinase C), release of ceramide and increase of metabolic ROS production [51].

In order to realize these programs it is necessary to have transcriptional activation of the part of a genome, whose structure, however, at this may be considerably damaged. This is a reason for evident complication for adaptive reactions to be realized by a cell as response to radiation exposure.

This effect may have two outcomes: 1) death mainly through apoptosis and 2) survival with (a) mutations in progeny, (b) cell returning to its initial state and (c) changes of functional state caused by adaptive processes, which make cells different from those that existed before having been irradiated. Such cells along with those mutant cells, which are completely recovered, are able to produce progeny, but with high possibility of accidental development of radiation induced genome instability in such progeny.

The reasonable question is: what is the role of ROS and networks that transfer signal generated by ROS, in these processes? This is not a new question for radiobiologists and was considered as a completely solved one. Hydrogen and water radicals induced by radiation damage of DNA and membranous lipid-containing structures, activate chain reactions in membranous lipid-containing structures with all the consequences, which are loss of part of easily oxidizing lipoic components, reduction of ability of the rest of components to be oxidized and to decaying of peroxidation process [52].

According to modern data and concepts that are being developed on their basis activation of metabolic ROS production occurs under short-term increase of radiation induced water and oxygen radical contents in cells in the same way as under influence of other stress agents. Only after several minutes after exposure to γ -radiation increase of production of ROS and active nitrogen oxide species occurred in culture of cells from five lines. However, this increase was observed not in all irradiated cells and as radiation dose increased, it spread to a greater number of these cells. Quantitatively increase in ROS formation in every cell did not depend on radiation dose in the range of 1 to 10 Gy. However, the observed activation effect of production of ROS and active nitrogen compounds was caused by the increase of number of cells in which this effect occurred: 20% at dose rate of 1 Gy to 80% at dose rate of 10 Gy [53, 54].

Herewith ROS production increase time coincided with the time of reversible radiation induced depolarization of mitochondrial membranes and change in calcium channel permeability. It is considered that by using radiation doses considerable part of mitochondria remains intact. However, space-time interactions of mitochondria assume such model of observed ROS production increase when oxidative event in mitochondrion is a result of localized release of Ca^{2+} , capture of its neighboring mitochondrion by depolarization of membrane and further transfer of this signal from one mitochondrion to another. Inhibitor of mitochondrial membrane permeability – cyclosporine A – canceled increase of ROS metabolic generation. Natural and synthetic compounds, which

provide bounding of intracellular calcium, also suppressed radiation induced ROS metabolic production increase.

Effect of hyperproduction of O_2^\bullet and H_2O_2 was observed also in such cases when cell nucleus or even a cell itself had not exposed to direct radiation effect. Non-irradiated cells enforced ROS production during incubation with medium, which contained serum and was exposed to α -particles or with supernatant of irradiated cell suspension [53] or were in contact with an irradiated cell [55].

ROS production increase after irradiation was a reason for observed MAPK cascades activation due to the fact that inhibition of ROS production cancelled post-radiation activation of MAPK cascades.

EVALUATION OF IMPORTANCE OF CELLULAR NETWORK OF TRANSMISSION OF REGULATORY SIGNALS CONNECTED TO ROS FOR FORMING OF RADIobiological EFFECT

Radiosensitivity

It was determined that content of ROS in tissue of animals plays a considerable role in determination of radiation sensitivity level due to the fact that the level of antioxidant activity of cells of critical organs has considerable importance for outcome of radiation damage and may be regarded as a test for evaluation of radioresistance of an organism [56]. Understanding of mechanisms of ROS participation in forming of radiosensitivity/radioresistance is impossible without consideration of their role in activation of intracellular network for conducting regulatory signals, which determine a cell response to damaging exposures. ROS may act not only as signal molecules of regulatory cascades but also as a factor of damaging of the most important intracellular structures. Development of signalling or damaging function of ROS is complicated not only with compartmentalization of their formation in a cell, physiological condition of a cell but mainly with constitutive peculiarities, which are determined genetically. This may have a considerable meaning in terms of basic of differences in radiosensitivity and not a simple picture of influence of anti- and pro-oxidants on formation of radiobiological effect.

Character of gene expression, which is initiated by MARK, is important for radiosensitivity, i.e. lethal effect or radioresistance (survival). This pattern

distinctly illustrates experiments with radiation exposure of mouse lymphoma B cells of two lines – sensitive to apoptosis of line Lyas (similar in terms of radiosensitivity to ataxia teleangiectasia cells) and resistant Lyar (similar in terms of radioresistance to Chinese hamster cells), which differ in normal condition in level of glutathione and protein blc-2 contents and expression of several gene, which take part in maintaining redox potential (particularly genes of tetrospanine CD53 and fructose-1,6-diphosphatase). Cells of radiosensitive line Lyas displayed fast expression of a cluster of genes, which proteins are involved in mitochondrial electron transport and facilitate dissociation of oxidation and phosphorylation and loss of membranous potential. Correspondingly, suppression of these biochemical paths and activation of a path, which facilitates maintenance of high redox potential through increase of glutathione level is observed in Lyar cells [57]. Increase of contents of reduced glutathione in Lyar cells, which was reduced after irradiation, by means of adding this compound esters reduced the number of cells, which die by apoptosis after irradiation [58]. The observed differences of post-radiation profile of gene expression in cells of Lyas and Lyar lines probably are conditioned by constitutive differences in character of activation balance of JNK- and Erk-cascades in them.

It is known that many cellular effects may display as considerably changed on level of an integral organism *in vivo*. Due to that, evaluation of the level of importance of effects observed during experiments *in vitro*, which are connected to changes in production and signal role of ROS by response criteria of an integral organism, is of great interest.

It turned out from *in vivo* experiments that state of systems of production reactive oxygen species and reaction cascades that are initiated by them as a response to radiation damage play an important role in determination of radiosensitivity/radioresistance of an organism. In order to evaluate the state of such systems in non-irradiated organism we used the injection of bacterial lipopolysaccharide – pyrogenal, which influences signal regulatory cellular network causing gene activation, which takes part in ROS production, particularly, by means of induction of nitric oxide synthase [59], which is able to activate apoptosis mechanisms and transfer of signal of programmed death to neighboring intact cells through tumor necrosis factor α [60].

It was determined that resistance of animals to ionizing radiation exposure (on survival ratio) in range of doses inducing animal death by bone marrow syndrome directly correlates (during investigation before irradiation) with ability mononuclear cells of their blood to react by changing production of

superoxide anion-radical after injection of pyrogenal as a test exposure (Figure 2) [61].

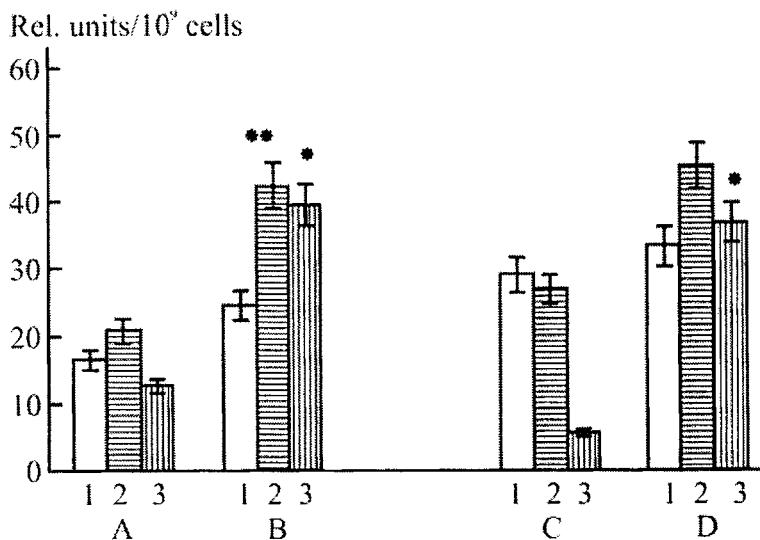


Figure 2. Latex induced oxidant potential of dogs blood mononuclear cells before and after pyrogenal injection to animals under different levels of their natural radiosensitivity (on survival in 45 days after whole body of ^{60}Co -irradiation in the dose of 7.64 Gy, dose rate 0.017 cGy/s) or induced radioresistance [61].

A – dyed animals; B – survived animals; C - animals groups after indometophen intjection in radiation-protection non-effective dose (10 mg/kg) and D – the same as in C, but under indometophen intjection in radiation-protection effective dose (30 mg/kg).

1 - before pyrogenal injection; 2 – after pyrogenal injection; 3 – average difference between individual values of oxidant potential before and after pyrogenal intjection (decrement). In datum line there are figures of nitrotetrazolium test in rel.units/ 10^9 cells.

* Differences between corresponding parameters in groups of radioresistant and radiosensitive animals are significant, $p < 0.05$;

** The same under $p < 0.01$.

It is obvious that in case when cells of organism possess an evident ability to react to the influence of stress-factors through their signal regulatory network

by triggering on genetic adaptive programs, which provide for activation of redox systems (persisting change of ROS production). This provides for high resistance of an organism to ionizing radiation exposure.

The given data prove the conclusion about importance of ROS in providing signals of adaptive rearrangement of an organism in the direction of its resistance to exposures of damaging environmental factors, including ionizing radiation. This signal function of ROS may be also important for initiation of a mechanism of antimicrobial resistance, which is based on ability to produce ROS.

Radiation-induced genome instability

Until recently it was considered as axiom in radiobiology that such biological effects of ionizing radiation as cell death, mutations, chromosomal aberrations and malignant transformation are results exclusively of irreversible DNA damages, which occur in cells during irradiation. However, in last decade the development of experimental radiobiology shook this opinion greatly. There appeared proofs of one more basis of the above-mentioned events. The received data display that part of cells that survived after irradiation and did not have definable structural changes of genotype turned to be different after irradiation and produced functionally mutated progeny, which in many generation display highly frequent spontaneous and sort of occasional de novo chromosomal aberrations and genetic mutations, which in some cases caused high cell death by apoptosis. These delayed manifestations of radiation effect do not have clone-type character and are called radiation-induced genome instability (RIGI) [62 - 64].

Unlike permanent genome instability, which typical for heritable diseases (ataxia teleangiectasia, Fanconi's anemia, the Blume syndromes, etc.) when genome instability is determined by structural genetically inheritable DNA defects radiation-induced genome instability have of an another basis. It is not based on changes of primary DNA structure, i.e. genetic code, but it is based on stable acquired change of functioning of cell as the whole; these changes are inherited by progeny through epigenetic mechanisms but not by means of genetic mechanisms of inheritance of radiation DNA damages of parental cells. This state may occur in cells, which were not irradiated, but stayed side by side with those that were and received damage signals from them. The phenomenon

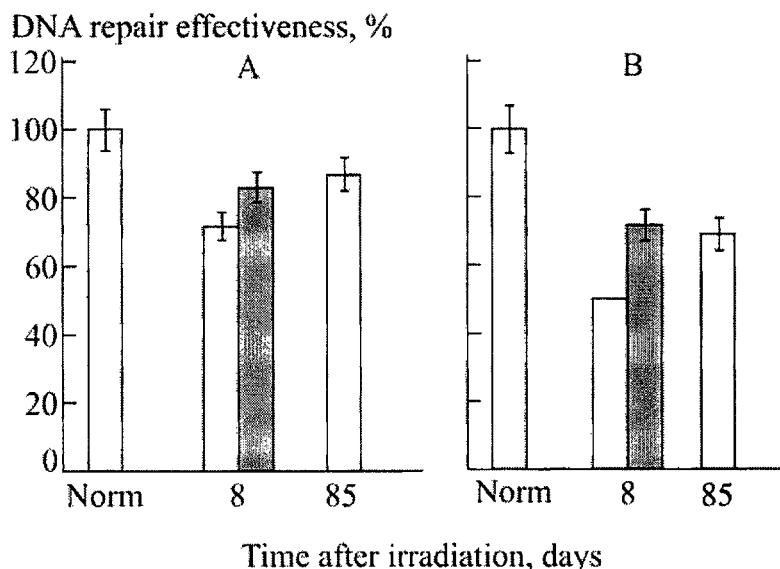


Figure 3. Repair effectiveness of spontaneous (A) and UV-induced (B) DNA damages in mice bone marrow cells before, on the 8th and 85th day after γ -irradiation in the dose of 1.5 Gy. The shaded columns – after repeated phenosane injection.

of transfer of the changed state from one cell to another (which was not changed) was called as “bystander” effect and realized in two ways: through direct intercellular contacts by means of p53-mediated mechanism or by humoral way. In both cases “bystander” cells and their descendant increase of ROS production. It is important to note that RIGI formation was experimentally determined in several cases after ionizing radiation exposure even in low doses – 1 cGy for α -particles and 10 cGy for γ -radiation. Prolonged maintenance of progeny of irradiated cells at such state increases risk of malignant transformation and, thus, investigation of RIGI problem and acquiring remedies and ways of overcoming unfavorable consequences of radiation-induced genome instability is turning into actual task for modern radiobiology.

It is obvious that RIGI formation is not governed by destructive component of ROS activity but a signal regulatory one. At the same time in order to realize RIGI value in a way of ROS compounds, which induce structural DNA damages is also obvious [65].

Significance of signal-regulatory function of ROS in RIGI formation are proved by data of phenosane influence (developed in the Institute of Biochemical Physics, Russian Scientific Academy) over redox homeostasis and DNA repair in bone marrow cells of irradiated animals. As for this compound it is known that in the taken pattern of spontaneous and initiated peroxidation of membrane lipids of liver endoplasmatic reticulum in concentration range of 10^{-18} to 10^{-3} mol/l it may develop both antioxidant (10^{-10} - 10^{-4} mol/l) and promoting peroxide properties (10^{-15} - 10^{-12} mol/l) [66].

Phenosane injection (10^{-15} mol/l once a day during 3 week-period) prevented raise of ROS contents in bone marrow cells of animals which was observed 15 minutes after irradiation at dose of 1.5 Gy, i.e. turned the ROS system into a state which differed from typical for irradiated mice, which did not receive phenosane injections [67]. Due to that signal network reacted to damaging influence and being connected to ROS system provided another changes in bone marrow cells in comparison with those changes that took place with only irradiated animals.

This version of phenosane injection improved the efficiency of DNA reparation on the 8th day after irradiation, i.e. at the moment when major part of irradiated cellular population had already been replaced by progeny (Figure 3). This data points out the possibility to put influence on ROS-dependent processes, which take part in formation and maintenance of genome stability by introducing compounds, which modify ROS production.

Prolonged introduction of phenosane to marrow cells could cause such changes of signal-regulatory systems, which contain ROS as an essential component, that turned out to be substantial enough for changing condition of Erk- and JNK-cascades of cell response to damaging exposures, including ionizing radiation. This process is also proved by evident raise of catalase activity, which is a important link in chain of signal transfer from H_2O_2 to soluble guanyl cyclase [67].

It is known that tumor suppressor p53 plays an important role in RIGI arise [68]. Stabilization and raise of protein p53 contents initiated by ionizing radiation may be changed through influence on ROS generation system. Suppression of activity of one of enzymes that take part in ROS formation NAD.H quinone oxidoreductase 1 by means of a specific inhibitor (dicumarol) cancelled raise of protein 53 and p53-dependent apoptosis in thymocytes, which was initiated under the influence of ionizing radiation [31]. The presented data display the ability to influence through ROS on regulatory chains of cell

response to damaging influences taking part in formation of different forms of pathology which develop after ionizing radiation exposure.

CONCLUSION

The result of intensive investigations that were carried out during the last decade have proved an earlier stated idea saying that evolution of living organisms in response to disturbing external influences (including damaging) of variable intensity in cells have formed modules of standard reactions, which provide adaptation of cellular communities to new conditions. Structure of these modules, consists of sensor (receptor) unit, cascade of mitogen activated kinases (MAP-kinases), transcriptional factors activating certain genome areas, products of gene expression – inhibitors and activators of biochemical reactions, that realized two direction of development - cellular growth/differentiation or apoptosis (programmed cell) death has been decoded.

An important role in cell networks of response to disturbing exposures, which are triggered by the above mentioned modules belongs to such reactive oxygen species as superoxide-anion-radical O_2^\bullet , hydrogen peroxide and nitrogen oxide (NO). In these networks the above mentioned ROS do not act only as signal factors but also as factors that damage DNA and certain components of regulatory networks. After exposure of ionizing radiation metabolic ROS production is determined by activation of p53-dependent mechanism that is activated by genome damage, which causes expression of the so-called p53-induced genes 1-14 (PIG1-14) controlling redox processes in a cell. The other factor, which influences ROS contents, is transfer of signals by means of intercellular contacts or humoral ways from an irradiated cell to non-irradiated ones (“bystander” effect).

From the point of view of recent knowledge the results of investigations of ROS role make it possible to develop fundamental conceptions, which have been earlier proposed by Russian scientists, about role of ROS system, pro- and antioxidants in cellular divisions, regulation of cell reproduction, malignant transformation developments [34] and, also, possible mechanisms of radiosensitivity where endogenous thiols and lipid antioxidant activity [56], particularly, play an important role. Comparison of ability of regulatory systems, which provide for reaction of mononuclear blood cells of laboratory animals to disturbing exposures, to be responsible for changes of superoxide

anion-radical with animal resistance to further irradiation with lethal dose (by survival ratio) demonstrated an important role of ROS in providing conditions of adaptive readiness of an organism to react to environmental damaging factors, including ionizing radiation.

Due to the fact that realization of another important radiobiological phenomenon – radiation-induced genome instability is both a damaging ROS activity component and also a signal regulatory one, investigations were carried out in order to study the influence of condition of a system of redox reactions, which take part in ROS formation, on DNA damaging within a long-term period after having been irradiated, in cases when its main source is metabolic processes. The observed data point out changes in redox processes of ROS production and elimination immediately and within a long period of time after irradiation. Preliminary influence to signal-regulatory cascades of cell response reactions to damages with the help of phenosane affecting redox processes influences significantly on processes of genome stability maintaining in late period after irradiation.

The presented results show the prospect in research of ROS signal role in cell regulatory chains for solving such important problems as radioresistance, genome instability and malignant transformation.

REFERENCE

1. Harwell L.H., Hopfield J.J., Leibler S., and Murray A.W., 'From molecular to modular cell biology', *Nature*, 1999, vol. **402**(6761), pp. C47 - C52.
2. Burlakova E.B., Mikhailov V.F., and Mazurik V.K., 'Redox homeostasis system in radiation-induced instability', *Radiats. Biol. Radioekol.*, 2001, vol. **41**(5), pp. 489 - 499. (Rus)
3. Turpaev K.T., 'Reactive oxygen species and gene expression regulation', *Biokhimia*, 2002, vol. **67**(3), pp. 339 - 352. (Rus)
4. Bray S.E., Schorl C., and Hall P.A., 'The challenge of p53: linking biochemistry, biology, and patient management', *Stem Cells*, 1998, vol. **16**(4), pp. 248 - 260.
5. de Toledo S.M., Azzam E.I., Keng P., Laffrenier S., and Little J.B., 'Regulation by ionizing radiation of CDC2, cyclin A, cyclin B, thymidine kinase, topoisomerase II alpha, and RAD51 expression in normal human

- diploid fibroblasts is dependent on p53/p21Waf1', *Cell Growth Differ.*, 1998, vol. 9(11), pp. 887 - 896.
- 6. Meek D.W., 'New developments in the multi-site phosphorylation and integration of stress signalling at p53', *Int. J. Radiat. Biol.*, 1998, vol. 74(6), pp. 729-737; Budd R.C., 'Death receptors couple to both cell proliferation and apoptosis', *J. Clin. Invest.*, 2002, vol. 109(4), pp. 437 - 442.
 - 7. Schaeffer H. and Weber M.J., 'Mitogen-activated protein kinases: specific messages from ubiquitous messengers', *Mol. Cell. Biology*, 1999, vol. 19(4), pp. 2435 - 2444.
 - 8. Ju S.T., Matsui K., and Ozdemirli M., 'Molecular and cellular mechanisms regulating T and B cell apoptosis through Fas/FasL interaction', *Int. Rev. Immunol.*, 1999, vol. 18(5-6), pp. 485 - 513.
 - 9. Cryns V. and Yuan J., 'Proteases to die for', *Gen. Devel.*, 1998, vol. 12(11), pp. 1551 - 1570.
 - 10. Haunstetter A. and Izumo S., 'Apoptosis. Basic mechanisms and implications for cardiovascular disease', *Circulat. Res.*, 1998, vol. 81(11), pp. 1111 - 1129.
 - 11. Zhang H., Xu Q., Krajewski S. et al., 'BAR: An apoptosis regulator at the intersection of caspases and bcl-2 family proteins', *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97(6), pp. 2597 - 2602.
 - 12. Budd R.C., 'Death receptors couple to both cell proliferation and apoptosis', *J. Clin. Invest.*, 2002, vol. 109(4), pp. 437 - 442.
 - 13. Pimentel-Muinos F.X. and Seed B., 'Regulated commitment of TNF receptor signalling: a molecular switch for death or activation', *Immunity*, 1999, vol. 11(6), pp. 783 - 793.
 - 14. Li P.F., Dietz R., and von Harsdorf R., 'P53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2', *EMBO J.*, 1999, vol. 18(21), pp. 6027 - 6036.
 - 15. Lotem J., Peled-Kamar M., Groner Y., and Sachs L., 'Cellular oxidative stress and the control of apoptosis by wild-type p53, cytotoxic compounds, and cytokines', *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93(17), pp. 9166 - 9171.
 - 16. Huang P., Feng L., Oldham E.A. et al., 'Superoxide dismutase as a target for the selective killing of cancer cells', *Nature*, 2000, vol. 407(6802), pp. 390 - 395.
 - 17. Peled-Kamar M., Lotem J., Okon E. et al., 'Thymic abnormalities and enhanced apoptosis of thymocytes and bone marrow cells in transgenic

- mice overexpressing Cu/Zn-superoxide dismutase: implications for Down syndrome', *EMBO J.*, 1995, vol. 14(20), pp. 4985 - 4993.
- 18. Tucker P.C., Griffin D.E., Choi S. *et al.*, 'Inhibition of nitric oxide synthesis increases mortality in Sindbis virus encephalitis', *J. Virol.*, 1996, vol. 70(6), pp. 3972 - 3977.
 - 19. Irani K., Xia Y., Zweier J.L. *et al.*, 'Mitogenic signalling mediated by oxidants in Ras-transformed fibroblasts', *Science*, 1997, vol. 275(5306), pp. 1649 - 1652.
 - 20. Clement M.-V. and Stamenkovic I., 'Superoxide anion is a natural inhibitor of FAS-mediated cell death', *EMBO J.*, 1996, vol. 15(2), pp. 216 - 225.
 - 21. Wang X., Martindale J.L. Liu Y., and Holbrook N.J., 'The cellular response to oxidative stress: influences of mitogen-activated protein kinase signalling pathways on cell survival', *Biochem. J.*, 1998, vol. 333(Pt. 2), pp. 291 - 300.
 - 22. Chung H.T., Pae H.O., Choi B.M. *et al.*, 'Nitric oxide as a bioregulator of apoptosis', *Biochem. Biophys. Res. Commun.*, 2001, vol. 282(5), pp. 1075 - 1079.
 - 23. Wang J.F., Jerrells T.R., and Spitzer J.J., 'Decreased production of reactive oxygen intermediates is an early event during in vitro apoptosis of rat thymocytes', *Free Radical Biol. Med.*, 1996, vol. 20(4), pp. 533 - 542.
 - 24. Buschmann T., Yin Z., Bhoumik A., and Ronai Z., 'Amino-terminal-derived JNK Fragment Alters Expression and Activity of c-Jun, ATF2, and p53 and Increases H₂O₂-induced Cell Death', *J. Biol. Chem.*, 2000, vol. 275(22), pp. 16590 - 16596.
 - 25. Fuchs S.Y., Adler V., Pincus M.R., and Ronai Z., 'MEKK1/JNK signalling stabilizes and activates p53', *Biochemistry*, 1998, vol. 95(18), pp. 10541 - 10546.
 - 26. Chandel N.S., Schumacker P.T., and Arch R.H., 'Reactive oxygen species are downstream products of TRAF-mediated signal transduction', *J. Biol. Chem.*, 2001, vol. 276(46), pp. 42728 - 42736.
 - 27. Polyak K., Xia Y., Zweier J.L. *et al.*, 'A model for p53-induced apoptosis', *Nature*, 1997, vol. 389(6648), pp. 300 - 305.
 - 28. Yu J., Zhang L., Hwang P.M. *et al.*, 'Identification and classification of p53-regulated genes', *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96(25), pp. 14517 - 14522.
 - 29. Hwang P.M., Bunz F., Yu J. *et al.*, 'Ferredoxin reductase affects p53-dependent, 5-fluorouracil-induced apoptosis in colorectal cancer cells', *Natl. Med.*, 2001, vol. 7(10), pp. 1111 - 1117.

30. Flatt P.M., Polyak K., Tang L.J. *et al.*, 'p53-dependent expression of PIG3 during proliferation, genotoxic stress, and reversible growth arrest', *Cancer Lett.*, 2000, vol. 156(1), pp. 63 - 72.
31. Asher G., Lotem J., Cohen B. *et al.*, 'Regulation of p53 stability and p53-dependent apoptosis by NADH quinone oxidoreductase 1', *Proc. Natl. Acad. Sci. USA*, 2001, vol. 98(3), pp. 1188 - 1193.
32. Gu Z., Gilbert D.J., Valentine V.A. *et al.*, 'The p53-inducible gene EI24/PIG8 localizes to human chromosome 1 1 q23 and the proximal region of mouse chromosome 9. Cytogenet', *Cell. Genet.*, 2000, vol. 89(3-4), pp. 230 - 233.
33. Burlakova E.B., Dzankiev B.G., Sergeev G.B., and Emanuel N.M., 'On specificity of irradiation influence in the process of generation of toxic products in fat', *Nauch. Dokl. Vyssh. Shk., Ser. Biol.*, 1960, vol. 1(1), pp. 145 – 150. (Rus)
34. Burlakova E.B., Alesenko A.V., and Molonkina E.M., *Bioantioxidants In Radiation Affection And Malignant Growth*, Moscow, Nauka, 1975. 211 p. (Rus)
35. Brash A.R., 'Lipoxygenases: Occurrence, Functions, Catalysis, and Acquisition of Substrate', *J. Biol. Chem.*, 1999, vol. 274(34), pp. 23679 - 23682.
36. Kamata H. and Hirata H., 'Redox regulation of cellular signalling', *Cell Signal*, 1999, vol. 1(1), pp. 1 - 14.
37. Furchtgott R.F., 'The 1989 Ulf von Euler lecture. Studies on endothelium-dependent vasodilation and the endothelium-derived relaxing factor', *Acta Physiol Scand.*, 1990, vol. 139(2), pp. 257 - 270.
38. Wolin M.S., Hintze T.H., Shen W. *et al.*, 'Involvement of reactive O₂ and N₂ species in signalling mechanisms that control tissue respiration in muscle', *Biochem. Soc. Trans.*, 1997, vol. 25(3), pp. 934 - 939.
39. Liu X., Miller M.J.S., Joshi M.S. *et al.*, 'Accelerated reaction of nitric oxide with O₂ within the hydrophobic interior of biological membranes', *Proc. Natl. Acad. Sci. USA*, 1998, vol. 95(5), pp. 2175 - 2179.
40. Gardner P.R., Nguyen D.H., and White C.W., 'Aconitase is a sensitive and critical target of oxygen poisoning in cultured mammalian cells and in rat lungs', *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91(25), pp. 12248 - 12252.
41. Gardner P.R., Costantino G., Szabo C., and Salzman A.L., 'Nitric oxide sensitivity of the aconitases', *J. Biol. Chem.*, 1997, vol. 272(40), pp. 25071 - 25076.

42. Chance B., Sies H., and Boveris A., 'Hydroperoxide metabolism in mammalian organs', *Physiol. Rev.*, 1979, vol. **59**, pp. 527 - 605.
43. Burke T.M. and Wolin M.S., 'Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation', *Amer. J. Physiol.*, 1987, vol. **252**(4), Pt. 2, pp. H721 - H732.
44. Keyse S.M., 'The role of protein phosphatases in the regulation of mitogen and stress activated protein kinases', *Free Radic. Res.*, 1999, vol. **31**(4), pp. 341 - 349.
45. Herrlich P. and Bohmer F.D., 'Redox regulation of signal transduction in mammalian cells', *Biochem. Pharmacol.*, 2000, vol. **59**(1), pp. 35 - 41.
46. Rao G.N., 'Hydrogen peroxide induces complex formation of SHC-Grb2-SOS with receptor tyrosine kinase and activates ras and extracellular signal regulated protein kinases group of mitogen activated protein kinases', *Oncogene*, 1996, vol. **13**(4), pp. 713 - 719.
47. Wei C., Kulmacz R.J., and Tsai A.-L., 'Comparison of branched-chain and tightly controlled reaction mechanisms for prostaglandin H synthesis', *Biochemistry*, 1995, vol. **34**(26), pp. 8499 - 8512.
48. Hemler M.E., Cook H.W., and Lands W.E., 'Prostaglandin biosynthesis can be triggered by lipid peroxides', *Arch. Biochem. Biophys.*, 1979, vol. **193**(2), pp. 340 - 345.
49. Paschen W., 'Dependence of vital cell function on endoplasmic reticulum calcium levels: implications for the mechanisms underlying neuronal cell injury in different pathological states', *Cell Calcium*, 2001, vol. **29**(1), pp. 1 - 11.
50. Green D.R. and Reed J.C., 'Mitochondria and Apoptosis', *Science*, 1998, vol. **281**(5381), pp. 1309 - 1312.
51. Walters D., 'Molecular mechanisms of ionizing radiation-induced apoptosis', *Immunol. Cell. Biol.*, 1999, vol. **77**(3), pp. 263 - 271.
52. Burlakova E.B. and Shishkina L.N., *Cell Membrane Repair And Its Significance In Radiation Affection. Natural And Modified Radiosensitivity Problems*, Moscow, Nauka, 1983, pp. 29 - 43. (Rus)
53. Narayanan P.K., Goodwin E.H., and Lehnert B.E., 'Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells', *Cancer Res.*, 1997, vol. **57**(18), pp. 3963 - 3971.
54. Leach J.K., Van Tuyle G., Lin P.-S. et al., 'Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen', *Cancer Res.*, 2001, vol. **61**(10), pp. 3894 - 3901.

55. Grosovsky A.J., 'Radiation-induced mutations in non-irradiated DNA', *Proc. Natl. Acad. Sci.*, 1999, vol. **96**(10), pp. 46 - 53.
56. Burlakova E.B., Ivanenko G.F., and Shishkina L.N., 'The role of endogenic thiols and lipid antioxidative activity in organism radioresistance determination', In Coll.: *Natural And Modified Radiosensitivity Problems*. Moscow, Nauka, 1983, pp. 21 - 29. (Rus)
57. Voehringer D.W., Hirschberg D.L., Xiao J. et al., 'Gene microarray identification of redox and mitochondrial elements that control resistance or sensitivity to apoptosis', *Proc. Natl. Acad. Sci. USA*, 2000, vol. **97**(6), pp. 2680 - 2685.
58. Vlachaki M.T., Meyn M.D., and Meyn R.E., 'Astro research fellowship: the role of bcl-2 and glutathione in an antioxidant pathway to prevent radiation-induced apoptosis', *Int. J. Radial. Biology*, 1999, vol. **42**(1), pp. 185 - 190.
59. Monick M.M., Carter A.B., Flaherty D.M. et al., 'Protein kinase C plays a central role in activation of the p42/44 mitogen-activated protein kinase by endotoxin in alveolar macrophages', *J. Immunol.*, 2000, vol. **165**(8), pp. 4632 - 4639.
60. Lindner H., Holler E., Ertl B. et al., 'Peripheral Blood Mononuclear Cells Induce Programmed Cell Death in Human Endothelial Cells and May Prevent Repair: Role of Cytokines', *Blood*, 1997, vol. **89**(6), pp. 1931 - 1938.
61. Mazurik V.K. and Mikhailov V.F., 'Some biochemical determinants and mammalian organism radioresistance markers', *Radiats. Biol. Radioekol.* 1997, vol. **37**(4), pp. 512 - 521. (Rus)
62. Little J.B., 'Radiation-induced genomic instability', *Int. J. Radiat. Biol.*, 1998, vol. **74**(6), pp. 663 - 671.
63. Seymour C.B., Mothersill C., and Alper T., 'High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation', *Int. J. Radial. Biol. Relat. Stud. Phys. Chem. Med.*, 1986, vol. **50**(1), pp. 167 - 179.
64. Baverstock K., 'Radiation-induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer', *Mutat. Res.*, 2000, vol. **454**(1-2), pp. 89 - 109.
65. Mazurik V.K. and Mikhailov V.F., 'Radiation-induced genome instability: phenomenon, molecular mechanisms, pathogenetic significance', *Radiats. Biol. Radioekol.*, 2001, vol. **41**(3), pp. 272 - 289. (Rus)
66. Palmina N.P., 'Features of effect of natural (α -tocopherol) and synthetic (potassium phenosane) antioxidants in a wide range of concentrations (10^{-18} - 10^{-3} M) to peroxide oxidation of lipids in biological membranes', *Thes.*

Rep. VI International Conference “Bioantioxidant”, Moscow, April 16-19, 2002, pp. 440 - 441. (Rus)

67. Mikhailov V.F., Mazurik V.K., and Burlakova E.B., ‘Signal molecules produced by cells in redox reactions: participation in adaptive processes and development of radiation effect, antioxidants influence’, *Thes. Rep. VI International Conference “Bioantioxidant”, Moscow, April 16-19, 2002, pp. 395 - 396. (Rus)*
68. Liang L., Shao C., Deng L. *et al.*, ‘Radiation-induced genetic instability in vivo depends on p53 status’, *Mutat. Res.*, 2002, vol. **502**(1-2), pp. 69 - 80.

Structural and functional changes induced by exposure to X-ray adaptive doses in human lymphocytes, both normal and defective, by DNA double strand break reparation

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ABSTRACT

In the present work it is shown that the phenomenon of interphase chromosome centromeric region displacement, previously revealed by the authors, is not realized in G₀-lymphocytes with heterozygous *BRCA1/2* gene mutations. The role of these genes in DNA double strand break (DSB) reparation is known. It is concluded that chromosome locus displacement is necessary for DSB repair, at least in the process of homologous recombination. In accordance with the authors' data, some features (pericentromeric cluster disintegration and displacement, the nucleus size increasing) characteristic for S- and G₀- lymphocytes are observed in normal G₀-lymphocytes treated with 3 and 10 cGy. However, the size of nucleus in G₀-lymphocytes is restored through 6 hours after irradiation as opposed to the process in dividing cells. It was proposed that some functions of G₀-lymphocytes typical of resting cells after inducing by adaptive doze of radiation are stopped as similarly as after stimulation of cells. It is of interest that the process of the induced chromosome loci displacement is correlated with the decreasing of DNA reparation possibilities under UV-irradiation. The induced apoptosis level also decreases when chromosome loci are displaced. The possible mechanisms of the revealed

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phenomenon are discussed. This research supported by RFBR grant (No. 01-04-49180).

Keywords: chromosome loci displacement, adaptive response, apoptosis, low doses of radiation, *BRCA1* and *BRCA2* genes, DNA reparation

The effect of low-dose ionizing radiation [1] or low concentrations of chemical agents in a biological system may initiate, at least, three types of changes: modification, epigenetic and genetic. Among them modification changes reflecting some cell reactions, initiated by external influences, are suggested to be the most save. Some of these reactions are considered to be a stress or excitation. Finally, they are aimed at support of cell homeostasis and their abundance, one of the essential conditions for normal functioning of biological systems. Among these reactions initiated by low-dose radiation, of great importance are adaptive response, DNA repair system developed in diploid eukaryotes, bystander-effect, etc. These reactions mainly are aimed at a decrease of genetic damages and elimination of cell damages. The bystander-effect functions are not clear yet.

Meanwhile, the fact that reactions themselves, aimed at homeostasis support, may be of importance for normal functioning of the entire biological system should not be excluded. For example, on the one hand, SOS-reparation is aimed at elimination of some DNA damages; on the other hand, it is the source of mutation events [2]. It is suggested [3] that cells from evolution or ontogenetic reserve are one of the sources for supporting the rate of genetic variance in diploid eukaryotes. At the same time, according to the hypothesis, these cells may be the ancestors of transformed clones.

Thus the results of realization of different strategies of cell homeostasis support cannot be unambiguously estimated without determination of their mechanisms.

**TOPOLOGICAL CHANGES OF CHROMOSOME LOCI
ORGANIZATION IN THE NUCLEUS UNDER LOW-DOSE
RADIATION EFFECT**

Apparently, the adaptive response (AR) is the most complicated cell protective reaction. The initiating stage of this reaction induced by adaptive doses makes cells ready for reparation of DNA double breaks (DB) after probable influence of high (damaging) dose. The complexity of this reaction is stipulated by the fact that in the case of DNA DB, another (homological) undamaged DNA molecule playing the role of a matrix for precise reparation, using the recombination process, is required. Thus realization of readiness to repair DNA DB, initiated by intensity-dependent low-dose radiation, requires displacement (approaching) of chromosome homologue in the cell nucleus and, apparently, a change of gene expression the products of which participate both in homologue displacement and DNA reparation.

It is known that transition to readiness after adaptive dose influence proceeds in 4 – 6 hours. This state may be preserved during several cell divisions [4] or even longer [5, 6]. Preservation of the “readiness” state during several sequential cell divisions implies its transition to a new epigenetic state or synthesis of the amount of protein that, despite dilution at the cell division, is still able to preserve this state.

In the previous studies [1, 7 – 9], it was primarily shown that chromosome centromeric loci displace towards center from periphery of human G₀-lymphocyte nucleus with respect to nonlinear dependence on γ -irradiation and X-ray doses. Therefore, two cases were considered: 1) near centromeric areas of chromosomes were identified using C-dyeing [7]; 2) using nonradioactive hybridization of pRT 301 clone that contained a recloned insertion specific for 1q12 satellite DNA. It is shown [7] that affected by adaptive X-radiation doses (3 – 10 cGy) all near centromeric chromosome loci, detected by C-dyeing, both displace, as mentioned above, and indicate one more principal feature.

It is common knowledge that the number of near centromeric areas detected by C-dyeing in interphase lymphocytes nuclei is below the number of chromosomes contained in the nucleus, because a part of these loci is associated with one another [10, 11]. In the work [7], 23 such complexes were detected. However, as influenced by 5 cGy doses, the number of detected areas increases to 28. The measures granule squares decreased from 310 to 220 relative units.

This indicates that adaptive doses of X-ray radiation induce the decay of the above-mentioned, associated near centromeric zones of chromosomes.

It is known simultaneously [12, 13] that such decay of centromeric clusters to individual centromers in human lymphocyte nuclei happens at cell transition to the S-phase. Similar process takes place in lymphocytes of mice [14], where using confocal microscopy and FITC-labeled samples specific to centromeric loci, on average, 10 and 12 - 14 centromeric clusters per nucleus were detected in rest and activated B-lymphocytes, respectively. However, in the present case, lymphocytes are in the G₀-state. Thus affected by adaptive radiation doses, G₀-lymphocytes obtain some features typical of stimulated cells.

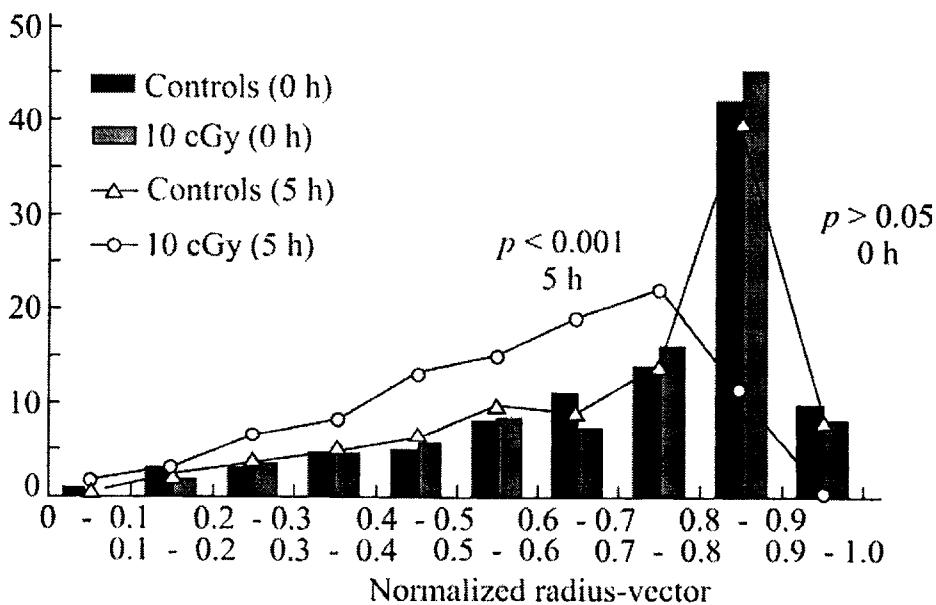


Figure 1. The frequency distribution of hybridized signal of chromosome 1 locus 1q12 by normalized radius-vector of the nucleus in irradiated (10 cGy) and intact (control) G₀-lymphocytes right after and 5 hours after irradiation.

Abscissa axis: normalized radius-vector (0 is the nucleus center);
Ordinate axis: hybridized signal frequencies, %

Further on, using nonradioactive hybridization *in situ*, X-radiation induced (3 - 10 cGy) displacements of centromeric chromosome 1 (1q12) loci in human G₀-lymphocytes was analyzed. The results of hybridization were

estimated using IBAS image analysis system. The data obtained are presented (Figure 1) by frequency distribution diagrams of hybridization signal from 1q12 by normalized radius-vector of the nucleus. Based on these data, locus 1q12 dispositions in the nucleus were 3D-reconstructed by an original algorithm. Figure 1 shows that already 2 - 5 hours after irradiation 1q12 displaces from periphery to the internal nucleus zone. Therefore, the only number of cells in which 1q12 is displaced is dose-dependent (in the studied dose range) rather than the displacement amplitude.

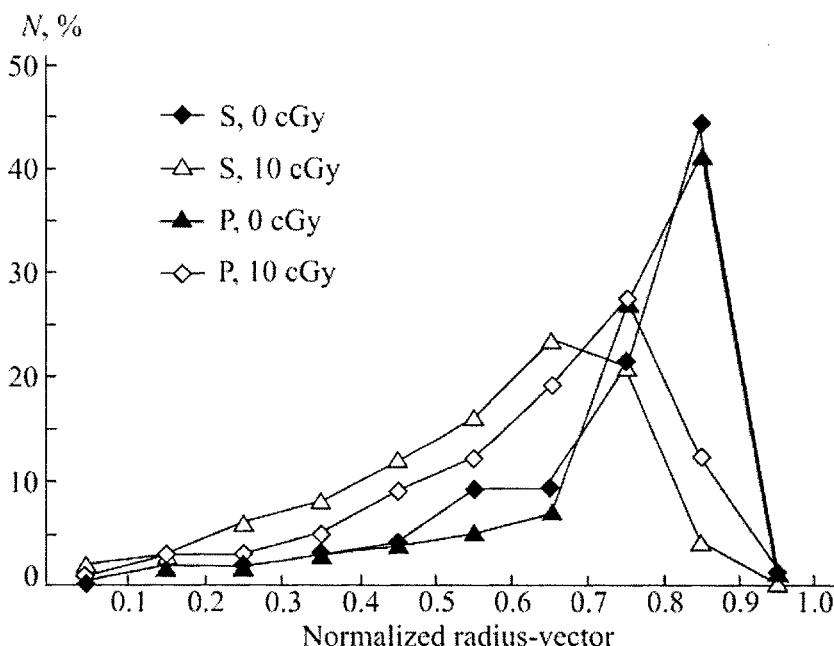


Figure 2. The frequency distribution of chromosome 1 locus 1q12 hybridized signal by normalized radius-vector of the nucleus in irradiated (10 cGy) and intact (0 cGy) G₀-lymphocytes of the proband (P) with mutated *BRCA2* gene and its nonmutated sister (S).
 Abscissa axis: normalized radius-vector (0 is the nucleus center);
 Ordinate axis: hybridized signal frequency, %

It must be emphasized that 1q12 is displaced similarly under lymphocyte stimulation at their transition to S- or G₂-phase of the cell cycle [15]. These data were obtained on a confocal scanning laser microscope. Similar data were obtained in lymphocytes in mice [16]. As before, in the discussion of centromeric loci, it should be emphasized that their displacement in G₀-

lymphocytes induced by adaptive doses is also typical of the analogous process at lymphocyte stimulation for division.

As suggested, displacement of separate loci in chromosomes under adaptive radiation doses' influence on G₀-lymphocytes is required for making cells ready for DNA DB reparation; therefore, in cells defect by this sign, this displacement must be limited or completely absent. This suggestion was checked in lymphocytes of patients with *BRCA1* and *BRCA2* gene mutations. These lymphocytes were obtained from probands possessing familiar genetic burden to breast cancer. It is suggested that products of these genes participate in DNA DB reparation, but mutations break this process [17 – 19]. Great proteins of these genes possess no homologous sequences. Protein in *BRCA2* gene interacts with *Rad51* which is *RecA* homologue and participates in recombination at DNA DB reparation in bacteria. Cells heterozygous to the mentioned mutations are specified by increased sensitivity to genotoxic agents, high chromosomal aberrations, etc. Figure 2 shows that in spite of healthy donor and proband sister nonmutated cells, in proband G₀-lymphocytes with identified *BRCA2* gene mutation (NT 3995A/C, 11 exon), X-radiation induced 1q12 displacement is absent or significantly limited in a majority of cells.

Figure 3 shows the full absence of radiation-induced 1q12 displacement in cell nuclei of another proband with identified *BRCA1* gene mutation (5328insC, 20 exon). Similarly, in nonmutated cells of the proband sister, 1q12 displacement was the same as in cell nuclei of healthy donors. The obtained data conform to the hypothesis that chromosome loci displacement in the cell nucleus is required for reparation of DNA double breaks. It may be suggested that products of a series of genes (*BRCA1*, *BRCA2*, *ATM*, *NBS*) participate in chromosome loci displacement. Validation of this statement requires further investigations.

One may suggest that position dynamics of chromosome loci in the cell nucleus represents one of the fundamental mechanisms of epigenetic switching of the gene activity. For example, it is stated that the chromatin structure, detected cytologically, represents the tissue "portrait" [20] which, properly speaking, is the nucleus phenotype reflecting the structural state and relative position of chromosomes in it. Apparently, different positions of chromosome loci of different structures themselves in differentiated cells [21 – 23] represent the image, called the tissue portrait by cytologists.

Meanwhile, differentiated cells are distinguished from one another by expressed protein spectra. What is it, simply a correlation between positioning of chromosome loci in the nucleus and spectra of expressed genes or

Data sequences to compare		<i>D</i>	α
0 cGy-S	10 cGy-S	0.3794	< 0.001
0 cGy-P	10 cGy-P	0.033	> 0.2
0 cGy-S	0 cGy-P	0.0447	> 0.2
10 cGy-S	10 cGy-P	0.3517	< 0.001
0 cGy-S	10 cGy-P	0.0487	> 0.2
10 cGy-S	0 cGy-P	0.3917	< 0.001

Statistical analysis of obtained data using the Kolmogorov-Smirnov criterion
D is the maximum of the frequency difference absolute value
 α is the error probability.

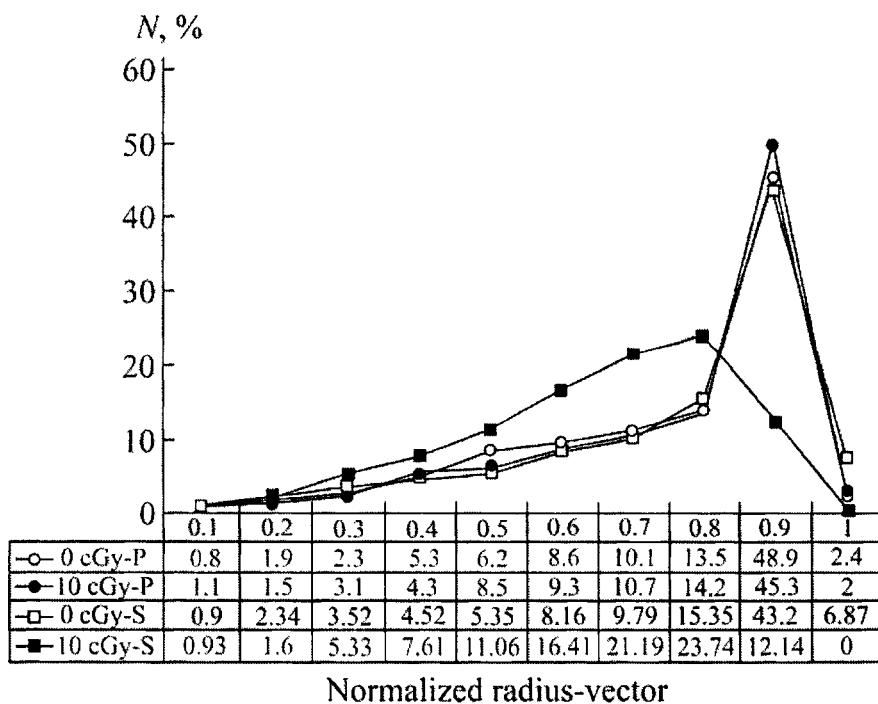


Figure 3. The frequency distribution of chromosome 1 locus 1q12 hybridized signal by normalized radius-vector of the nucleus in irradiated (10 cGy) and intact (0 cGy) G₀-lymphocytes of the proband (P) with mutated *BRCA1* gene and its nonmutated sister (S). Abscissa axis: normalized radius-vector (0 is the nucleus center); ordinate axis: hybridized signal frequency, %

interrelated events? If the last is true, what is the primary event? One may suggest that the positioning of chromosome loci in the cell nucleus provides for its specificity, i.e. the spectrum of expressed proteins. This suggestion is based on a principal ability of chromatin (chromosomes) to self-organization [24] and transition cooperation between chromatin steady states [3, 15, 26].

Naturally, this is just a scheme, not considering relationships of chromosomes and the nuclear matrix [27]. Moreover, every chromosome may apparently contact with the nuclear membrane. For example, it has been shown [28, 29] that in *D. melanogaster* germinal nuclei each chromosome contacts the nuclear membrane in 15 points. Apparently, the products of many above-mentioned genes participate in release of separate chromosome loci from the contact points with intra-nuclear structures and thus allow self-organization of these structures, i.e. displacement of their loci to corresponding areas of compartmentalization in the cell nucleus.

Of course these processes will be accompanied by some complicated events. Another fact is of importance: centromeric loci of chromosomes are typical examples of heterochromatin, with which nonspecific gene repressors (including *Ikaros*-proteins family) are co-localized, in lymphoid cells, in particular [14, 30]. Centromeric loci advance through the chromosome will correspond to activation and repression of appropriate genes. This scheme is opposite to that suggested in [30]. It is obvious that such a dynamic effect of the "location" may be implemented also in other heterochromatin areas [31]. Apparently, initiating signal to complicated chromosome transformations in the cell nucleus may be realized through the cell membrane [32 – 35].

Thus at least two parameters (decay of centromeric clusters and displacement of chromosome loci to a new location) of G₀-lymphocytes affected by adapting doses are identical to cells, entering the S-phase of the cell cycle. However, in the last case, cell size increase, progressive in the cycle [14] is observed. It has been shown [7] that the effect of adapting radiation doses, causing displacement of centromeric loci, increase nuclear size only during 3 hours, approximately. Five hours after irradiation, nucleus parameters are normalized, i.e. become typical of non-irradiated cells. Meanwhile, it has been shown that 5 hours after irradiation, new coordinates of displaced chromosome loci remain completely unchanged. Since nucleus expansion conforms to functional activity of nuclei [36], it may be suggested that during 2 – 3 hours after irradiation the increased activity of nuclei is directly focused at displacement of chromosome loci. However, preservation of this new location does not yet require special processes in the nucleus, i.e. it is apparently

preserved epigenetically. This conclusion conforms to the data and locations shown in [37].

ALTERATIONS OF SOME FUNCTIONAL PARAMETERS OF LYMPHOCYTES UNDER INFLUENCES INDUCING DISPLACEMENTS OF CENTROMERIC LOCI OF CHROMOSOMES

As mentioned above, adapting radiation doses initiate: a) displacement of centromeric loci in chromosomes and b) centromeric locus cluster decay in G₀-lymphocytes. However, the same processes are also typical of cell stimulation to division. As a consequence, some part of processes typical of cell advancing by the cycle is implemented also in G₀-lymphocytes under adaptive influences. Meanwhile, it is common knowledge that if division is not the function of differentiated cells themselves, the cell functioning (synthesis of specific proteins, enzymes, etc.) is implemented at rest only, i.e. in the absence of division. Then if cell division is stimulated, cell functions are suppressed, because cells begin implementing the new function, division. Thus performing normal function (preparation for DNA DB reparation) and using some processes for this purpose, developing in cells at stimulation of division, G₀-lymphocytes affected by adaptive radiation doses must partly lose the functions typical of rest cells.

As shown [8] (refer to Figure 4), the dose dependence of reparation index (the difference between the number of decays per minute (dpm) of radionuclide in irradiated and nonradiated lymphocytes related to dpm in control) possesses clear minimum. Thus affected by 3 cGy the cell includes much more T thymidine with the minimum at 10 cGy; then reparation index increases again with the dose. The most mystifying fact is that the cell "remembers" the influence of adaptive doses, because at further UV-irradiation the reparation minimum conforms to maximum at X-ray influence (3 cGy). Thus in the area of adaptive doses which cause displacement of centromeric loci in chromosomes the cell does not perform complete reparation, induced by further effect of higher UV doses (20 J/m²).

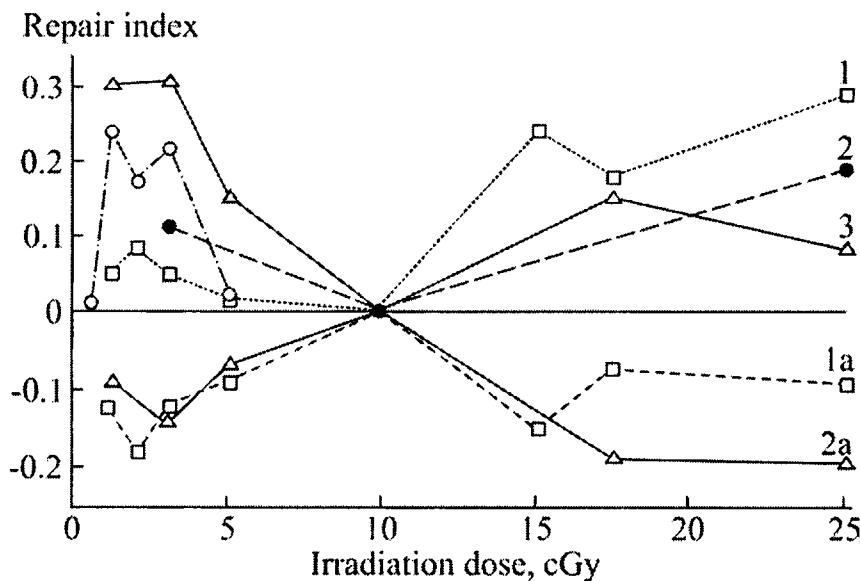


Figure 4. Alteration of intensity of DNA casual synthesis with X-irradiation dose and further effect of UV-irradiation (20 J/m^2).

1 – 4 – DNA casual synthesis intensity with X-irradiation dose in lymphocytes of four different donors.

1a – 2a - DNA casual synthesis intensity with X-irradiation dose in lymphocytes of donors 1 and 2 at further UV-irradiation effect (20 J/m^2).

Abscissa axis: irradiation dose, cGy;

Ordinate axis: reparation index $\left(\frac{dpm_{\text{test}} - dpm_{\text{control}}}{dpm_{\text{control}}} \right)$, normalized at 10 cGy dose

One of fundamental functions of cells is programmable reaction of their loss, the apoptosis. Figure 5 shows a histogram that represents the level of apoptosis induced by various methods (serum-free medium, long-term cell incubation under unfavorable conditions, Cr(IV) ions) in the range of doses causing displacement of centromeric loci in chromosomes. The apoptosis intensity was determined by the amount of nucleosomal, oligonucleosomal and other DNA fragments (below 20 thousand base pairs) in the cell incubation medium. The histogram clearly shows that 3 cGy dose inhibits (10 cGy to a lesser extent) preliminarily induced apoptosis.

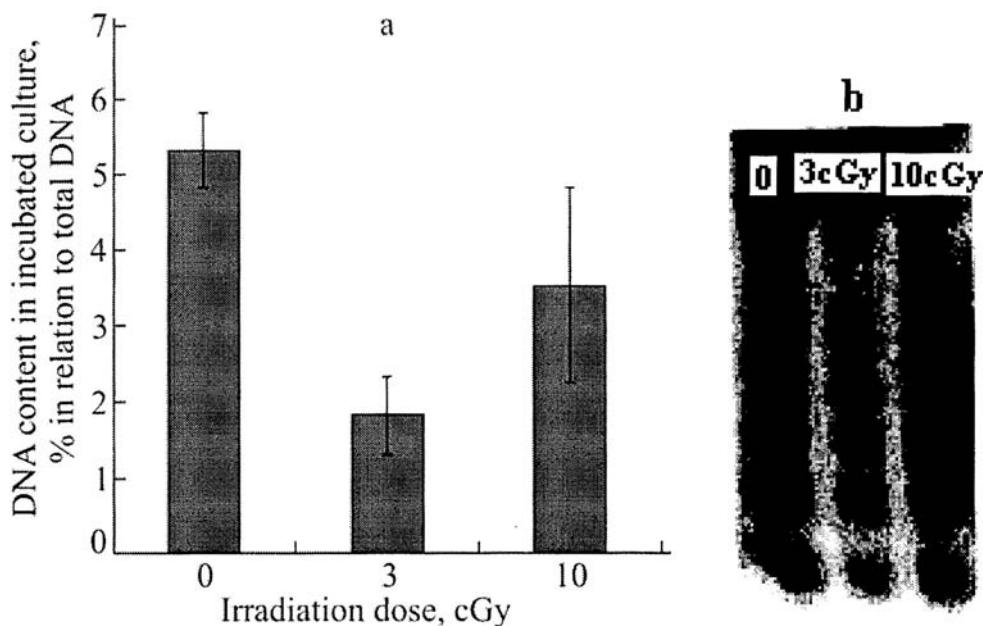


Figure 5. Inhibition of induced human G₀-lymphocyte apoptosis by adaptive X-irradiation doses.

a – the dependence of DNA fragment content (from nucleosomes to 20 thousand nucleotide pairs) in the cell incubation medium (after their precipitation) relative to the amount of total DNA (DNA in the incubation medium + cell nucleus DNA) on X-irradiation dose.

b – typical electrophoregrams of DNA fragments extracted from the incubation medium of G₀-lymphocytes after apoptosis induction, at further effect of different X-irradiation doses (3, 10 cGy) or in their absence (0 Gy).

Similar data were obtained in analysis of apoptosis (TUNEL technique) in extracted lymphocyte subpopulations [38]. The technique represents growth of fluorescent-labeled nucleotide chain, using terminal deoxynucleotidyl transferase, at the ends of DNA breaks in cell nuclei, occurred in apoptosis. The lymphocyte fractioning procedure allows obtaining of fractions possessing some different parameters (cell size, spontaneous thymidine inclusion, reparation index, etc.) [39]. By its average parameters, fraction 5 is similar to nonfractionated G₀-lymphocytes. Fraction 3 contains excessive CD25 of positive cells (17% in fraction 3 and 7.5% in fraction 5). Figure 6 shows data on the amount of apoptotic cells after fractionation (control) and further irradiation

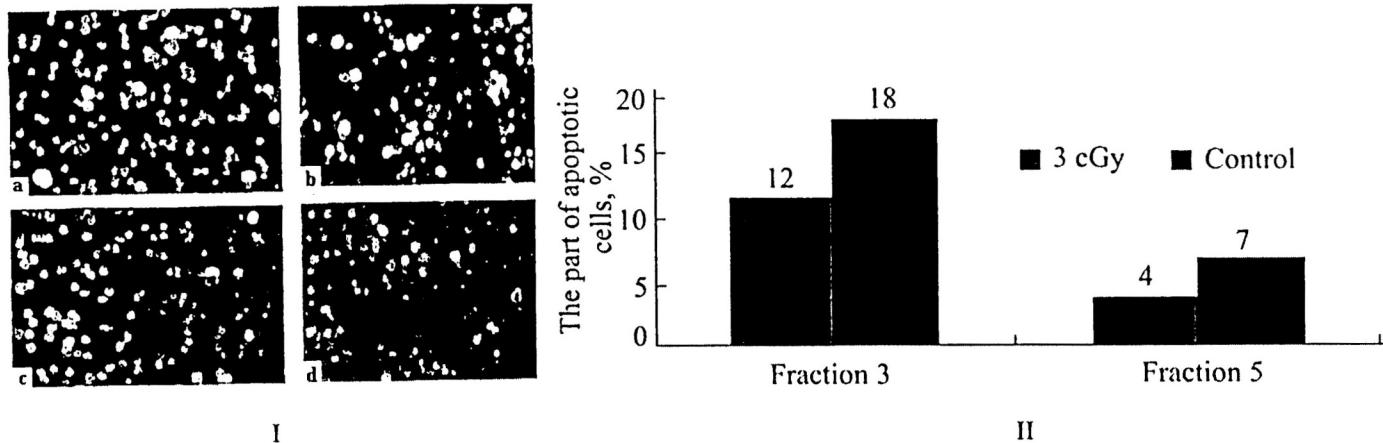


Figure 6. The study of low-dose ionizing radiation effect on spontaneous apoptosis of mononuclear leukocytes in fractions 3 and 5 by the TUNEL technique 5 hours after irradiation. Leukocytes were extracted on a step gradient of fraction 5 of bovine serum albumin.

I – Microphotographs obtained in the study (TUNEL technique) of fractions 3 and 5 mononuclear leukocyte apoptosis; leukocytes were incubated in the culture medium during 5 hours. Tests were performed at preliminary X-irradiation (3 cGy) (c, d) and without it (a, b). Apoptotic cells possess high fluorescence; 20 \times 16 magnification.

II – The part of fraction 3 and 5 apoptotic cells incubated in the culture medium during 5 hours at preliminary X-irradiation (3 cGy) and without it (control)

by 3 cGy. The histogram indicates a reliable decrease ($p < 0.03$) of the part of apoptotic cells after irradiation in fraction 3 and a tendency to decrease ($p = 0.06$) in fraction 5.

Thus the data of two independent methods applied to quantitative determination of apoptosis provide for a conclusion that adaptive doses that induce displacement of centromeric loci in chromosomes inhibit apoptosis in human G₀-lymphocytes. Up to now, the factors inducible by adaptive doses which cause the above-considered structural-functional transformation in G₀-lymphocytes are not clear yet. This problem became urgent with respect to the bystander-effect, discussed in many works [40 – 42]. This effect concludes in damaging of cells, not yet damaged by corresponded particles at irradiation of cell population by low-dose ionizing radiation. There are two main explanations of this fact:

1. Cells, through which several ionization tracks have passed, secrete a substance into the incubation medium. This substance affects non-irradiated cells.
2. Cells damaged and undamaged by ionization tracks contact with one another, and damaged cells transmit a signal to undamaged ones. It is not clear yet what mechanism is more valid or both mechanisms are probable.

Irradiation by α -particles is most often used in the analysis of bystander-effect. The influence of X- or γ -irradiation causes more complicated effects. This is connected to track structures in these types of irradiation and, therefore, to calculation of the amount of damaged cells. In the case of these kinds of irradiation, another approach is used. A suspension of cells is treated by low-dose irradiation (when, on average, one or several tracks pass through a cell). After incubation of treated cells the medium is purified from cells, and untreated cells are injected to it. Hence, it is expected that substances secreted by damaged cells are present in the medium and induce the bystander-effect in non-irradiated cells.

The last approach was also implemented in tests by the authors of the current review. G₀-lymphocytes (10^6 cell/ml) were irradiated by 3 and 10 cGy doses and then incubated during 3 hours. After cell removal (by centrifugation) from the medium non-irradiated cells were added to it and then incubated during 3 hours. Then cells were precipitated and DNA was extracted from the culture medium. After gel-electrophoresis the amount of fragments from nucleosomes and oligosomes (nucleosomal ladder, refer to Figure 5) to these sized below 20 thousand base pairs. Figure 7 shows histogram-shaped

experimental data. As indicated, the medium in which 3 cGy irradiated cells were incubated inhibited the induced apoptosis by tested parameter. However, it is not clear yet, if the data shown in Figure 7 reflect the bystander-effect or not. To answer this question, cell-by-cell apoptosis detection *in situ* is required.

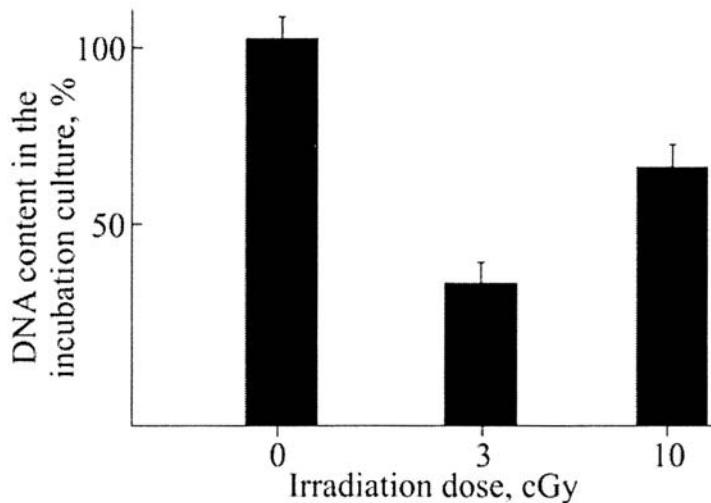


Figure 7. The influence of irradiated G₀-lymphocyte (3 and 10 cGy) incubation medium on concentration of DNA fragments at intact G₀-lymphocyte incubation in the medium.

Abscissa axis: irradiation dose (cGy);

Ordinate axis: relative concentration of DNA fragments in the incubation medium

In conclusion, it should be noted that adaptive ionizing radiation doses induce high structural-functional transformations in G₀-lymphocytes. Further investigations allow an observation if these transformations are of interim or epigenetic status. The mechanisms of described phenomena are not clear yet. This specially concerns induced displacements of centromeric loci. Since nonspecific repressors of some genes (*Ikaros*-complex capable of modifying chromatin structure [43] and PARP) are co-localized with the latter, delocalization in the cell nucleus as a part of fundamental cell processes [44] may become of high importance of normal functioning.

If in gene *BRCA1* and *BRCA2* mutations this delocalization is absent, DNA DB reparation system is disturbed. Therefore, inducing numerous genome damages, this may be the leading mechanism that causes malignant cell

transformation, i.e. transfer cells from heterozygous (aptitude) to homozygous (by these genes) state. Notice that implementation of the DNA DB reparation act, apparently, involving homological recombination mechanisms requires an additional displacement of corresponded chromosome loci in the nuclei. The impossibility of this process realization disturbs DNA DB reparation at simultaneous usual activity of all other components in the reparative complex. This might be the third way of reparation at gene *BRCA1* and *BRCA2* mutations indicated in the work [45].

REFERENCES

1. Spitkovsky D.M. and Kuzmina I.V., *Radiat. Biol. Radioekologija*, 2001, vol. **41**(5), pp. 399 – 405. (Rus)
2. Echils H., *Cell*, 1981, vol. **25**, pp. 1 – 2.
3. Spitkovsky D.M., *Radiobiologia*, 1992, vol. **32**, Iss. 3, pp. 381 - 400. (Rus)
4. Wolff S., *Environ. Health. Perspect.*, 1998, vol. **106**, Suppl. 1, pp. 277 – 283.
5. Semenets T.N., Semina O.V., and Saenko A.S., *Proc. 3rd Conference On Radiation Studies*, vol. **1**, Pushchino, 1997, pp. 163 - 164. (Rus)
6. Gil'yano N.Ya., Bolshakova O.I., Bikineeva E.G. *et al.*, *Radiat. Biol. Radioekologija*, 1999, vol. **39**(5), pp. 543 - 547. (Rus)
7. Talyzina T.A. and Spitkovsky D.M., *Radiobiologia*, 1991, vol. **31**, Iss. 4, pp. 606 - 611. (Rus)
8. Spitkovsky D.M., Ermakov A.V., Gorin A.I. *et al.*, *Radiat. Biol. Radioekologija*, 1994, vol. **34**(1), pp. 23 – 31. (Rus)
9. Spitkovsky D.M., *Radiat. Biol. Radioekologija*, 1999, vol. **39**(1), pp. 145 - 155. (Rus)
10. Stefanova E.V. and Chentsov Yu.S., *Molek. Biol.*, 1990, vol. **24**, pp. 501 - 506. (Rus)
11. Rattner J.B. and Lin C.C., *Heterochromatin. Molecular And Structural Aspects*, Ed. R.S. Verma, Cambridge, 1988, pp. 203 – 227.
12. Bartholdi M.F., *J. Cell. Sci.*, 1991, vol. **99**, pp. 255 – 263.
13. Weimer R., Haaf T., Proof M. *et al.*, *Hum. Genet.*, 1992, vol. **88**, pp. 673 - 682. (Rus)
14. Brown K.E., Baxter J., Graf D. *et al.*, *Mol. Cell*, 1999, vol. **3**, pp. 207 – 217.
15. Ferguson M. and Ward D.C., *Chromosoma*, 1992, vol. **101**, pp. 557 – 565.

16. Vourch C., Taruscio D., Boyle A.N. *et al.*, *Exp. Cell Res.*, 1993, vol. **205**, pp. 142 – 151.
17. Vu V.P.C.C., Koehler M., Steinlein C. *et al.*, *Genes And Development*, 2000, vol. **14**, pp. 1400 – 1406.
18. Tuff A., Gabriel A., Bertwistle D. *et al.*, *Curr. Biol.*, 1999, vol. **9**, pp. 1107 – 1110.
19. Xu X.L., Weaver Z., Linke S.P. *et al.*, *Mol. Cell*, 1999, vol. **3**, pp. 389 – 395.
20. Prokofieva-Belgovskaya A.A., *Molek. Biol.*, 1982, vol. **16**, pp. 771 - 775. (Rus)
21. Manuelidis L., *Proc. Natl. Acad. Sci. USA*, 1984, vol. **81**, pp. 3123—3127.
22. Manuelidis L. and Borden J., *Chromosoma*, 1988, vol. **96**, pp. 397 – 410.
23. Arnoldus E.P., Peters A.C., Bots G.T. *et al.*, *Hum. Genet.*, 1989, vol. **83**, pp. 231 – 234.
24. Andreev S.G. and Spitkovsky D.M., *Doklady AN SSSR*, 1983, vol. **269**, pp. 1500 - 1503. (Rus)
25. Andreev S.G. and Spitkovsky D.M., *Proc. III Intern. Symp. "The Mechanisms of Superlow Doses"*, Moscow, 2002, p. 237. (Rus)
26. Spitkovsky D.M.. and Andreev S.G., *Studia Biophysica*, 1981, vol. **86**, pp. 83 – 89.
27. Enukashvili N.I. and Podgornaya O.I., *Tsitologiya*, 2001, vol. **43**, pp. 52 - 60. (Rus)
28. Marshall W.F., Dernburg A.F., Harmun D. *et al.*, *Mol. Biol. Cell*, 1996, vol. **7**, pp. 825 – 842.
29. Pyrpasopoulou A., Meier J., Maison C. *et al.*, *EMBO J.*, 1996, vol. **15**, pp. 7108 – 7119.
30. Brown K.E., Guest S.S., and Smale S.T., *Cell*, 1997, vol. **9**, pp. 845 – 854.
31. Festenstein R. and Kioussis D., *Current Option in Genetics And Development*, 2000, vol. **10**, pp. 199 – 202.
32. Spitkovsky D.M., *Radiat. Biol. Radioekologiya*, 1995, vol. **35**, Iss. 3, pp. 346 - 348. (Rus)
33. Bondarchuk I.A., *Radiat. Biol. Radioekologiya*, 2002, vol. **42**(1), pp. 36 - 43. (Rus)
34. Kublik L.N., Eliseeva N.A., and Koryistov Yu.N., *Radiat. Biol. Radioekologiya*, 1993, vol. **33**, Iss. 3(6), pp. 870 - 878. (Rus)
35. Eidus L.Kh., *Membrane Biological Mechanisms Of Low Doses*, Moscow, 2001, 82 p. (Rus)
36. Kheisin Ya.E., *The Size Of Nuclei And Functional State Of Cells*, Moscow, Meditsina, 1967, 423 p. (Rus)

37. Bychkovskaya I.B., Stepanov R.P., and Fedortseva R.F., *Radiat. Biol. Radioekologiya*, 2002, vol. **42**(1), pp. 20 - 35. (Rus)
38. Gold R., Schmied M., Giegerich G. *et al.*, *Lab. Invest.*, 1994, vol. **71**, pp. 219 – 225.
39. Ermakov A.V., Pospekhova N.I., and Spitkovsky D.M., *Radiat. Biol. Radioekologiya*, 2000, vol. **40**(1), pp. 62 - 70. (Rus)
40. Mothersill C. and Seymour C., *Radiat. Res.*, 2001, vol. **155**, pp. 759 – 767.
41. Little J.B., Azzam E.I., Toledo S.M. *et al.*, *Radiat. Prot. Dosim.*, 2002, vol. **99**, pp. 159 – 162.
42. Prise K.M., Belyakov O.V., Newman H.C. *et al.*, *Radiat. Prot. Dosim.*, 2002, vol. **99**, pp. 223 – 226.
43. Kioussis D. and Ellmeier W., *Nature Reviews*, 2002, vol. **2**, pp. 182 – 188.
44. Choo K.H.A., *Trends In Cell Biology*, 2000, vol. **10**, pp. 182 – 188.
45. Wang H., Zeng Z.-C., Bui T.-A. *et al.*, *Cancer Res.*, 2001, vol. **61**, pp. 270 – 277.

Response of a cell population to low-dose irradiation

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ABSTRACT

The results of many-years studies of the efficiency of low-dose (up to 0.5 Gy) ionizing radiation carried out on cells in a tissue culture and on blood lymphocytes of humans (adults and children) are reviewed. It was found in laboratory studies and studies performed in areas contaminated with radionuclides as a result of the Chernobyl accident that the descendants of irradiated cells exhibit genome instability. This instability manifests itself as delayed cell death, an increased incidence of cells with micronuclei, a decrease in proliferative activity, the loss of capacity for adaptive response, increased sensitivity to subsequent irradiation, and increased frequency of sister chromatid exchanges. The results of studies of the adaptive response in a population of human blood lymphocytes are presented. It was found that, in all populations examined, there are individuals who do not develop adaptive response and individuals exhibiting an increased radiosensitivity after adaptive irradiation (0.05 Gy). On the basis of the own results and literature data, possible mechanisms of the effects of low-dose ionizing radiation are discussed. It is concluded that (a) irradiation with low doses may lead to the formation of a population having new properties and (b) the effects and the mechanisms of their realization may differ from those operating upon irradiation with high doses.

Keywords: low doses, genome instability, adaptive response, micronuclei, cells in a tissue culture, human blood lymphocytes

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The standard radiobiological model that explains the effect of high doses of radiation is based on the principle of a target, which is considered to be a DNA molecule. It is assumed that DNA lesions induced by these doses trigger all events leading to the biological effect, and the key role in this process is played by DNA double-strand breaks (DB). Unrepaired and misrepaired DB lead to mutations, malignant transformations, and cell death.

In the last few years, problems associated with the effect of low-dose radiation have become a matter of extensive study. A question arises what doses can be considered to be low and what changes they induce. According to the classification of the General Assembly, doses up to 20 cGy (200 mSv) are considered as low, although at a dose of 0.1 cGy, one track can pass across a nucleus 8 μm in diameter, inducing a lesion and the corresponding effects in the cell [1].

In recent years, a number of effects characteristic of low-dose radiation have been reported. They were called non-target effects because they may not be directly related to initial DNA lesions induced by radiation [2 - 4]. These are:

1. Adaptive response. This phenomenon consists in the fact that cells preliminarily irradiated with low doses become more resistant to subsequent exposure to higher doses of radiation (or another agent).
2. Hypersensitivity. This effect manifests itself in an increase in the initial portion of the dose-effect curve after irradiation with doses of up to 0.3-0.5 Gy.
3. Hormesis. This phenomenon is defined as a stimulating effect, which makes itself evident in a weakening of the radiation-induced effect compared with the linear square dose-effect dependence.
4. Genome instability. This effect shows up as genetic changes (chromosomal aberrations, mutations, delayed cell death, etc.) in survived descendants of irradiated cells.
5. The bystander effect in which damage is registered in cells that were not exposed directly to radiation.
6. Expression of genes that induce the synthesis of a number of proteins, transcription factors, etc., and the activation of enzymes.

It is not excluded that many of these processes may play a role in radiation-induced carcinogenesis.

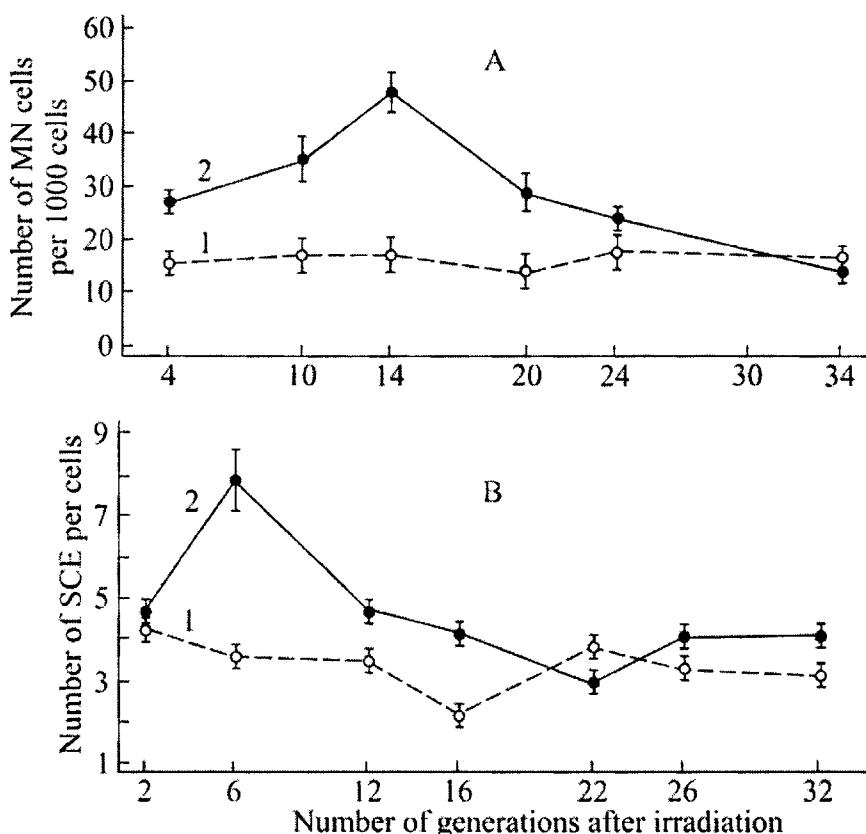


Figure 1. Frequency of cells with micronuclei (MN) (*A*) and sister chromatid exchanges (SCE) (*B*) in descendants of Chinese hamster cells irradiated with a dose of 0.5 Gy.

1, control; 2, descendants of irradiated cells.

Key: 1. *A*: Number of cells with MN per 1000 cells

2. *B*: Number of SCE per cell

3. The number of generations after irradiation

From the analysis of these effects it can be concluded that the regularities observed upon low-dose irradiation are different from those registered upon irradiation with high doses.

This report is a short review of the results of our previous studies on cellular manifestations of genome instability and responses of cells to irradiation

with low doses (up to 0.5 Gy). In addition, the results of experiments on cells irradiated with a dose of 0.05 Gy and with high doses (1--3 Gy) are compared.

Experiments were carried out predominantly on cells in a tissue culture and human blood lymphocytes. The efficiency of treatment was assessed using the following criteria: cell survival [5], frequency of cells with sister chromatid exchanges (SCE) [6], and the frequency of cells with micronuclei (MN) after the cytokinetic block with cytochalasin B and without it [6, 7]. In this short review we give no detailed description of the experiments and the methods because they were described previously [8, 9].

Under genome instability are meant genetic changes in survived descendants of irradiated cells. These may manifest themselves as delayed cell death, destabilization of chromosomes, somatic mutations, gene amplification, changes in radiosensitivity, etc. [10]. Genome instability can lead to malignant transformations [11].

We investigated some manifestations of genome instability in cells irradiated with low doses (0.1-0.5 Gy). Thus, it was shown that, in a population of cloned HeLa cells irradiated with doses of 0.2-0.4 Gy, the number of cells in the clone decreases during 7-10 generations and there appear "loose" colonies, which may indicate delayed cell death (Table 1) [12].

Table 1
Number of cells in a clone in different generations of HeLa cells after acute irradiation with different doses [12]

Number of generations	Number of cells in a colony			
	Radiation dose, cGy			
	0	10	20	40
3	155	114*	84*	64*
7	130	91*	90*	97*
10	231	212	227	171

Footnote: * Significant radiation effect.

In Chinese hamster cells irradiated with a dose of 0.5 Gy, an increased frequency of cells with MN was observed, with the peak of incidence of these cells, i. e., the appearance of *de novo* lesions, being registered by generation 14 after the treatment (Figure 1, A) [13].

In descendants of irradiated cells of the same line, an increase in the frequency of SCE was observed, which preceded in time the increase in the

number of cells with MN (Figure 1B). This may indicate that irradiation with rather low doses enhances metabolic processes leading to lethal events, in particular, the appearance of MN [13]. These experiments were performed on heterogeneous populations, which made the interpretation and analysis of the results difficult. However, a study of a population growing from one irradiated cell showed that the heterogeneity in survived descendants increased, and cells with MN also appeared (Table 2) [14].

Table 2
Variability in the number of cells with MN in the 14th generation of Chinese hamster cells after irradiation [14]

Parameter	Number of cells with micronuclei per 1000 cells	
	Control	Dose 0.5 Gy
Scatter of data	14 - 30	15 - 48
Average frequency	19.2 ± 0.9	29.8 ± 1.6*

Footnote: * Significant radiation effect.

Genome instability is observed after treatment with various agents. Its manifestations and duration depend on the type of induced damage and the mode of DNA repair. Thus, after treatment with methylnitrosourea (an alkylating and carbamoylating agent), delayed cell death was observed up to the 48th generation, whereas after treatment with cisplatin, which induces DNA-DNA and DNA-protein cross-links, enhanced cell death was registered only during six generations. It may be assumed that the lesions induced in the last case are poorly repaired and lead to a rapid cell death; those cells predominantly survive in which no genome instability arises [8].

Further it was shown that the descendants of Chinese hamster cells irradiated with a dose of 0.5 Gy possess enhanced radiosensitivity. After subsequent irradiation with a challenge dose of 3.0 Gy, the frequency of cells with MN in the 14th generation was twofold higher than in the control population (138.0 ± 4.1 compared with 72.0 ± 8.1) [13].

The descendants of irradiated HeLa cells exhibit no adaptive response (AR), a decrease in the frequency of cells with MN after irradiation with an adaptive dose of 0.05 Gy and subsequent (after 5 h) exposure to a challenge dose of 2.0 Gy. No AR was registered during about 20 generations after the treatment [13].

Thus, it can be assumed that genome instability is induced by those injuries that are transmitted from one generation to another and can lead to increased frequency of *de novo* SCE, MN, delayed death, etc.

At present it is known that genome instability can be induced by ionizing radiation, UV-light, chemical agents, hydrogen peroxide, the action of irradiated cells (the bystander effect), etc. As a result, even judging from our own data [8, 13], there arises a population that exhibits new properties, a different sensitivity to external influences, and no capacity for AR.

There are a great number of reports devoted to genome instability and its possible mechanisms [15, 16]. Among Russian publications, a very interesting expert review of V.K. Mazurik and V.F. Mikhailov merits special attention [17]. Possible mechanisms for radiation-induced genome instability are as follows: DNA DB, defective repair, microdeletions, disturbance of gene expression, disturbance of signal transduction pathways, the activation of recombination and exchange processes, changes in the status of the p53 protein, gene amplification, the appearance of complexes of the replicative protein A, and others. Here we listed only several of possible mechanisms. The current status of our knowledge does not enable one to propose a single general mechanism for the induction of genome instability (if this exists at all).

The mechanisms of the formation of adaptive response are also not yet completely understood. Since the publication of Olivieri *et al.*, the phenomenon of AR has been described comprehensively [18]. AR was discovered at different levels of biological organization by using a variety of criteria [19]. AR is an inducible reaction, which consists in the fact that cells and organisms preliminarily irradiated with low adaptive doses become more resistant to subsequent irradiation with higher challenge doses. AR was registered for many effects: survival, chromosomal aberrations, MN, mutations, and others. AR is induced by irradiation in the dose range of 0.05 - 0.20 Gy; it depends on the rate of the adaptive dose [19] and the value of the challenge dose [20]. AR is observed within a definite time after the adaptive irradiation. Its magnitude depends on the genetic constitution and functional state of cells. Upon AR, the anticarcinogenic effect is observed in tissue culture cells and animals; in tumor cells, AR can be absent. A cross-adaptation with various chemical and physical agents (hydrogen peroxide, bleomycin, alkylating agents, cisplatin, other antitumor drugs, hyperthermia, and others) was reported.

AR results from the induction and activation of many processes in the cell: expression and repression of genes, synthesis of proteins, metallothioneins, NO-synthase, and antioxidants, an increase in the activity of protein kinases, the

activation of repair of DNA, first of all, DNA DB (AR is not registered in the absence of repair of DNA DB). A more complete and faultless DNA repair, the activation of genes of programmed cells death, and a delay of the cell cycle in check points may also lead to AR [19, 21].

It should be emphasized that the magnitude of AR depends on the type of DNA lesions and the mode of their repair. Thus, the magnitude of AR to treatment with bleomycin, which induces DNA breaks, is considerably greater than to treatment with mitomycin, which gives rise to DNA-DNA cross-links (50 and 20%, respectively) [22]. The authors of [22] attribute this effect to prolonged and incomplete repair of cross-links, which have no time to be eliminated by the time of the application of the challenge dose.

It is assumed that AR is the inducible mechanism of protection against extreme influences, which has developed in the course of evolution. However, the question concerning the universality of this mechanism requires additional consideration.

We carried out a series of investigations on tissue culture cells and blood lymphocytes of adults and children living in different districts of Moscow and in areas contaminated by radionuclides after the Chernobyl accident. It was found that tissue culture cells (HeLa cells) exposed in the zone of the Chernobyl accident (total dose of 10 cGy; dose rate 0.3 R/h) showed an increased radiosensitivity after irradiation with an adaptive dose of 0.05 Gy followed by a challenge dose of 3 Gy. It should be noted that prolonged irradiation upon exposure in contaminated areas did not induce AR [23].

A study of AR on human blood lymphocytes in several groups of persons revealed a substantial variability in cell sensitivity to subsequent irradiation with high doses (1.0 Gy) after preliminary irradiation with low doses (0.05 Gy). In all groups being examined: in adults and children considered to be healthy, in children with various pathologies, and in children exposed to chronic low-dose radiation during the life in contaminated areas, there were individuals in which no AR was induced. Of critical importance is that, in all groups, as a rule, there were individuals who exhibited an increased radiosensitivity after adaptive irradiation. Thus, even among normal healthy children, up to 20-25% of individuals showed an increased radiosensitivity after irradiation with low doses [24]. Figure 2 gives the data on the variability of AR and an increase in radiosensitivity in different groups of children. It is evident that, in a group of children living in areas contaminated with radionuclides and in a group of children of chronic alcoholics, the number of individuals showing AR was strongly decreased; in children with oligophrenia, AR was absent.

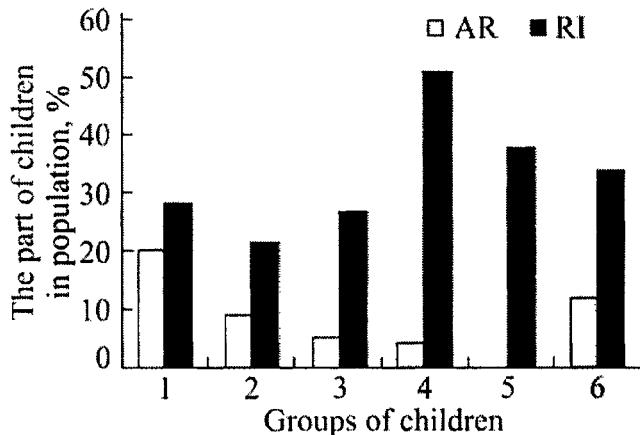


Figure 2. Percentage of children with significant adaptive response (AR) and significant radiosensitivity increase (RI) in different groups of children. Adaptive irradiation with a dose of 0.05 Gy and subsequent (after 5 h) irradiation with a challenge dose of 1.0 Gy.
 1, healthy children; 2, children of chronic alcoholics; 3, children living in areas contaminated as a result of Chernobyl accident (city Novozybkov); 4, 5, oligophrenic children; 6, children living in an ecologically hazardous district of Moscow.
 Key: 1. AR; 2. RI; 3. Percentage of children in a population; 4. Groups of children

Thus, it can be assumed that different individuals respond differently to irradiation with low doses. AR is induced not in all cases, and protective mechanisms operate not in all persons. There are several possible explanations for this phenomenon. These may be the defects of DNA repair, defects in the development of inducible processes mentioned above, the formation of AR before the time of examination, etc.

We also showed that the increase in radiosensitivity after the adaptive irradiation of human lymphocytes is related to the spontaneous damage to the genome (Figure 3): in most persons showing an increased incidence of cells with MN, an increase in radiosensitivity was registered after the irradiation with low doses [25].

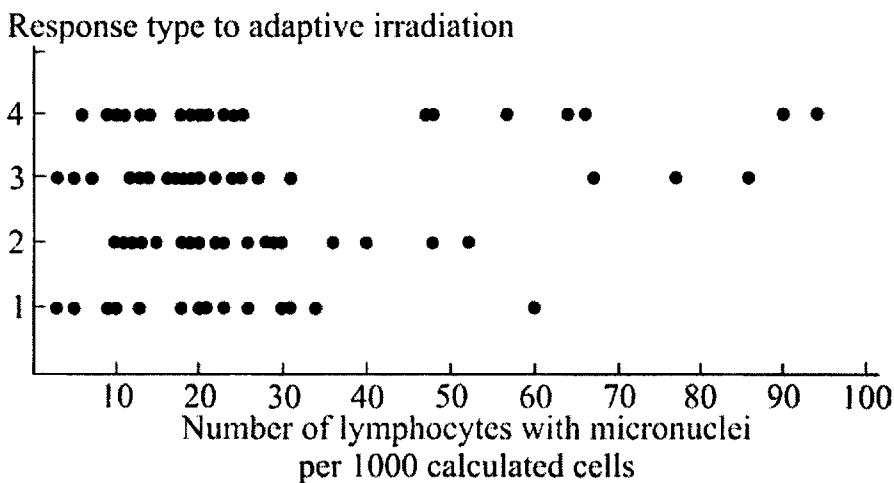


Figure 3. Relationship between the extent of spontaneous damage to the genome and the type of response to adaptive irradiation.

Type of response: 1, significant adaptive response; 2, insignificant adaptive response; 3, insignificant increase in radiosensitivity; 4, significant increase in radiosensitivity.

Key: Type of response to adaptive irradiation

It is noteworthy that one more mechanism of AR is possible, which was not described so far. We studied the capacity for AR in a population of human peripheral blood lymphocytes stimulated by phytohemagglutinin (adaptive dose 0.05 Gy, challenge dose 1.0 Gy 5 h later, and cytokinetic block by cytochalasin B). Simultaneously, the composition of the cell population (the number of mono-, bi-, and polynuclear cells, the number of all types of cells with MN, and the number of cells in the stage of mitosis) was examined. It was shown that AR may arise due to an increase in the number of undamaged binuclear cells rather than a decrease in the number of damaged cells [26].

What can be said now about the mechanisms of AR? They are complex and varied. All events begin to develop after irradiation with low adaptive doses: the presence and absence of AR and an increase in radiosensitivity make themselves evident only after subsequent exposure after a definite time interval. The consideration of possible mechanisms of AR leads one to conclude that AR is the result of the mobilization of all cellular systems and a simultaneous stimulation of numerous signal transduction pathways. As an example, we consider below one possible mechanism of AR [21]. An event that initiates AR

may be DNA damage (e. g., DB), which leads to the activation of protein kinase C, mitogen- and stress-activated protein kinases, and some other protein kinases. These kinases regulate the expression of early genes (*myc*, *fos*, *jun*) whose functioning results in the activation of late genes, the production of growth factors and cytokines, the induction of DNA repair, and the regulation of the cell cycle progression.

It should be particularly emphasized that the effects of irradiation with low doses are often hard to detect; however, in combination with other agents they may become more hazardous and enhance many reactions.

To summarize, it should be noted that irradiation with low doses gives rise to a variety of processes, events, and effects that are not registered after irradiation with high doses. Irradiation with low doses may induce genome instability, expression of genes, the synthesis of proteins, the activation of enzymes and transcription factors, gene mutations, chromosomal aberrations, the formation of reactive oxygen species, a decrease (adaptive response) or an increase in the sensitivity to subsequent treatments, the stimulation of DNA repair, the bystander effect, etc.

There is reason to suggest that the effects induced by irradiation with low doses are realized by mechanisms different from those operating upon high-dose irradiation. Therefore, the extrapolation from the high-dose to low-dose effects seems to be inapplicable. It seems reasonable to re-examine the dose—effect dependences. It may be stated that irradiation with low doses gives rise to a general stress response of the population: the stimulation of metabolic activity, the synthesis of RNA and proteins, the induction of transcription, the stimulation of antioxidant activity, changes in the effect of subsequent exposure to radiation and other agents. It can be stated that irradiation with low doses leads to the formation of a new population with its inherent peculiar properties.

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REFERENCES

1. *Biological Effects at Low Radiation Doses – Models, Mechanisms, and Uncertainties. Report to the General Assembly, 48th Session of UNSCEAR*. Vienna, 12—16 April, 1999.
2. Ward J.F., *Radiat. Res.*, Eds. M. Moriarty, C. Mothersil *et al.*, KS: Allen Press, 2000, pp. 379 - 402.
3. Prise L.M., Belyakov O.V., Newman H.C. *et al.*, *Radiat. Protect. Dosimetry*, 2002, vol. 99(1-4), pp. 223 - 226.
4. Upton A.C., *Crit. Rev. Toxicol.*, 2001, vol. 3(4-5), pp. 681 - 695.
5. Puck T.T. abd Marcus P.J., *J. Exp. Med.*, 1956, vol. 103, pp. 653 - 658.
6. Kato H., *Nature*, 1974, vol. 211, pp. 70 - 72.
7. Fenech M. and Morley A.A., *Mutat. Res.*, 1985, vol. 147(1-2), pp. 29 - 36.
8. Gotlieb V.Ya., Serebryanyi A.M., Chernikova S.B. *et al.*, *Tsitologiya*, 1996, vol. 38(9), pp. 974 - 982. (Rus)
9. Pelevina I.I., Aleshchenko A.V., Afanas'ev G.G. *et al.*, *Radiats. Biol. Radioekol.*, 2000, vol. 40(5), pp. 544 - 549. (Rus)
10. Gotlieb V.Ya., Toponainen N.Ya., and Pelevina I.I., *Radiobiologia*, 1985, vol. 25, Issue 4, pp. 435 - 443. (Rus)
11. Murnine J.P. and Sprung C.N., *Radioprotection*, 1997, vol. 32(Ch. 1), pp. 217 - 221.
12. Alferovich A.A., Gotlieb V.Ya., and Pelevina I.I., *Izv. RAN, Ser. Biol.*, 1995, No. 1, pp. 15 - 18. (Rus)
13. Pelevina I.I., Gotlieb V.Ya., Kudryashova O.V. *et al.*, *Tsitologiya*, 1998, vol. 40(5), pp. 467 - 477. (Rus)
14. Pelevina I.I., Gotlieb V.Ya., Kudryashova O.V. *et al.*, *Ontogenet.*, 2001, vol. 32(1), pp. 51 - 57. (Rus)
15. Sabatier L., Lebeau J., Pommier J.R *et al.*, *Radiat. Res. Congress Proc.*, 1995, vol. 2, pp. 509 - 512.
16. Little J.B., *Int. J. Radiat. Biol.*, 1998, vol. 74, pp. 663 - 671.
17. Mazurik V.K. and Mikhailov V.F., *Radiats. Biol. Radioekol.*, 2001, vol. 41(3), pp. 272 - 289. (Rus)
18. Olivieri G., Bodycote J., and Wolff. S., *Science*, 1984, vol. 223(4636), pp. 594 - 597.
19. Wolff. S., *Environ. Health Perspect.*, 1998, vol. 106, Suppl. 1, pp. 277 - 283.
20. Chernikova S.B., Gotlieb V.Ya., and Pelevina I.I., *Radiats. Biol. Radioekol.*, 1993, vol. 33, Issue 1(4), pp. 537 - 541. (Rus)

21. Stecca C. and Gerber G.B., *Biochem. Pharmacol.*, 1998, vol. **55**(7), pp. 941 - 951.
22. Schlade-Bartusiak K., Stembalska-Kozlowska A., Bernady M. *et al.*, *Mutat. Res.*, 2002, vol. **513**(1-2), pp. 75 - 81.
23. Pelevina I.I., Afanas'ev G.G., Gotlieb V.Ya. *et al.*, *Radiats. Biol. Radioekol.*, 1993, vol. **33**, Issue I(4), pp. 508 - 520. (Rus)
24. Pelevina I.I., Afanas'ev G.G., Aleshchenko A.V. *et al.*, *Radiats. Biol. Radioekol.*, 1999, vol. **39**(1), pp. 106 - 112. (Rus)
25. Pelevina I.I., Aleshchenko A.V., Antoshchina M.M. *et al.*, *Radiats. Biol. Radioekol.*, 2001, vol. **41**(5), pp. 573 - 579. (Rus)
26. Serebryanyi A.M., Aleshchenko A.V., Gotlieb V.Ya. *et al.*, *Tsitologiya*, 2003, vol. **44**(1). (Rus)

The results of cytogenetic observation of children and teenagers inhabited in zones radiation-polluted after Chernobyl accident

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ABSTRACT

The results of many-year cytogenetic investigations on children and teenagers inhabited in radiation-polluted zones after Chernobyl accident are shown. The average ^{137}Cs pollution density in two comparison inhabited zones equaled 111 and 200 kBq/m², and the average doses of the whole body external irradiation, accumulated in the period of 1986 – 2001, equaled 6.7 and 11.4 mGy, respectively. The average dose per thyroid gland for all age groups of the population in the second zone was found 1.5 times higher, approximately, than in the first zone. Hence, for the youngest ages (0 – 1 year) these doses equaled 114.3 and 174.3 mGy, respectively.

Cytogenetic observations performed during 17 years after the accident displayed increased level of the chromosome aberrations in peripheral blood lymphocytes for 30 to 60% of investigated persons, approximately. For the whole observation period, the average frequency of unstable aberrations (acentric fragments, dicentrics and centric rings) equaled about 0.4 per 100 cells (0.22 per 100 cells in the control); the level of aberrations – radiation impact markers (dicentrics and centric rings) remained increased within all periods of investigations and varied from 0.04 to 0.19 per 100 cells (0.03 in the control).

Simultaneous study of some people using the FISH-method of stable aberration frequency (mostly translocations) showed their level being 3 times above the observed concentration of dicentrics. Higher cytogenetic damages were detected in the groups of children prenatal (antenatal) irradiated during the accident.

Keywords: Chernobyl accident, polluted zones, prenatal (antenatal) irradiation, human lymphocytes, stable and unstable aberrations, chromatid aberrations, multiaberrant cells, risk of cancer

Since the Chernobyl accident is the largest nuclear catastrophe in the peaceful history of the humankind, over eighteen years passed. This catastrophe caused radioactive pollution of tremendously wide territories by long-living radionuclides, which were inhabited by millions of people who, consequently, were subject to chronic radiation impact after the accident. The unprecedented situation occurred, when ten and even hundred thousands of children (we are talking only about the population of Russia) were not only impacted by sharp irradiation directly at the Chernobyl accident, including incorporation of iodine radionuclides, but also are living impacted by long-term low dose irradiation. It is the well-known fact that the child's organism is the most sensitive to radiation impact, especially, during the antenatal life. Therefore, children represent the critical group of population, mostly injured in Chernobyl accident.

The tumor response to ionizing radiation was indicated many times in various epidemiological investigations. According to contemporary ideas carcinogenesis is bases on mutative changes in somatic cells of the organism [1 – 3]. The reliable correlation between the risk of cancer genesis and frequency of chromosome [4, 5] was illustrated on quite representative selection (about 3,000 people). Therefore, people with high structural damages of chromosomes may be related to the group of increased tumor risk [1]. Translocations [6] are the most frequent chromosome anomalies typical of hemoblastosis and some solid tumors. Good correlation between translocations in bone marrow cells and leukemia and solid tumors [7] was manifested for survivors in Hiroshima and Nagasaki.

As a consequence, the observed level of chromosome aberrations may be used as a forecasting test for early detection, e.g. before development of malignant changes in cells, of people with increase carcinogenic risk. The

authors consider this to be the main aim, necessity and significance of long-term cytogenetic monitoring of the population inhabited on radiation-polluted areas, at chronic impact of low radiation doses.

It should be noted that immediately after the Chernobyl accident the State Long Range Program on the Accident Management was established. This Program envisaged regular cytogenetic examination of accident liquidators and the population inhabited in the polluted zones. Unfortunately, the USSR disintegration and further problems in economic development of the Russian Federation were the reason for termination of the target financing of these investigations. Nevertheless, the systematic cytogenetic examinations of children and teenagers inhabited in radiation-polluted zones of Kaluga and Bryansk Districts, initiated in 1989 by the authors of the current review, are continued still. This review shows results of dynamic cytogenetic observations for the mentioned cohort inhabited in the polluted zones of two areas in Kaluga District.

TARGETS AND METHODS

The studied groups included children and teenagers inhabited in Zhizdrinsk and Ulianovsk Regions of Kaluga District. Totally, 333 children and teenagers were examined in Ulianovsk Region and 407 people - in Zhizdrinsk Region (740 people in both Regions). Among them 149 people were irradiated during antenatal life during the accident with the following distribution of the fetal age at the moment of the accident (April 26, 1986): 0 – 8 weeks – 36 children (24.2%), 9 – 28 weeks – 76 children (51%), and 29 – 40 weeks – 37 children (24.8%). The group of rest 591 examined patients included 540 children born before the accident, aged from 0 to 18 at the moment of the accident, and 51 children born after the accident, i.e. after March 07, 1987 (refer to Table 2). In the period of 1989 – 2003, sequential cytogenetic examinations were carried out in Ulianovsk Region - 5 times, and in Zhizdrinsk Region - 7 times. Hence, some children and teenagers were repeatedly examined from 2 to 6 times. The control group consisted of 87 children and teenagers (examined in 1991 and 1993), inhabited in pollution-free zones.

Blood (1 – 3 ml) was sampled from ulnar veins of patients, placed to sterile test tubes with heparin solution (200 un/ml of blood) and transported to a laboratory. Then lymphocytes were cultivated, chromosome preparations were made and analyzed at standard coloration (by Himse) of chromosomes and

using the technique of fluorescent in situ hybridization of cells (FISH-technique). The culture composition was the following: whole blood - 0.8 ml; MEM medium – 6.16 ml; inactivated calf serum – 1.6 ml; L-glutamine – 0.08 ml; antibiotic - 0.08 ml; phytohemagglutinin (PHA) – 0.15 ml. For cell incubation, flasks were exposed in a thermostat at 37°C during 48 hours. To block mitosis at the metaphase 2 hours prior to the incubation end, demecolcine solution (0.2 µg/ml of medium) was added to the flasks.

After the cultivation end, for cell sedimentation, the cell culture was poured to centrifugal test-tubes and centrifuged at 1,000 rot/min rate during 15 min. Supernatant fluid was removed by a water-jet pump then hypotonic solution (0.75 M KCl), preliminarily heated up to 37°C, was added, and the sediment was resuspended in it. Then the flasks with the cell culture are exposed on water bath (37°C) during 10 – 12 min. After making the medium hypotonic, it was again centrifuged under the same conditions with successive removal of the supernatant fluid.

The cells were fixed by resuspending the sediment in 1 – 1.5 ml of freshly prepared fixing agent (methyl alcohol and glacial acetic acid mixture in proportion 3:1) in a shaker and poured up to 10 ml. The fixing agent was substituted three times with further centrifugation.

Preparations were made immediately after the fixation end. Drops of the suspension of cells were placed to glass slides wetted by distilled water and cooled prior to placement. After drying preparations were hydrolyzed in 5 N hydrochloric acid solution during 10 min.

Chromosome preparations were colored by the Himse method.

Cytogenetic analysis was performed on a light microscope under immersion, magnification 90×10.

All types of chromosome aberrations were analyzed, distinguished with karyotyping. Aberrations of the **chromosome type** are acentric fragments (paired and point), centric rings and dicentrics. These are the so-called aberrations of the **unstable type**. Contemporarily, among them dicentrics and centric rings are aberrations- markers of the radiation effect. The presence of this aberration type in peripheral blood lymphocytes indicates the impact of radiation-type mutagens on the organism.

The standard method of metaphase analysis, i.e. using no karyotyping, also allows acquisition of a definite part of **stable aberrations**. They are the so-called anomalous monocentrics, which in the most cases result from reciprocal translocation. They were also considered in the current study. The latest data [8]

show that this method makes possible acquisition up to 20% of the initial translocation value.

Among aberrations of the **chromatid type**, acentric fragments (single deletions and isodeletions) and exchange aberrations (as a whole) were considered.

Determinations by the FISH-method used a mixture of biotin-modified (via PCR) DNA probes for chromosomes 2 and 3. Before hybridization, the preparations were treated by ribonuclease (100 µg/ml in 2xSSC) during 1 h at 37°C. For DNA denaturation, a solution of 70% deionized formamide, 2xSSC and 10 mM phosphate buffer (pH 7.0) was added to the preparation beneath the cover glass. The preparation was placed to a hot bench at 80°C for 4 min and then passed through a series of ethyl alcohol dilutions: 70%, 90%, and 100%. Then the preparations were dried. Chromosome DNA probes were mixed with hybridized buffer and denatured during 5 min at 70°C, then placed to ice-cold water for 5 min and, finally, exposed to the water bath at 37°C. The mixture of DNA probes for chromosomes 2 and 3 were applied on the preparation beneath the cover glass and exposed to a thermostat at 37°C for the night for hybridization. After hybridization, preparations were washed in 50% formamide, then in 0.1xSSC and 0.05% Tween 20. The next step was immunochemical coloring; to detect biotin-modified DNA probes Fluorescein Avidin D and Anti-Avidin D (Vector Laboratories) were used. FITS-Avidin D (5 mg/ml, 3×30 min) and Anti-Avidin D (5 mg/ml, 2×30 min) were successively applied on the preparation, which then was incubated at room temperature and washed in 0.05% Tween 20 (3×5 min). Finally, the preparations passed through a series of ethyl solutions (70%, 90%, and 100%), dried, and PI-Vectashield was applied on them.

Metaphases were analyzed on a fluorescent microscope (Zeiss Co.) with dual filter (AHF), specific for FITC and PI.

Stable chromosome aberrations were identified using PAINT (Protocol of Aberration Identification and Nomenclature Terminology) classification system [9]. At standard chromosome coloration (by Himsa) from 300 to 500 cells of each examined patient were analyzed, and from 350 to 1,500 cells were analyzed using FISH-technique. After estimation of the frequency of translocations, in which 2 and 3 colored chromosome pairs giving 14.9% of the genome, the translocation frequency per genome was calculated by the formula suggested by J.N. Lucas *et al.* [10].

Statistical data processing applied common parametric and non-parametric criteria realized in ORIGIN and MS EXCEL software.

RESULTS AND DISCUSSION

One of the main and necessary conditions for carrying out radiation epidemiological investigations is the knowledge about radiation situation and irradiation doses of population in the areas of such investigations. Ignorance of these data makes impossible the science-based assessment of radiation hazard and forecast of the far medical consequences of irradiation for examined human population. Only up to 2000, according to specified data accumulated doses of total irradiation and doses per thyroid gland were finally reconstructed for the population inhabited on the polluted territories after the Chernobyl accident. The doses were approved by Ministry of Health of the Russian Federation and coordinated with Russian National Committee on Radionuclide Pollution [11]. Irradiation doses for Kaluga District population were reconstructed in the Russian State Medical-Dosimetric Register (RSMDR, Ochninsk).

The average density of ^{137}Cs territory pollution in the zones under investigation and total accumulated doses per the whole body and doses per thyroid gland for the population inhabited in these zones are shown in Table 1. As is obvious, for the zones in Zhizdrinsk and Ulianovsk Regions the average densities of pollution by ^{137}Cs were as high as twice different (111 and 200 kBq/m², respectively), though due to irregularity in ^{137}Cs fallout the pollution density in inhabited localities was significantly different from the mean-zone values. In accordance with the radionuclide pollution density the average doses, accumulated per the whole body for the population of these regions since 1986 till 2001, were also two-fold different: 6.7 and 11.4 mGy, respectively.

In RSMDR, specified average, age-dependent and individual (if radiometry for 1986 is available) irradiation doses per thyroid gland were also reconstructed for the population of Kaluga District. As the calculations performed indicate, in 1986 the average doses per thyroid gland for the population of all age groups in Ulianovsk Region were 1.5 times higher, approximately, than for the inhabitants of Zhizdrinsk Region. Hence, maximal doses per thyroid gland in both regions were observed in the youngest inhabitants, aged 0 - 1 (174.0 and 114.3 mGy, respectively). This was caused by predominant radioactive iodine intake with milk. These doses reduced with the age and reached their minimum for people >18, on average, 29.2 and 19.1 mGy for Ulianovsk and Zhizdrinsk Regions, respectively.

*Table 1***Density of ^{137}Cs contamination and mean-region irradiation doses in Kaluga District***

Region	^{137}Cs contamination density, kBq/m ²	Irradiation dose of the whole body till 2001, mGy	Thyroid irradiation dose, mGy					
			Age, years					
			0-1	1-3	3-8	8-13	13-18	≥ 18
Zhizdrinsk	111	6.7	114.3	86.8	53.2	30.4	23.9	19.1
Ulianovsk	200	11.4	174.0	136.6	82.2	47.3	36.1	29.2

* – data from [11].

*Table 2***The cohort of examined children and teenagers in Kaluga District**

Region of inhabitance	Total number of examined (male - female)	Year of birth (min - max)	Total number of blood samples for the period 1989-2003	Persons distribution by number of examinations					
				1	2	3	4	5	6
Zhizdrinsk	407 (191-216)	1966 - 1989	717	196	140	48	19	3	1
Ulianovsk	333 (161-172)	1968 - 1989	543	181	102	42	8	-	-
For two regions	740 (352-388)	1966 - 1989	1260	377	242	90	27	3	1

Table 3

The average frequency of chromosome aberrations per 100 cells in children and teenagers living in contaminated regions of Kaluga District

Year of examination	Time after accident, years	Number of examined persons	Average age*, years	Number of cells	Cell aberrant**, %	% of persons with aber. cells above*** control
Zhizdrinsk Region						
1990	4.4	73	7.2	14600	0.58±0.04	61.6
1991	5.4	96	8.4	18226	0.47±0.04	48.0
1994	8.4	109	11.0	31870	0.27±0.02	27.8
1995	9.4	133	12.9	39867	0.44±0.02	36.8
1998	12.2	88	13.9	44000	0.40±0.02	55.6
2001	15.4	128	15.1	63491	0.37±0.02	49.2
2003	17.0	90	16.7	45000	0.40±0.02	52.2
All years		717		257054	0.40±0.01	
Ulianovsk region						
1989	2.7	17	13.1	4920	0.30±0.07	23.5
1991	5.0	146	9.1	28459	0.46±0.03	53.4
1992	6.0	192	13.3	36781	0.37±0.03	47.3
1994	7.8	83	11.8	24400	0.34±0.03	28.9
1998	12.5	105	15.1	52380	0.42±0.02	53.8
All years		543		147140	0.40±0.01	
Control		87	12.4	26878	0.23±0.03	

Table 3 (Continues)

Year of examination	Chromosome type					Chromatid type	MAK/ 1000 cells
	Acentrics	Centric rings	Dicentrics	Total ***	Anomalous monocentrics		
Zhizdrinsk Region							
1990	0,45±0,06	0,08±0,02	0,07±0,02	0,59±0,06	0	0,62±0,07	0
1991	0,30±0,04	0,04±0,01	0,07±0,02	0,41±0,05	0,07±0,02	0,56±0,06	0,21±0,11
1994	0,17±0,02	0,01±0,01	0,10±0,02	0,28±0,03	0,01±0,01	0,58±0,04	0
1995	0,33±0,03	0,05±0,01	0,07±0,01	0,45±0,03	0,02±0,01	0,45±0,03	0,03±0,03
1998	0,32±0,03	0,01±0,01	0,03±0,01	0,36±0,03	0,05±0,01	0,52±0,03	0
2001	0,26±0,02	0,03±0,01	0,11±0,01	0,40±0,03	0,03±0,01	0,86±0,04	0,54±0,09
2003	0,30±0,03	0,02±0,01	0,08±0,01	0,39±0,03	0,02±0,01	0,73±0,04	0,04±0,03
All years	0,29±0,01	0,03±0,01	0,08±0,01	0,40±0,01	0,03±0,01	0,64±0,02	0,12±0,06
Ulianovsk region							
1989	0,24±0,07	0,08±0,04	0	0,33±0,08	0	1,22±0,16	0
1991	0,36±0,04	0,05±0,01	0,05±0,01	0,46±0,04	0,02±0,01	0,45±0,04	0,14±0,07
1992	0,32±0,03	0,01±0,01	0,05±0,01	0,38±0,03	0,02±0,01	0,59±0,04	0,19±0,07
1994	0,29±0,03	0,02±0,01	0,04±0,01	0,35±0,04	0,004±0,004	1,16±0,07	0,08±0,06
1998	0,25±0,02	0,04±0,01	0,09±0,01	0,38±0,03	0,07±0,01	0,77±0,04	0,05±0,03
All years	0,29±0,01	0,03±0,01	0,06±0,01	0,39±0,02	0,04±0,01	0,73±0,02	0,09±0,04
Control	0,19±0,03	0,01±0,01	0,02±0,01	0,22±0,03	0,01±0,01	0,41±0,04	0,04±0,04

* – at the examination

** – with chromosome type aberrations

*** – 95% confidence

**** – with no respect to anomalous monocentrics

Table 2 shows data on the cohort of people, examined in two polluted regions of Kaluga District. The main cohort was formed by children and teenagers. The cytogenetic examination was performed for 740 people inhabited in Ulianovsk (333 people) and Zhizdrinsk (407 people) Regions. In the periods of 1989 – 1998 and 1990 – 2003, examinations were performed 5 and 7 times, respectively, in the first and the second Region. Hence, some children were repeatedly examined from 2 to 6 time (refer to Table 2).

Table 3 shows the main results of cytogenetic observations for the mentioned cohort of the population. The inhabitants in Ulianovsk and Zhizdrinsk Regions were examined 2.7 and 4.4 years after the Chernobyl accident for the first time and 12.5 and 17 year for the last time, respectively.

The 5-time successive cytogenetic examination, performed in Ulianovsk Region in the period of 1989 - 1998, involved 543 people, including those passed the repeated examinations (refer to Table 2). Meanwhile, almost 150,000 metaphases were analyzed, on average, about 300 metaphases from each individual. For the observation period, the quantity of individuals with increased (compared to control) amount of cells with chromosome aberrations varied from 28.9 to 53.8%. The mean frequency of unstable chromosome aberrations (acentric fragments, dicentrics and centric rings) varied within the range of 0.33 – 0.46 per 100 cells, which gave, on average, (0.39 ± 0.02) for the period of observations. This value reliably exceeds the control value: (0.22 ± 0.03) . Note also that the average level of radiation marker aberrations (dicentrics and centric rings) practically in all observation periods reliably exceeded the control value. It varied in the ranges of 0.04 – 0.09 and 0.01 and 0.08 per 100 cells compared with 0.02 and 0.01 per cells in the control, respectively.

In Zhizdrinsk Region, the 7 times repeated cytogenetic examinations involved 717 people, including those examined repeatedly. Therefore, over 250,000 metaphases were analyzed, on average, 350 metaphases per each individual. Whence, the amount of people with increased content of abberant cells (compared with the control) varied from 27.8 to 61.6% in the observation period, i.e. almost within the borders for inhabitants of Ulianovsk Region. The average frequency of unstable chromosome aberrations varied within the same range - 0.28 - 0.59 per 100 cells – which gave, on average, 0.40 ± 0.01 in the observation period that reliably exceeded the control level. Similar to inhabitants of Ulianovsk Region, the average frequency of aberrations-markers of the radiation impact (dicentrics and centric rings) in the current cohort always, within the observation periods, reliably exceeded the control level,

varying within the ranges of 0.03 – 0.11 and 0.01 – 0.08 per 100 cells, respectively.

Among the chromosome type aberrations, the so-called anomalous monocentrics mostly representing (by the formation mechanism) symmetrical reciprocal translocations were taken into account. They are related to the class of stable aberrations due to their non-prevention of indirect cell division and freely transfer to daughter cells. According to the information received [8] the standard method (without karyotyping) of metaphase analysis of chromosome aberrations allows identifying up to 20% of translocations. According to data in Table 3, the frequency of observed translocations always (in all observation periods) was reliably above the control level in the inhabitants of both regions, on average, 0.03 – 0.04 per 100 cells.

Both chromosome and chromatid aberrations were studied. They are commonly subdivided into two classes - fragments (deletions) and exchanges. In control cultures of human lymphocytes and in cultures of inhabitants in polluted zones, among chromatid deletions single fragments dominated, whereas paired one (isodeletions) were observed extremely infrequently. Meanwhile, among chromatid exchanges, symmetrical ones are predominant and asymmetrical ones are extremely infrequent. According to the data of multiple investigations, including the authors of the current review [12], in the control lymphocyte culture the proportion of aberrations of chromosome and chromatid types equals, on average, 1:1.5 – 2. Hence, as shown before [12], the frequency of chromatid aberrations increases with age and, thus, the mentioned proportion may shift greater towards chromatid aberrations.

According to data in Table 3, the increased level of chromatid aberrations was observed in inhabitants of both regions in all observation periods: 0.73 and 0.64 per 100 cells (0.41 in control) in inhabitants of Ulianovsk and Zhizdrinsk Regions, respectively. The ratio of chromosome and chromatid aberrations, both in control and inhabitants of Ulianovsk Region was practically equal (1:2 and 1:1.9), whereas for inhabitants of Zhizdrinsk Region it was somewhat lower (1:1.5).

The increased level of chromosome type aberrations in human lymphocytes is usually associated with the impact on the organism of radiation-induced mutagens, whereas chromatid aberrations are usually caused by impact of chemicals, including medicinal preparations, as well as viral infections (hepatitis, Biett's disease, flue, etc.). Table 3 also shows that in some years cytogenetic observations displayed much higher level of chromatid aberrations, which might be caused by any viral infections: adenoviral, flue, or some prophylactic measures, for example, vaccinations.

Finally, the latter cytogenetic index used in the current study is the occurrence of so-called multiabberant cells (MAC – in Russian) or rogue cells in the blood lymphocytes. Cytogenetically, the rogue cell phenomenon is manifested in the study of traditional number of metaphases (200 – 300 cells) by observation of a single, less frequently two cells in the individual containing many different chromosome aberrations. The cases also occur, in which similar to the current investigation, the whole cell genome is structurally damaged and involved complex restructurings. Therefore, in the rest cells chromosome aberrations are either absent or single cells are also obtained, usually, obtaining one aberration.

Primarily, the rogue cells were detected about 35 years ago, in blood lymphocytes of Latin American Indians with the frequency of 1/200 metaphases [13]. Since in the next two years of observations the occurrence rate of these cells significantly reduced (to 1/5000 metaphases), the reason for the rogue cell occurrence in the current cohort was suggested to be a tropical infection [14]. However, in the following years rogue cells were also observed in other regions of the Earth, including Europe. Since this question is the objective for much more profound analysis and discussion, we do not contemplate the detailed consideration of it in the current article. The authors only want to note that rogue cells were detected in various cohorts of patients already at the initial cytogenetic examinations, carried out after the Chernobyl accident. Naturally, the occurrence of these cells was associated with radiation-induced consequences of the accident [15] and, first of all, with incorporated impact of “hot” particles [16].

In the primary cytogenetic examinations of Chernobyl cohort, we have identified the rogue cell occurrence in some patients from various groups of the population: people evacuated from the accident zone, ones inhabited the polluted territories, and accident liquidators. However, the rogue cell occurrence in the blood lymphocytes was not unambiguously bound to the radiation-induced effect of the accident, including eventual impact of incorporated, “hot” particles [17]. Pure biological etiology of these specific structural damages in human cell chromosomes is also suggested [18, 19]. In this case, the reasons are direct viral infections or activation of latent retroviruses (transposones), contained in the cell genome. This hypothesis may also be confirmed by the examination results of inhabitants in Zhizdrinsk Region, carried out in 2001. The obtained data indicated the highest frequency of chromatid aberrations (0.86 per 100 cells), observed during the whole period of observation. It is common knowledge that chromatid aberrations may also be induced by viral infections, where the highest rogue cell frequency equals 0.54 per 1,000 cells

(Table 3). Unfortunately, this is just a suggestion, and no unified idea about rogue cell etiology and their importance for the organism is present.

Despite the fact that the density of ^{137}Cs pollution and average irradiation doses of the population in the mentioned regions are two-fold different (Table 1), reliable differences in the spectrum of chromosome aberrations and their total frequency were not observed (Table 3). Therefore, clearer dynamics of cytogenetic damages in patients inhabited in polluted zones was obtained by uniting results for both studied regions. As is obvious, the average frequency of dicentrics and centric rings (the aberrations-markers of the radiation-induced impact) weakly increases with time - the tendency, much clearer observed for chromatid aberrations. Hence, the frequency of acentric aberrations remained constant within all examinations.

In the recent 17 years, the observed increased frequency of aberrations-markers of the radiation-induced impact in people cohorts inhabited in the territories, polluted by the Chernobyl accident, has not displayed any tendency to decrease. This testifies about permanent irradiative, mutagenic impact of the environment on these populations. Data in Table 1 show that the densities of ^{137}Cs pollution in Ulianovsk and Zhizdrinsk Regions (200 and 111 kBq/m², respectively) and the average doses of total irradiation, accumulated by 2001 in these Regions (11.4 and 6.7 mGy, respectively), are not so high. Nevertheless, the observed average frequency of dicentrics and centric rings (totally, 0.09 and 0.11 per 100 cells) in blood lymphocytes of examined patients are as much as 3 and 3.7 times higher than the control value, respectively (Table 3).

On the one hand, the observed tendency for increasing frequency of chromatid aberrations in examined cohorts, untypical for radiation-induced mutagenesis, may testify about the increasing global anthropogenic, mostly chemical pollution of the environment. On the other hand, they may be partly caused by the radiation impact, because it is known that low radiation doses induce genome instability in descendant irradiated cells. This instability is detected shaped as aberrations, mostly as simple restructurings – the occurrence of cells with micronuclei, acentric fragments of the chromosome and chromatid type, and sister chromatid exchanges [20].

By now the prevailing idea is that the prenatal period is the most radiation-sensitive in the organism development. It consists of several subsequent periods of the human organism formation. Therefore, any negative effect on the embryo and developing fetus may cause either organism death in future or occurrence of congenital malformations or birth defects, formation of tumors in the childhood, etc. Of course, irradiation is one of the most hazardous among such impacts. Hence, sensitivity to such impacts and expressivity will

mostly be defined by the prenatal development period. Examinations of survivals in Hiroshima and Nagasaki indicated the highest risk of the prenatal irradiation in the pregnancy period of 8 – 15 weeks for the cerebrum development, which causes further mental deficiency or defectiveness [21, 22]. Though any strict dose dependence on mental deficiency was not detected, it was found that it occurred at doses about 1 Gy or higher.

It is common knowledge that structural damages of chromosomes are the earliest manifestations of the cell response to radiation impact, and they may be clearly quantitatively accounted. Therefore, of interest is comparative estimation of cytogenetic disturbances in patients, irradiated in the Chernobyl accident and after it in pre- and postnatal periods. In the period of 1989 – 2003, the united group of patients examined in both regions consisted of 149 people, irradiated in the prenatal period at the accident moment and continued inhabiting in polluted zones. According to the fetal age at the moment of the accident they were subdivided by three pregnancy periods: 0 – 8, 9 – 28 and 29 – 40 weeks (Table 4).

Table 4
The cohort of prenatal irradiated children, now living on examined radioactivity contaminated territories of Kaluga District

Fetus age (weeks)	Born in the period	Number of examined (male - female)	Number of blood samples in the period 1989–2003	Distribution of persons by numbers of examinations			
				1	2	3	4
0–8	13.12.1986 – 10.03.1987	36 (13–23)	61	15	18	2	1
9–28	26.07.1986 – 12.12.1986	76 (31–43)	139	24	42	9	1
29–40	26.04.1986 – 25.07.1986	37 (17–20)	98	4	10	18	5
Whole period	26.04.1986 – 10.03.1987	149 (61–86)	298	43	70	29	7

Table 5 shows comparison results of the main cytogenetic indices, impacted by the accident consequences in pre- and postnatal periods of development. As is obvious, general differences in cytogenetic effect between two groups were observed only in total frequency of dicentrics and centric rings (0.13 and 0.09 per 100 cells) and chromatid aberrations (0.76 and 0.64 per 100 cells, respectively). More intense cytogenetic effect of the accident

consequences had the effect at periods 1 and 2 of prenatal development (0 - 28 weeks) and was reliably lower in the third period (29 - 40 weeks). The frequency of aberrations observed in the groups of pre- and postnatal irradiated patients reliably exceeded the control value (Table 3).

Table 5

The average frequency of chromosome aberrations per 100 cells in children and teenagers irradiated in pre- and postnatal periods during the Chernobyl accident

	Groups of irradiated (period)				
	0-8 weeks	9-28 weeks	29-40 weeks	prenatal	postnatal
Number of persons	36	76	37	149	591
Number of blood samples	61	139	98	298	962
Number of cells	24353	62340	43771	129964	273230
Aberrant cells*, %	0.46±0.03	0.43±0.02	0.35±0.02	0.41±0.01	0.39±0.01
Chromosome type					
Acentrics	0.29±0.03	0.30±0.02	0.26±0.02	0.28±0.01	0.30±0.01
Dicentrics and rings	0.14±0.03	0.14±0.02	0.10±0.02	0.13±0.01	0.09±0.01
Total**	0.45±0.04	0.44±0.03	0.36±0.03	0.41±0.02	0.38±0.01
Anomalous monocentrics	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01
Chromatid type					
Deletions and isodeletions	0.57±0.05	0.64±0.03	0.74±0.04	0.66±0.02	0.56±0.01
Exchanges	0.12±0.02	0.10±0.01	0.06±0.01	0.09±0.01	0.08±0.01
Total	0.69±0.05	0.74±0.03	0.80±0.04	0.76±0.02	0.64±0.02

* – with chromosome type aberrations

** – with no respect to anomalous monocentrics

The increased frequency of cytogenetic disturbances (compared with the control) at prenatal irradiation of 42 children in the Chernobyl accident was observed in the far periods [23]. The additional method of chromosome differential G-staining, applied by the authors, indicated much higher frequency of stable aberrations (translocations, para- and pericentric inversions, terminal and interstitial deletions, and insertions) compared with unstable aberration output.

In the current observation, a part of examined patients (21 individuals) were also subject to additional analysis of stable chromosome aberrations using fluorescent *in situ* hybridization of cells (FISH-technique) for 2 and 3 chromosomes, which gave 14.9% of the cell genome. Unfortunately, patients irradiated in the prenatal period were not examined in this group. Data in Table 6 show that in 2/3 of cases (in 14 of 21 examined patients) any stable aberration was observed in blood lymphocytes. The average frequency of translocations in the group was reliably higher compared with the control - 0.37 and 0.11 per 100 cell genomes, respectively. Because the same patients were examined simultaneously at standard (routine) staining of unstable chromosome aberrations, of interest was comparison of frequencies of dicentrics and translocations (theoretically expected to be 1:1 at the radiation-induced effect) for the same patients. As shown in Table 6, only 14.3% of cases (3 of 21 patients) displayed dicentrics, whereas translocations were detected in 42.9% of cases (in 9 of 21 patients), which three times higher. This may be explained in the only way as follows. The lymphoid cell population in peripheral blood is regenerated with death of both normal and aberrant lymphocytes with equally probable elimination of cells with stable and unstable aberrations. Therefore, the observed increased frequency of translocations is associated with their occurrence in peripheral blood from irradiated stem cells. For the cohort of Chernobyl-irradiated patients, these results show prospect of long-term studying stable chromosome aberrations, which are preserved in the organism for a long time and represent potential source of cell malignant change.

As mentioned above, the examined cohort included three groups of people: born before the accident (before April 26, 1986), prenatal irradiated (since April 26, 1986 till March 10, 1987), and born after the accident (after March 10, 1987). Thus, the last group really consisted of children born almost 1 year after the accident, which escaped from additional irradiation by radioactive iodine. Therefore, it is possible to estimate the relative contribution of additional irradiation by radioactive iodine into the observed cytogenetic effect. This additional effect may be significant for patients from two initial groups only.

Table 6

The frequency of stable chromosome aberrations in children and teenagers living on contaminated territories of Ulianovsk Region (the examination of 1998)

Number of examined persons	Cell number	Aberrant cell number	Number of aberrations				Frequency of translocations to genome/100 cells	Frequency of dicentrics per 100 cells*
			Complete trans-locations	Incomplete translocations	Deleted chromo-somes	insertions		
1	1500	0	0	0	0	0	0	0
2	1500	0	0	0	0	0	0	0
3	1500	3	1	1	1	0	0,59±0,24	0
4	700	1	0	1	0	0	0,63±0,36	0,02±0,02
5	600	1	0	1	0	0	0,74±0,42	0
6	1500	1	0	0	1	0	0	0
7	1245	0	0	0	0	0	0	0
8	1000	2	0	2	0	0	0,89±0,36	0
9	353	1	0	0	1	0	0	0
10	616	5	1	3	1	0	2,89±0,71	0,02±0,02
11	1500	1	0	0	1	0	0	0
12	1500	0	0	0	0	0	0	0,40±0,28
13	1500	1	0	1	0	0	0,30±0,17	0
14	1500	2	1	1	0	0	0,59±0,17	0
15	1500	2	0	0	1	1	0	0
16	566	1	0	0	1	0	0	0
17	1076	0	0	0	0	0	0	0
18	570	0	0	0	0	0	0	0
19	464	0	0	0	0	0	0	0
20	684	1	0	1	0	1	0,65±0,37	0
21	1500	3	2	0	1	0	0,59±0,24	0
Average by group							0,37±0,04	0,02±0,01
Controls							0,11±0,05	0,02±0,01

* – the results are obtained at standard solid (routine) dying of chromosomes

To conclude the present review, we would like to note the following. Epidemiological examinations of the population of polluted zones of Belarus, Ukraine and the Russian Federation indicated stable increase of total morbidity. Thyroid diseases are most frequent. The data from the literature and the authors' results also indicate the increased frequency of cytogenetic disturbances in somatic cells of these cohorts. It is beyond the question that the main reason for development of both processes is induced by the consequences of Chernobyl accident and, first of all, with chronic effects of low doses of irradiation during living in the polluted zones. Though the effect of increased mutability of somatic cells on the human organism is not proved yet, data are increasingly accumulated on a definite contribution of somatic mutations in development of some diseases, such as diabetes, heart diseases, atherosclerosis, pulmonary emphysema, tumors, etc.

As mentioned above, by now clear confirmations of reliable correlation between the frequency of chromatid aberrations and the cancer risk were obtained. As concerns induction of cell malignant change, of special potential hazard are long-living stable aberrations, translocations, in particular. The above-shown results of the current investigation indicate their significant dominance in the polluted zones under consideration. This is the reason why regular investigations of the frequency of cytogenetic disturbances in somatic cells of human populations inhabiting on radionuclide polluted territories are of so high importance. They also may be used as the forecast test for early detection of individuals with increased tumor risk.

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REFERENCES

1. Sorsa M., Wilbourn J., and Vainio H., *Mechanisms of carcinogenesis in Risk Identification*, Lyon, International Agency for Research on Cancer, 1992, pp. 543 - 554.
2. Turusov V.S., *Kantserogenez*, Moscow, Nauchnyi mir, 2000, pp. 251 – 259. (Rus)
3. Wild C.P., Law G.R., and Roman E., *Mutat. Res.*, 2002, vol. **499**(1), pp. 3 - 12.
4. Hagmar L., Brogger A., Hansteen J.L. *et al.*, *Cancer Res.*, 1994, vol. **54**, pp. 2919 - 2922.
5. Hagmar L., Bonassi S., Stromberg U. *et al.*, *Cancer Res.*, 1998, vol. **58**, pp. 4117 - 4121.
6. Fleishman E.V., *Kantserogenez*, Moscow, Nauchnyi mir, 2000, pp. 342 – 360. (Rus)
7. Bond V.P., *Stem cells*, 1995, vol. **13**(Suppl. 1), pp. 21 - 29.
8. Nakano M., Kodama Y., Ohtaki K. *et al.* // *Int. J. Radiat. Biol.*, 2001, vol. **77**(9), pp. 971 - 977.
9. Lucas J.N., Awa A.A., Straume T. *et al.*, *Int. J. Radiat. Biol.*, 1992, vol. **62**, pp. 53 - 63.
10. Tucker J.D., Morgan W.F., Awa A.A. *et al.* // *Mutat. Res.*, 1995, vol. **347**, pp. 21 - 24.
11. Vlasov O.K., God'ko A.M., Shishkanov *et al.*, *The Chernobyl Heritage*, Iss. 3, Kaluga, Oblizdat, 2001, pp. 30 – 38. (Rus)
12. Sevankaev A.V., Kozlov V.M., Guzeev G.G. *et al.*, *Genetika*, 1974, vol. **10**(6), pp. 114 – 120. (Rus)
13. Bloom A.D., Neel J.V., Choi K.W. *et al.*, *Proc. Nat. Acad. Sci. USA*, 1970, vol. **66**(3), pp. 920 - 927.
14. Bloom A.D., Neel J.V., Tsuchimoto T. *et al.*, *Cytogen. Cell Genet.*, 1973, vol. **12**, pp. 175 – 186.
15. Pilinskaya M.A., Shemetun A.M., Dyibsky S.S. *et al.*, *Tsitologiya I Genetika*, 1994, vol. **28**(1), pp. 27 – 32. (Rus)
16. Domracheva E.V., Rivkind N.B., Shklovsky-Kordi I.E. *et al.*, *Problems of Hematology and Blood Transfusion*, 1997, No. 2, p. 12. (Rus)
17. Sevankaev A.V., Tsyb A.F., Lloyd D.C. *et al.*, *Int. J. Radiat. Biol.*, 1993, vol. **63**(3), pp. 361 - 367.
18. Neel J.V., Awa A.A., Kodama Y. *et al.*, *Proc. Nat. Acad. Sci. USA*, 1992, vol. **89**(15), pp. 6973 - 6977.

19. Lazutka J.R., *Mutat. Res.*, 1996, vol. **350**(2), pp. 315 - 329.
20. Pelevina I.I., Aleshchenko A.V., Antoshchina M.M. *et al.*, *Radiobiol. Radioekol.*, 2003, vol. **43**(2), pp. 161 – 166. (Rus)
21. Pochin E.E., *Radiat. Prot. Bull.*, 1988, No. 95, pp. 10 - 12.
22. Mole R.H., *Int. J. Radiat. Biol.*, 1990, vol. **57**(4), pp. 647 - 663.
23. Stepanova E.I., Misharina Zh.A., and Vdovenko V.Yu., *Radiobiol. Radioekol.*, 2002, vol. 42(6), pp. 700 – 703. (Rus)

Cytogenetic effects of low dose radiation in mammalian cells: the analysis of the phenomenon of hypersensitivity and induced radioresistance

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ABSTRACT

The induction of cytogenetic damage by irradiation of Chinese hamster cells and human melanoma cells within a dose range of 1-200 cGy was studied. The anaphase and metaphase analysis of chromosome damage and micronuclei (MN) test were applied. The hypersensitivity (HRS) at doses below 20 cGy and the increased radioresistance at higher doses (IR) were shown with all cytogenetic criteria for both cell lines. The phenomenon of HRS/IR was reproduced in synchronized as well as in asynchronous populations of Chinese hamster cells. This fact shows that HRS was caused by high radiosensitivity of all cells in the population and can not be explained by any differential sensitivity of cells in different phases of the cell cycle. The increasing radioresistance is probably determined by the triggering of the inducible repair processes in all cells. This conclusion was confirmed by the facts that HRS on dose-effect curves was not revealed after preliminary irradiation with low doses (1-20 cGy) which induces repair processes so that some part of preexistent damage was repaired. It can be concluded that the inducible repair processes similar in their nature underlie both HRS/IR and adaptive response phenomena.

Keywords: Chinese hamster cells, human melanoma cells, low dose, chromosomal aberrations, micronuclei, radiosensitivity, inducible repair

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Evaluation of biological effects of low dose ionizing irradiation is extremely important for prognosis of genetic and carcinogenic radiation risk. At present the task is solved through extrapolation of high dose effects to the low dose range and the results of estimation depend on the model chosen for extrapolation. A characteristic example of contradictions arising on the above-mentioned reason is prognosis of consequences of irradiation of large human population during Chernobyl catastrophe: Gofman [1] predicts 300,000 lethal outcomes from cancer, Laki [2] predicts the decrease on 20,000 cases in mortality from spontaneous cancers.

Non-threshold linear concept assuming the danger of any even the smallest exceeding of natural radiation background nowadays is generally recognized and serves the basis for ICRP recommendations [3, 4]. However numerous experimental data obtained recently contradict clearly with this concept and show the incompetence of linear extrapolation of high dose effects to small ones.

To estimate the biological efficiency of low dose irradiation the frequency of cytogenetic damage is commonly registered namely, induction of chromosomal rearrangements and micronuclei [5 - 10] or malignant transformation [11]. Using of modern precise methods [12] allowed accurate evaluation of the survival of mammalian cells in the range of very low doses [13 - 14] that was impossible to do by routinely used Puck-Marcus method [15]. By all mentioned criteria, complex non-linear dose-effect dependence has been shown in the low dose range characterized by the existence of dose-independent region or even inverse negative relationship on the dose at the range below 1 Gy. As a rule a higher efficiency per dose unit is observed in this range compare with the higher doses. Hypersensitivity (HRS) at low doses was clearly demonstrated using survival assay on more than 26 mammalian cell lines [13, 14, 16, 17].

The existence of HRS at low doses can be explained by several reasons. First possible explanation may be the existence of sub-population of genetically or phenotypically radiosensitive cells. Against the presence of genetically stable sensitive population serves the fact that in cells irradiated in vitro with adaptive dose 1 Gy and then in 24 hours by the same challenge dose HRS is revealed in the same extent as in the cells treated by 1 Gy without pre-irradiation [13]. Such quick restoration of radiosensitive fraction can hardly be expected after its "knockout" by pre-irradiation in the dose of 1 Gy.

Phenotypically, conditioned radiosensitivity can be connected to distribution of asynchronous population in the cell cycle and thus HRS can be the consequence of selective mortality of cells in radiosensitive cycle phase.

However, mathematical model based on the existence of two cell sub-populations with different radiosensitivity predicts that radiosensitive fraction should be approximately 4 % of the whole population and should be 60 times more radiosensitive than all other cells [14]. This looks unlikely while the maximum difference in radiosensitivity of different cycle stages of mammalian cell cycle in vitro does not exceed 10 [18]. Moreover, the same rate of relative biological efficiency is shown on partly synchronized and asynchronous Chinese hamster cells irradiated with X-rays and neutrons that indirectly proves the identical shape of dose-effect curve independent on the distribution of cells among cell cycle phases [13]. Nevertheless as several authors supposed [17, 19] the given arguments do not exclude apriori the fact that HRS at low doses may be the consequence of predominant cell mortality in radiosensitive stage of cell cycle, e.g. G₂/M.

Most investigators however give another explanation of the complex shape of dose-effect curve: HRS reflects high radiosensitivity of all cells on the base of constantly functioning constitutive repair; increase of radioresistance of cell population is a result of switching on of inducible repair at the definite level of cell damage [13-21]. It means that biological response to irradiation changes with the dose so that extrapolation of high dose effects to low dose range is incorrect.

The goal of the present investigation was to study the dose dependence of cytogenetic damage induction and analysis of its mechanisms on two mammalian cell lines. Anaphase and metaphase analysis of chromosomal aberration were applied as well as micronucleus test. Experiments were conducted on asynchronous and synchronized cell populations.

MATERIALS AND METHODS

V-79 Chinese hamster cells and BRO human melanoma line were studied. For anaphase and micronucleus assays the exponentially growing populations of both cell lines were irradiated. In details the culture conditions and slide preparation are described elsewhere [22, 23]. In our experiments the duration of V79 cell cycle was about 12 hours, of human melanoma cells – 20-24 hours.

γ -rays ⁶⁰Co at dose rate 18 cGy/min were used for irradiation. For anaphase method V79 and BRO cells were fixed at 8 and 11 hours postirradiation

correspondingly, for micronucleus test – at 20 and 30 hours after single irradiation. The percentage of cells with bridges and acentric fragments or the amount of cells with MN per 1,000 cells was determined. For each dose 4 dependent samples were used and several thousands anaphases and not less than 10,000 interphase cells for MN test were analyzed.

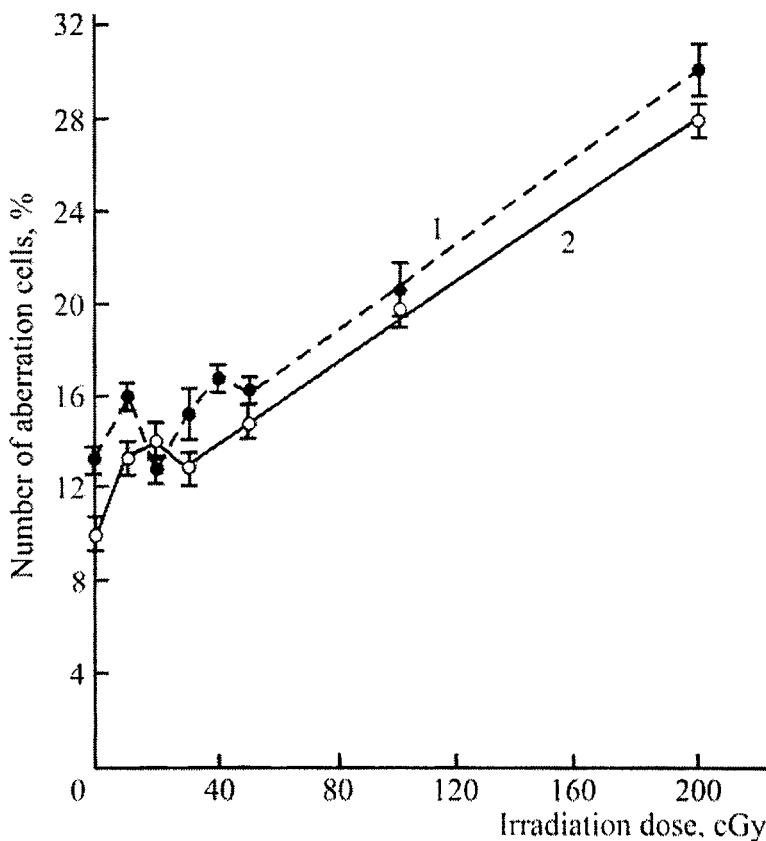


Figure 1. Dependence of frequency of aberrant cells on irradiation dose.
1 – human melanoma cells; 2 – Chinese hamster cells.

The analysis of chromosomal aberrations in metaphase cells was done on synchronized Chinese hamster cells. Synchronization was performed using mitotic shake-off method based on selective mechanical detachment of dividing cells from monolayer. 2×10^7 cells were seeded in roller bottles 750 cm^2 and cultured for 24 hours. The mitotic cells were collected in 25-minute intervals,

centrifuged, and $(2\text{-}4) \times 10^5$ cells were seeded in cultural flasks. In 2 hours after seeding when according to the flow cytometry data not less than 95 % cells were in G₁-phase, the cells were irradiated with X-rays (250 keV, 20 mA, filter 1 mm Cu and 1 mm Al, dose rate 20 cGy/min). 12-14 hours after irradiation cells were fixed preceded by 2 hours of colcemide treatment (0.1 mkg/ml). Slide preparations and staining were done according to the standard procedure. 200-500 metaphases were analyzed per dose while for low dose range (1-50 cGy) not less than 1,000 cells were recorded per dose. All slides were coded and analyzed blind.

RESULTS AND DISCUSSION

Figure 1 shows the dependence of amount of aberrant cells on irradiation dose in asynchronous populations of Chinese hamster and human melanoma cells. Dose-effect curves have similar pronounced non-linear character. Percentage of aberrant cells increases in the range 0-10 in human melanoma and 0-20 cGy in Chinese hamster cells (HRS region), then it decreases rapidly. Percentage of cells with aberrations in melanoma cells at the dose of 20 cGy is lower than in control, i.e. inverse negative relationship on dose takes place. Dose dependence becomes linear with dose increasing over 30-50 cGy (IR), and the slope of the curve decreases significantly in comparison to initial one.

These curves show the reaction to irradiation of cells divided at 8 and 11 hours post-irradiation (Chinese hamster cells and human melanoma, correspondingly). The amount of cells with MN was recorded twice: on the same preparations used for anaphase analysis (Figure 2a) reflecting the cytogenetic damage in cells divided at this time (8 and 11 h, correspondingly); and at 20 and 30 hours post-irradiation when nearly all cell of population divided thus, the reaction of the whole population can be estimated (Figure 2b). MN test reveals the same features as chromosomal aberration analysis: HRS is clearly expressed at early time after irradiation especially in melanoma cells, then inverse negative relationship on dose (melanoma cells) or dose-independent region (Chinese hamster cells) takes place followed by linear increase of effect with the final slope of the curve in an order lower than the initial one in HRS region.

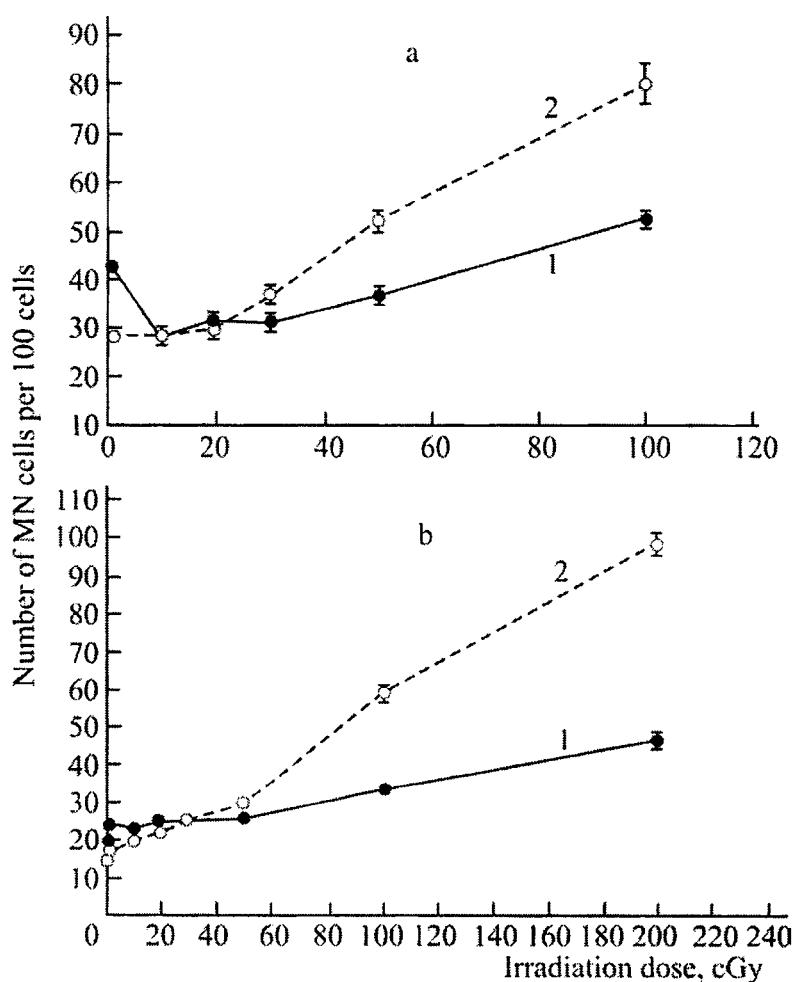


Figure 2. Dose dependence of frequency of micronucleated cells after irradiation in human melanoma cells (a) and Chinese hamster (b).
 a: 1-11 hours after irradiation; 2-30 hours after irradiation;
 b: 1-8 hours after irradiation; 2-20 hours after irradiation.

Similar dose dependences were received on induction of chromosomal aberrations in synchronized Chinese hamster cells using metaphase method (Figure 3, curves 1 and 2). The metaphases of the first postirradiation cycle were analyzed to evaluate the percentage of aberrant cells and the amount of

aberration per 100 cells. In the latter case the most pronounced non-linearity of dose-effect curves was revealed (Figure 3, curve 2).

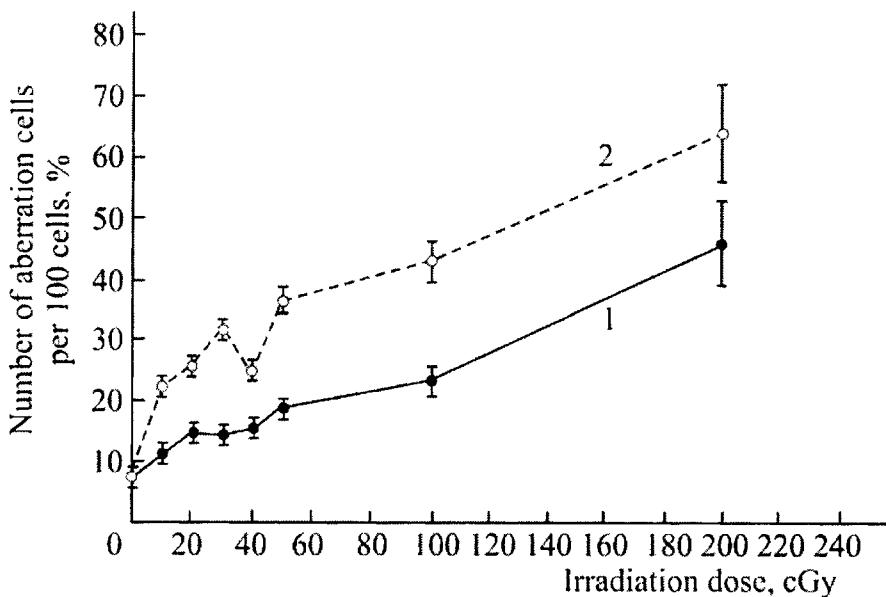


Figure 3. Percentage of cells with chromosome aberrations (1) and amount of aberrations for 100 cells (2) after irradiation of synchronized cells of Chinese hamster

The shape of the curves coincides pretty well with those obtained by anaphase and MN assays on asynchronous cells. The fact that HRS phenomenon is demonstrated both in asynchronous and synchronized cell populations confirms the idea that it is caused by the high radiosensitivity of the whole cell population in the narrow range of low doses and is not connected to mortality of definite cell fraction being in radiosensitive stage of the cell cycle. With dose increase all cells become more radioresistant probably because of repair processes induction. Thus, another more realistic explanation of non-linearity of dose-effect curves and higher efficiency of irradiation per dose unit at low dose range is that at certain level of DNA damage inducible repair systems are switched on causing the decrease of curve's slopes, i.e. increase of cell radioresistance.

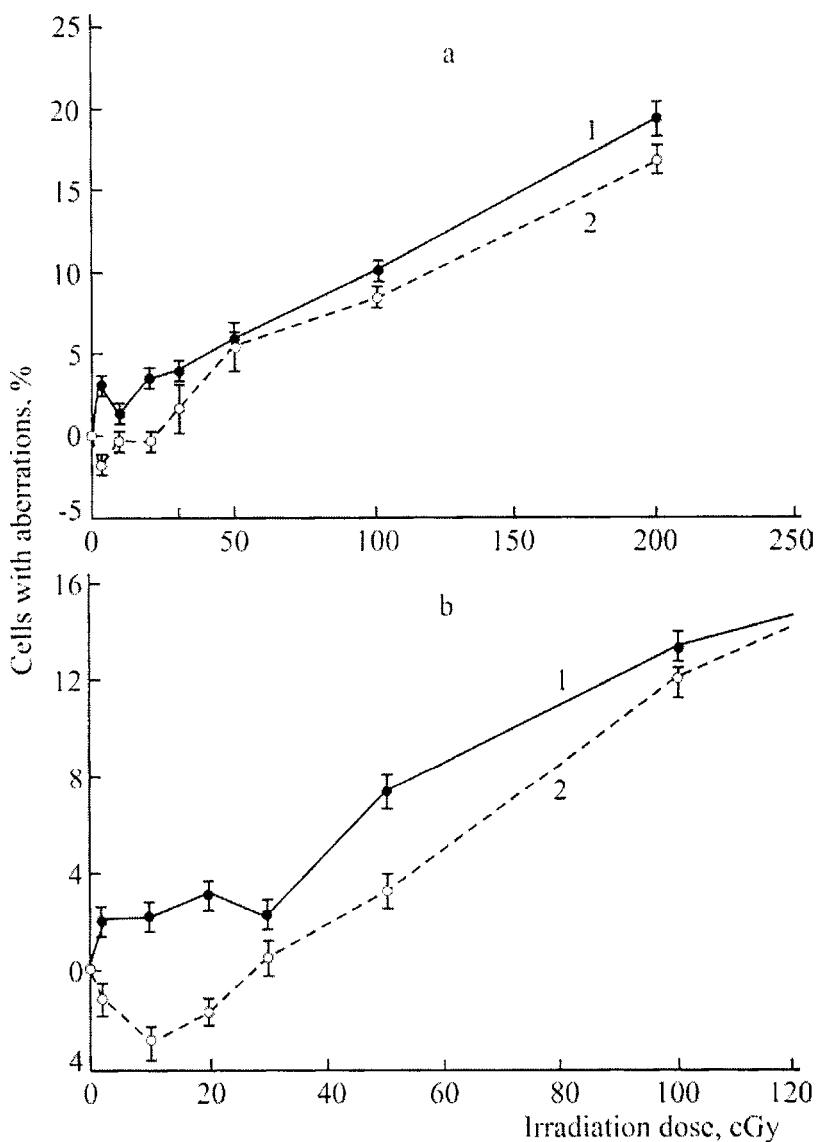


Figure 4. Dose dependence of chromosome aberrations induced in Chinese hamster cells (a) and human melanoma (b) by single irradiation (1) and pre-irradiated by adaptive doses (2).

It is probable that HRS/IR phenomenon after single irradiation is similar in its nature to adaptive response (AR) observed after double irradiation and that is also connected with inducible repair systems [24-28]. If this hypothesis is true, than the shape of dose-effect curves should change due to induction of repair processes by pre-irradiation – HRS region should not be displayed. To check this assumption Chinese hamster cells and human melanoma were pre-irradiated with doses optimal for AR induction – 20 and 1 cGy, correspondingly [29], and in 5 hours - with challenge doses 1-200 cGy. The results are presented on figure 4. As a zero point the frequency of aberrations in control cells was chosen for single exposure (fig. 4, a, b, curves 1), and the amount of aberrant cells induced by adaptive dose for double exposure (fig. 4, a, b, curves 2). In both cell lines HRS is observed in the range 0-1 cGy after single exposure (fig. 4, a, b, curves 1). IR in Chinese hamster cells is observed at the doses \geq 50 cGy. In human melanoma cells chromosome damage is practically independent on dose in the range of 1-30 cGy; IR is observed at the doses over 30-50 cGy. Dose dependence of induction of aberrant cells with pre-irradiation (fig. 4, a, b, curves 2) has no HRS region at all, moreover, there is a decrease of aberrant cell percentage below zero level, i.e. restoration of some part of pre-existing (spontaneous or induced by the adaptive dose) chromosomal rearrangements. This decrease is most pronounced in human melanoma cells. After double exposure IR is expressed in Chinese hamster cells starting from 70 cGy, in melanoma cells – from 10 cGy. It proves that IR processes in human melanoma cells “work” more effectively than in Chinese hamster cells that coincides with results received earlier in AR study on these cell lines.

Thus from the data presented here the relation between HRS/IR after single exposure and AR is assumed. The use of synchronized Chinese hamster cells allows us to exclude the assumption that HRS is caused by high radiosensitivity of small cell fraction in a certain cell cycle stage; it is more probably the result of high radiosensitivity of the whole cell population. When the definite level of chromosome damage is reached the radioresistance of all cells increases. It is logic to suppose that the same mechanisms underlying the increase in radioresistance after single exposure are responsible for AR. As it was established AR is the consequence of repair processes induction by low doses making cells less sensitive to further irradiation [24-28, 30]. The fact that HRS is not displayed in the dose-effect curve in cells pre-irradiated by low doses gives the reason to say that repair mechanisms induction by the adaptive dose protects initially radiosensitive cells increasing their radioresistance. It proves that inducible repair processes similar in their mechanisms underlie both

HRS/IR and AR phenomena. The further investigation of signal pathways and molecular mechanisms [30-32] of inducible processes may open new possibilities for irradiation protection, increase of effectiveness of cancer radiotherapy and estimation of genetic risk of irradiation.

REFERENCES

1. Gofman D.P., *Chernobyl accident: radiation consequences for present and future generations*, Minsk: Vysshaya shkola, 1999. (Rus)
2. Luckey T.D., *Radiation Hormesis*, Boca Raton, Florida, USA: CRS Press, 1991.
3. International Commission on Radiation Protection. 1990 Recommendation of the ICRP, Pub. 60. Annals of the ICRP, vol. 21(1-3), Oxford: Pergamon Press, 1991.
4. *Radiation Safety Standards (RSS-99)*, Moscow: Health Ministry of Russia, 1999. (Rus)
5. Pohl-Rulling J., Fisher P., Haas O. et al., *Mutat. Res.*, 1983, vol. 110, pp. 71 - 82.
6. Lloyd D.C., Edvards A., Leonard A. et al., *Int. J. Radiat. Biol.*, 1992, vol. 61, pp. 335 - 343.
7. Sevanjkaev A.V. and Luchnik N.V., *Genetika*, 1977, vol. 35(3), pp. 524 - 532. (Rus)
8. Gerasjkin S.L., *Radiats. Biol. Radioekol.*, 1995, vol. 35(5), pp. 563 - 571. (Rus)
9. Zaichkina S.L., Aptikaeva G.F., Akhmadieva A.Kh. et al., *Radiobiologia*, 1992, vol. 32(1), pp. 38 - 41. (Rus)
10. Mitchell J.C. and Norman A., *Int. J. Radiat. Biol.*, 1987, vol. 52, pp. 527 - 535.
11. Oftedal P., *Proceeding of the Workshop on Genetic Effects of Charged Particles*, Dubna, 1990, pp. 11 - 13. (Rus)
12. Palcic B. and Jaggi B., *Int. J. Radiat. Biol.*, 1986, vol. 50, pp. 345 - 352.
13. Marples B. and Joiner M.C., *Radiat. Res.*, 1993, vol. 133, pp. 41 - 51.
14. Lambin P., Fertil B., Malaise E.P. et al., *Radiat. Res.*, 1994, vol. 38, pp. 32 - 36.
15. Puck T.T. and Marcus P.J., *J. Exp. Med.*, vol. 103, pp. 653 - 666.

16. Wounter B.J. and Skarsgard L.D., *Radiat. Res.*, 1997, vol. **148**, pp. 435 - 442.
17. Short S., Mayes C., Boulton P. *et al.*, *Int. J. Radiat. Biol.*, 1999, vol. **75**, pp. 1341 - 1348.
18. Sinclair W.K., *Curr. Top. Radiat. Res.*, 1972, vol. **7**, pp. 264 - 285.
19. Lambin P., Marples B., and Fertil B., *Int. J. Radiat. Biol.*, 1993, vol. **63**, pp. 639 - 650.
20. Skov K.A., *Mutat. Res.*, 1999, vol. **430**, pp. 241 - 253.
21. Joiner M.C., Marples B., Lambin P. *et al.*, *Int. J. Radiat. Oncol. Biol. Phys.*, 2001, vol. **49**, pp. 379 - 389.
22. Shmakova N.L., Fadeeva T.A., Krasavin E.A. *et al.*, *Nukleonika*, 1999, vol. **44**, pp. 539 - 548. (Rus)
23. Shmakova N.L., Fadeeva T.A., and Krasavin E.L., *Radiats. Biol. Radioekol.*, 1998, vol. **38**(6), pp. 841 - 847. (Rus)
24. Olivieri G., Budicote J., and Wolff S., *Science*, 1984, vol. **223**, pp. 549 - 597.
25. Wienke J.K., Afzal V., Olivieri S. *et al.*, *Mutagenesis*, 1986, vol. **1**, pp. 375 - 380.
26. Wolff S., Afzal V., Wienke J. *et al.*, *Int. J. Radiat. Biol.*, 1988, vol. **53**, pp. 39 - 48.
27. Shadley J.D. and Wolff S., *Mutagenesis*, 1987, vol. **2**, pp. 95 - 96.
28. Shadley J.D. and Wienke J., *Int. J. Radiat. Biol.*, 1988, vol. **56**, pp. 107 - 118.
29. Shmakova N.L., Abu Zeid O., Fadeeva T.L. *et al.*, *Radiats. Biol. Radioekol.*, 2000, vol. **40**(4), pp. 405 - 409. (Rus)
30. Stecca C. and Gerber G.B., *Biochem. Pharmacol.*, 1998, vol. **55**, pp. 941 - 951.
31. Hendrikse A.S., Hanter A.J., Keraan M. *et al.*, *Int. J. Radiat. Biol.*, 2000, vol. **76**, pp. 11 - 21.
32. Sadecova S., Lehnert S., and Chow T.J.K., *Int. J. Radiat. Biol.*, 1997, vol. **72**, pp. 653 - 660.

A possible mechanism for the adaptive response induced in mammalian cells by low doses of radiation

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ABSTRACT

The mechanism offered in this paper is purposed to explain the Radiation-induced Adaptive Response (RAR) in mammalian cells. The start point of RAR in the frame of this mechanism is activation of receptors for growth factors due to increasing the microviscosity of the plasma membrane containing the oxidative damage. The main components of the mitogen-activated signal transduction pathway participating in the subsequent processes are protein kinases PKC, MAPK and JNK. They carry out a posttranslation modification of the DNA metabolism enzymes and of the transcription factors p53 and c-Jun/AP-1, and among the c-Jun/AP-1 and p53 targets there are genes taking part in the excision repair and apoptosis. C-Jun/AP-1 and p53 can turn out to be direct participants at the stage of excision repair when the DNA damage is recognized. Thus, the proposed scheme of events removes the contradiction between two hypotheses which explain RAR, i.e. between the intracell DNA repair induction and cell selection in the culture of mammalian cells.

Keywords: radioadaptive response, plasma membrane oxidative damage, receptors for growth factors, signal transduction, transcription factors c-Jun/AP-1 and p53.

INTRODUCTION

The Radiation-induced Adaptive Response (RAR) is a decrease of the damage effect of high doses of radiation in cells pretreated by a low (relatively nontoxic) dose of ionizing radiation with a low linear energy transfer (LET) (γ -, X-rays, β -particles with different energy from incorporated isotopes ^3H , ^{14}C and ^{32}P). RAR has been observed in many types of mammalian cells: human lymphocytes, established cell lines and cells of several mouse tissues [1-3]. It is defined by the induction of DNA breaks, chromosome aberrations, mutation, micronuclei and cell killing. The adaptation is a rapid process which reaches its maximum in 4-6 hours after irradiation and is going on for several hours, days [1], months [4, 5] and even one year [6].

Though there are many publications dedicated to RAR of mammalian cells, the molecular mechanism and signaling pathway, which effect the regulation of RAR, have not been determined yet. The basic hypotheses explaining RAR are either intracell DNA repair induction after a low-dose radiation exposure [1], or cell selection in the culture of mammalian cells [7-9]. In favor of each hypothesis there is a corresponding experimental confirmation, but there are no arguments which of them to prefer.

The origin of the sensitive target where the damage initiates RAR, has not been defined yet. Experiments with restrictases have shown that the DNA damage may turn out to be a start point for RAR [1]. However, there are some objections to this hypothesis and the thesis that RAR is initiated by the damage of cell membranes, is supported in [2], where it is emphasized that only the start point of RAR depends on this damage, but a specific scheme of subsequent events leading to the induction of radioresistance is beyond the frame of this publication. To establish this scheme is important from the scientific point of view as well as from the point of view of practical application.

By the present moment the total set of enzymes participating in this process has not been fully studied yet. The aim of this paper is to summarize the available information and to trace the pathway of induction of radioresistance of the mammalian cells after their exposure to a low dose of radiation from the appearance of plasma membrane damage up to the DNA repair activation or cell selection.

PLASMA MEMBRANE OXIDATIVE DAMAGE AND RECEPTORS FOR GROWTH FACTORS ACTIVATION

It is known that two phenomena: response of cells on the influence of growth factors and response on the influence of low doses of radiation, are very similar [2]. Both of them can stimulate the proliferation activity of mammalian cells of various types. The stimulatory effect has been observed at the same dose range as RAR. So, it is reasonable to use this parallel as a key to understand the origin of RAR.

The stimulatory effect of low doses of radiation has been explained by the modification of the receptors for growth factors on the cell surface [10]. Such a modification is a dimerization of homologous subunits of the receptor and subsequent activation of the protein tyrosine kinases (PTK) contained within them, that is usually considered to accompany the receptors activation by the appropriate ligand [11]. Besides the attachment of the appropriate ligand on the receptor, there is different physical and chemical influence which induces formation of the homodimer and, finally, activates the receptor [12]. This influence is expected to facilitate the interprotein crosslinks and/or to increase the microviscosity of the membrane that results in changing the diffuse of neighboring receptor subunits.

It is known that the microviscosity of the plasma membrane increases if there is the oxidative damage in it caused by the exposure of cells to the low doses of radiation which induce RAR [13]. This damage is cumulative and is not repaired [13]. The cells irradiated in the absence of oxygen do not show any membrane oxidative damage [13], and the cells irradiated under the conditions of hypoxia, do not demonstrate RAR [5].

The calculations have shown that, probably, the triggering signal for the RAR program is realized in such a cooperative system as the cell membrane [14]. Actually, the cooperative properties of the membrane clearly manifest themselves while changing the microviscosity of the membrane caused by the lipid oxidative damaging.

The lipid oxidative damaging stimulates the formation of hydrogen peroxide leading to the decrease of fluidity of the membrane [15]. The low concentration of hydrogen peroxide stimulates the cell division [16, 17] and protects the cell against the X-rays influence following [18].

All the mentioned above confirms the idea that the damage of the plasma membrane could induce RAR. Moreover, the activation of receptors for growth factors could be a start mechanism for RAR.

MITOGEN-ACTIVATED SIGNAL TRANSDUCTION PATHWAY AND INDUCTION OF RADIRESISTENCE

Since the activation of receptors for growth factors may turn out to be a start mechanism for RAR, it should be expected that components of mitogen-activated signal transduction pathway take part in the subsequent processes leading to radioresistance induction.

The mitogen-activated signal transduction pathway begins from the binding of different proteins with specific tyrosine autophosphorylation sites on the receptor-PTKs and from subsequent activation of these proteins, phosphoinositide 3OH-kinase (PI3K), phosphoinositidase C (PIC), Ras and some others among them [11]. The amount of (3,4,5)-phosphorylated phosphoinositide ($\text{PI}(3,4,5)\text{P}_3$) and diacylglycerol (DG) produced by PI3K and PIC, correspondingly, grows within few seconds after activation of the receptor. These products activate protein kinase C (PKC) [11]. PKC regulates the cell metabolism by means of transcription factor c-Jun/AP-1 (activator protein-1) [19]. Protein c-Fos mediates transduction of the signal from PKC to c-Jun/AP-1 [20, 21].

Increasing of the c-Fos [22], c-Jun/AP-1 [23] and PKC [24] activity was observed in the mammalian cells exposed to low doses of radiation. Expression of the genes encoding c-Fos and PKC has been induced only in the cells irradiated with a low LET [25]. It is in agreement with the experimental data which have demonstrated that high-LET radiation is not able to induce RAR in mammalian cells [1, 2].

Attachment of Ras to the activated receptor initiates the cascade of successive activations of several protein kinases, including the activation of mitogen-activated protein kinase (MAPK). This cascade is completed with the activation of c-Jun N-terminal kinase (JNK) [26]. JNK belongs to the family of homologous enzymes activated by the cellular stress [27]. There are no data obtained either on the induction of JNK in the cells irradiated with low doses or on participation of JNK in RAR. Nevertheless, it is known that the influence of cycloheximide during the first two hours after adapting irradiation may strengthen

RAR [28]. The cells exposed to hypertermia [3] or treated by cytokines become more radioresistive [29]. The influence of hypertermia, cytokines or cycloheximid stimulates the JNK activity [27]. So, it is possible to suppose that JNK activation is an essential stage in the process of induction of radioresistance.

Recently the involvement of PKC and MAPK in regulation of RAR has been shown in the experiments [30]. Moreover, it has been shown that MAPK physically associates with PIC participating in the induction of RAR in frame of the mechanism offered in this paper. The heat-shock protein Hsp70 participates in RAR while the PKC activity increasing when the expression of Hsp70 grows [32]. The involvement of PKC in signaling for RAR has been confirmed by the observation that RAR is totally blocked by PKC inhibitors and that a small dose of phorbol ester, an activator of PKC, imitates the pretreatment by low-dose X-rays [31].

The authors of the review [33] on possible mechanisms for RAR have also postulated that the initial elements could be various kinases, such as PKC and JNK. However, some years ago there was a lack of information about enzymes whose activity increases after the low dose irradiation. In the light of the latest data it seems possible to follow all the steps of RAR induction mechanism and this is the purpose of the chapters below.

CELL FUNCTIONS OF PKC, C-JUN/AP-1 AND THEIR SUBSTRATES

The activated PKC is translocated from cytoplasma to the cell nuclear [34], where it is associated with the nuclear matrix [35]. PKC performs the posttranslational modification of DNA polymerase α , topoisomerase I, histone H1 [36] and of the enzymes regulating the transcription, specifically p53 [37], APE-1 [38] and c-Fos [20]. What is the role of these substrates of PKC in the induction of cell radioresistance?

Topoisomerase I can facilitate the function of DNA repair enzymes through decreasing of the DNA supercoiling, i.e. of decompactization of chromatin [39, 40]. The involvement of topoisomerase I in the repair of DNA radiation damage was carried out in [41]. It is known that phosphorylated histones like other phosphorylated proteins become more acid. So, the connection of histones with DNA becomes less strong. Since histone H1 packs

chromatin, the relaxation of its connection with DNA should be accompanied by decondensation of chromatin and increasing of cell radioresistance [40].

Probably, DNA polymerase α takes part in the excision DNA repair [42]. It is confirmed by the observation of a close physical contact between DNA polymerase α and poly(ADP-ribose)polymerase (PARP) participating either in DNA breaks monitoring while DNA replication, or in the base excision repair. The involvement of PARP and DNA polymerase α in RAR has been suggested from the observation of a block of RAR either by PARP inhibitor 3-AB or by DNA polymerase α inhibitor Ara-C [1-3].

Also, PK C phosphorylates another enzyme participating in the base excision DNA repair – apurinic/apyrimidinic endonuclease (APE-1) [38]. Moreover, APE-1 regulates the DNA-binding activity of transcription factors p53 [43] and AP-1 [44].

A preliminary post-translation modification of c-Jun/AP-1 is required to activate different c-Jun/AP-1-dependent genes. This modification may include phosphorylation of c-Jun/AP-1 by means of the activated JNK [27] (it is a more probable mechanism for induction of radioresistance by means of JNK activation) and formation of the complex between c-Jun/AP-1 and c-Fos preliminary phosphorylated by PKC [20, 21].

C-Jun/AP-1 is involved in regulation of diversity of genes and in initiation of DNA replication and regulation of cell proliferation, differentiation, apoptosis and induction of tumorigenesis [45]. There is the c-Jun/AP-1-responsive site in the gene encoding p53 [46]. Recently it has been shown that in cells of the lymphoblast cell line TK6 the low-dose radiation exposure induced the elevated p53 level and that RAR with endpoint of cell death took place [47]. The relation of the RAR induction with the existence of intact p53 gene in the cell has been also confirmed in [48].

P53 is in the inactive state in cells and PKC activates its latent function [37]. It leads to the transcription activation of the p53-dependent genes whose products stimulate the DNA repair activity and cause the cell cycle delay to provide the extra time for DNA repair, as well as the induction of apoptosis that eliminates the damaged cells from the population [49]. Actually, the experimental data have shown the induction of the p53 target-genes GADD45 and CDKN1A which participate in DNA repair and the cell cycle delay, correspondingly, after the exposure of mammalian cells to the low dose radiation [50]. A more gradual induction of some other p53-dependent genes – MDM2, BAX and ATF3 – was also observed. Moreover, other experiments have

confirmed that p53 directly takes part in the nucleotide excision repair, probably, while recognizing the DNA damage [51].

It is possible that the c-Jun/AP-1-responsive site is contained in genes encoding enzymes which participate in the base excision repair. Among them there are DNA polymerase β gene and some others also activated by PKC [52]. Moreover, it is known that MAPK activation after attachment of Ras to the activated receptors for growth factors [26] is accompanied by activation of transcription factors (for example, c-Jun/AP-1 is activated through JNK activation by MAPK) and subsequent induction of the DNA polymerase β gene expression. DNA polymerase β is required in mammalian cells for the predominant pathway of the base excision repair involving the DNA synthesis of a single nucleotide gap. The DNA polymerase β is associated with PARP as the DNA polymerase α [42].

There is the c-Jun/AP-1-responsive site in the promoter of the metallothionein (MT) gene [45]. MT mRNA expression or MT synthesis was found to be induced by the exposure of cells *in vitro* or tissues *in vivo* to the ionizing or UV radiation. The induced synthesis of MT is considered as one of the mechanisms involved in RAR [53]. It is involved not only in metal detoxification and homeostasis, but also in scavenging free radicals while the oxidative damage.

Except that c-Jun/AP-1 may participate in RAR through its target genes, c-Jun/AP-1 can take part in the increase of cell radioresistance more directly. Actually, the existence of cooperative interaction between c-Jun/AP-1 and TATA-binding protein (TBP) is known [20]. TBP recognizes the specific supercoiling of TATA-sequence. Similar supercoiling is observed in DNA locuses containing the damage. In response to the influence of the DNA damaging agents the TBP is attached to these sites with high affinity [54]. It leads to a temporary transcription arrest and initiation of the nucleotide excision repair. Thus, c-Jun/AP-1 may take part in the initiation of the excision repair by means of either DNA damage recognizing or of the inhibitory effect on transcription. Actually, it has been shown experimentally that the novel specific c-Jun/AP-1-responsive site in the damaged site of DNA leads to a partial suppression of transcription of the gene containing this site. So, c-Jun/AP-1 may function either as an activator or inhibitor of the transcription [55].

C-Jun/AP-1-responsive sites are in c-jun [20] and c-fos [56] genes. Intensification and prolonged conservation of the radioresistance state of cells are caused, probably, by stimulation of the c-jun and c-fos genes expression. Since c-fos gene is negatively regulated by its own product – protein c-Fos [56],

it means that the increase of c-Fos in the cell, finally, should lead to inhibition of the c-fos gene transcription and subsequent decrease of the adaptive reaction. The negative feedback regulation is often a physiological mechanism for gradual switching off various cellular processes, SOS-repair of bacteria among them [57].

The half life-time of c-Jun/AP-1 is about 6 hours [20]. It corresponds to the conservation of the adapted cell state during several hours or cell cycles that depends on life-time and amount of the enzymes synthesized in the result of activation of transcription by c-Jun/AP-1. If c-Jun/AP-1 leads to synthesis and/or activation of cell proteins, for example, various receptors or growth factors which cause long-lasting or even irreversible changes in cells, then the adapted state should be also preserved during a long period of time, for example, several months [4, 5] or one year [6]. Actually, it has been demonstrated that the gene encoding mitogen heparin-binding epidermal growth factor-like growth factor (HB-EGF) of fibroblast and epithelial cells, contains a composite c-Jun/AP-1/Ets site [58].

COMMON SCHEME OF VARIOUS RAR MECHANISMS

Let us try to demonstrate that the proposed scheme could lead to realization of either proposed mechanisms for RAR – induction of DNA repair, or cell selection in culture (see the Figure).

Several authors suppose that the populations of mammalian cells are heterogeneous with respect to radiosensitivity [8, 9]. If it occurs, more radiosensitive cells (or subpopulation of slowly repaired cells) should be killed after the first “adapting” irradiation. The rest of the cells should imitate the view of increased radioresistance. The form of death of radiosensitive cells is supposed to be apoptosis because it is the form resulted from deleterious influence that was not strong enough to cause coagulation changes in cells [59]. This supposition is in agreement with observation of the increased amount of apoptotically died cells after adapting irradiation [7].

As we have seen from the mentioned above, c-Jun/AP-1 activates transcription of the p53 gene [46] inducing apoptosis, and PKC performs post-translation activation of p53 [37]. PKC is activated by DG – the product of PIC that was activated through the activation of receptors for growth factors [11]. Another PIC product – Ins(3,4,5)P₃ – regulates releasing of Ca²⁺ from intracell

compartments. Increasing of concentration of free intracell Ca^{2+} activates the endonuclease degrading DNA, it is the key event of apoptosis [26].

Thus, activation of receptors for growth factors leads to apoptosis. It seems strange that receptors for the activation of cell proliferation lead to the programmed cell death. However, up to now the signal transduction pathway specific for apoptosis has not been found. Actually, cascades leading to apoptosis are activated through various cell physiological processes, for example, proliferation and differentiation. It is related with the following: the apoptosis is the most probable “physiological” death providing elimination either of the cells produced in excess, or of the cells incorrectly developing, or of the cells with damaged DNA [26]. If the most radiosensitive and, consequently, the most damaged cells of population receive stimulus to proliferation, then it could lead to the excess production of abnormal cells in population. It would be more preferable for the population to eliminate these cells by apoptosis.

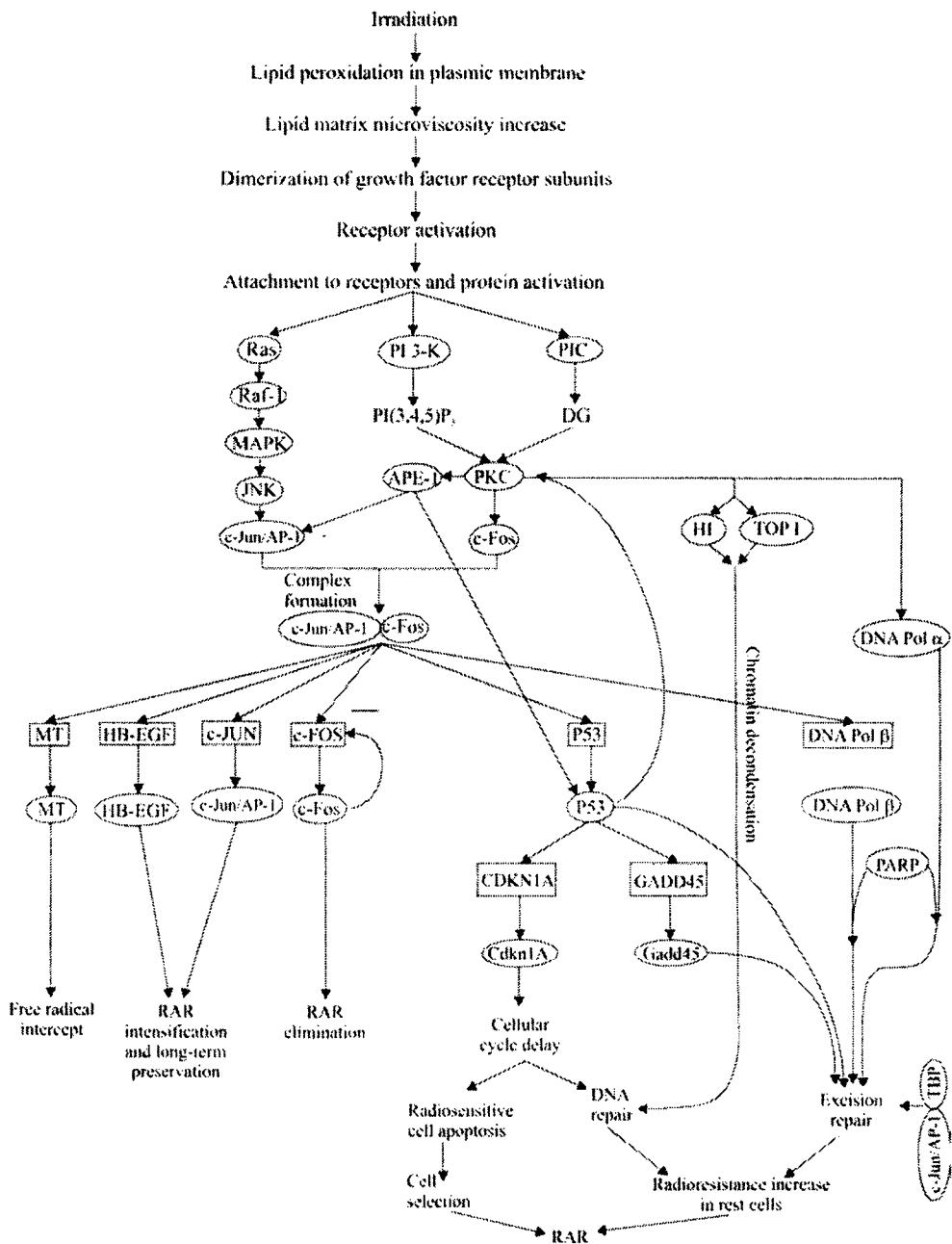


Figure. The scheme of cascade of events started in the low-dose irradiated mammalian cells, which leads to RAR induction. The regulator proteins are shown as ellipses, the genes - as rectangles. The arrows denote one of the following: successive activation of corresponding membrane processes; post-translation activation of one protein by another; activation of gene transcription; synthesis of corresponding proteins.

In the rest cells of the population the activation of receptors for growth factors promotes the reparative ability increasing. It is reached by several ways. Thus, decondensation of chromatin through phosphorylation of topoisomerase I and histone H1 by PKC facilitates the function of DNA repair enzymes. P53-dependent cell cycle delay provides some extra time for DNA repair. The activation of APE-1, DNA polymerase α , DNA polymerase β , p53 and of c-Jun/AP-1, directly participating in the excision DNA repair (see above), proves that the excision repair plays the key role in the induction of RAR. It is actually confirmed by experimental data [60-63].

A specific mechanism for RAR of mammalian cells depends on many factors. Among them, probably, there is a stage of cell cycle, or of proliferation, or of differentiation when the cells in the population have already been irradiated. Also, heterogeneity of the cell population in regard to these stages contributes to a concrete scheme of RAR.

It is known that intensity of oxidative damaging of the membrane lipids and fluidity of the membrane depend either on the stage of the cell cycle, or on the level of the cell differentiation, or on the cell age [64]. Since "old" cells are more resistant to oxidative damaging of the membrane lipids [13], then in the frame of the mechanism proposed in this work they can not exhibit RAR. Actually, RAR was absent in lymphocytes from donors older than 65 years [65].

Expression of c-fos and c-jun genes depends on the stage of the cell growth and differentiation [66]. Moreover, c-fos gene is negatively regulated by its own product – c-Fos protein [56]. So, the concentration of c-Fos in the cell during irradiation influences the activation of the transcription factor c-Jun/AP-1 and, finally, the expression of the genes regulated by c-Jun/AP-1. Also, association of PARP with DNA polymerase α depends on the cell cycle stage [42].

CONCLUSION

The mechanism explaining RAR initiation in mammalian cells is offered after analysis of the experimental data. Activation of receptors for growth factors by means of radiation-induced plasma membrane damaging is considered to be a start point of RAR. The key enzymes leading to RAR are likely to be transcriptions factors c-Jun/AP-1 and p53. Cytoplasmatic protein kinases (MAPK, JNK, PKC) are the key mediators taking part in the transduction of the

signal from the activated receptors to these factors. C-Jun/AP-1 and p53 participate in apoptosis and excision DNA repair either directly or through their target genes. Thus, the proposed scheme of events removes the contradiction between two hypotheses which assume that intracell DNA repair induction or cell selection in the culture of mammalian cells may cause RAR.

Of course, the proposed scheme can not be taken as the final design of the mechanism for induction of RAR. It requires further investigations.

REFERENCES

1. Wolff S., *Environ. Health. Perspect.*, 1998, vol. **106**, Suppl. 1, pp. 277 - 283.
2. Eidus L.Kh., *Radiats. Biol. Radioekol.*, 1996, vol. **36**(6), pp. 874 - 882. (Rus)
3. Zasukhina G.D., *Radiats. Biol. Radioekol.*, 1999, vol. **39**(1), pp. 58 - 63. (Rus)
4. Klokov D.Ju., 'Regulations of the generation of the radiation adaptive response in mice bone marrow cells in vivo', *Doctor Thesis*, Moscow, 2000. (Rus)
5. Semenez T.N., Semina O.V., and Saenko A.S., *3rd Congress on Radiation Research. Putschino Report Thesis*, vol. **1**, 1997, pp. 163 - 164. (Rus)
6. Giljano N.Ja., Bolshakova O.I., Bikineeva E.G., and Noskin L.A., *Radiats. Biol. Radioekol.*, 1999, vol. **39**(5), pp. 543 - 547. (Rus)
7. Giljano N.Ja., Bolshakova O.I., Lavrova G.A. *et al.*, *Radiats. Biol. Radioekol.*, 1998, vol. **38**(5), pp. 663 - 671. (Rus)
8. Spitkovsky D.M., *Radiobiologia*, 1992, vol. **32**(3), pp. 382 - 400. (Rus)
9. Wojcik A., Sauer K., Zolzer *et al.*, *Mutagenesis*, 1996, vol. **1**(3), pp. 291 - 296.
10. Kublik L.N., Eliseeva L.N., and Korystov Ju.N., *Radiats. Biol. Radioekol.*, 1993, vol. **33**(3b), pp. 874 - 878. (Rus)
11. Stephens L.R., Jackson T.R., and Hawkins P.T., *Biochim. Biophys. Acta*, 1993, vol. **1179**, pp. 27 - 75.
12. Herrlich P., Bender K., Knebel A. *et al.*, *C. R. Acad. Sci. III*, 1999, vol. **322**(2-3), pp. 121 - 125.
13. Giusti A.M., Raimondi M., Ravagnan G. *et al.*, *Int. J. Radial. Biol.*, 1998, vol. **74**(5), pp. 595 - 605.

14. Spitkovsky D.M., *Radiats. Biol. Radioekol.*, 1995, vol. **35**(3), pp. 346 - 348. (Rus)
15. Kameda K., Imai M., and Senjo M., *New Horizons Lipid Biochem.*, Ed. J. Imai, Sapporo, 1984, pp. 211 - 217.
16. Murrell G.A.C., Francis M.J.O., and Bromley L., *Biochem. J.*, 1990, vol. **265**, pp. 659 - 665.
17. Planel H., Caratero C., Croute F. et al., *Low Dose Irradiation and Biological Defensive Mechanisms*, Eds. T. Sugahara, L.A. Sapan, T. Aoyama, Amsterdam: Elsevier, 1992, pp. 13 - 20.
18. Marples B. and Joiner M.C., *Radiat. Res.*, 1995, vol. **141**(2), pp. 160 - 169.
19. Angel P., Allegretto E., Okino S. et al., *Nature*, 1988, vol. **332**, pp. 166 - 171.
20. Angel P., Hattori K., Smeal T., and Karin M., *Cell*, 1988, vol. **55**, pp. 875 - 885.
21. Nakabeppu Y., Ryder K., and Nathans D., *Cell*, 1988, vol. **55**, pp. 907 - 915.
22. Martin M., Crechet F., Ramounet B., and Lefaix J.-L., *Radiat. Res.*, 1995, vol. **141**, pp. 118 - 119.
23. Woloschak G.E. and Chang-Liu C.M., *Cancer Lett.*, 1995, vol. **97**, pp. 169 - 175.
24. Hasan N.M., Parker P.J., and Adams G.E., *Radiat. Res.*, 1996, vol. **145**, pp. 128 - 133.
25. Woloschak G.E. and Chang-Liu C.M., *Int. J. Radiat. Biol.*, 1991, vol. **59**(5), pp. 1173 - 1183.
26. Novikov V.S., Bulavin D.V., and Zygan V.N., *Programmed Cell Death*, Ed. Novikov V.S., Saint-Petersburg, Nauka, 1996, pp. 30 - 50. (Rus)
27. Fritz G. and Kaina B., *J. Biol. Chem.*, 1997, vol. **272**(49), pp. 30637 - 30644.
28. Youngblom JM., Wiencke J.K., and Wolff S., *Mutat. Res.*, 1989, vol. **227**(4), pp. 257 - 261.
29. Neta R., *Radiat. Res.*, 1995, vol. **141**, p. 120.
30. Shimizu T., Koto T., Tachibana A., and Sasaki M.S., *Exp. Cell Res.*, 1999, vol. **251**, pp. 424 - 432.
31. Sasaki M.S., *Int. J. Radiat. Biol.*, 1995, vol. **68**, pp. 281 - 291.
32. Park S.M., Lee S.J., Chung H.Y. et al., *Radiat. Res.*, 2000, vol. **153**(3), pp. 318 - 326.
33. Stecca C. and Gerber G.B., *Biochem. Pharmacol.*, 1998, vol. **55**(7), pp. 941 - 951.
34. Kiss Z., Delli E., and Kuo J.F., *FEBS Lett.*, 1988, vol. **231**(1), pp. 41 - 46.

35. Capitani S., Girard P.R., Berezney R. *et al.*, *Biochem. Biophys. Res. Commun.*, 1987, vol. **142**, pp. 367 - 376.
36. Hallahan DE., Virudachalam S., Schwartz J.L. *et al.*, *Radiat. Res.*, 1992, vol. **129**, pp. 345 - 350.
37. Chung L.F, Cooper R.M., Yan P. *et al.*, *Biochem. Biophys. Res. Commun.*, 1987, vol. **145**(3), pp. 1376 - 1383.
38. Yacoub A., Kelley M.A., and Deutsch W.A., *Cancer Res.*, 1997, vol. **57**, pp. 5457 - 5459.
39. Zbarsky I.B., *The Organization Of Cell Nucleus*, Moscow, Medicine, 1988, 368 p. (Rus)
40. Krutjakov V.M., Belyakova N.V., Kravezkaya T.P., and Naryzhny S.N., *Structural And Functional Aspects Of Replication And Repair Of DNA*, Putschino, 1983, pp. 113 - 122. (Rus)
41. Musk S.R. and Steel G.G., *Brit. J. Cancer*, 1990, vol. **62**, pp. 364 - 367.
42. Dantzer F., Nasheuer H.-P., Vonesch J.-L. *et al.*, *Nucl. Acids Res.*, 1998, vol. **26**(8), pp. 1891 - 1898.
43. Jayaraman L., Murthy K.G., Zhu C. *et al.*, *Genes Dev.*, 1997, vol. **11**, pp. 558 - 570.
44. Tomicic M., Eschbach F., and Kaina B., *Mutat. Res.*, 1997, vol. **383**, pp. 155 - 165.
45. Kaina B., Haas S., and Kappes H., *Cancer Res.*, 1997, vol. **57**, pp. 2721 - 2731.
46. Ginsberg D., Oren M., Yaniv M., and Piette J., *Oncogene*, 1990, vol. **5**, pp. 1285 - 1290.
47. Hendrikse A.S., Hunter A.J., Keraan M. *et al.*, *Int. Radiat. Biol.*, 2000, vol. **76**(1), pp. 11 - 21.
48. Wang B., Ohyama H., Haginoya K. *et al.*, *Radiat. Res.*, 2000, vol. **154**(3), pp. 277 - 282.
49. Hartwell L. and Kastan M., *Science*, 1994, vol. **26**, pp. 1821 - 1827.
50. Amundson SA., Do K.T., and Fornace A.J., *Radiat. Res.*, 1999, vol. **152**, pp. 225 - 231.
51. Smith M.L., Chen I.-T., Zhan Q. *et al.*, *Oncogene*, 1995, vol. **10**, pp. 1053 - 1059.
52. Chen K.-H., Yakes F.M., Srivastava D.X. *et al.*, *Nucl. Acids Res.*, 1998, vol. **26**(8), pp. 2001 - 2007.
53. Cai L., Satoh M., Tohyama C., and Cherian M.G., *Toxicology*, 1999, vol. **132**(2-3), pp. 85 - 98.

54. Vichi P., Coin F., Renaud J.-P. *et al.*, *EMBO J.*, 1997; vol. **16**(24), pp. 7444 - 7456.
55. Tulchinsky E.M., Georgiev G.P., and Lukyanidin E.M., *Dokl. RAN*, 1996, vol. **349**(4), pp. 553 - 556. (Rus)
56. Schontal A., Herrlich P., Rahmsdorf H.J., and Ponta H., *Cell*, 1988, vol. **54**, pp. 325 - 334.
57. Little J.W. and Mount D.W., *Cell*, 1982, vol. **29**, pp. 11 - 22.
58. Park J.M., Adam R.M., Peters C.A. *et al.*, *Amer. J. Physiol.*, 1999, vol. **277**(2, Pt. 1), pp. 294 - 301.
59. Majno G. and Joris I., *Amer. J. Pathol.*, 1995, vol. **146**(1), pp. 3 - 15.
60. Ikushima T., *Mutat. Res.*, 1987, vol. **180**(2), pp. 215 - 221.
61. Le X.C., Xing J.Z., Lee J. *et al.*, *Science*, 1998, vol. **280**(5366), pp. 1066 - 1069.
62. Ramana C.V., Boldogh L., Izumi T., and Mitra S., *Proc. Natl. Acad. Sci. USA*, 1998, vol. **95**, pp. 5061 - 5066.
63. Kurihara Y., Rienkjkarn M., and Etoh H., *Radiat. Res.*, 1992, vol. **33**, pp. 267 - 274.
64. Buzhurina I.M. and Panova M.A., *Scientific And Technical Results: General Problems of Physical And Chemical Biology*, vol. 3, M.L VINITI, 1986, 258 p. (Rus)
65. Gadhia P.K., *Mutagenesis*, 1998, vol. **13**(2), pp. 151 - 152.
66. Angel P. and Karin M., *Biochem. Biophys. Acta*, 1991, vol. **1072**, pp. 129 - 157.

The peculiar long term cellular alterations under low doses of ionizing radiation

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ABSTRACT

The present work reviews the long-standing results of investigations by authors and literary data. The peculiar form of a tissue post-irradiative reaction characterizing by massive, dose-independent transition of cell populations to the steady state modification with the essential raise of cell damage and cell loss probability as compared with the probability level of the same alterations in control was examined. Some other demonstrations of this type of cellular transformations were described. It was found that the indicated cellular condition occurred both in active and slowly proliferating tissues. The reaction occurred at relatively low doses of irradiation. Some nonmutagenic factors may also induce such effects. The authors' experimental data allow a supposition of epigenetic mechanisms taking part in the induction and preservation of these alterations. The discovered form of the cellular reaction manifested in different biological objects may be considered as a general biological tendency. The importance of the studied reaction for pathogenesis of late consequences of low dose irradiation is discussed.

Keywords: ionizing radiation, low doses, long term cellular effects, unicellular organisms, mammal cells

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The peculiar form of post-irradiative cellular reaction is of interest in the course of the problem of low dose irradiation effects [1 - 7]. It represents a jump-like transition of the cell population or the overwhelming majority of it to a new steady state. Cell damage and cell loss probability increase was found the most typical of the new state. Experimental data testifying about some other demonstrations of such alterations were obtained. The transition to the new steady state occurs already at low doses of irradiation and does not increase with dose. At higher doses alterations remain masked by traditional dose-dependent cellular effects. The alterations under consideration were found quite stable, and state recovery was not observed during the whole period of investigations. Moreover, the effect occurred with no respect to proliferation. The nonspecific feature of the phenomenon for irradiation effect was also indicated. Similar alterations may be caused by radiation-free (nonmutagenic) factors.

Massive alterations at low impacts, dose independence in a broad irradiation range and nonspecific type of mutagens' action are the specific features of the current phenomenon versus typical dose-dependent effects, caused by genetic structure damage. Most probably, the phenomenon is based on epigenetic alterations due to inheritable changes in gene activity rather than their structure [8].

Different objects (amebas, infusorians, yeast, mammal tissues) displayed the same general type of reaction, which is transition to the new steady state that attaches general biological significance to this reaction form.

Long term massive, dose-independent cellular alterations (threshold and dose plateau) still are not considered by the majority of radiobiologists. This may be caused by technical problems in studies of such alterations. They are clearly observed only when traditional cytogenetic cellular effects masking them are minimal. Moreover, independent of the phenomenology, all examples of the cell "memory" of irradiation impacts are traditionally classified to the same type of alterations induced by genome instability [9, 10]. Therefore, a group of dose-dependent effects that may also be initiated by primary DNA damages are added by dose-independent ones of different origin. This suppresses further investigations of the phenomenon and prevents determination of its role in pathogenesis of the late radiation effect.

In this review, on the basis of experimental data, the reality of regularities under considerations is discussed.

UNICELLULAR TESTS

The phenomenon of dose-independent inheritable cell loss increase

This phenomenon was observed in objects possessing different phylogenetic types, the genetic magenta (amebas, infusorians, yeast).

There are experimental data confirming the phenomenon existence [1 – 4]. The most demonstrative results were obtained for amebas (*Amoeba proteus*) and two infusorian species (*Paramecium caudatum* and *Climacostomum virens*), incubated in the form of so-called individual lines in the Lozin-Lozinsky microaquarium [11], when only one of proliferating specimen are remained in each cell (sister cells were quite identical, thus the remaining one was randomly preserved). If the remained cell was lost, the individual line terminated.

As some cells are regularly lost, with no respect to the loss frequency, all individual lines will be lost, because sooner or later a lost cell will be met. That is why the cell loss intensity in the population was judged by the line loss frequency. After relatively low dose impacts, Protozoa were lost via lysis, which was sometimes preceded by alteration of their shape and behavior. Anyway, a day before loss cell seemed to be normal.

As was found, amebas and two species of infusorians displayed unityypical reactions on single irradiation impacts, namely, transition to a new steady state which is cell loss increase versus the control. The effect was detected shortly after the impact and did not decrease with time. Figure 1 shows the results of tests in amebas irradiated by broad range of X-ray doses. The individual line curves indicate some cell loss in control (spontaneous loss). The frequency of ameba loss at irradiation by 0.5, 1, 1.5 and 3G doses is approximately the same. When the threshold doses of 4 - 5 G are reached (they are approximately two orders of magnitude lower than these, traditional cytogenetic irradiation effects in Protozoa), the cell loss frequency is jump-like increased.

It is essential that the cell loss sharply increases with an insignificant dose increase from 3 to 4 – 5 G, but the future effect does not change even with dose increase to 400 G.

In this dose range, the ameba loss increased on the background of visual safety of the main amount of cells. The average duration of the cellular cycle remained equal the control (about 2 days).

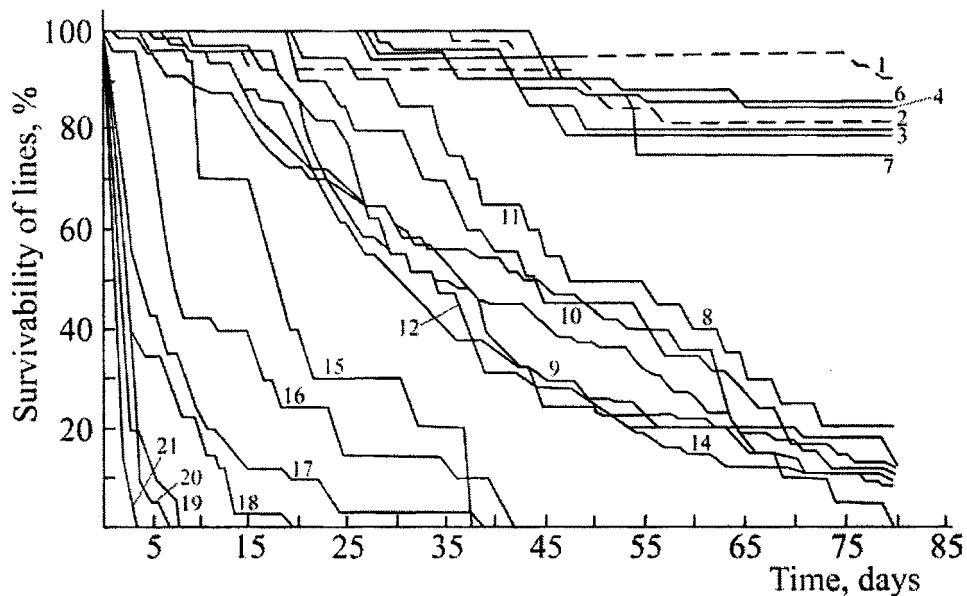


Figure 1. Dynamics of ameba individual line loss in control (dashed lines 1 and 2) and after X-ray irradiation (continuous lines) by doses: 0.5 (3), 1 (4), 1.5 (5), 2 (6), 3 (7), 4 (8), 5 (9), 20 (10), 50 (11), 100 (12), 200 (13), 400 (14), 600 (15), 900 (16), 1,000 (17), 1,200 (18), 1,500 (19), 2,000 (20), and 4,000 (21) G.

The number of individual lines for each curve equals: 100 (1 and 10), 80 (9 and 14), 60 (13), 50 (12), 30 (18 and 19), 20 (2 – 8, 11, 15, 16, 20 and 21)

Clearly, Figure 1 shows that dose-dependent lethal effects are more intensively expressed in amoebas and occur at higher doses (600 G or more). Hence, the so-called “lethal sectoring” [12, 13], reproductive loss and nonproliferated cell loss [14] with the dose increase were observed. Contrary to the above-described effects, these were accompanied by cell proliferation and morphology.

Thus besides traditional forms of irradiation damage of cells determined for individual lines of amoebas, untypical dose-independent effects, jump-like occurring after irradiation impacts low for Protozoa were observed.

Basically, for two species of infusorians the same effects were observed, differing only by cell loss frequency and effective dose values [3, 4]. Statistical confidence of these and other data in tests in Protozoa were also proved [15].

Peculiarity of described alterations allowed a supposition that the effects are induced by products of culture aqueous medium radiolysis rather than irradiation itself (i.e. radiation acts indirectly). Therefore, experiments were performed, in which Protozoa were not irradiated, but treated by culture medium (or tridistilled water) preliminarily irradiated by doses of previous tests [2, 4].

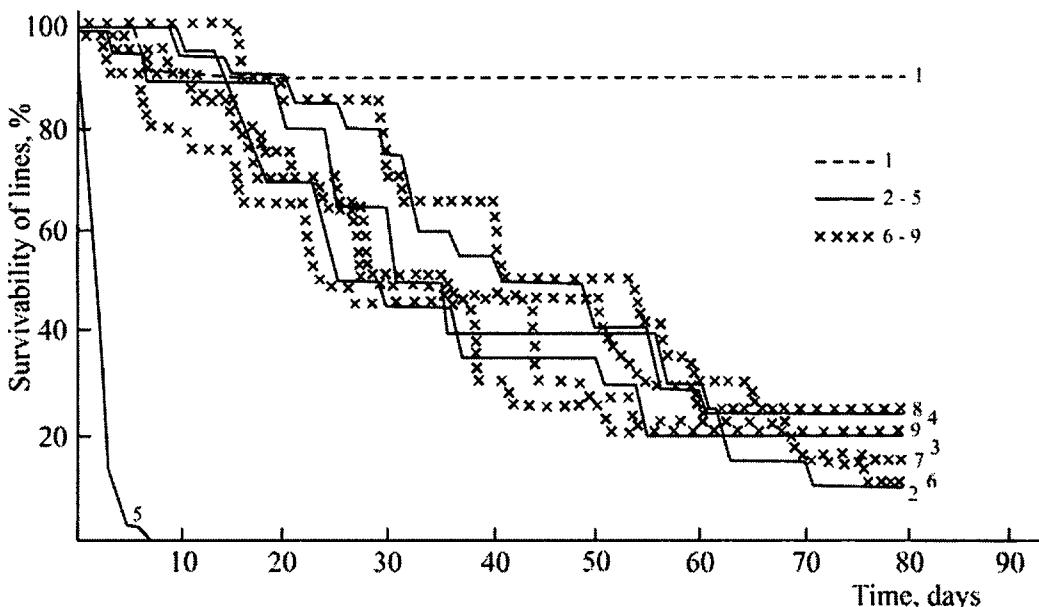


Figure 2. Dynamics of ameba individual lines loss in health (1), after X-ray irradiation (2 – 5) and after treatment by a culture medium irradiated by the same doses (6 – 9). The radiation doses, G: 5 (2, 6), 20 (3, 7), 400 (4, 8) and 2,000 (5, 9); 40 individual lines (20 in each test version) were used in control

The heat treatment effect was also studied, when amoebas and infusorians were exposed in a medium at 29°C during different times (for amoebas, this temperature exceeds the optimum level by 4 - 5°C [16]), and then tested [4, 17]. It is found that these impacts may also cause a jump-like transition of cells in the population to the state with higher cell loss frequency compared to control (Figures 2 and 3). These alterations were, both qualitatively and quantitatively, similar to the above-described irradiation effects. The action of different agents displayed no additive property [4].

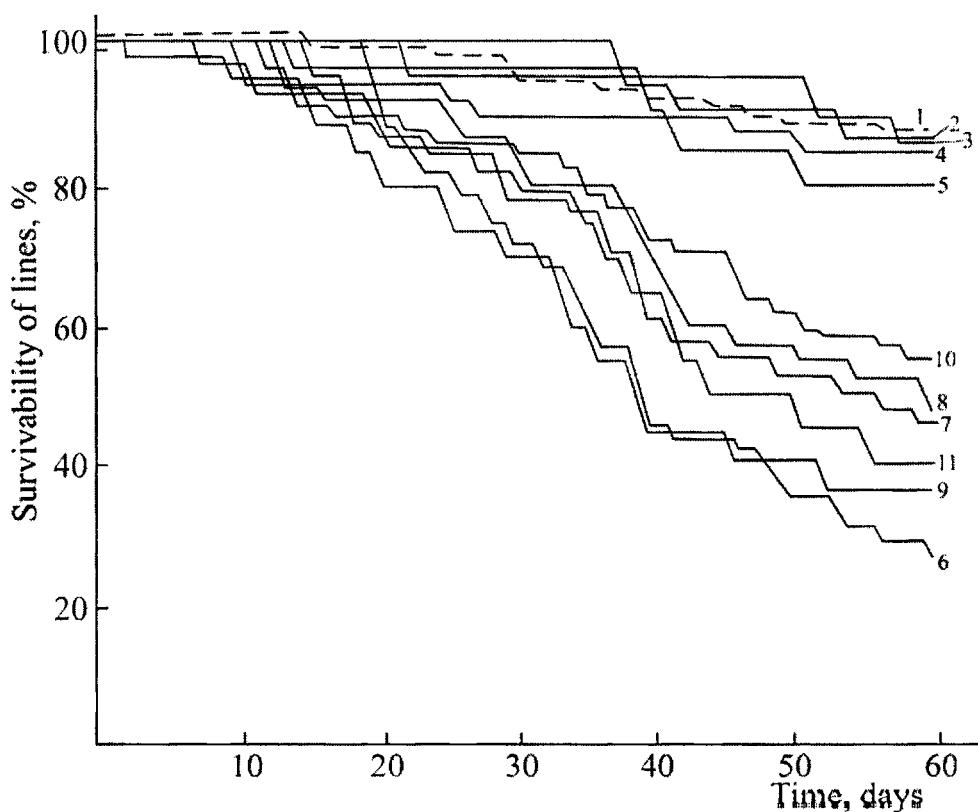


Figure 3. Dynamics of ameba individual line loss after heat treatment (29°C) during different times: 1 (dashed line) – control; 2 – 11 – tests; 2 – 5 – ineffective influences (by exposure duration): 2, 3, 4 and 5 h, respectively; 6 – 11 – super threshold influences: 6, 7, 8, 24, 48 and 72 h, respectively. In each case, 20 individual lines were tested

Of interest is that the tests with radiation-free agents displayed the dose independence zone much greater than at cell irradiation. For example, at indirect irradiative influence the dose-independent zone spreads up to the maximum of doses used (2,000 G), whereas in the case of cell irradiation to 600 G only (refer to Figures 1 and 2). For indirect radiation influence, this may be explained by the absence of lethal effects of different origin, which might mask the above-described alterations.

The dose independence is most pronounced at tests with low heat influence, which is not pathogenic at all (in the ordinary sense of the term) [4].

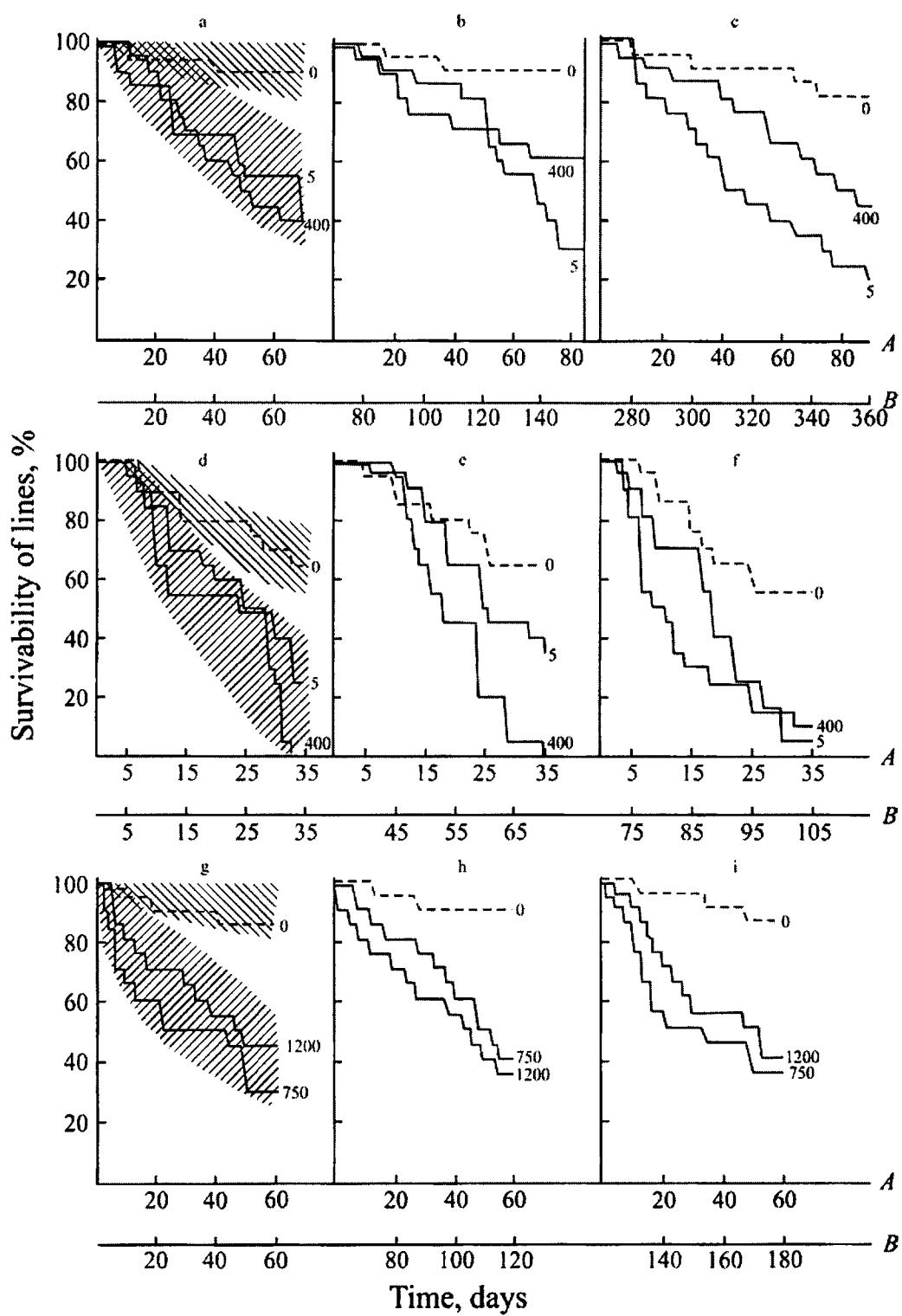


Figure 4. The dynamics of restarted individual lines loss in Protozoa: *A. proteus* (a – c), *P. caudatum* (d - f) and *C. virens* (g – i) in control (dashed lines) and after irradiation (continuous lines).

a, d, g – the loss dynamics of individual lines formed right after the impact; b, e, h – after initial and c, f, i – second restart, respectively. Numbers at curves give the irradiation dose in G. In every test 20 individual lines were studied.

Abscissa axis: upper (A) – time after individual line formation, days; lower (B) – time after irradiation, days. Ordinate axis: survivability, %.

b – c between 160 and 270 days (B) amebas were exposed in massive cultures. Shared area borders correspond to the limiting cases of curve spreading in repeated tests

One more feature of the phenomenon under consideration is that the loss frequency of studied objects (both in control and in test) did not correlate with their proliferation intensity, which was clearly manifested in ameba tests. Different feeding schedules were used that significantly affected the cellular cycle duration in the current object. It was found that the cell loss intensity did not alter even at a significant (3-fold or more) slow-down of their proliferation [18]. This indicates the absence of the cause-effect relation between these processes.

Further on, an ingenious method was used, in which individual lines were restarted from sister cells. This allowed extension of observations for cells in individual cultures indefinitely [4]. Such procedure was performed in long post-effect time, when a low amount of individual lines remained in the test.

Both in control and in tests, restarted lines were initiated by sisters of specimens preserved in cages or their closest generation. It proved to be the case that independent of the fortune of preserved cells, the overwhelming majority of their sisters were viable.

Figure 4 shows results of observations for individual lines of amebas and two species of infusorians, formed right after irradiation by different doses and in long postirradiative times.

It appears that for all objects, both in control and in test (at both doses used), the loss intensity equals for restarted and initial individual lines. At further restarts of lines, similar effects were observed. If between tests the objects were exposed in mass cultures (tests in amebas), the effect remained invariable.

The same type of restarted individual lines loss was observed in tests in radiation-free (radiochemical and thermal) effects (tests in *P. caudatum* and *A. proteus*, *C. virens* and *A. proteus*) [2, 4, 17].

The maximum term of overseeing restarted individual lines equaled three years, during which hundreds of cell generations superseded (tests in amebas). The difference with control always remained constant [4], though cell loss frequency during so long observations varied sometimes (usually, because of alterations in incubation conditions).

To the authors' point of view, the unique possibility to interpret these results is the suggestion that after reaching the effective dose used agents induce stable, dose-independent alterations, inherited in indefinitely long sequence of cell generations. These alterations manifest themselves in cell loss frequency increase compared to the spontaneous level. The data under consideration showed that inherited alterations regarded practically all cells in the population,

because at a sample large enough any of them (if not lost) produced progeny higher inclined to loss.

The effect was detected in individual cell cultures and, consequently, information storage and realization (cell loss) were autonomous processes (other cells of the population had no effect on them).

In different experiments [4, 19, 20] other investigation techniques were used (the study of colony formation effectiveness and their propagation rate), which gave similar results for yeast cells (diploid *Saccharomyces ellipsoideus* Megry 139-B and haploid *Saccharomyces cerevisiae* 180/113). For inductive agents (direct and indirect irradiation impacts), the effect was nonspecific. The effectiveness already at low doses, dose independence, no relation to mitosis, and irreversibility for indefinitely long period were detected.

For yeast, the experiments on cloning and subcloning showed that the mechanisms providing more intense cell loss versus in control were initiated in each cell of the population and inherited by progeny. This may testify about the nonspecific property of the described processes for studied objects. Obviously, these processes may be interpreted as massive, dose-independent transition of cells in the population to a new state, in which cells are more inclined to loss.

Inherited decrease of adaptation to unfavorable environmental conditions

The data similar to the above-described ones were obtained by I.F. Kovalev on individual infusorian cultures (*P. caudatum*) irradiated by different doses of ^{60}Co [21]. In seemingly unsatisfactory experiments, in which optimum incubation conditions were disturbed (overpopulation, abrupt medium alteration) at different post-irradiation stages, and then at deliberate disturbance of these conditions mass loss of experimental cells and abrupt suppression of proliferation were detected. This was post-irradiative effect at low doses (200 G) for *Paramecium*, and did not increase with the dose (up to 600 G). It was detected even after 100 postirradiative generations. As a consequence of the above-described effects, the latter is stipulated by the occurrence of dose-independent massive alterations inherited at cell proliferation.

Thus the regularities under discussion may be detected by both changes in cell loss frequency and disturbance of adaptation to unfavorable vital conditions. Therefore, it may be expected that both phenomena are stipulated by the same cellular alterations, because postirradiative increase of cell loss intensity in weakened ameba and infusorian cultures induced by frowy feeding is sometimes very significant and terminated experimental populations [4].

TEST RESULTS ON MAMMALS

Stable, dose-independent increase of vascular endothelial cells damage and loss

These alterations [5 – 7] were detected by R.P. Stepanov [22] in long-term electron microscopy studies of blood vessel walls in rats. White nondescript rat males were X-ray irradiated in different doses and effect conditions (irradiation doses were: 0.5, 2.25 and 4.5 G for general irradiation, 9 and 30 G myocardium zone irradiation and 9 and 18 G for stomach partial irradiation, respectively). Test material was sampled right after irradiation (1 – 48 hours), in longer (4 – 30 days) and late (3 – 18 months) dates after irradiation. For control, rats of the same age exposed under test conditions were taken.

As shown in the literature [23 – 25], irradiation impact may significantly increase frequency of endotheliocytes with ultrastructure disturbances compared to cell concentration in control. Most frequently, disturbances were classified to cytoplasmic category. Both extensive alterations, finally, seizing the cell nucleus, and small local defects, including damaging of separate organoids, were observed. Among normal cells, damaged endotheliocytes were disseminated.

Time and dose dependencies of cell output with the signs of most frequently observed cytopathology elements (edematose degeneration, isolated damage of mitochondria, local cytosis) were analyzed [5 – 7]. These types of damages are developed independently [22].

At consideration of every kind of cytopathology, the same responses to radiation impacts as in tests with unicellular organisms (transition to a new steady state). The latter differed from the initial state by an increase of cell occurrence with damages under consideration.

Similar to unicellular organisms, the effect occurred already at low dose irradiation. The vessel endothelium is classified to radioresistant tissues [26]. Meanwhile, the studied alterations were observed already at animal irradiation by the dose as low as 0.5 G (Figure 5). They are detected in consideration of cells with different damages. Figure 5 indicates an unusual dynamics of alteration development with time. In all cases, differences with control occur already in initial hours after irradiation and are then preserved during the whole observation periods (12 months).

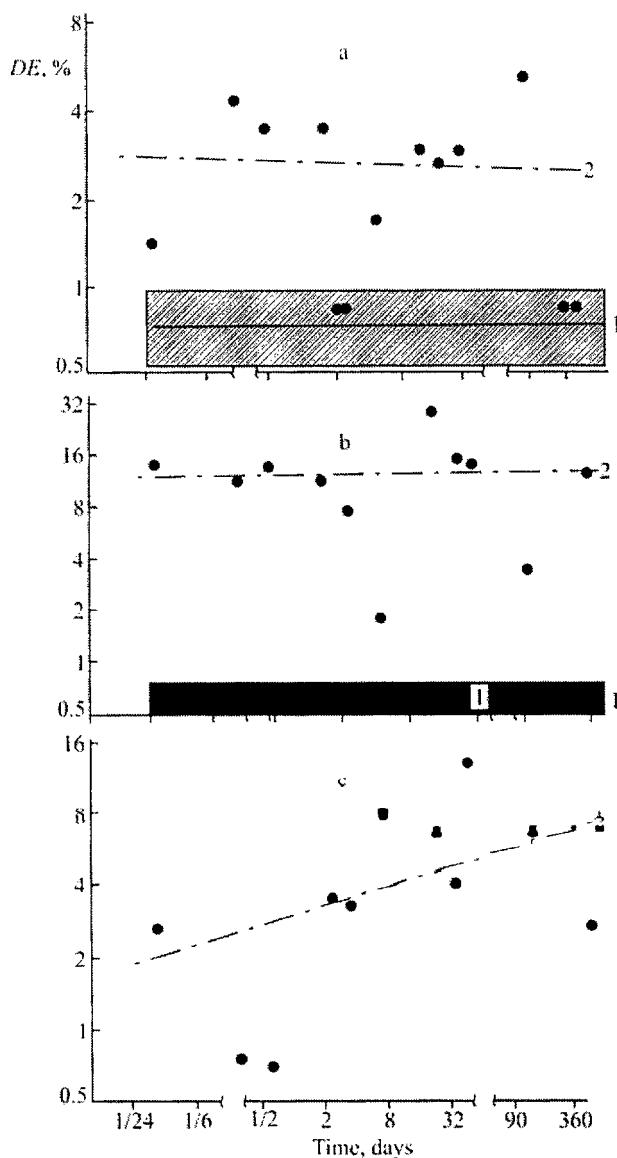


Figure 5. The frequency of occurrence of damaged endotheliocytes (DE) (and corresponded regression lines for the "time-effect" dependence) in myocardium capillaries in rats in different periods after irradiation by 0.5 G dose. Cells with signs of edematose degeneration (a), isolated damage of mitochondria (b) and intracellular lysis (c).

1 – in control (the average value with confidence limits – crosshatched and shaded zones); 2 – irradiation

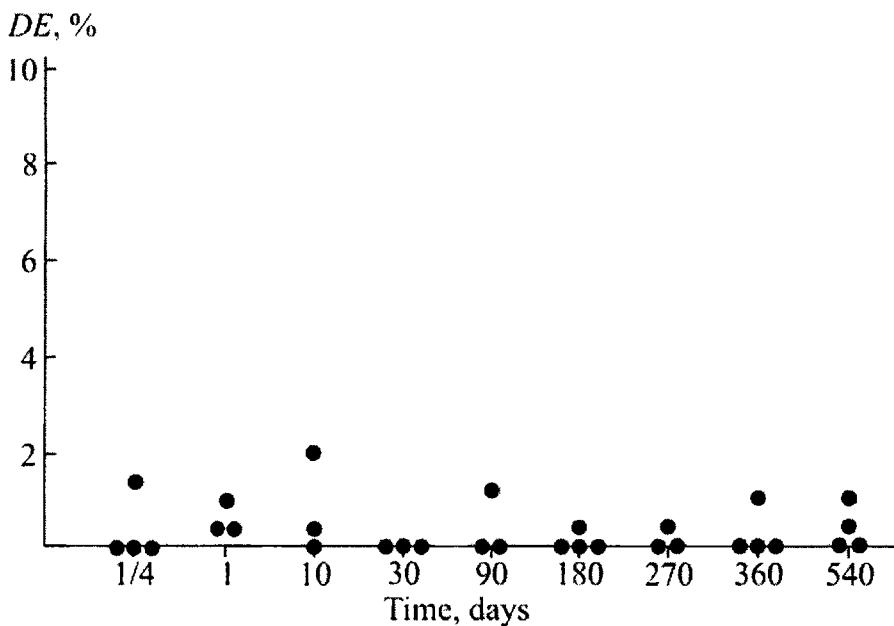


Figure 6. The frequency of occurrence of unviable endotheliocytes (*DE*) in myocardium capillaries in intact rats. Each point corresponds to data on a single animal

As illustrated [5, 7], alterations occurring at irradiation by higher doses (total irradiation of animal by 2.25 and 4.5 G and heart zone irradiation by 9 and 30 G) are of the same type. Hence, no clear effect dependence on the irradiation dose was detected. N.P. Lebkova also reports about the absence of the output dependence of cells with ultrastructural pathology on the irradiation dose [24]. She carried out many-month electron microscopy investigations of myocardium capillary endothelium in rats after the heart zone irradiation by doses as high as 6.9 and 20 G.

The dose independence is the feature, fundamentally distinguishing the alterations described from the known ones.

It is demonstrated [5, 22] that similar to experiments with unicellular organisms, the studied effect is nonspecific for irradiation action. It was detected in tests with direct and distant radiation influence at partial stomach irradiation by 9 and 18 G doses. In this case, it was suggested that the effect is caused by an action of toxic products injected to blood in irradiated intestine.

In studies [5 – 7] the destiny of cells with considered damages is most frequently unclear. They may either be lost from damages (especially in the case

of edematose degeneration or preserved in the tissue for a long time, or damages may be repaired. That is why in subsequent works [15, 27] the regularities of cell output with irreversible ultrastructure damages which *a fortiori* caused cell loss were analyzed. In these observations, the frequency of occurrence of endotheliocytes with the signs of generalized organoid degradation, damaged plasmolemma and nuclear structure destruction was considered.

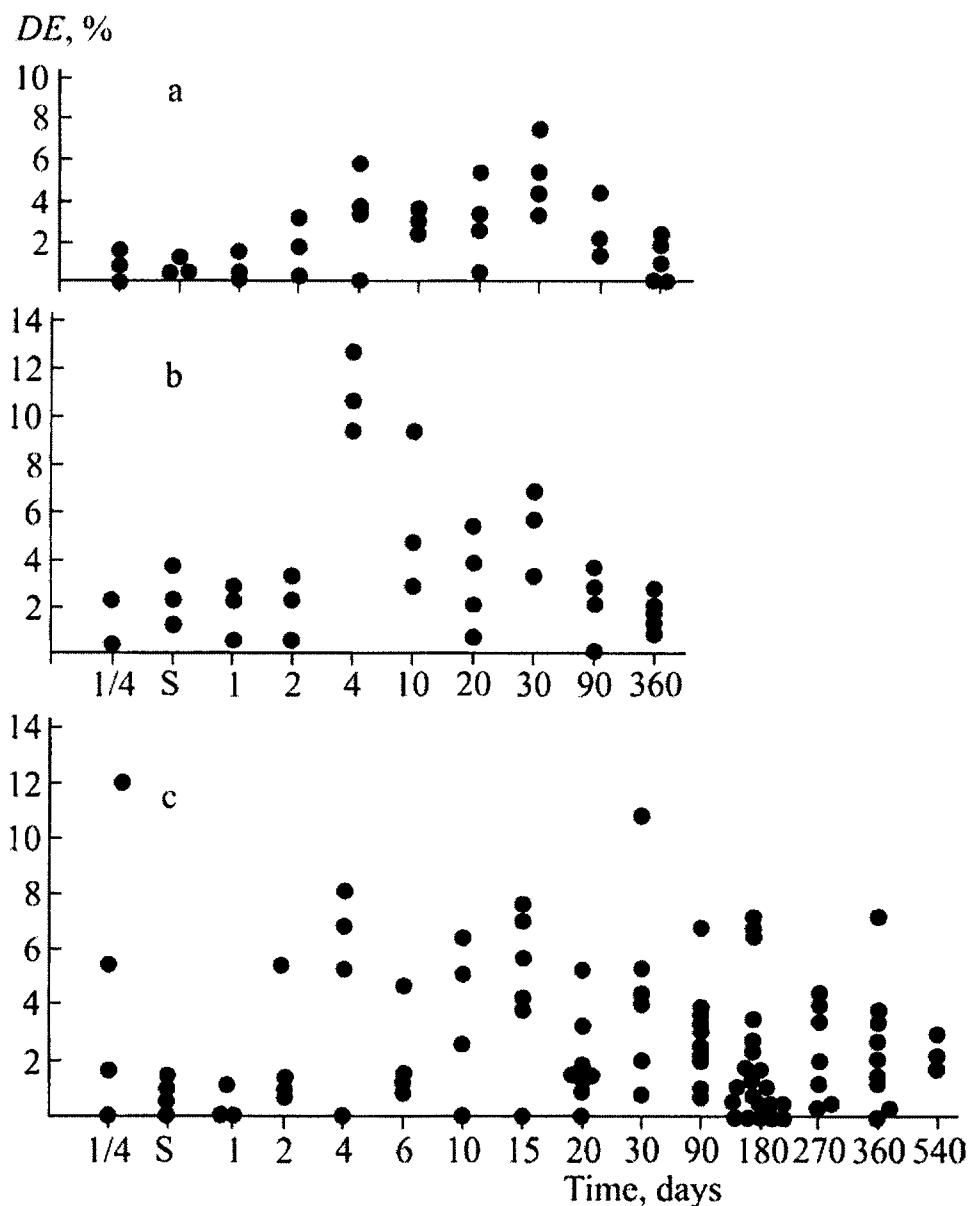


Figure 7. The frequency of occurrence of unviable endotheliocytes (DE) in myocardium capillaries in rats at different times after total irradiation by doses: 0.5 (a), 2.25 (b) and 4.5 (c) G. Each point corresponds to data on a single animal

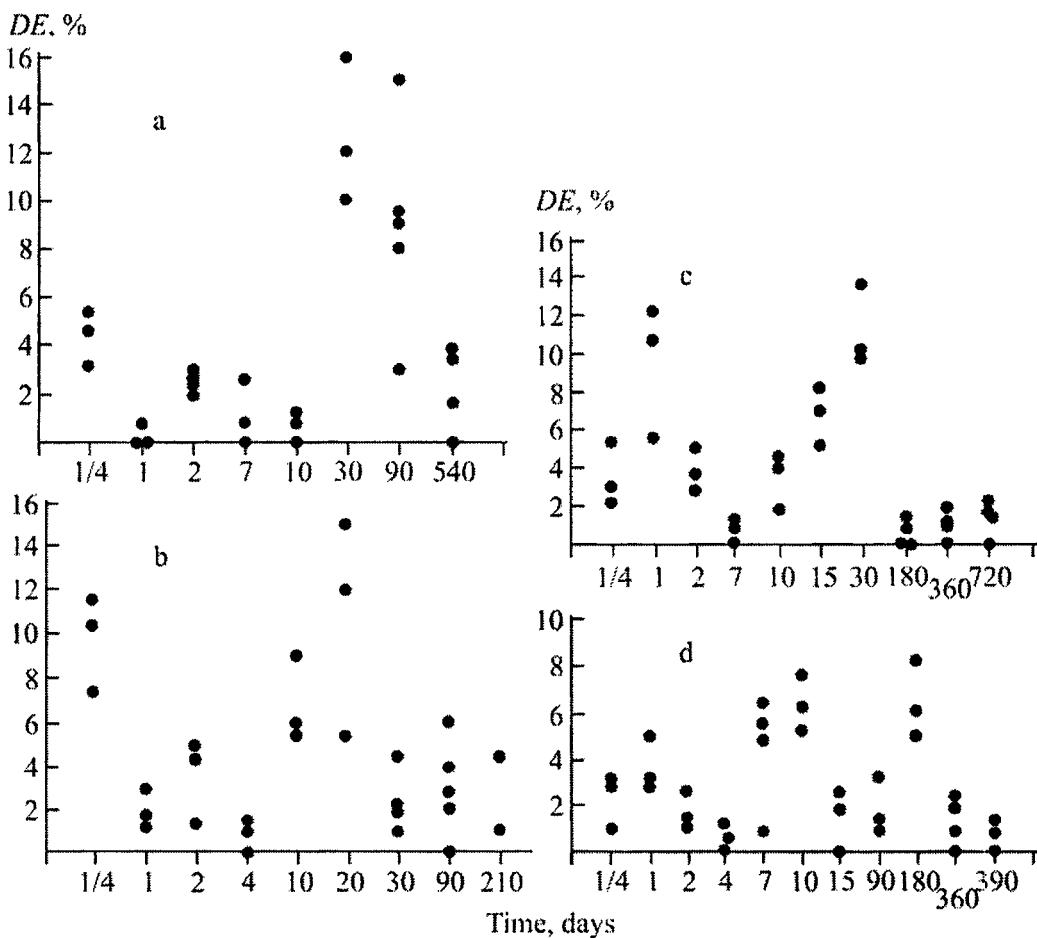


Figure 8. The frequency of occurrence of unviable endotheliocytes (E) in myocardium capillaries in rats at different times after heart zone irradiation by 9 (a) and 30 G (b) doses, and after intestine zone irradiation by 9 (c) and 18 G (d) doses. Each point corresponds to data on a single animal

In this case, the type of alterations similar to estimation of the frequency of occurrence of cells with relatively lower, mostly local damages was also observed. Figure 6 shows concentration of unviable endotheliocytes for every tested animal at different times in control. Figures 7 and 8 show the same parameter after all types of impact. Clearly all types of irradiation (including relatively low dose of 0.5 G) display a significant increase of unviable endotheliocytes' output compared to control. Dynamics of alterations was found

the same as shown above. The effect occurred shortly after irradiation and was observed during a long time. In a wide range of used doses (0.5 – 30 G) no significant increase of considered alterations happened. Thus the effect was observed in tests with both direct and distant action of irradiation (refer to Figure 8c, d).

Unviable cells with acute ultrastructural pathology are desquamated and eliminated [22]. In this connection, after all applied impacts endothelial lining defects in endothelium of myocardium capillaries in rats were observed. They were then repaired by extended spreading of neighbor undamaged cells. This caused thinning of particular endothelium zones, the number of which increased with time [22].

The foregoing allows a suggestion that during long months unviable cells regularly occur *de novo*. This is possible in the only case, when applied impacts induce mass stable alterations in cell populations, manifesting themselves as increased susceptibility of cells to loss. As mentioned above, persistence of alterations providing for an increase of cell loss probability and their massive behavior were also detected in tests with unicellular organisms.

Post-irradiative increase of damaged endotheliocytes' occurrence frequency was observed against extremely low mitotic activity typical of vessel endothelium. Historadiography with tritiated thymidine has shown that only one per 10,000 cells enters the mitotic cycle per day, and the cycle duration exceeds 4.5 months [22]. This indicates that similar to unicellular organisms the effect manifestation is independent of mitosis.

Now then in rats, an unusual response to irradiation impacts (both direct and distant), in many ways resembling the reaction of various unicellular organisms, is typical of endothelium cells of myocardium capillaries. As follows from [22, 28], similar regularity of post-irradiative alterations is also typical of endothelium of great vessels (aorta).

Long preserved alterations in myocytes of vessel walls

As shown by electron microscopy studies of post-irradiative effects on aorta wall [22, 28, 29], both endotheliocytes and muscular cells receive and store alterations caused by radiation, manifested as stable increase of cell damage probability compared to control. This was essentially concerned with relatively low cytoplasmic damages (the occurrence of zones with membrane myelin-like degeneration, lysis or fractural decomposition of a part of cytoplasmic structures, isolated damage of mitochondria, and excessive hydration of

cytoplasm in cells). Higher damage and loss of myocytes were rather infrequent. Deviations from control were detected already in the first day after irradiation and at low dose impact (0.5 G). The increased concentration of myocytes with different structural defects was preserved without a noticeable decrease during the major part of animals' life.

According to quantitative analysis by R.P. Stepanov in tests with local irradiation of the aorta zone in animals by 9, 14, 20, 30 and 40 G doses, the frequency of myocyte occurrence with various structural damages was found independent of the irradiation dose (tests were performed 10, 20, 80, 90, 180 and 360 days after irradiation) [29].

Thus it may be considered that smooth musculature cells are also subject to unusual alterations.

On the long-term alterations in connective tissue cells

Some authors [30 – 34] have described the so-called tumor bed effect, consisting in a decrease of engraftment of non-irradiated tumor cells and the growth rate of tumors inoculated subcutaneously and intramuscularly to preliminarily irradiated zones (usually, to one of extremities of tested animal). These zones (particularly, subcutaneous tissue) obtain the mentioned antitumor properties already after the radiation effect and then preserve them during many months or even years. The most detailed study of the dose dependence of the above-described alterations is given in works [33, 34]. One of the extremities of nondescript, white mice-males was affected by X-ray doses of 0.25, 1, 3, 5, 6, 8, 10, 15, 20, 30 and 40 G. Then a definite amount of Ehrlich's ascites carcinoma nonradiated cells were inoculated to this extremity or one of intact animals. Comparison of grown up tumors and the amount of successful subinoculations in test and control has indicated that the "tumor bed effect" occurs after a definite dose threshold (between 1 and 3 G). After that a broad plateau is observed: the effect does not increase even at 20-fold dose raise. It was registered at all doses already a day after irradiation and then preserved, approximately, at the same level during the whole observation period (up to 5 months). All the above-discussed indicate the induction of massive, dose-independent and stable alterations in future bed tissues by X-ray radiation. The same (both qualitatively and quantitatively) was observed at distant irradiation effect at both short-term [30] and long-term [34] observations. In these tests, subtotal or partial irradiation of animals was performed, and the tissues of future "bed" were screened.

On the whole, the "tumor bed effect" may be understood as an alteration of intracellular interactions. Thus in this situation as compared to the previous cases, manifestations of different massive long-term alterations are observed. At the same time, general phenomenology was similar that allows a suggestion about principal similarity of described alterations. It is still unclear what the cell elements in normal tissues participate in this process. As mentioned above, the "tumor bed effect" consists in suppression of both growth and engraftment of tumor cells, which is also observed for their subcutaneous inoculation. Thus a suggestion can be made that the phenomenon under consideration (even if partly) results from long-term alterations occurring in the connective tissue.

This is conformed to still unexplained data on the "radiation memory" of connective tissue manifested as rapid loss of leukocytes migrated to it [35] and the features of dermal homotransplant engraftment at distant post-irradiation periods [36]. Of special interest are direct proofs of the "radiation memory" preservation by connective tissue cells, which are subcutaneous fibroblasts [37]. As microcolonies of these cells are sowed 3, 7, 12 and 16 months after irradiation of rats by neutrons (3 G dose), it was found that at all periods the decrease of sowed cells' sensitivity to growth factors added to the culture medium and high probability of microcolony growth termination were observed. The intensity of these alterations was approximately the same in all observations. Other deviations from the control were not observed (growth of explants and the state of "thick" suspension cultures were studied). At cloning of subcutaneous fibroblasts, reproducibility of results and normal distribution of microcolonies by sizes were demonstrated. This gave the authors [37] an opportunity to conclude about homogeneity (the mass character) of corresponded, practically irreversible cellular alterations providing for the observed effect.

Thus connective tissue cells subject to relatively low irradiation effect may transit to a new steady state.

The authors have considered quite enough number of facts testifying favorably that unusual regularities of the cell reaction discovered in tests on unicellular organisms may be transferred to mammal cells. These facts were obtained nonrandomly in observations for cells of slowly regenerating tissues (endothelial, muscular, and connective). Due to low mitotic activity, cytogenetic post-irradiative effects are infrequently observed in tissues (or not detected at all). They noticeably occur at provocation of regeneration only [22] and, therefore, do not impede detection of studied changes under normal conditions.

Unfortunately, in tests in mammals it was impossible to clear out if the alterations, providing the studied phenomenon, are reproduced at mitosis.

Endotheliocytes, myocytes and fibroblasts divide very rarely. Therefore, the case in hand may be only possible transition of corresponded state to the nearest cell descendants, but not to a broad sequence of cell generations, as it happens to actively dividing unicellular organisms (in tests with fibroblasts, at microcolony formation, the number of cell divisions was also limited). Nevertheless, it seems to us that considered data relate to the same class of events, the more so in unicellular organisms the regularity under consideration may manifest itself against extremely low mitotic activity [4].

The results involving mammal cells are mainly obtained *in situ*. In this connection, the question is brought up about significance of the state of organism for studied processes. Vascular endotheliocytes and myocytes observations showed that as irradiation methods (total, local and remote) and radiation dose were changed, e.g. in different states of organism, the situation did not change, in general. In fibroblasts, mass long-term alterations were preserved *in vitro* at their cloning. All this suggests that cellular rather than organism reactions are of the greatest importance in studies events. So far as concerns the importance of the organism state, it most likely consists in quantitative modification of the effect, e.g. decay or intensification of its manifestation.

In this connection, data from [22] are of interest. They show that hemodynamic load sharply increases the frequency of occurrence of damaged endotheliocytes both in control and after impacts which induced a stable increase of cell aptitude to damage or loss.

ON THE POSSIBILITY OF STUDIED ALTERATIONS' MANIFESTATION AS AN INCREASE OF CELL OUTPUT WITH GENETIC DAMAGES

The most detailed studies of alterations in cell populations were performed on the example of stable (as compared to control) probability of cell damage and loss. Therefore, damaged cells had no signs of structural mutations. Let us now discuss data from the literature which indicate that the cell output with damaged genetic structures may also obey the described laws.

In this relation, the results obtained in studies of genetic damage output at low dose irradiation are of the greatest interest [9, 38]. In this case, the regularities are similar to those described in above Sections. The effect jump-like occurs then, according to the majority of experimental data, is dose-

independent in a definite range, in which (it is reflected by the plateau on "dose-effect" curves) cells with genetic damages occur more frequently than expected at linear or linear-quadratic extrapolation from high dose effects. At irradiation by higher doses, genetic damage output increases with the dose.

In the area of low doses, the above-described dose dependence of genetic damage output was observed for different biological objects: germinated seeds, various mammal and human cells (both healthy and tumor), and was considered as a general biological regularity [9, 38].

It is of interest that such type of genetic damaged cell output is nonspecific for low dose irradiation action. This was clearly shown in tests of radiotoxin simulator (oxidized oleic acid) effect on Ehrlich's carcinoma cells. It was found that compared to control, the "radiotoxin" induces a jump-like raise of chromosome aberration output, which level did not change even with a noticeable increase of the compound concentration. In this case, genetic effects, the occurrence probability of which increases with the agent dose, were not observed at all [39]. According to data obtained in tests on human lymphocytes [40, 41], 5-fluorodeoxyuridine, monoiodoacetic acid, adenosine diphosphate, sodium cyanide and sodium fluoride are acting. All studied substances induced nearly similar effects, which remained unchanged with their concentration (up to a thousand-fold increase). The combined effect of the agents was, approximately, the same as for their separate influence.

There are works showing that chromosome damages in lymphocytes of human peripheral blood may occur after blood plasma (serum) influence, which was taken from irradiated patients [42]. Hence, no clear dependence of the effect on the dose value was observed, too [43].

A series of experiments with the culture of human tumor cells (HEp-2) displayed an increase of cell output with chromosome aberrations after treatment by reduced (suboptimum) temperature and low concentrations of caffeine and propyl gallate [44, 45]. The applied factors reliably increased the amount of aberration cells with preservation of the average number of damages per damaged cell. It is untypical of the agent action which caused local damage of DNA [40]. All these factors ("pseudo-mutagens" in N.V. Luchnik terms) [40, 41] induced nearly the same effect, and no additive effect at their combined application (tests with reduced temperature and caffeine) was observed [44, 45]. One has an impression that damages induced by various nonmutagenic factors may not exceed a definite maximal level typical of the object used. This conforms to a conclusion about dose independence of alterations described above.

Usually the dose independent output of structural mutations in the low radiation dose area is explained by initiation of SOS repair mechanisms or inhomogeneity of the cell population [9, 38]. The possibility to induce dose-independent effects by weak nonmutagenic factors can be hardly explained on the basis of the above-mentioned concepts. However, these signs are typical of alterations under study. The important reason for the benefit of a supposition that dose-independent genetic effects obey the above-described regularities would be data on their persistence.

Already in 1960-70's, in the analysis of long-term effects of atomic explosion and various emergency situations [46] a possibility of long preservation of cytogenetic damages induced by low radiation effects was shown. At present, it is decisively demonstrated by epidemiological studies performed after the accident at ChNP [47].

Of importance is that some of these studies, performed in a definite range of low doses, may indicate the dose independence of the effect [48, 49]. Of special interest are retrospective studies of blood in children and teenagers living in radiation polluted areas in Bryansk Region[49]. They showed a plateau located in the same zone as the plateau for human lymphocytes irradiated *in vitro*. It is postulated [9] that the effect is fundamentally different from dose-dependent alterations and represents a specific cell reaction to external influences. It is suggested that as relatively high irradiation doses are reached, dose-independent alterations are not eliminated but masked by dose-dependent effects. This very situation was observed in experiments on unicellular organisms studying long-term increase of probability of cell loss [4].

At the present time, by cloning of irradiated mammal cells it is proved that chromosome damages may occur *de novo* after some stages of mitosis. Transmission of such damages during many cell generations was also shown [50 – 52].

Experimental materials on the dose dependence of chromosomal instability are short and ambiguous. In some works it is reported about effect increase with the dose [53], and others indicate its dose independence [54 – 57].

Conclusions on the target of these alterations are ambiguous, too. Some authors [56] state the target location in the cell nucleus and other [58] suggest the target size exceeding the nucleus. In accordance with [59] persistent chromosome damages may also be induced by indirect radiation effect on cells. It is decisively indicated in experiments on bone marrow cell irradiation by α -particles in mice through a lattice and further cloning of viable cells.

Such ambiguity is due to a collective meaning "chromosomal instability" notion. There are data allowing a suggestion that it also includes the above-

described regularities. The studies containing the following information are meant: 1) data on dose independence (in some dose range) of persistent cytogenetic effects [54 - 57]; 2) on their independence of the dose intensity [51]; 3) on low impact effectiveness [54 – 57]; 4) on massive cell involving into the reaction [58, 60]; 5) on nonspecific character of ionizing irradiation for direct effect [59]. These features are typical of the event under consideration.

Let us now dwell on the well-known facts of post-irradiative raise of cell sensitivity to mutagens. They were obtained in a series of experiments by Frank and Williams [61, 62]. In these tests, V-79 cells were X-ray irradiated either once by doses 3.8, 6 and 9 G or fractionally by 0.15 G/day during 60 days (total dose 9 G). Then sensitivity of irradiated cell progeny to PUVA (8-methoxypyralen combined with long-wave UV-radiation) was determined in equal time periods. The mutation frequency in hypoxanthine guanine phosphoribosyl transferase (HgPRT) locus was determined. It is found that already on the first post-irradiative day the cells become more sensitive to PUVA. At low doses of the agent (PUVA) a sharp increase of the number of mutations induced by it was observed (20-fold as compared to nonirradiated cells).

The observed effect was practically independent of either irradiation dose or the irradiation method (single or fractionated). For a long time, it was preserved at its maximum which was searched for in exponentially growing cultures during 108 days. Hence, the background of spontaneous mutation did not change. It was also found that the progeny of irradiated cells mutated after treatment by some chemical agents, which are nonmutagenic for healthy cells. Later on, similar effect was observed for preliminary UV-irradiation [63]. No further investigations of this problem were found in the literature.

Thus there are cases for that besides the enumerated manifestations cells subjected to the above-mentioned alterations may become disposed to damage of the genetic apparatus and hypersensitivity to some mutagens.

ON THE MECHANISM OF STABLE, DOSE INDEPENDENT CELL EFFECTS INDUCED BY EXTERNAL IMPACTS

As shown on different examples discussed in the previous Sections, irradiation and some radiation-free factors, including mutagenic ones (in low doses), may induce transition of cell populations to a new steady state. Therefore, it was concluded that the transition happened due to a jump-like

occurrence of exclusive alterations, nonspecific and dose independent for the current agent, proceeding in all or the overwhelming majority of cells. Effect of this type were registered both in tests with different unicellular organisms and in observations for mammal tissue cells *in situ*. For actively proliferating Protozoa, it is proved that such alterations may be inherited in the sequence of cell generations.

The phenomenology of this effect (first of all, mass occurrence of alterations after low impacts and dose independence) indicates its non-mutation origin. In this connection, of interest are materials [64 – 66] obtained for amebas, on which the nucleus transfer technique was developed [67 – 68]. The studies of nuclear-cytoplasmic relationships in induction and inheritance of cell loss stable increase displayed that these alterations can be transferred both with the nucleus and cell cytoplasm. The nucleus transfer itself did not induce these alterations.

Since in amebas the nucleus is transplanted without an addition of cytoplasm, this amazing result may be obtained in two cases: 1) the factors stipulating it are permanently localized both in the nucleus and cytoplasm; 2) these factors, whatever their origin may be, are not constantly localized in the cell and are capable of migrating between the nucleus and cytoplasm. The second supposition was found valid. This was proved in experiments with nucleus retransfer between "hybrids" which showed that when contacted with corresponded components of altered cells, the "normal" nucleus and/or cytoplasm altered themselves. In fact, reconstructed "normal" cells carried and transferred alterations stipulating the discovered phenomenon to cell progeny. This proves that the factor capable of transferring information from one cell component to another, migrating between the nucleus and cytoplasm, participate in genetic control (anyway, in amebas). This factor stability (about 3 days), high migration rate and the possibility to induce studied alterations at the influence on denucleated cytoplasm were proved. The latter fact shows that direct impact of inductive agents on a "target" located in the nucleus is not obligatory.

The data shown in this Section prove once again that the effects under consideration are not caused by nuclear gene mutations or chromosome aberrations, as well as cytoplasmic mutations. Therefore, the role of nuclear-cytoplasmic relationships in control for the studied hereditary sign is emphasized that essentially supply previously discussed materials.

Searched for features of genetic phenomenology of the sign under discussion may be explained under a supposition of its connection to inheritable activity alteration of any nuclear gene. Such hereditary variance, which may be highly frequent not only after mutagenic impacts, is called epigenetic variance

[69]. Hence, the existence of a regulator factor, migrating in the cell may be assumed [67, 68].

CONCLUSION

In this review, the experimental data by many authors are present, showing that relatively low radiation and some other impacts, including non-mutagenic ones, may transfer cell populations to the new stage of existence. The effect was performed by type of trigger switchover in all or the overwhelming majority of impacted cells.

It was found that the reaction of this type was inherent to objects of various organization, phylogenetically separated from one another (amebas, infusorians, yeast, different mammal tissue cells), which may be considered as total biological regularity. Different manifestations of this state were displayed. Stable increase of cell damage and loss probability was most frequently detected. The long-term disturbances of adaptation ability under unfavorable conditions (tests in infusorians) and intercellular interactions (mammal cell observations) were described. Data implying that alterations of this type may also manifest themselves as a stable increase compared to spontaneous output of genetic damages and an increase of cell sensitivity to some mutagens are discussed.

The complex of considered data (primarily obtained in tests in unicellular organisms, in which the effect inheritance in the cell generation sequence was proved) testifies about stipulation of this unusual phenomenon by initiation on intracellular mechanisms (programs) in almost all cells. These mechanisms provide a long-term homeostasis setting and may also be initiated by interactions, which are not usually considered as pathogenic ones (for example, low and short-term temperature increase). Hence, in any experiment no population recovery was observed.

The behavior of alterations under consideration (first of all, their mass induction already by low influences and dose independence) does not allow their classification either as the mutation result or the consequence of a trivial damaging action of applied agents. Most likely, it represents a physiological process, to describe which adequate terms can hardly be found. In connection with the fact that the acquired changes manifest themselves mostly as an increase of cell damage and loss, a suggestion is induced that this phenomenon is connected to programmed cell loss. However, the specific aspect of this

problem should be emphasized, which is not the initiation of loss program in separate cells (in accordance with one morphological scenario or another [70 – 73]), but induction of genetically determined increase of aptitude to structural damages and loss in all (or the overwhelming majority) of cells in the population [15].

Particular molecular-genetic mechanisms of the described phenomenon remain unknown. The authors associate them (in the most general form) with epigenetic variance.

The question of biological meaning of the regularity under discussion remains open, too. Detection of this regularity in so phylogenetically separate objects suggests its fundamental meaning. It is not inconceivable that the case in hand is some previously unknown mechanisms, which are of importance for the evolution.

The problem of hormesis – the low-dose radiation action manifesting itself as adaptive response, proliferation stimulation, activation of different biological processes, etc. was not considered.

In the studies under consideration, no hormesis phenomenon was observed. Judging by the data from the literature [74 – 76], it is recorded in a narrow range of weak interactions. Effects of this type occur as the effective dose is reached, therefore, an original shape of dose dependence (the effect maximum at a definite dose and its decrease on either side of these values). At the lowest doses, the reverse dependence of the effect on the dose intensity was observed. These alterations, the same as those analyzed in the current review, are nonspecific for the radiation action. They were also induced by nonmutagenic factors. There are data showing that adaptive alterations in cells may be inherited in the cell generation sequence [77].

Similar to the above-described, the phenomenology of these alterations gives no opportunity to associate them with primary damage of genetic structures.

The case if both phenomena are independent of one another (in this case, unfavorable alterations under consideration may mask the hormesis effect), or there is a relationship between them is still unclear. This problem must be specially investigated.

The effects determined in mammal cells are of importance for medicine and ecology, primarily, relative to the problems of radiation safety. Low values of effective doses (alterations in vascular endotheliocytes were observed already at irradiation by 0.5 G dose), steadiness of the state obtained, a sharp quantitative difference from the control, and the probability of damage

occurrence in both propagating and post-mitotic cell populations may affect the state of the whole organism.

Recently, the problem if low-dose irradiation causes damages of tissues, organs and organisms or not was seriously discussed, because usually studied cytogenetic alterations, the frequency of occurrence of which after low influences is low, may not cause such consequences. The authors' attitude in this discussion is uncomplying [6, 7]. They suggest that the regularities under consideration form the real basis for development of an acute somatic pathology. In this case, slowly recovering tissue systems in which regular cell loss cannot be compensated by proliferation are critical. Vascular endothelium, in which at long-term post-irradiative periods the areas thinned due to increased spreading of endotheliocytes surrounding regularly occurring defects of endothelial lining [22] were observed, and data on gradual, dose-independent decrease of cellularity [78] provide the examples of this.

The data discussed in the review disclose new approaches to the study of possible weakening of long-term low-dose radiation consequences. This may be shaped as both researches on a possibility to modify the effect manifestation and searching for methods which may "breakdown" the intracellular program stipulating the increased cell aptitude to damage and loss. The latter requires decoding of particular, not yet determined molecular-genetic mechanisms of discussed alterations.

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REFERENCES

1. Bychkovskaya I.B. and Ochinskaya G.K., *Radiobiologia*, 1973, vol. **13**(2), pp. 211 - 215. (Rus)
2. Bychkovskaya I.B. and Ochinskaya G.K., *Radiobiologia*, 1973, vol. **13**(5), pp. 680 - 685. (Rus)
3. Bychkovskaya I.B. and Komarov E.I., *Radiobiologia*, 1990, vol. **30**(4), pp. 467 - 476. (Rus)
4. Bychkovskaya I.B., *The Problem Of Long-Term Radiation-Induced Cell Loss*, Moscow, Energoatomizdat, 1986, 158 p. (Rus)
5. Bychkovskaya I.B., Stepanov R.P., Antonov P.V. et al., *Radiat. Biologija. Radioekologija*, 1996, vol. **36**(6), pp. 926 - 931. (Rus)

6. Bychkovskaya I.B., Stepanov R.P., Antonov P.V. *et al.*, *Meditina Katastrof*, 1998, No. 3 – 4, pp. 23 - 27. (Rus)
7. Bychkovskaya I.B., Stepanov R.P., and Fedortseva R.F., *Med. Radiol. Radiat. Bezop.*, 2000, vol. **45**(1), pp. 26 - 35. (Rus)
8. Nanney D.L., *PNAS USA*, 1958, vol. **44**, pp. 712 – 717.
9. Geraskin S.A. and Sevankaev A.V., *Radiat. Biologia. Radioekologiya*, 1999, vol. **39**(1), pp. 35 - 40. (Rus)
10. Pelevina I.I., Gotlib V.Ya., Kudryashova O.V. *et al.*, *Radiat. Biologia. Radioekologiya*, 1996, vol. **36**(4), pp. 546 - 560. (Rus)
11. Zaar E.M. and Lozina-Lozinsky L.K., *Botanichesky Zh.*, 1964, vol. **49**(10), pp. 1455 - 1457. (Rus)
12. James A.P., *Brookhaven Symposia in Biology No. 20*, Brookhaven National Laboratory Associated Universities, Inc. USA, 1968, pp. 77 – 97.
13. Friedrich Freska H. and Kaudewitz F., *Zeitschrift fur Naturforschung*, 1953, Bd. **86**(7), S. 343 – 355.
14. Bychkovskaya I.B., Ochinskaya G.K., and Erokhina G.N., *Radiobiologia*, 1976, vol. **16**(3), pp. 390 - 394. (Rus)
15. Bychkovskaya I.B., Stepanov R.P., and Fedortseva R.F., *Tsitologia*, 2000, vol. **42**(11), pp. 1082 - 1093. (Rus)
16. Sopina V.A., *Tsitologia*, 1968, vol. **10**, pp. 207 - 215. (Rus)
17. Bychkovskaya I.B. and Ochinskaya G.K., *Tsitologia*, 1980, vol. **22**(7), pp. 841 - 849. (Rus)
18. Bychkovskaya I.B. and Ochinskaya G.K., *Radiobiologia*, 1974, vol. **14**(6), pp. 852 - 861. (Rus)
19. Bychkovskaya I.B. and Stazhevskaya T.I., *Radiobiologia*, 1978, vol. **18**(2), pp. 210 - 213. (Rus)
20. Bychkovskaya I.B. and Stazhevskaya T.I., *Radiobiologia*, 1980, vol. **20**(2), pp. 189 - 193. (Rus)
21. Kovalev I.F., *Functional Mechanisms Of Radiobiological Effect Development*, Moscow, Atomizdat, 1985, 309 p. (Rus)
22. Vorobiev E.I. and Stepanov R.P., *Ionizing Radiation and Blood Vessels*, Moscow, Energoizdat, 1985, 124 p. (Rus)
23. Khan M.J. and Ohanian M., *Am. J. Pathology*, 1974, vol. **74**(1), pp. 125 – 131.
24. Lebkova N.P., *Arkh. Patologii*, 1976, vol. **38**(1), pp. 33 - 41. (Rus)
25. Lyubimova N.V. and Svad'bina I.V., *Radiobiologia*, 1999, vol. **39**(4), pp. 413 - 417. (Rus)

26. UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation). *Ionizing Radiation: Sources And Biological Effects*, 24th Session of the General Assembly, New York, 1982, Add. 45A/37/45.
27. Bychkovskaya I.B., Stepanov R.P., and Fedortseva R.F., Proc. II Intern. Cong. On "Weak and Superweak Fields And Irradiation In Biology And Medicine", St. Petersburg, 2000, pp. 44 – 46. (Rus)
28. Stepanov R.P. and Shishko T.T., In TsNIRRI Coll.: *Problems Of Experimental And Clinical X-ray Radiology*, Leningrad, 1991, pp. 99 – 101. (Rus)
29. Stepanov R.P., Bychkovskaya I.B., Fedortseva R.F. et al., *Proc. Sci. Meeting on "Histochemical Analysis Of Variance And Regeneration Of Tissues"*, St. Petersburg, IEM, 1997, p. 84. (Rus)
30. Goldstein L.M., *The Value Of Local And Total X-ray Effect For Tumor Treatment*, Leningrad, 1941, 263 p. (Rus)
31. Summers W., Clifton K., and Vermund H., *Radiology*, 1964, vol. 82(4), pp. 691 – 703.
32. Urano M. and Snit H.D., *Radiat. Res.*, 1971, vol. 45(1), pp. 41 – 49.
33. Shiffer I.V., Strelin G.S., Bychkovskaya I.B. et al., *Med. Radiologia*, 1978, No. 1, pp. 31 - 33. (Rus)
34. Shiffer I.V., Strelin G.S., Bychkovskaya I.B. et al., *Med. Radiologia*, 1982, No. 3, pp. 35 - 44. (Rus)
35. Garshin V.T., *Vestn. Rentgenol. Radiol.*, 1938, vol. 19, pp. 546 - 554. (Rus)
36. Matyushkova R.M., *Problems Of Clinical And Experimental Radiology: Thes. Kyrgyzian RI On Oncology and Radiaology*, vol. 2, Frunze, 1965, pp. 217 - 222. (Rus)
37. Fritish P., Lombard N., and Beauvallet M., *Radiat. Protect. 8th Symp. Microdosim.*, Jul. 27 – Sept. 1, 1982, Luxemburg, 1982, pp. 845 – 855.
38. Geraskin S.A., *Radiats. Biologija. Radioekologija*, 1995, vol. 35(5), pp. 563 - 571. (Rus)
39. Labzina N.G., Kudryashev Yu.B., and Luchnik N.V., *Radiotoxins, Their Origin And Importance For Irradiative Damage Development*, Moscow, Atomizdat, 1966, pp. 176 - 179. (Rus)
40. Poryadkova N.A., Izmailova N.N., and Luchnik N.V., *Genetika*, 1981, vol. 17(12), pp. 2212 - 2219. (Rus)
41. Luchnik N.V., *Symp. Monitoring Occupat. Exposure Genotoxicants*, Helsinki, June 30 – July 2, 1985, p. 29.
42. Emerit I., Levy A., and Khan S., *Free Radical, Lipoproteins And Membrane Lipids*, New York, 1990, pp. 99 – 104.
43. Goh K.O., *J. Medicine*, 1975, vol. 6(1), pp. 51 – 60.

44. Poryadkova N.A., Kuzmina E.G., and Luchnik N.V., *Vopr. Onkologii*, 1973, vol. **19**(3), pp. 80 - 83. (Rus)
45. Poryadkova-Luchnik N.A. and Kuzmina E.G., *Radiats. Biologa. Radioekologiya*, 1996, vol. **36**(6), pp. 840 - 847. (Rus)
46. Beer J.Z., *Adv. Radiat. Biol.*, 1979, vol. **8**, pp. 364 – 416.
47. Aleksakhin R.M., *Radiats. Biologa. Radioekologiya*, 1996, vol. **36**(4), pp. 451 - 458. (Rus)
48. Goh K.O., *Radiat. Res.*, 1968, vol. **35**(1), pp. 155 – 159.
49. Sevankaev A.V., Zhloba A.A., Potetnya O.I. et al., *Radiats. Biologa. Radioekologiya*, 1995, vol. 35(5), pp. 607 - 611. (Rus)
50. Linoli C.I. and Morgan W.F., *J. Radiat. Oncol. Invest.*, 1997, vol. **5**(3), pp. 124 – 128.
51. Lambert B., Holmberg K., Hackman P., and Wennborg A., *Mutat. Res.*, 1998, vol. **405**(2), pp. 161 – 170.
52. Wright E.G., *J. Pathol.*, 1999, vol. **187**(1), pp. 19 – 27.
53. Linoli C.I., Corcoran J.J., Milligan J.R. et al., *Radiat. Res.*, 1999, vol. **151**(6), pp. 677 – 685.
54. Kosichenko L.N., *Tsitologiya I Genetika*, 1989, vol. **23**(1), pp. 14 - 20. (Rus)
55. Little J.B., Nagasawa H., Pfenning T. et al., *Radiat. Res.*, 1997, vol. **148**(4), pp. 299 – 307.
56. Kaplan M.I. and Morgan W.E., *Radiat. Res.*, 1998, vol. **150**(4), pp. 382 – 390.
57. Salomaa S., Holmberg K., Lindholm C. et al., *Int. J. Radiat. Biol.*, 1998, vol. **74**(6), pp. 771 – 779.
58. Manti I., Jamali M., Prise K.M. et al., *Radiat. Res.*, 1997, vol. **147**(1), pp. 22 – 28.
59. Lorimore S.A., Kadhim M.A., Pocock D.A. et al., *Proc. Nat. Acad. Sci. USA*, 1998, vol. **95**(10), pp. 5730 – 5733.
60. Trott K.R. and Teibe A.T., *J. Radiat. Environ. Biophys.*, 1998, vol. **37**(7), pp. 173 – 176.
61. Frank J. and Williams J.R., *Science*, 1982, vol. **216**(4543), pp. 307 – 309.
62. Williams J.R. and Frank J., *Radiat. Res.*, 1982, vol. **91**(2), pp. 368 – 371.
63. D'Arpa P. and Williams J.R., *Environ. Mutagens*, 1983, vol. **5**(3), pp. 411 – 413.
64. Bychkovskaya I.B., Ochinskaya G.K., and Yudin A.L., *Genetika*, 1980, vol. **16**(11), pp. 2018 - 2028. (Rus)
65. Bychkovskaja I.B., Ochinskaja G.K., and Yudin A.L., *Arch. Protistenk.*, 1980, vol. 123, pp. 1 – 11.

66. Yudin A.L., Bychkovskaja I.B., and Ochinskaja G.K., *Progress In Protozoology: Abstr. 6th Int. Congr. Protozool.*, Warshawa, 1981, p. 398.
67. Yudin A.L., *Nuclear-Cytoplasmic Relationships And Cell Inheritance in Amebas*, Leningrad, Nauka, 1982, 198 p. (Rus)
68. Yudin A.L., *Int. Rev. Cytol.*, 1979, Suppl. 9, pp. 63 – 100.
69. Ephrussi B.S., *J. Cell. Compar. Physiol.*, 1958, vol. **52**, Suppl. 1, pp. 35 – 53.
70. Beaulaton J. and Lockshin R., *Int. Rev. Cytol.*, 1982, vol. **79**, Suppl. 9, pp. 215 – 235.
71. Ellis R.E., Yuan Y., and Horvitz H.R., *Ann. Rev. Cell. Biol.*, 1991, vol. **7**, pp. 663 – 698.
72. Vaux P.L. and Kornsmeyer S.J., *Cell*, 1999, vol. **96**, pp. 245 – 254.
73. Hanson K.P., *Uspekhi Gerontol.*, 1999, Suppl. 3, pp. 103 – 110. (Rus)
74. Eydus L.Kh., *Radiats. Biologija. Radioekologija*, 1994, vol. **34**(6), pp. 748 – 758. (Rus)
75. Eydus L.Kh., *Radiats. Biologija. Radioekologija*, 1996, vol. **36**(6), pp. 874 – 881. (Rus)
76. Eydus L.Kh., *Radiats. Biologija. Radioekologija*, 1999, vol. **39**(5), pp. 12 – 15. (Rus)
77. Giliano N.Ya, Bolshakova O.I., Bikisheva E.G. et al., *Radiats. Biologija. Radioekologija*, 1999, vol. **39**(6), pp. 543 – 547. (Rus)
78. Eydus L.Kh., Lubimova N.V., Levitman M.K. et al., *Radiobiologia*, 1990, vol. **30**(2), pp. 266 – 268. (Rus)

Analysis of epidemiological data on radiocarcinogenic effects and approaches to determining the low-dose upper limit in terms of existence of biologically harmful effect threshold of ionizing radiation

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ABSTRACT

Analysis of epidemiological data on cancer mortality among the Japanese A-bomb survivors and the Chernobyl cohort of emergency workers_exposed to different doses of ionizing radiation made it possible to suggest with assurance that there is a threshold of the radiocarcinogenic effect in the region about 200 mGy (mSv). Analysis of the excess of deaths due to solid cancer over the expected value for the dose range 5-200 mSv observed in the Japanese cohort led us to the conclusion that this excess presents a (quasi)plateau and is presumably of a non-radiogenic nature. The epidemiological data from the Chernobyl cohort enabled the conclusion that the dose dependence of leukemia risks in a limited low level radiation (LLR) dose range cannot be used to determine real coefficients of excess absolute or relative risk when the level of adequate control is not taken into account. To corroborate the principle of single particle hit per cell nucleus as the basis for the microdosimetric definition of low radiation doses, an approach was proposed to objective separation of delimitation regions between low, intermediate, and high radiation doses on the dose scale. By this approach, the low dose upper limit of sparse ionizing radiation for a cell nucleus of 8 μm in diameter was evaluated at 0.65 mGy. It can be used in assessing dose rate thresholds in respect to safe environmental chronic radiation exposure for man.

Keywords: Low doses, radio-carcinogenic effect, threshold of radio-carcinogenic effect, radio-epidemiological studies

The conception of existence of a threshold for the harmful effect of ionizing radiation on biological objects has been substantiated previously [1, 2]. It was mainly based on the analysis of radiobiological data on both the molecular-cellular level and higher levels up to the whole-organism level. Besides, we have considered the arguments "for" and "against" for both the presence and absence of a threshold of the harmful effect of radiation, these arguments being in the form of axiomatic prerequisites for interpreting investigation results or choosing mathematical models to describe ionizing radiation effects. It has been shown that the axiomatic basis for a threshold model of the harmful effect of radiation significantly exceeds that for the no-threshold conception. Nevertheless, all actual mathematical approaches to the interpretation of data on the influence of radiation on biological objects have been based on assumptions of a linear or linear-quadratic dependence, but in any case on non-threshold dose-effect dependence. This tradition has been especially established in the field of radio-epidemiological investigations. This is connected with the convenience of using a no-threshold linear model, with the sparseness of samples for appropriate epidemiological studies and, finally, with the overestimation of the primordial damaging action of any high-energy particle on a biological structure. Undoubtedly, it is of interest to analyze available radio-epidemiological data on carcinogenic effect as to how much they are in favor of the no-threshold effect of ionizing radiation. Perhaps they at least give us a chance to raise the question about the competence of building other models that would suggest the presence of a threshold in the biologically harmful action of radiation, primarily in its carcinogenic effect.

We have also analyzed microdosimetric approaches to define the upper low dose limit. They could be of practical importance in revealing a threshold for the biologically harmful effect of low dose rates of chronic radiation influence.

ANALYSIS OF EPIDEMIOLOGICAL DATA FROM THE JAPANESE COHORT OF A-BOMB SURVIVORS

The studies of [3 - 6] are included in this analysis. Of primary interest is the character of the dose response used to approximate data of many years on fatality rate for solid cancer and leukemia. This point was most thoroughly discussed in [3]. The authors came to the conclusion that the best approximation

for cancer data is a linear dependence and for leukemia a linear-quadratic dependence. The findings of different authors on the dose dependence of relative risk (RR) of death due to leukemia (Figure 1) and solid cancer (Figure 2) generally agree with the above conclusion. Though, the results by Kitayama et al. are rather in favor of presence of a threshold, but here we must admit that their data fall on a later time interval of observation of the exposed cohort (1968-1997) when the bulk of leukemia have already been realized. This thought is supported by the data on cancers which, unlike leukemia, are usually realized throughout the organism's life and for which the data by Kitayama et al. practically coincide with the results of two other research groups.

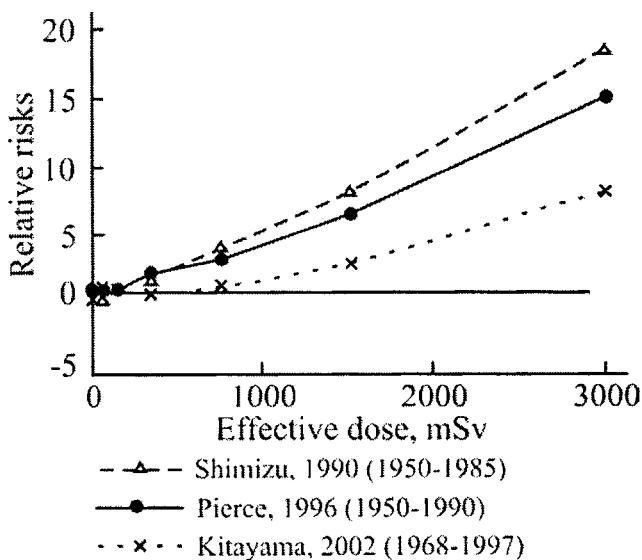


Figure 1. Dose dependence of relative risk (RR) of death due to leukemia in persons of the Japanese cohort according to the data of various authors (in brackets are observation periods).

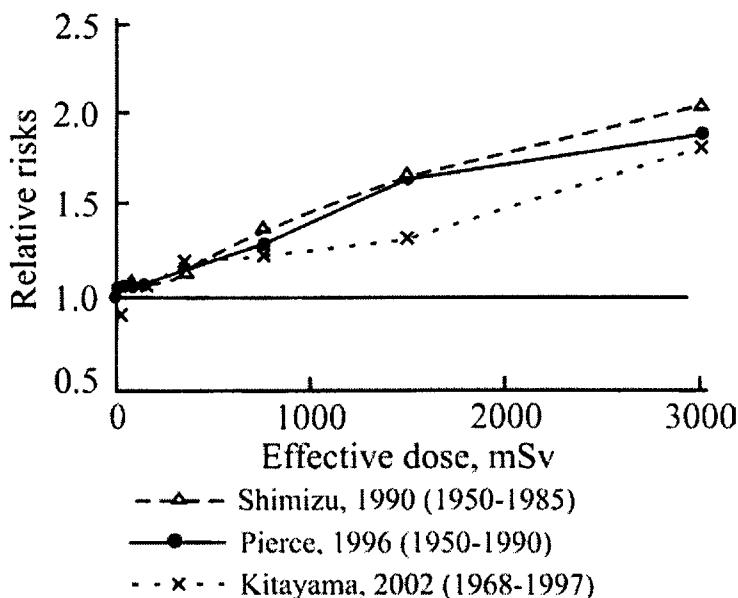


Figure 2. Dose dependence for the relative risk (RR) of death due to solid cancer in persons of the Japanese cohort, according to the data of various authors (in brackets are observation periods).

However, Figures 1 and 2 evidently show an extremely great density of points in the dose range below 200 mSv. Because of this it is impossible to form a clear picture of this part of the curve on such a small scale. Figures 3 and 4 present only the left portions of these curves, below 400 mSv. It is seen that the points for leukemia below 200 mSv mostly lie at the level of or below the control, whereas the points for solid cancer in the same dose region lie above 1 and form a plateau in the range from 20 to 200 mSv (here we present limiting values of the dose intervals for which only average values are given in the curves). Although each of these points, as judged from the errors given for them in the works cited above [3 - 6], shows no statistically significant difference with 1, the fact that all of them exceed the control level makes us believe that this excess is not casual. If we increase the sampling for this dose range, for instance at the expense of the Chernobyl cohort, and if in doing so all points will also lie above 1, their difference with the control may become significant and then the limit of detectable carcinogenic risks will substantially shift to a lower dose region.

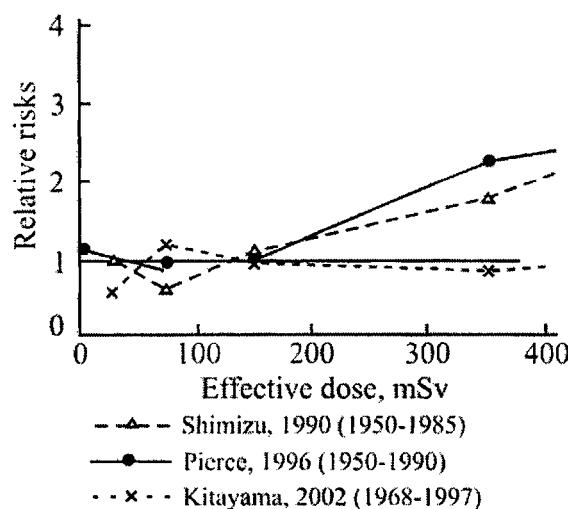


Figure 3. Dose dependence for the relative risk (RR) of death due to leukemia in the range of low doses in persons of the Japanese cohort, according to the data of various authors.

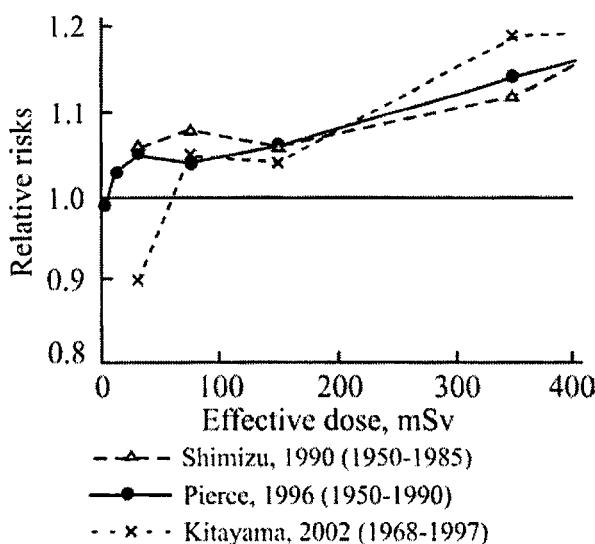


Figure 4. Dose dependence for the relative risk (RR) of death due to solid cancer in the range of low doses in persons of the Japanese cohort, according to the data of various authors.

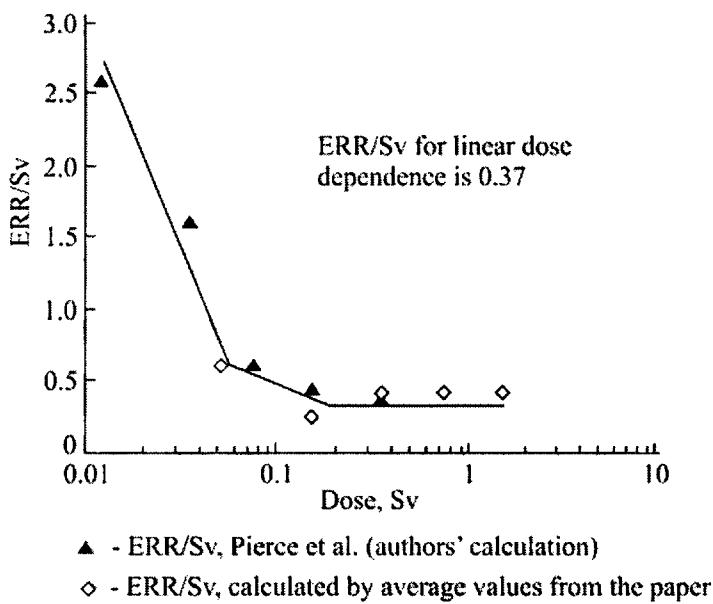


Figure 5. Dose dependence of ERR/Sv for solid cancer in the Japanese cohort (according to [3]).

1. ERR/Sv for a linear dose dependence is 0.37; 2. Dose, Sv; 3. ERR/Sv, Pierce et al. (calculated by the authors); 4. ERR/Sv, calculated from the average values given in the article; 5. ERR/Sv.

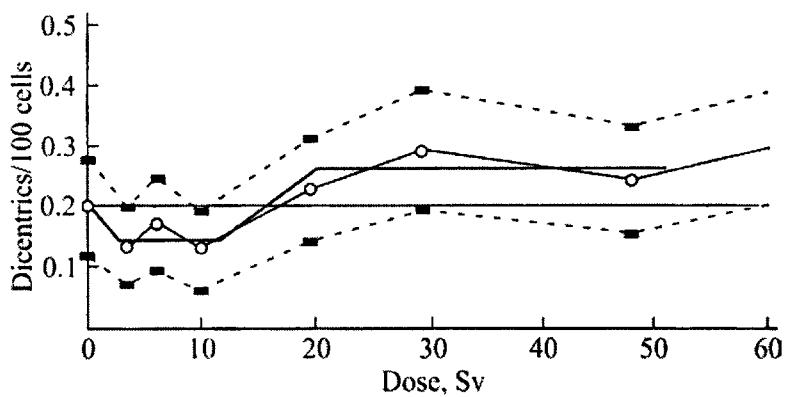


Figure 6. Dose dependence of dicentrics (with a 95% confidence interval) in human lymphocytes (according to [7]).

1. Number of dicentrics per 100 cells; 2. Dose, mGy.

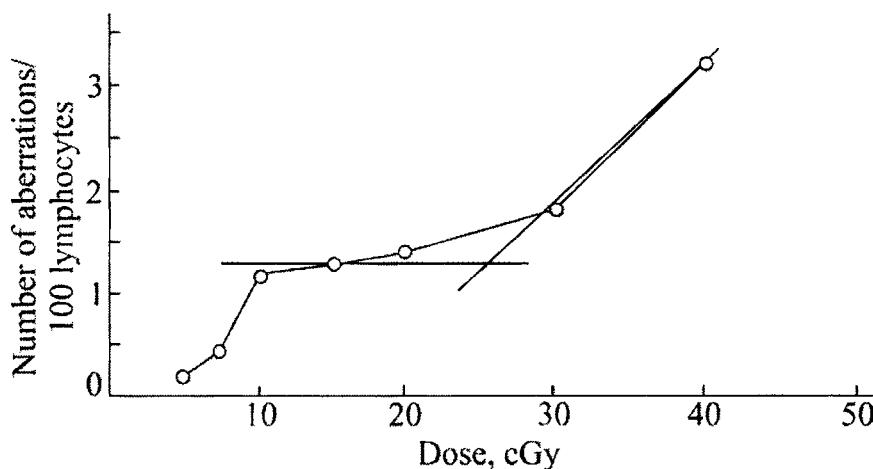


Figure 7. Dose dependence of chromosome aberrations in human lymphocyte culture (according to [8]).

1. Number of aberrations per 100 lymphocytes; 2. Dose, cGy.

There are two arguments in favor of the assumption of a plateau in the dose curve of cancer mortality. The first argument concerns the dependence of excess relative risk per dose unit (ERR/Sv) on dose. It is seen in Figure 5 that in the dose range below 0.2 Sv the value of ERR/Sv grows with decreasing dose. It appears logical to relate this fact to the invariability of risk value in this dose range. Some authors interpret the increase in ERR/Sv as an increase in the efficiency of damaging action of low doses as compared to higher doses but this viewpoint is in conflict with the decrease in low-dose damaging effect in absolute units (here, in standardized death rate). The second argument is based on the analogy with the evidence from experimental radiobiology where dose curves with plateau regions just in the low-dose range have been obtained and statistically validated on a great amount of experimental material. For instance, Figures 6 and 7 show dose curves for dicentrics in human lymphocytes [7, 8]. Plateau regions are well seen in both Figures: in the range 20-50 mGy in Figure 6 and in the range 10 - 25 cGy in Figure 7. The data on chromosome aberrations enable the assumption, as mentioned earlier [1, 9], that in the totality of cells there may exist minor populations of increased radiosensitivity and that these populations differ in radiosensitivity from the main population by a dose interval equal to the length of the plateau. For the data given in Figures 7 and 8, the size of these minor populations relative to the main population is 0.3% and 1.3%,

respectively. It seems that this concept of plateau can well be extended to the dose dependence of cancer death rate in the Japanese cohort.

Now it is appropriate to introduce one more reason to our consideration. It is well known that it is just leukemia that is believed to be the most sensitive indicator of radiation effect, not solid cancer. This is due in part to the fact that the spontaneous incidence rate of leukemia is markedly lower compared to that of cancers.

If this is the case the low incidence rate of leukemia should increase the chance of revealing the radiation factor of risk. In reality the situation is quite different: the tendency toward the increased risk of solid cancer incidence or death manifests itself at lower radiation doses than the analogous risk for leukemia. Then the radiogenic nature of the above tendency may be questioned. It must not be ruled out that the tendency toward increased cancer death at very small doses revealed in the Japanese cohort is concerned with factors other than radiation. And it is just for this reason that the above tendency looks like a (quasi)plateau in the dose curve of carcinogenic risk.

Interestingly, the authors of [3] defined a minimum dose resulting in a significant excess risk of death due to solid cancer and found it to be 0.05 Sv (probably calculated from a no-threshold linear dependence with regard to slope error). But they abandoned their attempt to estimate an analogous value for leukemia because of difficulties connected with the linear-quadratic dose dependence. To approach to this problem, we applied the method of regression analysis based on a linear approximation of the dose dependence for the data on leukemia. Certainly, it was a very simplified approach since only averaged data could be used. However, we thus got a view, though a rough one, of what will be the result with the use of a no-threshold linear dependence. The analytical dependence of RR of leukemia death on radiation dose was:

$$y = 0.77 + 3.73 x,$$

where x is the dose (Sv) and y is the effect (RR). If $y = 1$, then $x = 0.06$ Sv. Thus, a rough approximation of data on leukemia by a linear regression yields a rough value of threshold dose which almost coincides with the minimal dose significantly efficient to produce a carcinogenic effect for solid cancers (for which the incorrectness of the linear dose dependence in the LLR, i.e. the presence of a (quasi)plateau in the dose range up to 200 mSv is also, though not so evidently, ignored). Conversely, if we take into account the stepped structure of the dose dependence of low-dose carcinogenic effect for solid cancer (the

step/plateau is slightly above 1) and leukemia (the step/plateau is somewhat below 1) then in both cases the threshold of the carcinogenic effect (the end of the step/plateau) is also practically the same and nearly equal to 200 mSv (150 mSv on the plots but this is the average value for the range 100-200 mSv.).

Clearly, the value of a minimal dose that permits detection of the carcinogenic effect of radiation is an analog of threshold. But because this value was calculated on the basis of a non-threshold linear approximation of data, which ignores the actual shape of dose curve in the low-dose range, it reflects the sensitivity of the method for assessment and sampling rather than a real threshold of the carcinogenic effect of radiation. In order to determine the value of a real threshold, allowance must be made for not only the significant difference between the real points and the control but also, and above all, the stepped character (presence of a plateau) of the dose curve in the low-dose range.

ANALYSIS OF EPIDEMIOLOGICAL DATA ON CARCINOGENIC RISKS IN THE EMERGENCY WORKERS (LIQUIDATORS) OF THE CHERNOBYL ACCIDENT

At present, the monograph by V. K. Ivanov et al. [10] is the most comprehensive work to summarize the results of examination of the Chernobyl liquidators (CL) cohort. The monograph presents generalized estimates of both the somatic (different nosological forms) and oncological morbidity/mortality rate for CL surveyed in the years between 1986 and 1996 within the framework of the Russian National Medical and Dosimetric Registry (RNMDR) according to the criteria adopted in the world literature. Our analysis will be concerned with the data on leukemia. This choice is explained by that a substantial part of radiogenic leukemias have already been realized within the time elapsed after the Chernobyl accident, as distinct from radiogenic solid cancers whose peak will fall on the period between 30 and 35 years after the accident, as calculated by the authors.

Table 1

Incidence rate for leukemia in liquidators of the Chernobyl accident, according to the data of cohort studies for the periods 1986-1993 and 1986-1996 within the framework of RNMDR [10]

Characteristics of cohorts studied	SIR	EAR/ 10^4 PY Gy		ERR/Gy	
		All leukemias	Leukemias without CLL	All leukemias	Leukemias without CLL
Period 1986-1983 (including departmental registries)	1.52	1.45		4.7	
Cohort population 142000					
Person-year number 1026944					
Average dose 0.11 Gy					
Period 1986-1996 (without departmental registries)	1.4 (1.0-1.8)	1.5 (0.9-2.6)		3.8 (2.1-6.2)	
Cohort population 93849					
Person-year number 903230					
Average dose 0.11 Gy					
Period 1986-1996 (without departmental registries)		0.9 (-0.3-2.0)	1.2 (0.1-2.3)	3.7 (-1.7-8.4)	9.9 (1.1-18.6)
Cohort population with determined doses 68199					
Person-year number 648917					
Average dose 0.13 Gy					

Footnote: Calculation of risk coefficients was made by the formulae cited in the monograph (see the footnote in Table 2). In brackets: 95% confidence interval.

In view of great number of liquidators examined, the representation of predominantly low-dose radiation loads, and the more precise, in spite of numerous gaps, evaluation of these loads, as compared to the Japanese cohort,

the data on leukemia in liquidators can substantially help in solving the problem of presence/absence of a threshold in the carcinogenic effect of radiation.

The estimates given by the monograph's authors for the incidence rate of leukemia in liquidators point at first glance to the absence of a threshold and thus are seemingly inconsistent with the data from the Japanese cohort. Indeed, the tables of the monograph (here reduced to Table 1) give positive values obtained both by the criterion of standardized incidence ratio (SIR), which is the ratio in % of the number of new cases of disease to the number of expected ones, and by the criteria of excess absolute risk (EAR) and excess relative risk (ERR). As it follows from Table 1, all variants of investigation gave positive average values of estimation criteria, although some of these values showed no significant difference from 0. Hence, despite the low average doses obtained for all examined subcohorts of liquidators, an increase in carcinogenic risk has been noted in each individual subcohort. In principle, this could be explained as follows. The average dose for the whole cohort of liquidators gives no idea on the real dose loads in the cohort because of a great scatter of individual doses received by its members: from nearly background values to 500 mSv. According to the data given in the monograph, about 20% of liquidators received doses above 200 mSv, i.e. doses that, according to the data for the Japanese cohort, significantly increase the incidence rate of both leukemia and solid cancer. It is just this group of liquidators that could contribute to the positive average values of excess carcinogenic risk coefficients for the cohort as a whole.

In order to gain a fuller understanding of the presence/absence of dose threshold for the carcinogenic effect in liquidators, the dose dependence of their incidence rate of leukemia should be analyzed. These data are quoted in the monograph. Moreover, the leukemia risk coefficients were probably obtained as values of dose curve slopes. These coefficients are presented in Table 1 for that part of the subcohort of 1986-1996 which includes only persons with documented doses.

Thus, the authors have used two approaches to assessment of risks, namely approaches with an external and internal control. In the first case, the risks were determined as a ratio of registered and verified cases of leukemia (designate them as observed leukemia) to the expected number of leukemias calculated for the same cohort with regard to its age-sex structure on the basis of medical statistics data for the incidence rate of leukemia among persons of analogous sex and age. In the second case, the risk coefficients were derived from a mathematical model for the dose dependence of leukemia incidence rate,

where the control was a subcohort with the lowest dose load provided the latter was small (for instance, it was below 5 mSv in the Japanese cohort).

Table 2
Carcinogenic risk estimates from the incidence rate for all leukemias for the cohort of liquidators with documented risks (according to the data of [10])

Dose, Gy (D)	Num- ber of PY (NPY)	Number of observed leuke- mias (NOL)	Number of expected leukemias (NEL)	Observed incidence rate per 10^4 PY	Expected incidence rate per 10^4 PY	EAR/ 10^4 PY Gy	ERR /Gy
0.02	154226	5	6.8	0.32	0.44	-5.8	-13.2
0.08	211726	5	9.3	0.24	0.44	-2.5	-5.8
0.15	170453	6	7.5	0.35	0.44	-0.6	-1.3
0.23	111074	6	4.9	0.54	0.44	0.4	1.0
Below are summarized data for the total cohort							
0.13	647479	22	28.3	0.34	0.44	-0.7	-1.7

Footnote: Calculation of risk coefficients was done by the formulae cited in the monograph. $\text{EAR}/10^4 \text{ PY Gy} = (\text{NOL} - \text{NEL})/(\text{NPY} \times \text{D})$; $\text{ERR/Gy} = \text{EAR}/10^4 \text{ PY Gy} \times \text{NPY}/\text{NEL}$.

Figure 8 shows dose curves for the incidence rates of all leukemias and leukemias minus the chronic lymphoid leukemia (CLL) (the latter is believed to be of non-radiogenic nature), all curves being constructed on the basis of data from the tables given in the monograph. In spite of the great confidence intervals for the points (not given in the plot), one is under the impression that there is a manifest tendency toward such a dependence, if not a reality of it, and hence an evidence in support of the linear no-threshold conception. Let us try however to apply both of the above-mentioned approaches to estimation of excess carcinogenic risks using the data of the tables mentioned above as well as calculated data for the external control for this cohort. These regression dependence curves we have calculated from average values and their analytical expressions, as well as external control values, are given in Figure 8.

Table 2 gives both epidemiological and calculated data on the incidence rate of all leukemias. The calculation of leukemia risk coefficients for individual dose groups and for the cohort as a whole has been performed with the help of the calculated index of external control that was used by the monograph's

authors to calculate risks for whole cohorts of liquidators (i.e. including liquidators with not documented doses). The table shows that, except for the subcohort with the dose 0.23 Gy, the coefficients of excess risks are negative values, i.e. the threshold of the leukemiagenic action is between 0.15 Gy and 0.23 Gy, which correlates with the data from the Japanese cohort.

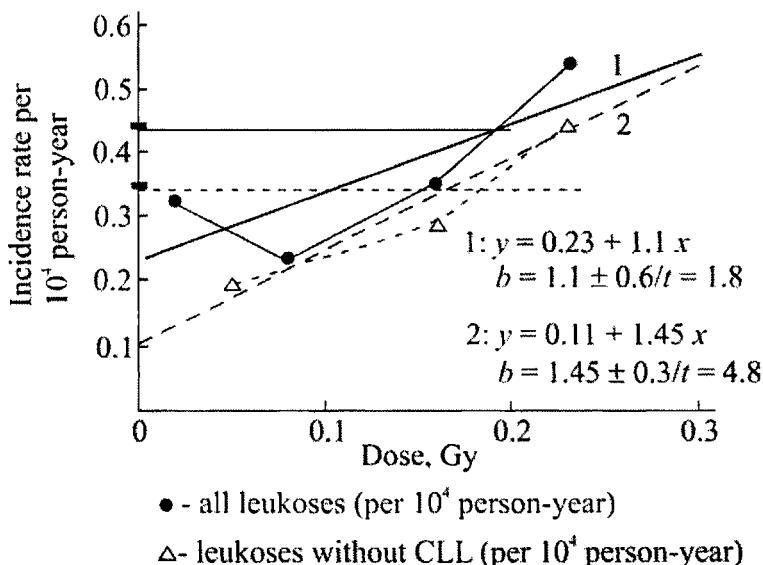


Figure 8. Dose dependence of incidence rate for leukemia in liquidators of the Chernobyl accident for the years 1986-1996 (according to [10]).
 1. Incidence per 10⁴ PY; 2. Dose, Gy; 3. All leukemias (per 10⁴ PY);
 4. Leukemias without CLL (per 10⁴ PY).

This conclusion was checked as follows. Using average dose values and their corresponding values of observed incidence rate, we calculated a regression dependence of the type

$$y = a + bx,$$

where y is the incidence rate per 10⁴ person-year (PY), a is the incidence rate in a subcohort with the dose 0 (analog of internal control), x is the dose (Gy). The following expression was obtained:

$$y = 0.23 + 1.1x.$$

This expression is identical to the linear model of the absolute risk:

$$\lambda = \lambda^0 + \text{EAR}/10^4 \text{ PY} \cdot \text{Gy} \times D$$

and, hence,

$$\text{EAR}/10^4 \text{ PY} \cdot \text{Gy} = 1.1; \lambda^0 = 0.23/10^4 \text{ PY}.$$

Hence, using the expression

$$\text{ERR/Gy} = \text{EAR}/10^4 \text{ PY} \cdot \text{Gy} / \lambda^0,$$

we obtain

$$\text{ERR/Gy} = 4.8.$$

Table 3
Carcinogenic risk estimates from incidence rate for leukemias minus CLL for the cohort of liquidators with documented doses (according to the data of [10])

Dose, Gy (D)	Number of PY (NPY)	Number of observed leukemias (NOL)	Number of expected leukemias (NEL)	Observed incidence rate per 10^4 PY	Expected incidence rate per 10^4 PY	$\text{EAR}/10^4 \text{ PY} \cdot \text{Gy}$	ERR /Gy
0.05	365952	7	12.8	0.19	0.35	-3.2	-9.1
0.15	170453	5	6	0.29	0.35	-0.4	-1.1
0.23	111074	5	3.9	0.45	0.35	0.4	1.2
Below are summarized data for the whole cohort							
015	647479	17	22.7	0.26	0.35	-0.6	-1.7

Footnote: Two lowest-dose groups were combined to one group by the monograph authors; calculation of risk coefficients was done by the formulae cited in the monograph (see the footnote for Table 2).

The excess risk values thus obtained are very close to those cited by the authors of the monograph (the minor difference is due to that we used a cruder approach, but it is not difficult to obtain values for the above parameters that would meet those quoted in the monograph and given in Table 1). However, the incidence values for the external and internal control differ significantly (0.44 and 0.23, respectively). From this it follows that the parameter "a" in the regression dependence is purely formal and cannot serve as a control adequate for the results of dose groups. A similar situation is with parameter "b", since, with allowance made for the actual control level adequately given by the external control value, almost the whole of the "dose curve" appears below this level (see Figure 8) and hence it must be concluded that there is a threshold in the leukemiagenic action of radiation.

In the same manner we treated the data for leukemias of radiogenic nature, i.e. all leukemias minus CLL. These data are presented in Table 3. The regression analysis of data presented in Table 3 yielded the analytical expression

$$y = 0.11 + 1.45x,$$

whence it follows that

$$\text{EAR}/10^4 \text{ PY·Gy} = 1.45; \lambda^0 = 0.11/10^4 \text{ PY·Gy}; \text{ERR/Gy} = 13.2.$$

These values however, though close to the coefficients given in the monograph, are also of no medical significance and are **purely formal parameters**.

Here one more important point should be stressed. According to the data of the monograph's authors, the average value of ERR/Gy for leukemias of radiogenic origin (evaluated at 9.9) appeared to be significantly different from 0, as distinct from the analogous index for all leukemias [3, 7] for which the low limit of confidence interval was a negative value (see Table 1). Our calculation of regression dependence curves and their parameters gave analogous results, which is also presented in Figure 8. But here the question arises as to whether the increase in the statistical significance of the coefficient ERR/Gy is actually connected with the exclusion of CLL cases from calculation. When calculating the parameters of dose dependence for leukemias of radiogenic nature, the monograph's authors integrated two groups with lowest doses into one. We used a similar procedure in our calculation (using average values) of the dose curve for all leukemias. The regression dependence in this case took the form:

$$y = 0.17 + 1.47x$$

and

$$\text{EAR} / 10^4 \text{ PY·Gy} = 1.47 \pm 0.54$$

and $t = 2.7$, i.e. this parameter became significantly different from 0. Thus, it cannot be excluded that the monograph's authors were able to approximate data on incidence rate of leukemias of radiogenic nature in liquidators of different dose groups by a line with parameter "b", which is significantly different from 0, because of the integration of two groups with lowest dose loads into one group, rather than due to the exclusion of CLL cases from calculation. The main thing is however not whether there is a tendency to a dose-dependent increase in incidence rate in this dose range but how the points being approximated by this dependence (or being not approximated) relate to the control level which in this case seems to be more adequately represented by the external control.

THE UPPER LOW-DOSE LIMIT IN TERMS OF MICRODOSIMETRY AND THE RESULTING PRACTICAL APPLICATION

In terms of microdosimetry, low doses are those at which only one particle/track of radiation passes through a certain sensitive volume of the cell usually associated with the cell nucleus [11-13]. The definition of this limit is understandable: most of the fatal consequences for a cell result from two-hit events.

The above-formulated definition of low doses is of a conceptual character and gives scope for the quantitative estimation of their upper limit. The thing is that because of the random character of hitting of particles on cell nuclei, there can exist no actual dose at which all cell nuclei would receive one hit at the same time, and some portion of nuclei, though an incomparably less one, would receive more than one hit. The point is: what the ratio between the nuclei with one hit and those with more hits is.

The authors of [11-13] gave an approximate estimation of the actual upper limit of low doses noting that it lies below a dose at which the portion of cell nuclei that received simultaneously some hits equals 0.2. They, however, did not undertake the task of determining the ratio between nuclei that received one hit and those with more than one hit. A different definition for the low-dose upper limit was proposed in [14, 15], namely one hit per nucleus on the average

for all cell nuclei. According to Poisson distribution, in this case 37% of nuclei have no hit, further 37% one hit, and 26% two and more hits. Clearly, in the first case [11-13] the estimation criterion is not definite enough, whereas in the second case [14, 15] this criterion is in conflict with the conception of low dose, since here the proportion of nuclei with one hit is comparable with the proportion of nuclei that received more than one hit at a moment.

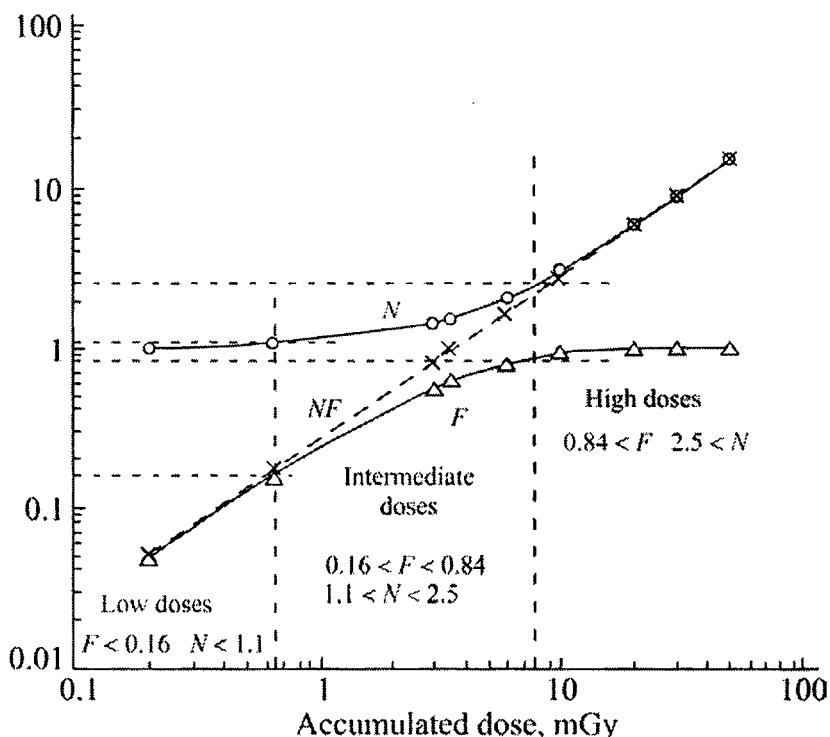


Figure 9. Absorbed dose dependence curves of microdosimetric parameters for sparsely ionizing radiation (according to the data of [11])
 F is the part of cell nuclei ($d = 8 \mu\text{m}$) with one or more hits;
 N is the average number of hits for nuclei having hits;
 NF is the average number of hits for all cell nuclei

It seems that the way out of this situation may be found in a more specific definition of the calculation criterion for the low-dose upper limit, the principle of one hit preference being undeniably retained. In Figure 9 we present

absorbed dose dependence curves for the microdosimetric parameters for the effect of ^{137}Cs radiation on cell nuclei $8\mu\text{m}$ in diameter. Two of these parameters (N and F) were taken from [11] and the third, NF , was calculated. Addition of this calculated parameter makes visual boundaries demarcating the low, intermediate, and high doses. Apparently, they must lie in the regions where the N and F curves approach/recede from the straight line NF or the horizontal line at level 1, and it is only necessary to define, even if arbitrarily, the acceptable degree of this approaching/receding. It is seen in Figure 9 that the range of low doses includes a region in which the proportion of nuclei with single hits preferentially grows, whereas the range of high doses forms a region with a preferential growth of the number of hits per nucleus. It only remains for us to set specific values of definite parameters. In line with the conceptual definition of low dose, let us prefer parameter N . Set $N \leq 1.1$. Using the Poisson distribution, find that this requirement of low dose upper limit is met by the absorbed dose 0.65 mGy ($N = 1.09$; $F = 0.156$; $NF = 0.17$) and hence $F \leq 0.16$. For the lower limit of a high dose, $F \geq 0.84$ can symmetrically be set, and in this case the absorbed dose will be about 8 mGy . Note once more that the above values of boundary doses are valid only for the sparsely ionizing radiation and for cell nuclei $8\mu\text{m}$ in diameter.

In terms of mathematics, the solution proposed here seems less exact than the establishing of a boundary between low and high doses at the level of crossing of a direct line NF and the line of Level 1, which corresponds to a dose of one hit per nucleus on the average for all nuclei. But the first of these solutions falls better into the conception of low dose. Indeed, for $NF = 1$ the absorbed dose is 3.5 mGy and the portion of nuclei having two and more hits is 26.5% (against 1.3% for $N \leq 1.1$). Besides, with the approach one hit per one nucleus on the average, the gradation of intermediate doses disappears at all, which is hardly correct in terms of general methodology (there is always an intermediate zone between principally different categories).

At first glance, the difference in the approaches to defining the upper limit of low doses is of extremely academic character and, above all, the superiority of one of them cannot in principle be proved in a radiobiological experiment. Nevertheless, it would be wrong to agree with this point of view. The quantity under discussion is related to determination of the threshold of the biologically harmful effect of low intensity chronic radiation. All kinds of radiation damage at the molecular/subcellular level have some "lifetime" and by the end of this interval they are either repaired or fixed as residual injuries, carrying definite health risks for the organism. As a conservative estimate of this

time interval, let us take 6 hours, in accordance with (13). Then, provided the test object is exposed to acute radiation at a low dose every 6 h, a dose potentially capable (or not capable) of producing some biological effect can be accumulated after a more or less lasting time. For instance, exposure to a dose of 0.65 mGy every 6 h for one year would yield a total dose of 0.95 Gy, and for a dose of 3.5 mGy, it would be 5.1 Gy. The resulting doses are large enough in order to try to detect an effect or to discriminate between two effects in an experiment. As a result, an answer may be obtained to the question if there is/there is not a threshold of the biologically harmful effect of exposures imitating chronic irradiation at dose rates at the beginning and at the midpoint of the third order above the background level (2 mSv/year), respectively, as well as if a 20-fold difference in proportions of cells receiving two and more hits at a time is significant in this situation.

CONCLUSION

The analysis of epidemiological data for the Japanese and Chernobyl cohorts of persons exposed to different doses of radiation presented here permitted us to suggest with confidence the presence of a threshold of the carcinogenic effect of radiation in the region about 200 mGy (mSv). This is primarily evidenced by direct data on incidence/mortality for leukemias of radiogenic nature, and now not only for the Japanese cohort but also for the Chernobyl cohort of liquidators, which have already passed the pick of leukemias. The situation is not so simple with solid cancers about which in the Japanese cohort for the dose range 5-200 mSv it has been noted that the actual number of deaths due to cancer exceeds the expected deaths. Although this excess in each dose group appears statistically insignificant, the tendency toward increased risk is still clearly pronounced. However, analysis shows the presence of a (quasi)plateau in this region of the dose curve of relative risk. This circumstance, together with the absence of an analogous tendency toward increased risk for leukemias (which are more sensitive indicators of radiation effect), permits the suggestion that the observed tendency towards increased carcinogenic risk of solid cancers in the low-dose range is of a non-radiogenic nature and, hence, the solid cancers also have their specific threshold of radiation influence. An alternative to this explanation may be the suggestion that

the (quasi)plateau may reflect the presence of a minor supersensitive subpopulation in the cohort under study.

Analysis of epidemiological data for the Chernobyl cohort showed that a direct dose dependence of carcinogenic risk (or a tendency for its presence) by itself cannot be an absolute argument in favor of existence of a real risk, since this dependence may actually be situated in the sub-threshold region. In this case the external control may appear a more adequate criterion for the significance of dose dependence than the internal control.

In the low dose region, the coefficients of excess absolute or relative risk (EAR/ 10^4 PY·Gy or ERR/Gy) are of a purely formal character and cannot be a measure of effectiveness of low dose radiation action. By and large, the range of low (from the medico-biological point of view) doses of radiation must be analyzed apart from the main body of data, otherwise its specificity connected in particular with the presence of a stepped (plateau) dose dependence can be lost in modeling radiation effects for the large range of doses of radiation influence.

In defining the low-dose upper limit by the microdosimetric criterion, the principle of one hit per one cell nucleus seems to be preferable. A workable approach to meet this principle is one according to which the proportion of cell nuclei that receive at least one hit of quantum/particle at a moment is 0.16 (in this case the relative proportion of cell nuclei that receive more than one hit at a moment is 1.3%). With this approach, the upper low-dose limit for sparsely ionizing radiation and for nuclei of 8 μm in diameter is 0.65 mGy and the possible threshold for the biologically harmful chronic radiation influence can correspond to a dose rate exceeding the usual radiation background by two orders.

REFERENCES

1. L.M. Rozhdestvensky, 'A conception of the biological action of low-level ionizing radiation: analysis of the problem in terms of thresholds and the radiosensitivity/radioreactivity of the organismic structures of different organizational levels', pp. 181-209: In: *Low Doses Of Radiation: Are They Dangerous?*, Ed. E.B. Burlakova, Nova Science Publishers, New York, 2000.
2. L.M. Rozhdestvensky, 'Pro and contra regarding the threshold/non-threshold mutagenic (cancerogenic) action of low level ionizing radiation', *Radiatsionnaya Biologiya. Radioekologiya*, 2001, vol. 41(5), pp. 580 - 588. (Rus)
3. D.A. Pierce, Y. Shimizu, D.L. Preston, M. Vaeth, and K. Mabuchi, 'Studies of the mortality of atomic bomb survivors. Report 12. Part 1. Cancer: 1950-1990', *Radiat. Res.*, 1996, vol. 146(1), pp. 1 - 27.
4. Y. Shimizy, H. Kato, and W.J. Schull, 'Studies of mortality of A-bomb survivors. 9. Mortality, 1950-1985: Part 2. Cancer mortality based on the recently revised doses (DS86)', *Radiat. Res.*, 1990, vol. 121(2), pp. 120 - 141.
5. H. Katayama, M. Matsuura., S. Endo, M. Hoshi, M. Ohtaki, and N. Hayakawa, 'Reassessment of the cancer mortality risk among Hiroshima atomic bomb survivors using a new dosimetry system, ABS2000D, compared with ABS93D', *J. Radiat. Res.*, 2002, vol. 43(1), pp. 53 - 63.
6. M.P. Little and C.R. Muirhead, 'Derivation of low-dose extrapolation factors from analysis of curvature in the cancer incidence dose response in Japanese atomic bomb survivors', *Int. J. Radiat. Biol.*, 2000, vol. 76(7), pp. 939 - 953.
7. D.C. Lloyd, A.A. Edwards, A.Leonard, G.L. Deknudt, L. Verschaeve, A.T. Natarajan, F. Darroudi, G. Obe, F. Paliti, C. Tanzarella, and E.J. Tawn, 'Chromosomal aberrations in human lymphocytes induced *in vitro* by very low doses of X-rays', *Int. J. Radiat. Biol.*, 1992, vol. 61(3), pp. 335 - 343.
8. A.V. Sevankaev, 'Quantitative estimation of the cytogenetic effects of low-level radiation: current aspects', *Radiobiologia*, 1991, vol. 31(4), pp. 600 - 605. (Rus)
9. D.M. Spitkovsky, 'Concept of the effect of low doses of ionizing radiation on cells and its possible use for the interpretation of medical-biological consequences', *Radiobiologia*, 1992, vol. 32(3), pp. 382 - 400. (Rus)

10. V. Ivanov, A. Tsyb, S. Ivanov, G. Souchkevitch *et al.*, ‘Low doses of ionizing radiation: health effects and assessment of radiation risks for emergency workers of the Chernobyl accident’, Eds. G.N. Souchkevitch, M.N. Repacholi, In: *Protection of the Human Environment Occupational and Environmental Health*, Geneva, 2001.
11. V.P. Bond, L.E. Feinendegen, and J. Booz, ‘What is a ‘low dose’ of radiation?’, *Int. J. Radiat. Biol.*, 1988, vol. 53(1), pp. 1 - 12.
12. J. Booz and L.E. Feinendegen, ‘A microdosimetric understanding of low-dose radiation effects’, *Int. J. Radiat. Biol.*, 1988, vol. 53(1), pp. 13 - 22.
13. L.E. Feinendegen, V.P. Bond, J. Booz, and H. Muhlensiepen, ‘Biochemical and cellular mechanisms of low-dose effects’, *Int. J. Radiat. Biol.*, 1988, vol. 53(1), pp. 23 - 37.
14. D.M. Spitkovsky, ‘Novel biophysical and biological aspects of eukaryotic cells response to low doses of low-LET radiation’, *Radiatsionnaya Biologiya. Radioekologiya*, 1999, vol. 39(1), pp. 145 - 155. (Rus)
15. S.A. Geraskin, V.G. Dikarev, A.A. Udalova., and N. S. Dikareva, ‘Regularities of cytogenetical disturbances induction by low doses of radiation in barley germ root meristem cells’, *Radiatsionnaya Biologiya. Radioekologiya*, 1999, vol. 39(4), pp. 373 - 383. (Rus)

Emotional stress and the problems of radiation medicine

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ABSTRACT

In connection with the problems of radiation medicine, consideration is given in this report to the possibility of influence of emotional stress on the behavior and occupational activity of man, on the development of posttraumatic stress disorders and other diseases associated with the actual or hypothetical effect of ionizing radiation, as well as on the course of diseases induced by radiation injuries. It is shown that the problems of emotional stress and radiation medicine and safety are closely related. Their study is essential in solving the tasks confronting the extreme medicine.

Keywords: Ionizing radiation, emotional stress, human behavior, somatic disorders, pharmacological correction

Information about the ionizing radiation as a factor capable of being hazardous to human safety and health may be the cause of an emotional stress. Among non-radiation factors, it is just the mental strain that most naturally follows the action of ionizing radiation on the human organism. The central point of emotional stress is the feeling of anxiety, the perception of situation as a threatening one, as a signal of trouble and hazard. This feeling can arise in response not only to the actual influence of ionizing radiation but also to a “hypothetical” hazard of exposure. The emotional stress reaction is undoubtedly an individual phenomenon: some psychic factor may be a stressor for one individual and be absolutely indifferent to another one; that is this reaction is based on a subjective attitude of an individual to a stress factor. The goal of

emotional stress is to adapt the organism to the action of stressor, but the radiation medicine is primarily concerned with the negative consequences of stress for human health. These consequences depend on the intensity and duration of stress reaction and are determined by phenotype. Under extreme conditions, the role of individual predisposition is substantially reduced, though its significance persists.

The radiation medicine provides (1) the medical supervision of the occupational activity of man that is associated with the use of ionizing radiation, (2) the health protection of people who have got into the area of possible radiation influence as well as (3) the prophylaxis and medical treatment of radiation-induced injuries and (4) must take proper account of the effects of acute and chronic emotional stress. These effects may combine with the action of ionizing radiation on the organism, be an emotional response to radiation exposure as well as to emergency conditions. When the influence of ionizing radiation is possible but does not actually take place or the exposure is very small, which is consistent with the notion of admissible risk, the consequences of emotional strain come to the fore.

According to the goals of radiation medicine, we shall consider the influence of emotional stress on the behavior and occupational activity of man under extreme conditions and on the development of posttraumatic stress disorders due to real or hypothetical action of ionizing radiation, assess the role of emotional stress effects in the genesis of somatic disorders as well as take into account the influence of emotional overstrain on the course of radiation disease.

The possibility of development of an acute emotional stress and its effect on man behavior are particularly significant in accidents, including radiation-induced ones, and in other extreme situations. In the occupational activity, this is primarily the psychotechnological problem of interaction of man with complex aggregates, which requires increased attention and maximum readiness to control the system. In emergency situations, significant decisions may be taken in the conditions of lack of regulatory instructions, discrepant information, shortage of time, which is accompanied by an extreme mental strain and is a background for emotional stress development. Under these conditions, the people, primarily persons that are unstable to mental stress factors, may show disorders in orientation and behavior, commit grave errors in the occupational activity, up to a complete inability to behave adequately in emergency conditions. This by itself may not only hamper activities associated with emergency measures but also aggravate the accidental situation. It should be kept in mind that an acute emotional stress is characterized by vegetative

reactions (tachycardia, giddiness, sickness, dyskinesia of the alimentary canal etc.), which also can hamper the occupational activity. It has been found that 60% to 80% of all accidents and crashes in the industry occur because of human errors, and an error may be caused by the negative consequences of emotional stress [1]. The development of an emotional stress, which negatively affects the efficiency of a person, depends on human phenotype, which determines the predisposition of a person to stress reactions, and on the health condition of this person during occupational activity. Therefore, it is necessary to improve the system of medicopsychological selection of people for the work in areas of special importance, to develop individual programs (if required) for correction of their emotional sphere. It has been well known that disorders in the mental adaptation may also be revealed in the professionals whose occupational activity suggests an obligatory preliminary medicopsychological selection [2]. According to the efficiency criteria and behavioral reactions, healthy persons subjected to an emotional stress can exhibit three types of mental strain: (1) an operational (stress-stable) type which demonstrates adequate reactions, stability or even an increase in operator activity; (2) an emotional (stress-unstable) type for which deadaptive neurotic reactions and a decreased efficiency are characteristic; and (3) an intermediate type with mean parameters [3, 4]. Human reactions have a genetic background and at the same time they are significantly influenced by socially conditioned subjective factors and professional knowledge, which determine the accordance between requirements imposed and possibilities to satisfy them [5]. In the system of professional selection, special markers for phenotypically different human stress reactions should be used, the initial values of which permit assigning an individual response to one or another type of reaction with a high degree of probability. The idea that emotional instability and anxiety play a role in the decrease of adaptation potential retains its significance. At the Institute of Pharmacology, RAMS, under the supervision of S.B. Seredenin and B.A. Badyshtov, a complex of parameters have been recently defined that enable the activity of man under emotional stress to be predicted. Most informative methods and the hormonal-biochemical characteristics for psychological testing have been identified. Of principal importance are the data that indicate that the selection with a 100% probability of people persistent to stress factors is only possible on the basis of characteristics determined in blood, such as the level of cholinesterase, cAMP, cGMP as well as cAMP/cGMP, and cortisol/insulin ratios [6]. A similar degree of discrimination can also be obtained by using selected mental characteristics that reflect the structural-typological peculiarities of a human individual [6].

These findings are of importance not only in the perfection of the system of professional selection. On the basis of the tests developed, one can predict the response of experienced specialists to tranquilizers and perform individual pharmacological correction.

Special consideration must be given to situations when experienced specialists have been for a long time occupied in the atomic industry and are of undeniable benefit to the society but their health condition is somewhat disturbed. It has been known that the disorders of mental adaptation under emotional overstrain are highly pronounced in persons with neurocirculatory dystonia, hypertension and the ulcer disease of stomach and duodenum. In these persons, not only the general health condition but also the emotional sphere must be under constant medical supervision and, if necessary, measures should be taken to correct the latter. Examination of the state of mental adaptation in the personnel of an NPP enabled detection of disorders in 12.4% of persons: from the prenosological rudimentary forms to the clinically manifested neurotic and other mental pathologies and recognition of groups with risks of deadaptation development [7].

The consequences of acute emotional stress are not confined to immediate reactions of the organism within hours or days. The durable changes in behavior, the disorder of the mental status may develop as a prolonged or delayed reaction after emotional stress, which has been reflected in the notions of posttraumatic stress disorders (PTSD) included in the International Classification of Diseases (ICD) [8]. PTSD are a psychiatric syndrome characterized by three groups of symptoms: 1) symptoms of "re-feeling", i.e. repeated reproduction in memory of events associated with the trauma; 2) symptoms of avoiding: attempts to evade any mentioning of the trauma, the feeling of keeping away and estrangement; 3) persistent symptoms of hyperexcitation, i.e. difficulties with going sleep, sleep disturbances, overanxiousness, and unhealthy hyperreactions to negative information [9]. The disorders in memory and cognition and the integration of information and emotions observed in persons with PTSD resemble the state that is termed in neurology "dissociation" of consciousness [10]. PTSD is a form of manifestation of emotional stress and does not depend on what particular traumatic event served as an etiological factor. A peculiar feature of PTSD is that for it to occur a single strong influence capable of inducing a shock is sufficient. The shock is fixed and is repeatedly revived in memory, creates a constant expectation of events fraught with health risk and is transformed to some kind of behavioral programs a man under mental stress unconsciously follows. Persistent

recollections of traumatic events accompanied by repeated emotions, depression, melancholy, anxiety and decreased interest in earlier significant aspects of life are characteristic of the PTSD pattern. Affective emotions associated with radiation damage risk as well as the formation of a psychological aim at expectation of negative consequences may also be among PTSD symptoms. Typical in this case are depressive phenomena and vegetative-humoral changes intrinsic in the anxiety state. Examination of "liquidators" of the Chernobyl accident revealed that about 20% of them suffered from PTSD: they believed they had been severely injured by radiation, although no signs of radiation damage were detected in them. These persons had experienced in the accident area deep negative emotions and anxiety about their health. Subsequently, they considered any indisposition to be related with radiation exposure [11]. The development of PTSD depends on the subjective perception of risk degree and on the individual features of person. But if the intensity of stress influence is very high, individual features of people do not play any decisive role. The information about the mechanism of PTSD is rather scanty. The neurochemical mechanisms underlying the "stagnant" negative emotions remain to be interpreted. Patients with PTSD show an increased activity of the sympathetic nervous system [12]. The intensive emotional reactions are accompanied by a substantial increase in daily excretion of catecholamines with urea, by tachycardia, and arterial hypertension. It is believed that of considerable importance in the development of PTSD is the "conditioning" or the conditioned fear which, acting via the noradrenergic mechanisms, continuously supports the "stagnant" negative emotions [13]. Patients with PTSD show changes in the activity of the hypothalamic-hypophyseal-adrenal axis. The level of ACTH secretion in response to stimulation, for example, by means of CRF, decreases. The level of cortisol in blood and urea is sharply reduced whereas the number and sensitivity of glucocorticoid receptors of lymphocytes are increased. The decrease in cortisol level directly depends on the severity of PTSD [14-17]. In patients suffering from PTSD, along with psychopathological reactions, somatic disorders are also observed. These disorders are characteristic of the disease pattern of PTSD but at the same time they can be the result of chronic emotional stress. Such patients frequently come into the view of physicians of a very wide spectrum of specialization but the treatment of accompanying diseases without correction of the emotional sphere is not always efficient.

According to the psychological, psychophysiological and endocrinological examinations, the phenomena of the chronic emotional stress and, as a consequence, somatic disorders have been registered in a part of the

population who have suffered from the Chernobyl accident and in the "liquidators" of this accident exposed to low doses of radiation [18-27]. In these persons, the development of emotional strain is likely to be associated with the fear of the consequences of exposure to radiation and with the changes in the social conditions of their life. The stress factors associated with the Chernobyl accident and the post-accident situation need analysis with due regard for their interaction [28]. The emotional stress may be a factor of etiology and pathogenesis for a variety of pathologies (stress diseases) and also potentiate diseases already available. The health condition of persons who have experienced a mental stress in connection with exposure risks and/or with an actual exposure to low-dose ionizing radiation is primarily determined by the stress-induced disturbances, which deserve special consideration.

The chronic emotional stress frequently causes neurotic states and personality decompensations, which are manifested as depressive and anxiophobic disorders [29-30]. The incidence rate of mental disorders in the liquidators of the CNPP accident gradually grew up to 1991 to be then stabilized at a high level. In 1993 the incidence rate of mental pathologies in liquidators exceeded the All-Russian level 9.6 times [31, 32]. The mental disorders in liquidators are characterized by polymorphism. They are represented by asthenic, psychovegetative, affective, and intellectual-mnestic disorders in combination with various forms of psychosomatic pathology, primarily with the diseases of the cardiovascular system [33].

The emotional stress may provoke and be an important factor in the development of the ischemic disease of heart, myocardium infarction, arrhythmia, arterial hypertension, and sudden cardiac death. A pronounced adrenergic component of the stress reaction may lead to a spasm of anatomically intact coronary vessels and cause a secondary ischemic affection of heart as well as potentiate blood coagulation and thrombosis of coronary vessels [34, 35]. A stress-induced hypercholesterolemia and atherogenic dislipoproteinemia have been described [36, 37]. The trigger of sudden cardiac death is frequently an emotional stress [38]. The chronic action of intensive emotional stressors is a significant factor of etiology and pathogenesis of the hypertension disease the basis of which is the stagnant activation of the adrenergic component of vessel tonus regulation. From 1987 to 1993, the incidence of blood circulation organs disorders in the liquidators of the Chernobyl accident gradually increased and in 1993 its value exceeded the all-Russia level 4.3 times [31, 32, 39]. Among the cardiovascular pathologies in liquidators, the ischemic disease of heart and the hypertension disease are most frequent. [40].

There is good reason to believe that in many cases there is a direct connection between the exacerbation or arising of the ulcer disease of stomach and duodenum in a patient and the presence of chronic action of negative emotions in his anamnesis [41]. In the liquidators of the Chernobyl accident, the incidence rate of digestion organ diseases in 1993 was 3.7 times higher than in the population of Russia as a whole [31, 32]. Among these diseases in liquidators, chronic gastritis, stomach and duodenum ulcer were prevailing [42]. An intensive and long stress reaction is accompanied by a prolonged suppression of the immune response up to the development of an immune deficiency state. In the case of a long-term emotional strain, a persistent decrease in the activity of normal killers was reported, which can well be a factor of risk of malignant pathologies [43]. A prolonged stress reaction also decreases the level of immunoglobulins in blood as well as of what is called normal antimicrobic antibodies [44, 45]. An emotional stress may provoke pathologies having an allergic or autoimmune component, rheumatoid arthritis, multiple sclerosis etc. [46]. Here an important conclusion is that a severe chronic emotional stress may aggravate immune system disorders that have been induced by other factors. In the liquidators of the CNPP accident, an increase in the incidence rate of diseases related to immune system disorders (infectious and autoimmune ones) was observed [47, 48].

Thus, the role of emotional stress factors should be allowed for in assessing the health state and in assaying the morbidity of persons who have resided in the area of possible radiation influence. The representatives of WHO have come to the conclusion: "Scientists who have had no wealth of experience in assessing the consequences of radiation exposure have explained various biological and medical abnormalities in exposed persons by the action of radiation. But these abnormalities are most probably the consequence of mental stress" [49].

The emotional stress after irradiation may aggravate the course of radiation-induced diseases. At early times after low-dose γ -irradiation, the emotional stress decreases the adaptive and compensatory potentialities of the haemopoietic system of animal organisms. The emotional stress after a lethal-dose irradiation inhibits the post-radiation recovery of haemopoiesis, aggravates the course of acute radiation disease, and decreases the efficiency of the radioprotector indralin. These disorders are especially pronounced upon a prolonged and intensive stress [50, 51]. The use of the selective anxiolytic aphobazol (a compound from the group of mercaptobenzimidazol) made it possible to arrest completely disturbances in the development of adaptive

reactions to stress in the blood system and to normalize its compensatory potentialities in animals under the conditions of combined influence of intensive long-term emotional stress and low-dose irradiation. In the case of lethally irradiated animals, the treatment of stressed animals with aphobazol favorably influenced the course of acute radiation disease, enhanced the recovery processes in the blood system, and increased the animal's survival. Under these conditions, the use of aphobazol completely removed the negative effect of emotional stress in indralin-protected animals [52-53]. Thus, the pharmacological correction of the emotional stress caused by different doses of radiation is not only a symptomatic but also a pathogenetically justified form of treatment.

In order to correct the emotional-stress pathology, an aggregate of methods are used. The psychopharmacological means are rather efficient in the prophylaxis and correction of the emotional stress associated with the action of ionizing radiation on the organism. The emotional stress may develop after irradiation and in most cases, as with other kinds of pathology, its action on the organism is unfavorable. The arrest of emotional stress is justified after exposures to lethal, sublethal and low doses. The mental depression that develops during acute radiation syndrome in man had dictated at one time the expedience of using barbiturates and sedative means and these recommendations have been included in the first manual on radiobiology [54, 55]. The use of pharmacological preparations permits elimination of the emotional strain and thus improves the general state of patients.

The cutting off a direct emotional stress is necessary for prophylaxis of posttraumatic stress disorders associated with radiation hazard in accidents, especially in persons who assess the risk as a very high one and exhibit pronounced emotions. The psychopharmacological treatment may also be used in the therapy of PTSD [56].

Worth of special consideration is the problem of arresting emotional stress in persons who are occupied in especially important areas of work and must take adequate solutions in emergency situations. Under these conditions it is most advisable to treat such persons with the agonists of the benzodiazepine receptors, which activate the stress-limiting inhibitory function of the GABAergic system and produce an anxiolytic effect. However, there are some limitations, primarily the possibility of development of side effects: soporific, sedative, myorelaxant ones, memory disturbances and, what is most essential, a decrease in "active mental adaptation" [57]. At present, benzodiazepines with distinct anxiolytic action able to optimize the operator's activity at low side

effects are being actively and successfully searched. These are preparations such as hydazepam, day-action tranquilizers, and the preparation aphobazol developed at the Research Institute of Pharmacology, RAMS, and now being studied in clinics [58]. A tranquilizer able to cut off emotional stress in persons unstable to stress factors, to normalize their operator activity, and at the same time not to decrease the efficiency in stress-stable persons – if such a preparation will be found (and there are all prerequisites for that) – will find a wide application in the radiation medicine. At present it is advisable to recognize the phenotypes of emotional-stress reactions and apply tranquilizers individually with due regard for phenotype.

A too strong and/or prolonged influence of a stressor may lead to cell damage and to the development of stress pathology. Because of the shortage of information, there is still no definite conception of the mechanisms by which the organism responds to the combined action of low-dose ionizing radiation and emotional stress, so this problem requires special analysis. Here we must point to some mechanisms responsible for the damaging effects of stress reaction. An excessive increase in Ca^{2+} and Na^+ in the cell results in its damage. Activation of lipases, phospholipases, and free-radical oxidation as well as an increase in fatty acid amount may lead to lesions in biomembranes. Under the conditions of a chronic intensive stress reaction, the mobilization of energetic and structural resources leads to a progressive exhaustion of the organism.

It has been determined experimentally that after a prolonged and intensive stress, the mobilization of the blood system gives way to a suppression of haemopoiesis. In the bone marrow, the number of myelocaryocytes decreases, the thymocyte content in the thymus sharply drops, the peripheral blood shows signs of leukopenia, netrophilopenia, lymphopenia, the number of monocytes decreases 2.5 times [59].

The biological effects of low-dose ionizing radiation can probably show up most strikingly at the background of emotional overstrain of the organism. The adaptation reaction or (under definite conditions) the damage in response to any irritant including an emotional one are effected by a system of secondary messengers, primarily cyclic AMP and GMP, through the activation of proteinkinases and Ca^{2+} , the universal regulator of intracellular processes. The system of intracellular intermediary agents of the neurohumoral signal was found to be very sensitive to low-dose ionizing radiation [60-62]. The character of the combined effect of radiation and emotional stress will depend on the individual sensitivity to these factors, dose, exposure conditions, the intensity of

emotional stress influence as well as the temporal interaction of radiation and emotional stress effects.

Thus, there is a background for the summation of the damaging effects of ionizing radiation and emotional stress, but in this case the processes of post-radiation recovery should be taken into account. This problem requires special investigation, although there is reason to believe that the effects of low-dose ionizing radiation are primarily associated with the stress pathology.

The role of prolonged emotional strain seems to be apparent in the development of somatic disorders or PTSD in connection with possible risks of exposure to or under the action of low-dose ionizing radiation on the organism. If correction of the emotional sphere is not included in the schedule of medical treatment, the efforts of physicians, among them cardiologists or gastroenterologists, may appear not to be sufficiently effective. Patients with emotional stress need psychotherapeutic and psychoneurologic help frequently including the methods of pharmacological correction with anxiolytics, antidepressants, and nootropics. Of special interest in the medical treatment of consequences of the combined effects of emotion stress and ionizing radiation is the use of antioxidants as well as complex preparations with antioxidant and anxiolytic activity which not only restrict unfavourable emotional reactions but, probably, also reduce the damaging effects of radiation on the cells [63]. The treatment of such patients requires special knowledge in the field of radiation medicine and psychoneurology, and of great importance in this case are the personal features of the physician, the patient's confidence in the personnel, and a proper orientation of the medical staff. The loss of confidence in the physicians and the authorities significantly promoted the disturbances in the mental status of people after the accident at the Chernobyl nuclear power plant.

The problems of emotional stress and radiation medicine and safety are closely interrelated. Their study is essential in performing the tasks confronting the extreme medicine.

REFERENCES

1. O'Hare D., Wiggins M., Batte R., and Morrison D., *Ergonomics*, 1994, vol. 37(11), pp.1865 - 1869.
2. Berezin F.B., *Mental and Psychophysiological Adaptation of Man*, Leningrad, Nauka, 1988, 243 p. (Rus).
3. Naenko N.I., *Mental Strain*, Moscow, Moscow State University, 1990, 186 p. (Rus).
4. Arshavsky V.V. and Rottenberg V.S., *Uspekhi Fisiologicheskikh Nauk*, 1978, No. 3, pp. 49 - 52. (Rus)
5. Sokh T., *Stress*, Moscow, Meditsina, 1981, 330 p. (Rus)
6. Seredenin S.B., Badyshtov B.A., Neznamov G.G., Makhnycheva A.L., Kolotilinskaya N.V., and Nadorov S.A., *Experimental and Clinical Pharmacology*, 2001, vol. 64(1), pp. 3 - 12.
7. Neznamov G.G., Syunyakov S.A., Morozov I.S. et al., 'Modern Approaches to Medical and Occupational Rehabilitation of Life Savers', *Proceedings of the First International Scientific Congress*, September 29-30, 1999. Moscow, 1999, pp. 176 - 177. (Rus).
8. *The International Statistical Classification of Diseases and Health Problems*, Tenth Revision, Geneva, WHO, 1995.
9. Putman F.W., *Am. N. Y. Acad. Sci.*, 1995, vol. 771, pp. 708 – 715.
10. Carlson E.B. and Putman F. W., *Dissociation*, 1993, vol. 6, pp. 16 – 27.
11. Tarabrina N.V., Lazebnaya E.O., and Petrukhin E.V., *The Chernobyl Trace. Medico-Psychological Consequences of Radiation Effect. Collection of scientific papers*, Part 1, Moscow, IzdAT, 1992, pp. 192 - 237. (Rus).
12. Southwick S.M., Kristal J.H., and Morgan C., *Arch. Gen. Psychiatry*, 1993, vol. 50, pp. 267 – 274.
13. Bremner J.D., Kristal J.H., Southwick S.M., and Charney D.S., *Synapse*, 1996, vol. 23(1), pp. 28 – 38.
14. Yehuda R., Giller E.L., Southwick S.M., Lowy M.T., and Mason J.W., *Biol. Psychiatry*, 1991, vol. 30, pp. 1031 – 1048.
15. Yehuda R., Southwick S.M., Kristal J.H., and Charney D.S., *Am. J. Psychiatry*, 1993, vol. 150, pp. 83 – 86.
16. Yehuda R., *Arch. Gen. Psychiatry*, 1995, vol. 52, pp. 583 – 593.
17. Yehuda R., *Science*, 1997, vol. 275, pp. 1662 – 1663.

18. Ushakov I.B., Arlashchenko N.I., Dolzhanov A.Ya., and Popov V.I., *Chernobyl: Radiation Psychophysiology and Ecology of Man*, Moscow, State Research Institute of Aviation and Space Medicine, 1997, 246 p. (Rus)
19. Romanenko A.E., Nyagu A.I., and Kalinauskas I.N., *Problems of Radiation Medicine. Republican Interdepartmental Collected Papers*, Kiev, Zdorovya, 1991, Issue 3, pp. 3 - 7.
20. Nyagu A.I., Ferents V.P., Garnets O.N. et al., *Medicinal Consequences of the Accident at the Chernobyl Nuclear Power Plant. Information Bulletin*. Kiev, Scientific Center of Radiation Medicine, AMS of the USSR, 1991. pp. 256 - 267. (Rus)
21. Krasnov V.N., Yurin M.M, Petrenko B.E. et al., "Liquidators" of the Chernobyl Accident. *Health Condition*, Moscow, IzdAT, 1995, pp. 98 - 107. (Rus).
22. Mitryaeva N.A., *Med. Radiol. i Radiats. Bezopasn.*, 1996, vol. 41(3), pp. 19 - 23. (Rus)
23. Malygin V.L. and Tsygankov B.D., *Mental Disorders of Radiation Genesis (clinics, pathophysiology, therapy)*, Tula, Tulsky Polygrafist, 2000, 231 p. (Rus).
24. Moroz B.B. and Deshevoy Yu.B., *Radiats. Biol. Radioekol.*, 1999, vol. 39(1), P. 97-105.
25. Moroz B.B. and Deshevoy Yu.B., *Low Doses of Radiation: Are They Dangerous?*, Ed. E. Burlakova, N.-Y., Nova Press Publishers, 2000, Chapt. 10, pp. 127 - 140.
26. Aleksandrovsky Yu.A., Rumyantseva G.M., Shtchukin B.P. et al., *Zh. Nevropatol. i Psichiatrii*, 1989, vol. 89(5), pp. 111 - 117. (Rus)
27. Girard P. and Heriard Debreuil G., *J. Radiol. Prot.*, 1996, vol. 16(3), pp. 167 - 180.
28. Heriard Debreuil G., *Revue Francaise de Radioprotection*, 1994, vol. 29, pp. 363 - 376.
29. Sokolova E.B., Berezin F.V., Barlay T.V., *Materia Medica*, 1996, No. 1 (9), pp. 5 - 21.
30. Berezin F. V. and Miroshnikov M. P., *Materia Medica*, 1996, No. 1 (9), pp. 29 - 56.
31. 'Materials of the Russian National Medical and Dosimetric Registry', *Radiation and Risk: Bulletin of the National Radiation and Epidemiological Registry*, Moscow, Obninsk, 1995, Issue 5, pp. 16 - 47. (Rus).
32. Tsyb A.F., Ilin L.A., and Ivanov V.K., 'Radioecological, Medicinal and Socioeconomic Consequences of the Accident at the Chernobyl NPP.

- Rehabilitation of Territories and Population', *Report Theses of the All-Russia Conference*, May 21-25, 1995, Moscow, Scientific and Methodical Center, Golitsino, 1995, pp. 37 - 52. (Rus)
33. Melnikova T.S., Krasnov V.N., and Marinenko K.E., 'Radioecological, Medicinal and Socioeconomic consequences of the Accident at the Chernobyl NPP', *Rehabilitation of Territories and Population Report Theses of the All-Russia Conference*, May 21-25, 1995, Moscow, Scientific and Methodical Center, Golitsino, 1995, p. 102. (Rus)
34. Simons M. and Downing S.E., *Am. Heart. J.*, 1985, vol. 109, pp. 297 - 305.
35. Krantz D.S., Kop W.J., Santiago H.T., and Gottdiener J.S., *Cardiol. Clin.*, 1996, vol. 14(2), pp. 271 - 281.
36. Polozhentsev S.D. and Rudnev D.L., *Fiziologiya Cheloveka*, 1986, No. 12, pp. 151 - 155. (Rus)
37. Clarkson T.B., Kaplan J.R., Adams M.R., and Manuck S.B., *Circulation.*, 1987, vol. 76, Pt. 2, pp. 1 - 29.
38. Myers A. and Dowaer H.A., *Brit. Heart*, 1975, vol. 37, pp. 1133 - 1146.
39. Mikhalsky A.I., Ivanov V.K., Maksyutov M.A. et al., *Radiation and Risk: Bulletin of the National Radiation-Epidemiological Registry*, Moscow, Obninsk, 1996, Issue 8, pp. 38 - 46. (Rus)
40. Shamarin V.V., Shalnova S.A., Kukushkin S.K. et al., *Kardiologiya*, 1996, vol. 36(3), pp. 44 - 46. (Rus)
41. Piper D.W. and Tennants C., *J. Clin. Gastroenterol.*, 1993, vol. 16(3), pp. 211 - 214.
42. Shamarin V.M., Shalnova S.A., Vinokurov B.K. et al., "Liquidators" of the Chernobyl Accident. Health Condition, Moscow, IzdAT, 1995, pp. 108 - 113. (Rus).
43. Kiecolt-Glaser J.K., *Psychosom. Med.*, 1984, vol. 46, pp. 7 - 14.
44. Kryzhanovsky G.I., Migayeva S.V., and Makarov S.R., *Neuroimmunopathology*, Moscow, Meditsina, 1997, 197 p. (Rus).
45. Kuz'min S.N. and Pershin B.B., *Sov. Med.*, 1988, No. 8, pp. 108 - 111. (Rus)
46. Solomon G.F., *Stress, Immunity and Aging*, Ed. Marsel Dekker. N.Y., 1984, vol. 10, pp. 1 - 10.
47. *Health Effects of the Chernobyl Accident. The Results of the Pilot Projects of IPHECA and their Associated National Programs: Scientific Report of the World Health Organization*. Geneva, 1996, pp. 174 - 215.
48. Kiseleva E.P., Kositskaya L.S., Freidlin I.S., Rudenko I.Ya., Kuzmenok O.I., *Radiats. Biol. Radioekol.*, 2000, vol. 40(1), pp. 32 - 36. (Rus)

49. *Chernobyl Project. Examination of Radiological Effects and Appraisal of Protective Measures. Closing Brochure.* IAEA, Austria, August, 1991, 42 p.
50. Moroz B.B., Deshevoy Yu.B., Lebedev V.G., Lyrshchikova A.V., and Vorotnikova T.V., *Radiats. Biol. Radioekol.*, 1997, vol. 37, Issue 4, pp. 581 - 589.
51. Moroz B.B., Deshevoy Yu.B., Lebedev V.G., Lyrshchikova A.V., Vorotnikova T.V., *Radiats. Biol. Radioekol.*, 1999, vol. 39(4), pp. 434 - 437. (Rus)
52. Moroz B.B., Deshevoy Yu.B., Seredenin S.B., Lyrshchikova A.V., Lebedev V.G., *Radiats. Biol. Radioekol.*, 2001, vol. 41(1), pp. 5 - 9. (Rus)
53. Deshevoy Yu.B., Moroz B.B., Seredenin S.B., Lyrshchikova A.V., and Lebedev V.G., *Radiats. Biol. Radioekol.*, 2003, vol. 43(1), pp. 56 - 59. (Rus)
54. Bak Z. and Aleksandr P., *Principles of Radiobiology*, Moscow, Inostrannaya Literatura, 1963, 427 p. (Rus)
55. Torubarov F.S. and Chinkina O.V., *Clinicheskaya Meditsina*, 1991, vol. 69(11), pp. 24 - 28. (Rus)
56. Tarabrina I.V., Sokolova E.D., Lazebnaya E.O., and Zelenova M.E., *Materia Medica*, 1996, No. 1 (9), pp. 57 - 68.
57. Voronina T.A., *Hydazepam*, Kiev, Naukova Dumka, 1992, pp. 63 - 75. (Rus)
58. Neznamov G.G., Sinyukov S.A., Chumakov D.V. et al., *Eksperimentalnaya i Klinicheskaya Farmakologiya*, 2001, vol. 64(2), pp. 15 - 19. (Rus)
59. Gorizontov P.D., Belousova O.I., and Fedotova M.I., *Stress and Blood System*, Moscow, Meditsina, 1983, 240 p. (Rus)
60. Kudryashov Yu.B. and Parkhomenko M.P., *Radiobiologiya*, 1987, vol. 27, Issue 3., pp. 297 - 302. (Rus)
61. Kotterov A.N. and Nikolsky A.V., *Radiats. Biol. Radioekol.*, 1999, vol. 39(6), pp. 648 - 662. (Rus)
62. Kotterov A.N., *Med. Radiologiya i Radiats. Bezopasnost'*, 2000, vol. 45(5), pp. 5 - 20. (Rus)
63. Voronina T.A. and Smirnov A.D., *New Technologies and New Medicines Used in Emergency Situations of Natural and Technogenic Origin*, Moscow, IzDAT, 1999, pp. 3 - 9. (Rus).

Time- and dose-dependent post-irradiation changes of Fe³⁺-transferrin and Cu²⁺-ceruloplasmin pools in blood, influence on ribonucleotide reductase activity in animal tissues and the effects of radioprotectors

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ABSTRACT

The time- and dose-dependent changes of Fe³⁺-transferrin (Fe³⁺-TF) and Cu²⁺-ceruloplasmin (Cu²⁺-CP) pools, superoxide dismutase activity and the inhibitory activity of α_2 -macroglobulin in blood as well as changes in biosynthesis rates of deoxyribonucleotides, DNA and proteins in organs (spleen, liver, bone marrow, thymus) of mice and dogs total body irradiation have been studied, using ESR spectroscopy, radioisotope techniques and biochemical determination of enzymatic activity. The experimental data have allowed revealing the sequence of organism's response reactions against irradiation and their modifications by radioprotectors. Changes in blood Fe³⁺-TF pool is one of the most informative, highly radiosensitive and rapidly reactive marker against irradiation and drug administrations. This iron-transport protein controls a rate-limiting iron-dependent stage for DNA synthesis – the synthesis of dNTP,

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catalyzed by iron-containing ribonucleotide reductase (Fe^{3+} -RR). It is shown that time-dependent post-irradiation changes of Fe^{3+} -TF pool in blood are characterized by three distinct stages: 1) the prompt increase of pool (SOS-type response) playing the important role in protecting cell genetic apparatus from damage; 2) the decrease of its pool within 3-18 h after irradiation resulting in the loss of Fe^{3+} -RR activity in tissues of blood-forming organs that make more stronger radiation-induced damage; 3) the following phase-dependent increase in Fe^{3+} -TF pool at the 2nd, 6th and 10-17th days after irradiation due to an increase in transferrin synthesis. This increase may be considered as a compensatory reaction of blood-forming organs aimed at restoring blood and organ cells. The time-dependent courses of the reactions are independent of radiation doses indicating the universal and nonspecific response of the organism against irradiation. Hence, the intensity of this compensatory-adaptive response at the 2nd and 6th days increases with radiation dose to the lethal level, and the organism response becomes abnormal and physiologically hypertrophic. The prolonged "stressful syndrome of biochemical tense state" should be attributed to negative effects for the organism, since it may result in the failure of compensatory adaptive organism reactions and animal killing. The radioprotectors prevent occurrence of this dangerous state. Dogs with initial individual characteristics of blood which were typical of "suppressed" or "activated" states had abnormal response to irradiation by low doses of 0.25 or 0.5 Gy. In these cases, the intensity of organism response reactions was essentially increased and markedly deviated from linear dose dependence. The phase-dependent increase of Fe^{3+} -TF pool in blood in post-irradiation time resulted in the increase of Fe^{3+} -RR activity in blood-forming organs. The key event ensuring the development of compensatory adaptive reactions is the increase of capacity of protein-synthesizing apparatus, the activation of biosynthesis of dNTP and DNA against the treatment by damaging factors.

Keywords: Fe^{3+} -transferrin (Fe^{3+} -TF), Cu^{2+} -ceruloplasmin (Cu^{2+} -CP), ribonucleotide reductase, deoxyribonucleotide and DNA biosynthesis, dynamics and dose dependence, compensatory adaptive reactions

The iron-transport blood plasma protein Fe^{3+} -transferrin (Fe^{3+} -TF) is the key compound providing for pool and function of iron-containing proteins and enzymes [1, 2]. Apo-transferrin binds and transfers Fe^{3+} ions to various tissues, especially of the reticuloendothelial system, where iron is released without changing the protein-transporter structure. Hemoglobin synthesis and iron-containing ribonucleotide reductase (RR) activity (the enzyme regulating the rate-limiting iron-dependent stage of DNA synthesis) are the most highly sensitive processes to iron content variations [3 – 5]. Iron demand for actively dividing cells during DNA synthesis is defined by that of M2 subunit of PP enzyme which catalyzes synthesis of four types of deoxyribonucleotides (deoxynucleoside triphosphates, dNTP). On this very stage, balanced and continuous dNTP delivery is provided for reparative and replicating DNA synthesis, the quality and rate of which are determined by pool sizes of four dNTP types and their ratio. PP activity is controlled by *de novo* synthesis of PP subunit M2, containing two iron ions [6]. This is the pathway, by which Fe^{3+} -TF, the iron-transport protein, regulates PP activity and, as a consequence, dNTP synthesis, on which the level of proliferative cell activity and reproductive ability of the blood system are highly dependent. Iron ions are transferred to a cell by Fe^{3+} -TF complex formation with transferrin (TF) receptor [7, 8]. TF receptor blockade led to DNA synthesis inhibition. In hematopoietic cells, interleukin-2, interleukin-6 and cyclic nucleotides are the factors, affecting gene expression in TF receptors [8]. Fe^{3+} -TF also participates in macrophage formation [9], synthesis of iron-containing CET proteins in mitochondria, cytochrome P-450, etc. As Fe^{3+} -TF pool defines synthesis of dNTP, DNA and iron-containing proteins in blood, its changes may be the marker of the cell system conditions in vitally important organs. Besides the iron-transport function, the ability of transferrin to be the controlling agent of peroxide oxidation of lipids is known. Fe^{3+} -TF is the prooxidant: at one-electron reduction, Fe^{2+} ions which are active reagents of peroxide oxidation escape. The reducers may be catechol amines, ascorbic acid, and tocopherol. Antioxidant properties are stipulated by the transferrin apo-shape capable of binding Fe^{3+} ions. For transferrin, which main biological function is the support of cells with iron for vital activity sustaining, they are side reactions.

Inclusion of iron ions into apotransferrin controls ceruloplasmin, the blood plasma protein, possessing ferroxidase activity and oxidizing Fe^{2+} ions to Fe^{3+} . Copper-transport protein Cu^{2+} -ceruloplasmin (Cu^{2+} -CP) defines synthesis of copper-containing proteins and, being the main blood antioxidant and biogenic amines' level regulator, possesses superoxide dismutase, peroxidase and amine oxidase activity [10].

Discussed in the present work are radiation-induced changes in Fe³⁺-TF and Cu²⁺-CP concentrations in blood and organs of animals and their dependence on irradiation dose and radioprotectors' administration. The objective of the current investigation is the study of the effect of changes detected in peripheral blood on ribonucleotide reductase activity of tissues of vitally important organs (spleen, bone marrow, thymus, liver). Moreover, possibilities of the use of studied metabolic values of peripheral blood for radioprotector efficiency estimation by their ability to increase radiation resistance of the organism.

MATERIALS AND METHODS

Experimental animals

The following species of laboratory animals were tested: first generation mice-hybrids (CBA x C₅₇ BL)F₁ – males weighted 20 – 24 g; nondescript white rats-males weighted 130 – 150 g; nondescript dogs (males and females) weighted 9 – 24 kg, aged 1 to 8.

Irradiation

Small animals (mice and rats) were generally γ -irradiated by 0.25 – 8 G doses on GUBE-1500 (⁶⁰Co) and IGUR (¹³⁷Cs) devices with dose intensity 3.5 and 1.6 G/min, respectively. Dogs were irradiated on EGO (⁶⁰Co) device with dose intensity 0.26 G/min in the dose range of 0.25 – 16 G and on LUE-8 device with the dose intensity, at least, 5 G/min.

Long-term, low-intense dose γ -irradiation of mice (CBA x C₅₇ BL) F₁ ($n = 600$), rats ($n = 88$) and dogs ($n = 23$) was carried out on GUB-I (¹³⁷Cs) device with 0.96 cG/min dose intensity or on TsU (¹³⁷Cs) device with 0.28 cG/min dose intensity during 96 hours up to total dose 16 G.

According to clinical-hematological data, severity of radiation sickness in dogs was characterized as follows. At 16 G dose, intestinal acute radiation sickness was developed. Times of animals' death were independent of irradiation dose intensity (EGO-2 and LUE-8). Differently expressed bone-marrow syndrome was observed in dogs irradiated by doses in the range of 8 – 3.2 G. In this case, animal deaths happened within 9 – 31 post-irradiation days in the presence of hemorrhagic syndrome and acute infective complications. In

dogs, irradiated by sublethal doses (2.5 G or lower), clinical displays were either lower expressed (2.5 G) or practically absent (1.0 – 0.25 G). However, according to ESR data, peripheral blood reaction happened at irradiation even at 0.25 G dose.

Sample preparation

Biological materials of mice and rats were sampled after animal killing by translocation of cervical vertebrae. Blood, spleen, bone marrow, thymus and liver were sampled during 30 - 60 s and frozen up in liquid nitrogen. Samples were studied during 10 – 15 min to 30 days after irradiation or administration. In tests in mice, 10 – 12 animals per every time period were studied.

In tests in dogs, 17 series of experiments were carried out. Animals were divided to groups, relatively equal by mass, sex and age with respect to irradiation dose. Animals were tested after quarantine and detection of constancy of measured metabolic blood markers. The studies lasted from 15 min after irradiation to animal death or till 90 days for survived dogs. Individual animal reaction to irradiation, estimated by changes in blood markers, depended on their initial values (before irradiation). Blood was samples from vein and frozen up in liquid nitrogen. Blood samples, frozen, were placed to a resonator of ESR radiospectrometer, where measurements were implemented.

In every series of tests, the following groups of animals were studied: intact; after adrenalin or indometafen radioprotectors administration; irradiated; irradiated after radioprotector administration.

Methods

DNA and protein synthesis intensity was studied by the radioisotope technique, using specific activity index of these molecules after injection of labeled predecessors to animals in different times after irradiation or administration. For labeling proteins and DNA, respectively, a blend of [¹⁴C]-leucine and [³H]-thymidine was injected intraperitoneally (i/p) to animals 1 hour before killing. The dose of [³H]-thymidine equaled 10 µCi per mouse or 100 µCi per rat (specific activity equaled 25 Ci/mmol). The dose of [¹⁴C]-leucine equaled 5 µCi per mouse or 50 µCi per rat (specific radioactivity equaled 1 µCi per mg of protein). Sampled organs were homogenized in cooled down 0.14 M

NaCl solution, and homogenates were treated by a standard technique of proteins and DNA chemical fractionation, described before [11]. Radioactivity of samples was measured in standard toluene scintillation counter SL-30 (Intertechnique, France). The number of pulses per 1 min from [³H]-DNA or [¹⁴C]-proteins related to their corresponded weight amounts (specific activity) represented the biosynthesis intensity measure of these macromolecules. Specific radioactivity of proteins and DNA, experimentally obtained, were related to corresponded check values. Radioisotope compounds were implemented by Isotope Co. (Russia).

ESR spectra of the whole blood samples and undestroyed tissues of organs were measured at 77 K on ER-220D spectrometer, Bruker Co. (Germany), using a standard technique of spectra accumulation and analysis on a mini-PC Aspect-2000. Table 1 shows radiospectroscopic parameters of ESR signals of studied metabolic centers in blood and organs.

Activity of proteinase α_2 -macroglobulin inhibitor in blood serum of dogs was estimated spectrophotometrically, using α -benzoyl arginine ethyl ester [13]. Activity was expressed in conditional inhibitor units, for which the inhibitor activity of the serum amount inactivating a single unit of trypsin was accepted.

Table 1
Radiospectroscopic parameters of ESR spectra for a series of metabolic centers of peripheral blood and organs under study

Metabolic indices	ESR signal parameters [12]
Fe ³⁺ -transferrin in blood and organs	Anisotropic signal with $g_{\perp} = 4.3$ and $g_{\parallel} = 9.3$
Cu ²⁺ -ceruloplasmin in blood	$g_{\perp} = 1.056$ and $g_{\parallel} = 2.209$; $A_{\parallel} = 155 - 200$ G
Low-molecular iron-albumin complexes in blood	Anisotropic signal with $g_{\perp} = 4.52$
Adrenalin radicals in blood	Singlet, $\Delta H = 14$ G, $g = 2.003$
Adenochrome radicals in blood	Singlet, $\Delta H = 9$ G, $g = 2.004$
Methemoglobin in blood and organs	Anisotropic signal with $g_{\perp} = 6.0$ and $g_{\parallel} = 2.0$
Fe-ribonucleotide reductase in organs	Doublet, superfine splitting 20 G, $g = 2.005$

Cu/Zn-superoxide dismutase (SOD) activity in whole blood was determined spectrophotometrically by inhibition level of tetranitrosole blue reduction by superoxide radicals to formazans at pH 10.2 [14]. The authors are thankful to L.S. Ter-Vartanyan, L.G. Nagler, S.M. Gurevich and A.I. Kozachenko for dog serum and peripheral blood sample measurements performed.

Indraline, the emergency action radioprotector, was one-shot injected, intramuscularly, to dogs in the dose 15 mg per 1 kg of body mass 10 – 15 min before irradiation and intraperitoneally to mice in the dose 100 mg/kg.

Indometafen, the long-acting radioprotector, was injected to dogs and mice orally in the dose 30 mg/kg 5 days before irradiation that gave the highest survival of animals [15, 16]. These radioprotectors were developed and presented for studies by SSC RF –Institute of Biophysics, Ministry of Health (Moscow).

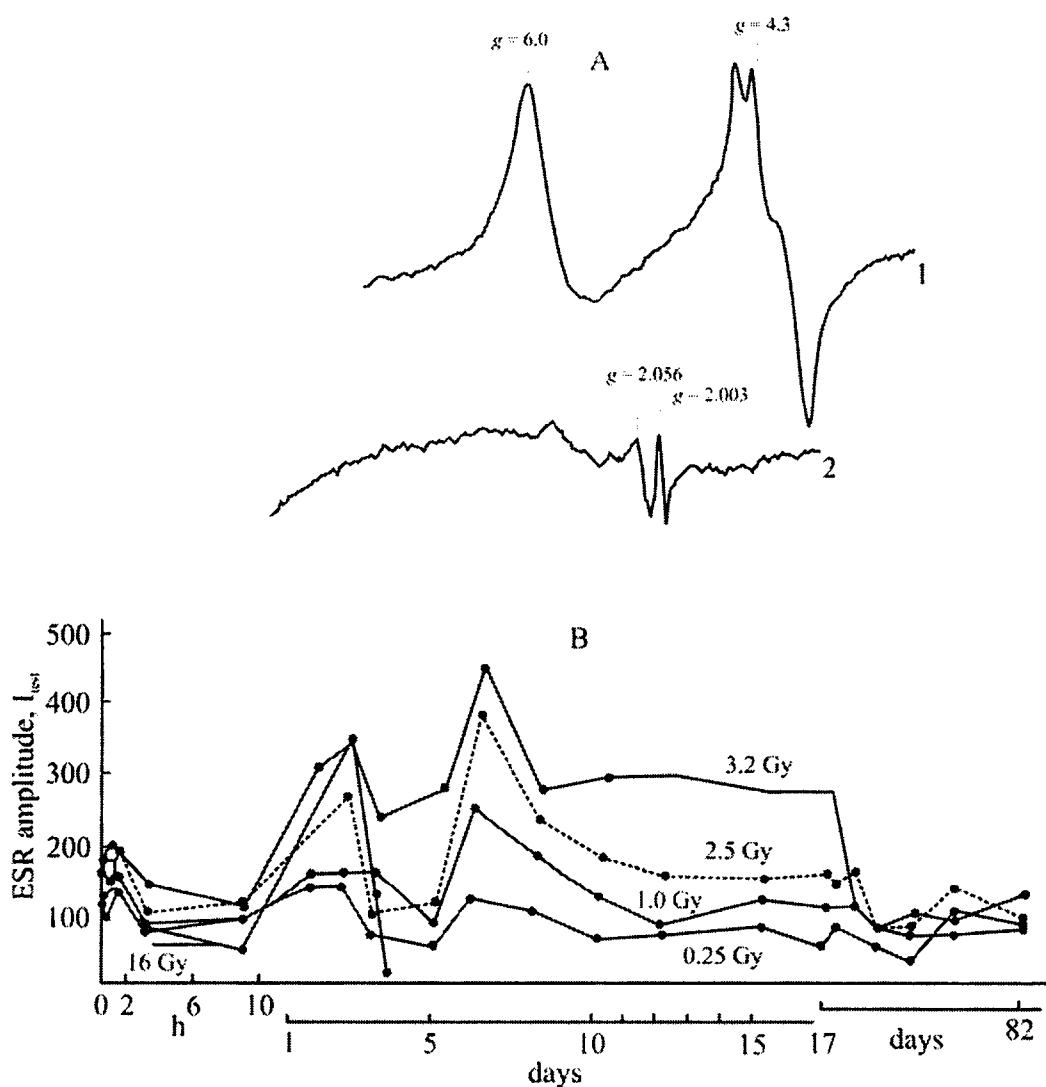
The effect of antioxidant α_2 -tocopherol on metabolic values of blood and organs in mice-males (CBAXC₅₇ BL) F₁ was studied on three groups of animals: 1) intact; 2) after one-shot intragastric injection of α_2 -tocopherol in twine-80 in the dose 17 mg per kg, using a sonde (0.255 mg dose per mouse of 15 g); 3) after daily one-shot per os α_2 -tocopherol administration during four days (total dose equaled 1.02 mg per mouse).

Two dogs, No. 101 and 132, received orally one-shot 20 mg dose of α_2 -tocopherol. Dogs N^os. 108 and 856 received 20 mg α_2 -tocopherol daily during 6 days.

RESULTS AND METHODS

Dynamics and dose dependence of changes in Fe³⁺-transferrin and Cu²⁺-ceruloplasmin concentration in blood at total body γ -irradiation of animals

ESR spectrum of dog's whole blood is almost equal to that of a mouse of a man (Figure 1A). In low magnetic field, Fe³⁺-TF and methemoglobin (MetHb) ESR signals were recorded. In some blood samples, ESR signal of Fe³⁺-TF interfered with that from low-molecular iron complexes with $g_{\perp} = 4.52$, the technique for separating which is described in [12]. ESR signals of Cu²⁺-CP



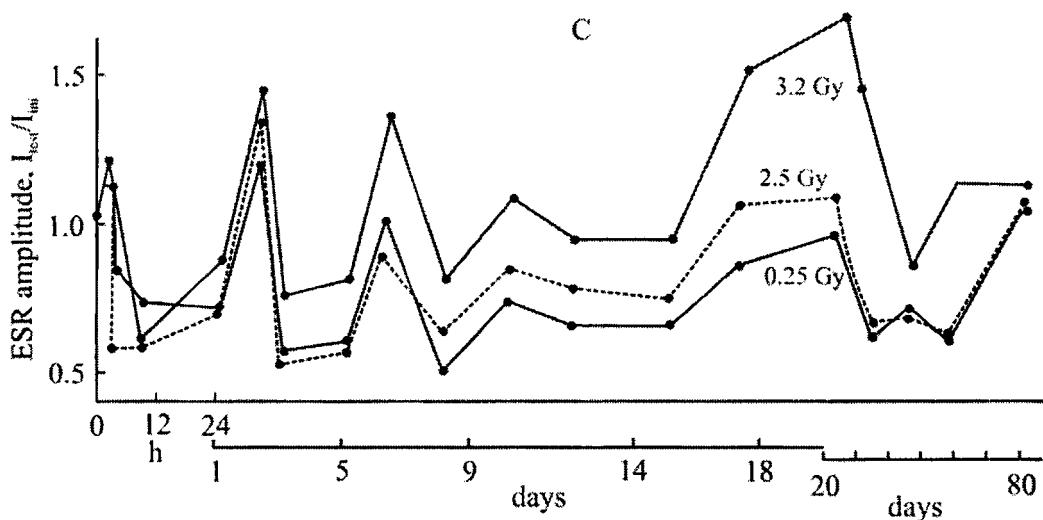


Figure 1. ESR spectrum of dog's blood (A) and dynamics of Fe^{3+} -transferrin (B) and Cu^{2+} -ceruloplasmin (C) pool variations in blood of dogs from the initial state (before irradiation) to animal death (16 G irradiation dose) or to 80 days after irradiation for survivals (irradiation doses: 3.2, 2.5, 1.0 and 0.25 G).

Data on a single series of experiments are shown, each curve for one dog. The arrow marks the dog death time.

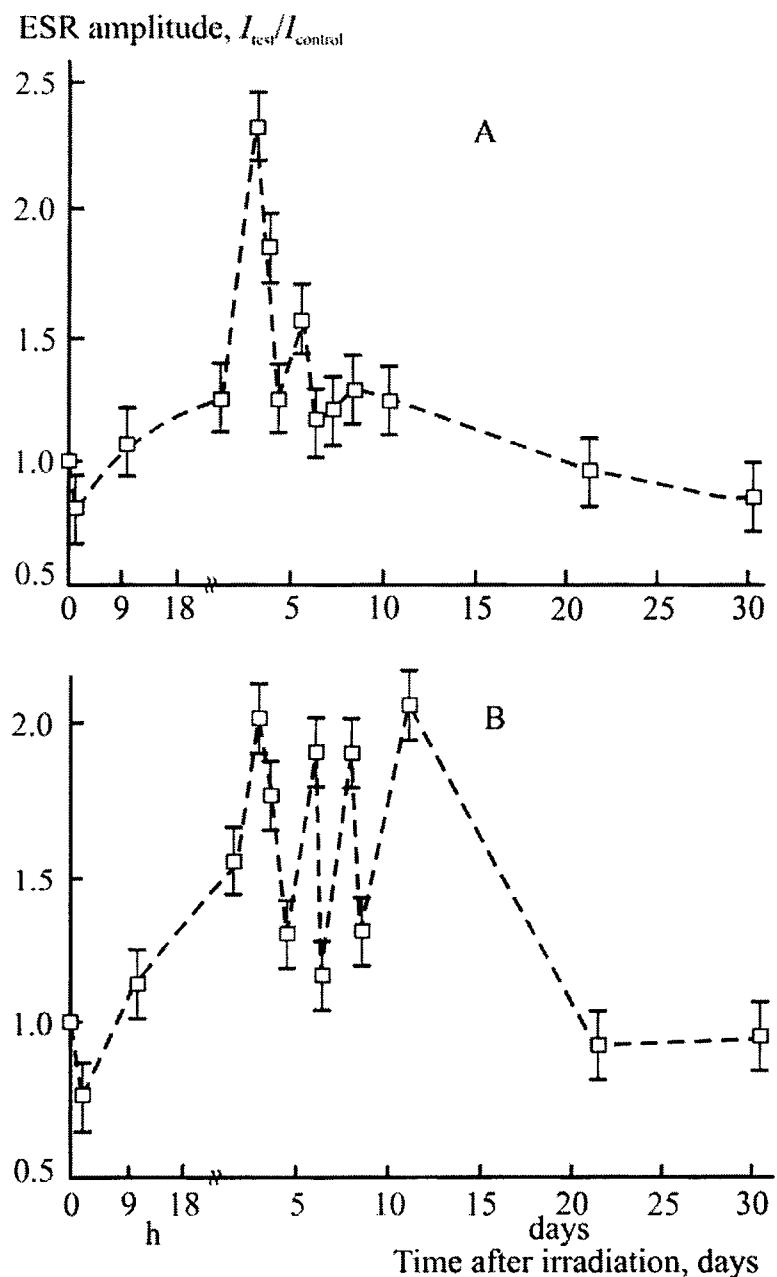
A (1) – methemoglobin and Fe^{3+} -transferrin ESR signals with $g_{\perp} = 6.0$ and $g_{\parallel} = 2.0$ and $g_{\perp} = 4.3$ and $g_{\parallel} = 9.3$, respectively, in the low-pole area are recorded.

Registration conditions: $\Delta H = 1,000$ G; $H_c = 1,350$ G; $P_{\text{SHF}} = 200$ mW.

A (2) – in the free-radical region, Cu^{2+} -ceruloplasmin ($g_{\perp} = 2.056$ and $g_{\parallel} = 2.209$, $A_{\parallel} = 155 - 200$ G) and adrenalin radical (a singlet with $\Delta H = 14$ G, $g = 2.003$) ESR signals are recorded.

Registration conditions: $\Delta H = 1,000$ G; $H_c = 3,250$ G; $P_{\text{SHF}} = 200$ mW.

Ordinate axis: B – ESR signal amplitude in conditional units, normalized by the control signal; C – $\frac{I_{\text{test}}}{I_{\text{ini}}}$ ratio, where I_{ini} is the ESR signal amplitude before irradiation of a dog; I_{test} is the post-irradiation ESR signal amplitude.



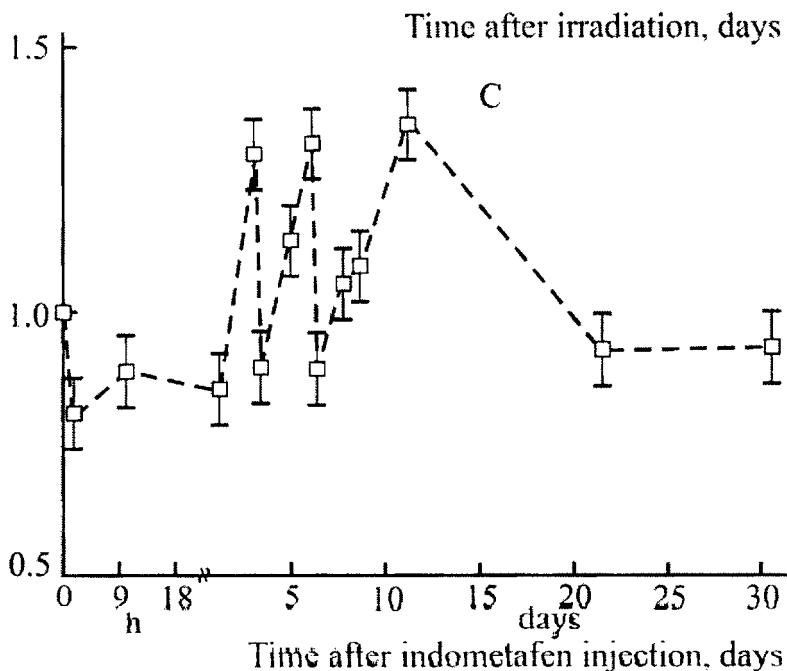


Figure 2. Dynamics of Fe³⁺-transferrin pool change in blood of mice after irradiation by 6.5 G dose (A), after irradiation by 6.5 G dose with administrated 30 mg/kg dose of radioprotector indometafen (B), and after administration of 30 mg/kg indometafen to intact mice (C).

Ordinate axis: ESR signal amplitude of Fe^{3+} -transferrin ($\frac{I_{\text{test}}}{I_{\text{control}}}$ ratio).

Abscissa axis: time after irradiation (A and B) and indometafen injection (C).

and adrenalin radicals are recorded in the free-radical region. At damaging impacts or injection of some biologically active compounds to animals, additional ESR signals of various chemichromes, adrenochrome, nitrosoyl complexes of hemoglobin, inactivated forms of SOD and ceruloplasmin are recorded [12].

The type of time and dose dependencies of changes in Fe^{3+} -TF and Cu^{2+} -CP pools in blood of irradiated dogs may be judged by the results of one of 7 series of experiments, shown in Figures 1B and 1C. Clearly untypical, dose-independent phase-type changes in Fe^{3+} -TF and Cu^{2+} -CP pools were developed as a response to irradiation. Maximal Fe^{3+} -TF pools were recorded on the 2nd and the 6th days after irradiation. Subsequent maximums were less intensive, extended in time, recorded on 10th to 17th, 25th to 27th, and 45th to 60th days. Data in Figure 2 show phase changes in Fe^{3+} -TF pool in blood of mice, induced by irradiation by 6.5 G dose (A), 6.5 G dose radioprotector indometafen administration (B) or one-shot indometafen administration to intact animals (C). For irradiated dogs, the phase dependence of changes was also detected for superoxide dismutase activity, antiproteolytic activity of α_2 -macroglobulin and MetHb pool in blood (Figure 3). Data obtained testify about nonspecific and universal type of response reactions of organism to radiation damage. Time dependence of changes in Fe^{3+} -TF pool in blood of irradiated dogs was divided into three periods, when the following took place: 1) emergent changes (SOS-type response), aimed at elimination of DNA damages; 2) intensification of radiation damage by post-irradiation inhibition of a series of molecular-cell processes; 3) further development of compensatory-adaptive reactions.

Emergent changes in Fe^{3+} -TF pool during initial thirty minutes were individual and dose-dependent. For the majority of dogs at doses below 1G, a decrease of this index was observed, whereas at higher doses it increased. Short-term increase of Fe^{3+} -TF pool in blood of dogs irradiated by doses above 1 G is considered as a component of the SOS-reaction of organism, aimed at mobilization of the cell system protective functions responding to DNA damages. The necessity of emergent repair of increased DNA damage scope stipulates early (up to 30 min after irradiation) emergent increase of ribonucleotide reductase activity of hematopoietic organ tissues (spleen, bone marrow, thymus), which was indicated in works [15, 17]. The reason is that iron-containing PP catalyzes synthesis of four types of dNTP required for DNA damage repair [17 – 23]. As follows from the data, there is a threshold radiation dose, at which a SOS-type response is developed, because the cell responds by activation of a complex of protective reactions to occurrence of a definite scope

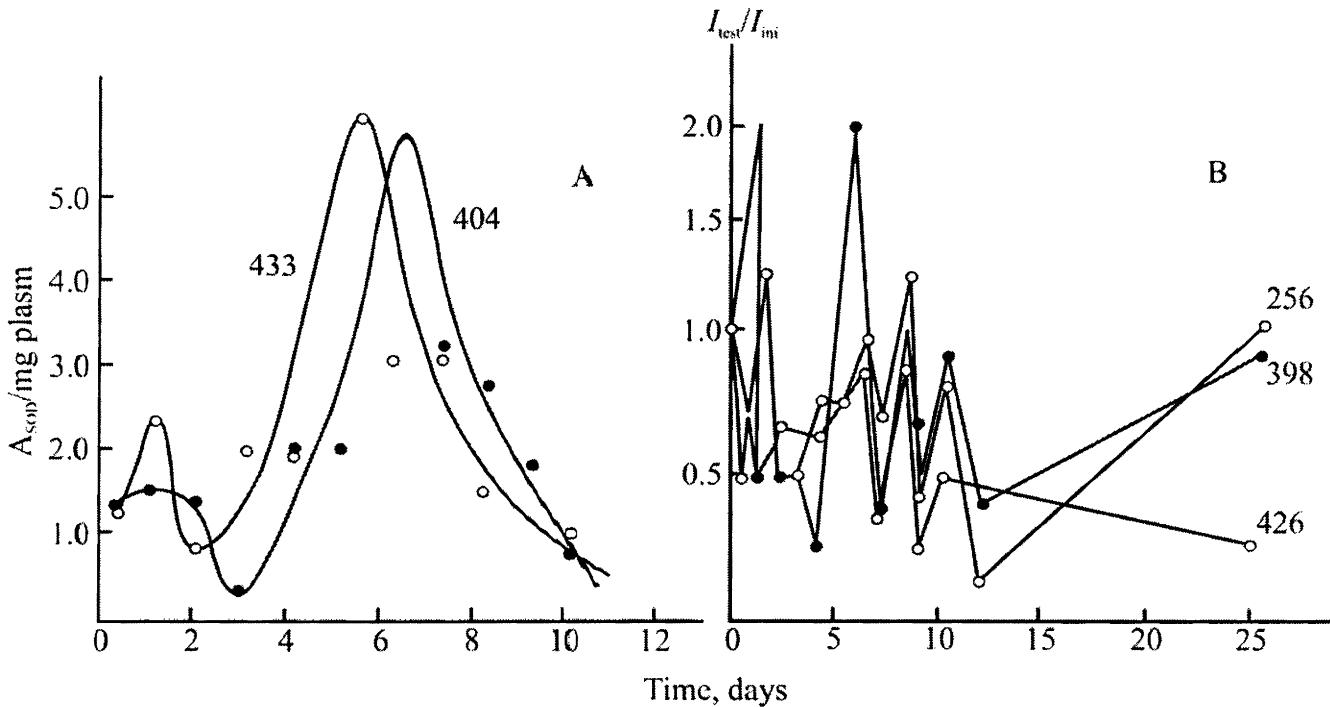


Figure 3. Post-irradiation changes of superoxide dismutase activity (A) and inhibiting activity of α_2 -macroglobulin (B) in blood plasma of dogs irradiated by 7.64 (A) and 2.0G (B) doses with the dose intensity 0.96 cG/min.

Ordinate axis: superoxide dismutase activity ($A_{SOD}/\text{ml of plasma}$) for dogs No. 433 and 404 (A) and anti-proteolytic activity of α_2 -macroglobulin ($\frac{I_{\text{test}}}{I_{\text{ini}}}$, where I_{ini} is the activity before irradiation) for dogs No. 256, 398 and 426 (B).

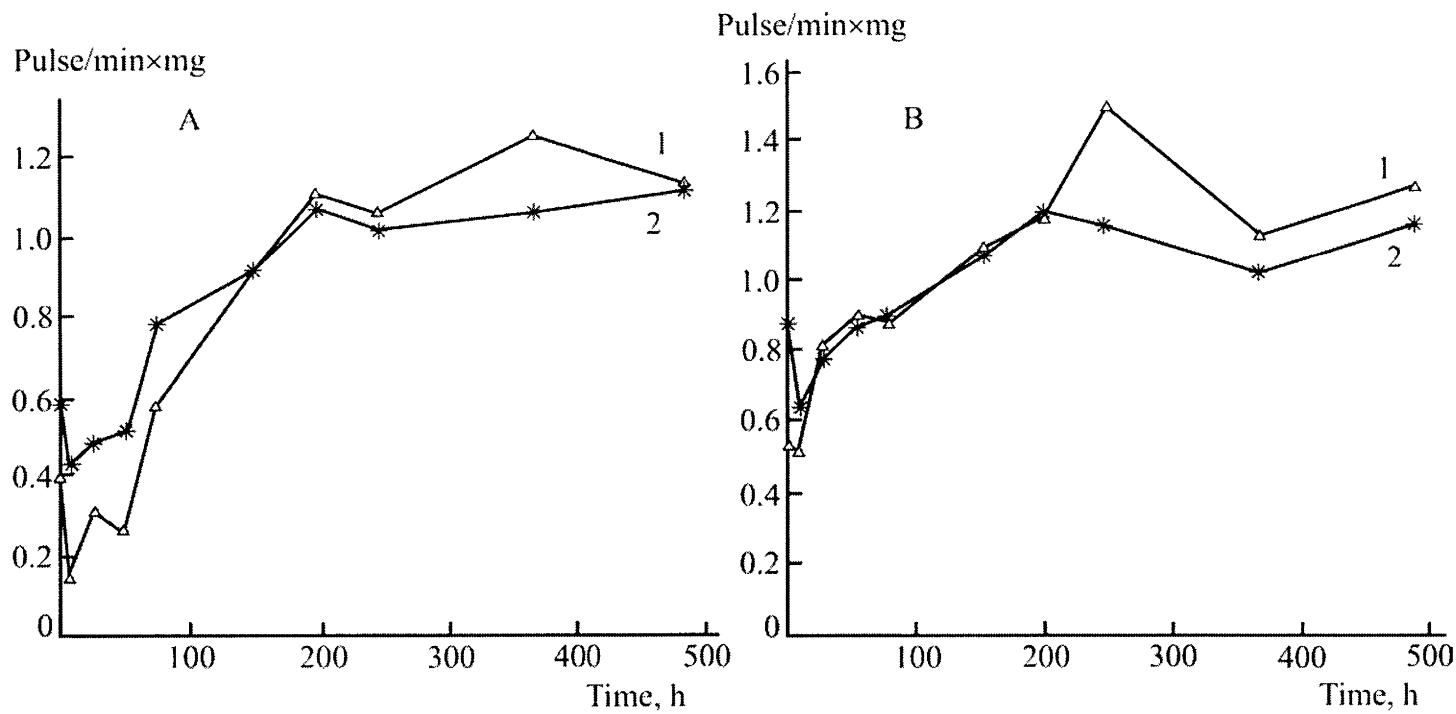
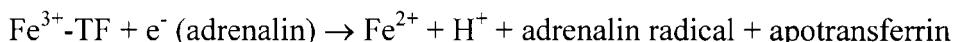


Figure 4. Reactions of DNA (1) and protein (2) synthesis systems in spleen (A) and liver (B) of mice to irradiation in 6.5 G dose.

Ordinate axis: the ratio of specific radioactivity in experiment to control. Intensity of radioactive marker inclusion (pulse/min·mg) was measured. For the control, intact animals were taken. Each experimental point is the average of 15 mice.

of DNA damages. For dogs, this dose equals 1 G. For the majority of dogs at lower doses, Fe^{3+} -TF pool decreased. This was stipulated by adaptive Fe^{2+} ions release with apotransferrin and low-molecular iron complex formation, ESR signal amplitude of which ($g_{\perp} = 4.52$) significantly increased in blood in this period. Catechol amines, which level in blood rapidly increased as a result of radiogenic stress, were iron ion reducers in Fe^{3+} -TF.



Adrenalin radicals were accumulated in blood with Fe^{3+} -TF and Cu^{2+} -CP pools' decrease, simultaneously (Table 1). Of importance is that iron ion release from Fe^{3+} -TF is accompanied by medium acidulation. Analogous effect of pH decrease in isolated hepatocytes of rats due to NADN were observed before (refer to the review [2]); hence, Fe^{2+} ions were released and NAD^+ occurred. pH decrease changes physicochemical properties of transferrin receptors (conformation changes, increase of transferrin linking strength) and affects H^+/e^- ratio, Na^+/H^+ exchange transport and Ca^{2+} , as well [1, 2].

Early post-irradiation changes of Fe^{3+} -TF pool in blood of dogs since 1 to 9 h are caused by 20 – 50% decrease of its initial value with respect to irradiation dose (Figure 1B). During this post-irradiation period, radiation damage is intensified by inhibition of many molecular-cell processes and activation of lipid peroxide oxidation [12]. Low Fe^{3+} -TF pool values were preserved up to 24 h that testifies about inability of apoTF formed to link Fe^{3+} ions again. The reasons may be the loss of HCO_3^- or CO_3^{2-} anions by this apo-protein, which are required for strong linking of iron ions [1, 2, 24] and its degradation by lisosomal enzymes, which are activated in this period in conditions of decreased pH. Simultaneously, biosynthesis of total protein was inhibited in spleen by 60% and in liver by 40% (Figure 4). Deep inhibition of translation was preserved during 6 h. In this period, DNA biosynthesis must also be inhibited.

Later (since the first day and further on) changes in Fe^{3+} -TF pool are defined by its iron-transport function associated with the synthesis of iron-containing proteins and, primarily, hemoglobin and ribonucleotide reductase which regulates the key iron-dependent stage in the DNA synthesis [2, 3]. In this post-irradiation period, in irradiated animals untypical changes in Fe^{3+} -TF and Cu^{2+} -CP pools with time were developed, characterized by the phase dependence and radiation dose independence. As mentioned above, intensity of these changes in periods, when they were most highly expressed (on the 2nd and

the 6th days) was linearly dose-dependent: the higher the dose was, the greater were increases of Fe³⁺-TF and Cu²⁺-CP pools (Figure 5). The response reaction in dogs to different radiation doses was estimated by Fe³⁺-TF pool increase above its minimum on the 2nd (or 6th) day. For the minimum, the value measured 9 h after irradiation was meant. Thus

$$\Delta I = I_{\max} - I_{\min},$$

where I_{\min} is the ESR signal amplitude of Fe³⁺-TF 9 h after irradiation; I_{\max} is the ESR signal amplitude of Fe³⁺-TF on the 2nd or the 6th day after irradiation.

Figure 5 shows that ΔI linearly depends on the radiation dose up to the lethal level. At higher doses, no further increase of ΔI was observed, i.e. protective potential of the organism was fully realized. In this case, the limiting "physiological and biochemical stress" state occurs, long-term preservation of which causes exhaustion of bioenergy, biosynthetic, neurohumoral and immune reserves, and brings the organism to a dangerous limit, after which even an insignificant deviation from conditions providing preservation of cellular homeostasis causes the organism death. Systemic nonspecific reaction of the organism to a damaging impact is aimed at reduction of the blood system and the function of vitally important organs, i.e. it is of the compensatory-adaptive type. Lethal or higher radiation doses induce break-down of this reaction at a rate estimated from the rate of Fe³⁺-TF pool decrease (by tangent of the dependence). The cross-point of the line characterizing Fe³⁺-TF pool decrease and the abscissa axis (showing time after irradiation) allows quite accurate forecasting of animal death time (refer to Figure 1B, 16 G dose). Development of compensatory-adaptive reactions is promoted by reduction and then a significant increase of biosynthesis intensity of proteins, the key process leading to normalization of the cellular composition of blood and organs and their functions (Figure 4).

At irradiation by 0.25 and 0.5 G doses, the compensatory-adaptive reaction was less expressed, though increased Fe³⁺-TF pool in blood of dogs was registered during initial 10 days. Further on, till the 90th day, Fe³⁺-TF pool decreased to 50% of the initial value. This testifies about the remote effect of low-dose irradiation which is inhibition of cell renovation and hematopoietic tissue recovery [2, 24].

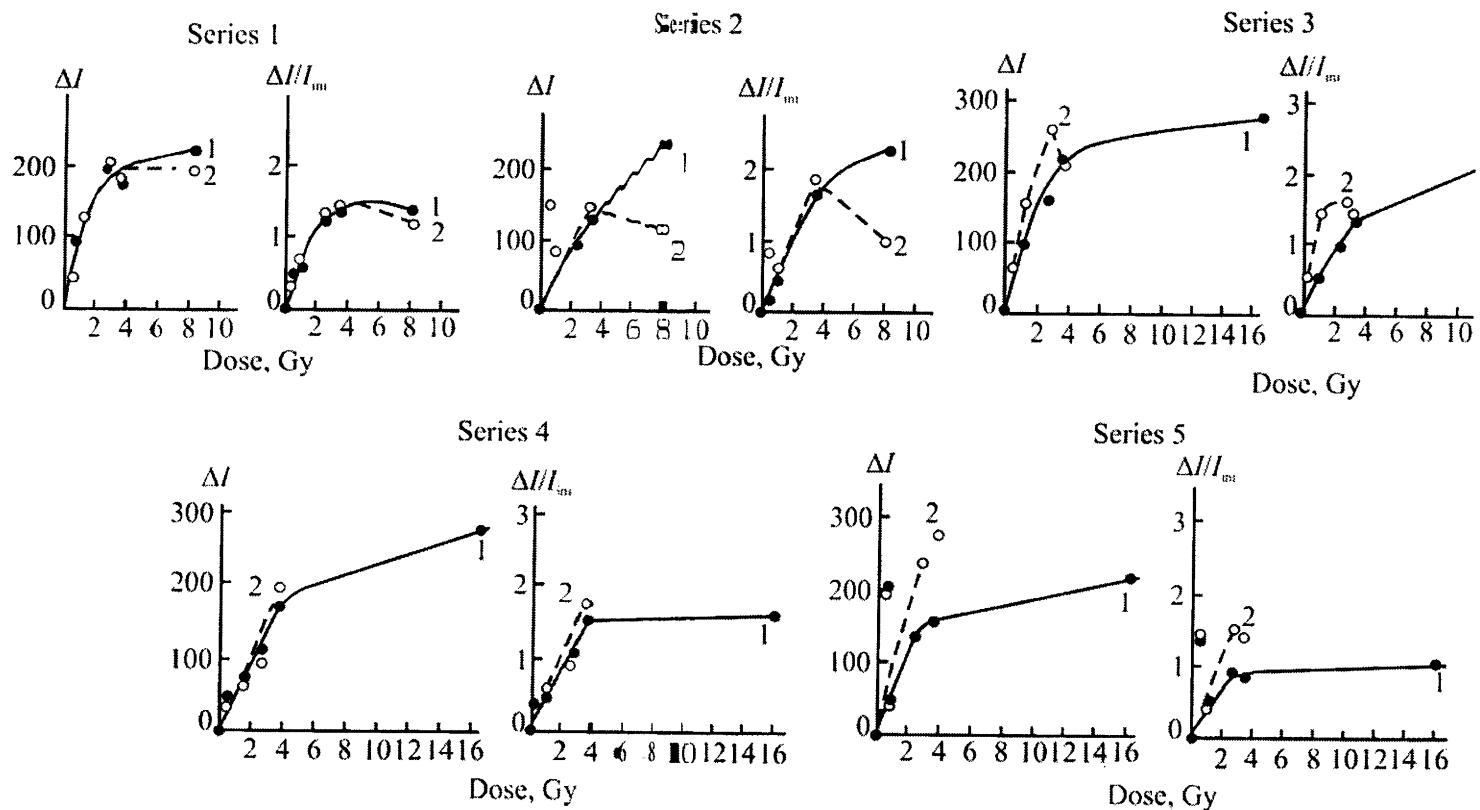


Figure 5. Dose dependencies of Fe^{3+} -TF pool increase in blood of irradiated dogs in periods of maximal compensatory-adaptive reaction: on the 2nd (1) and the 6th (2) days. Five series of experiments are shown. Each experimental point relates to one dog.
Ordinate axis: on the left

$$\Delta I = I_{\max} - I_{\min},$$

where I_{\max} is the ESR signal amplitude of Fe^{3+} -TF in blood of dogs on the 2nd (or the 6th) day after irradiation; I_{\min} is the ESR signal amplitude of Fe^{3+} -TF in blood of dogs 9 h after irradiation, when it is minimal.

On the right - $\frac{\Delta I}{I_{\min}}$ ratio.

Abscissa axis: irradiation dose, G.

Primary individual parameters of the systems defining the potential of compensatory abilities of the organism are sufficient for displays of the radiobiological effect in the entire organism. The interrelation between primary reactivity of the organism and its individual radiosensitivity was determined [25]. Figures 6 and 7 show that the type of response reaction in dogs to low-dose irradiation is defined by individual differences in primary conditions of animals. To characterize the primary conditions, the relation $(\text{Fe}^{3+}\text{-TF/MetHb})_{\text{prim}}$ in blood was chosen. This relation allows estimation of both synthesis and loss of functional hemoglobin. If this relation fell within the range of 0.9 – 1.6 (healthy dogs), the increase of Fe^{3+} -TF pool in blood on the 2nd and the 6th days as a response to irradiation by 0.25 G dose was approximately equal for all dogs (Figure 6). If $(\text{Fe}^{3+}\text{-TF/MetHb})_{\text{prim}}$ was higher (the ‘activation’ state) or lower (the ‘suppression’ state), a sharp increase of the response reaction intensity was observed, i.e. Fe^{3+} -TF pool increase in blood responding to irradiation by 0.25 G dose was the same as in apparently healthy dog after irradiation by much higher dose. Irradiation by 1 G dose gave the lower dispersion of response reactions, i.e. Fe^{3+} -TF pool increase was independent of initial individual blood values. Irradiation by 3.2 G dose or higher induced lower Fe^{3+} -TF pools in dogs with $(\text{Fe}^{3+}\text{-TF/MetHb})_{\text{prim}}$ values typical of ‘activated’ or ‘suppressed’ state versus animals with apparently healthy parameters. By Fe^{3+} -TF pool increase in blood of dogs, the ‘dose/organism

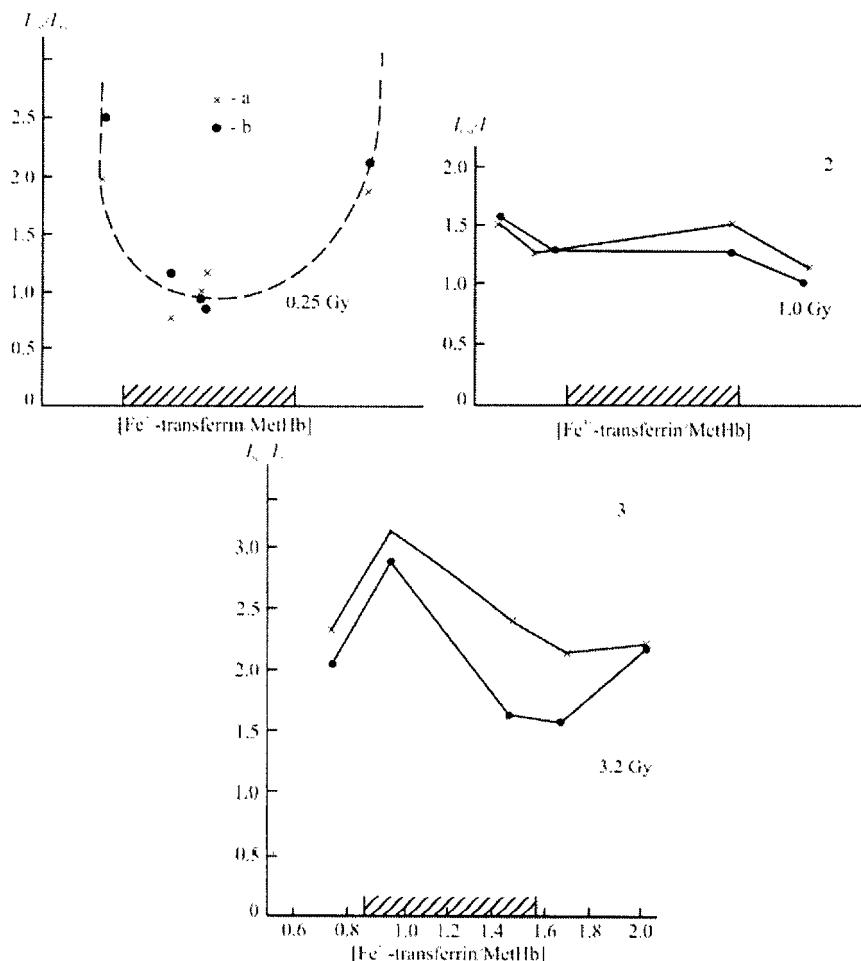


Figure 6. Dependence of response reaction on initial individual blood values in dogs to radiation doses of 0.25 (1), 1 (2) and 3.2 G (3). The response reaction was estimated by Fe^{3+} -TF pool increase in blood on the 2nd (a) and the 6th (b) days; the initial state was estimated by the ratio of Fe^{3+} -TF pools to methemoglobin (MetHb).

Ordinate axis: ESR signal amplitude of Fe^{3+} -TF ($\frac{I_{\text{test}}}{I_{\text{ini}}}$). Each experimental point relates to one dog.

Abscissa axis: $\left[\frac{Fe^{3+} - TF}{MetHb} \right]_{\text{ini}}$.

'response' dependence on the 2nd (A) and the 6th (B) day was plotted (Figure 7). Both minimal and maximal responses by Fe³⁺-TF pool increase in blood in groups of tested dogs displayed linear dependencies on the irradiation dose up to 3.2 G which was lethal for dogs. However, for 0.25 and 0.5 G, some deviations from linearity were observed, i.e. cases of sharp Fe³⁺-TF pool increase. Such deviations are typical of dogs in 'suppressed' or 'activated' conditions before irradiation, i.e. the ones with changed primary resistance of the organism and inadequate response reaction to irradiation dose. The latter increases the risk of organism transition to 'physiological and biochemical' stress conditions. Such animals are classified to the group of increased radiation risk.

The initial ceruloplasmin pool in blood is the most important individual parameter. Owing to superoxide dismutase and peroxidase activities [10, 26], its level defines the antioxidant statute of the blood plasma. However, changes of this parameter must not exceed definite limits, because owing to amine oxidase activity Cu²⁺-CP participates in neutralization of catechol amine pool in blood, excessive for the organism. As follows from the authors' experiments, Cu²⁺-CP pool increase must not exceed 40% of its initial value, because higher increase may cause a significant decrease of catechol amines' pool, which is injurious for the organism. Every individual organism has a definite basic level of ceruloplasmin that provides for antioxidant protection of cellular systems and control of the optimum concentration of active catechol amines. The latter relates to immunomodulatory effect of ceruloplasmin [27].

At irradiation by lethal or higher dose Cu²⁺-CP pool sharply increased (by 2 – 2.5 times versus the initial value) in blood before the animal death or in the most acute stage of radiation disease, namely, 15 – 20 days after irradiation (Figure 1C). Such significant increase of Cu²⁺-CP pool in blood during that period is caused by intense Cu¹⁺-CP oxidation and the change of their pool ratio due to complete exhaustion of deoxidizer depot and accumulation of products with high oxidative activity. A significant increase of Cu²⁺-CP pool indicates the exhaustion of the reserve capacity of the hemoral system in irradiated organism and suppression of adrenal gland activity in this period and may be one of the reasons for compensatory-adaptive reaction break-down. Under these conditions, transmission of iron ions from Fe³⁺-TF to cells is hindered, because it requires hormones-deoxidizers. For this reason, utilization of iron ions by cell for the purposes of biosynthesis of vitally important iron-containing proteins is disturbed.

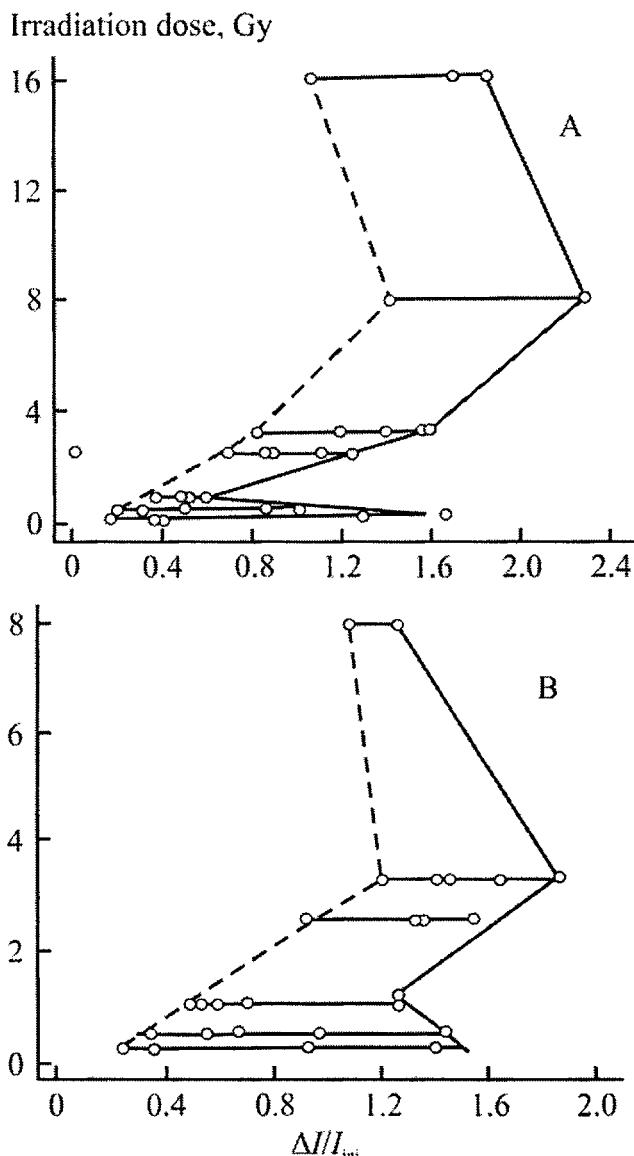


Figure 7. The dose-effect dependence determined by Fe^{3+} -TF pool change in blood of dogs on the 2nd (A) and the 6th (B) post-irradiation days. Ordinate axis: radiation dose, G.

Abscissa axis: $\frac{\Delta I}{I_{ini}}$ ratio, where $\Delta I = I_{\max} - I_{\min}$ is the Fe^{3+} -TF pool increase in blood of dogs.

Dynamics and dose dependence of blood values in dogs at extended irradiation with 0.96 cG/min dose intensity

By severity of radiation pathology, experimental dogs were characterized as follows. In dogs irradiated by 7.64 G (LD_{80}) dose, the marrow failure of various severities was observed. At 2 G irradiation dose, clinical-hematological changes in animals were less expressed, and at 0.5 G dose they were practically absent. Hematopoietic system is the important element of hemostasis and deviations in it are realized at formation of early and remote effects of extended, low-dose radiation impact. At long irradiation of animals in a broad range of doses, proliferative activity of cells during radiation impact initially increased in the pool of stem hematopoietic precursors (KOEc) [28 - 30]. In many ways, proliferative activity of cells is determined by dNTP pools and their synthesis is the rate-limiting iron-dependent process in DNA synthesis [3]. Ribonucleotide reductase activity of tissues is controlled by Fe^{3+} -TF pool in blood and presence of conditions for iron ion transmission to cell for the purposes of biosynthesis. Figure 8 shows data on Fe^{3+} -TF concentration change in blood of dogs irradiated by 7.64 G dose with 0.96 cG/min intensity. Directly after irradiation by lethal dose, Fe^{3+} -TF pool in blood of all dogs was significantly increased (initial measurement was made 30 min after irradiation end). During 8 – 10 days this parameter remained increased. Further on, an abrupt decrease of Fe^{3+} -TF pool in blood of all dogs was observed, which was caused by development of the critical stage of the radiation disease that led to death of three dogs: No. 898, 913 and 914. One of two survived dogs (No. 641) showed a response, expressed in recurrent increase of Fe^{3+} -TF pool between days 21 and 26. This period is critical for hematopoiesis recovery: the type of compensatory-adaptive reaction development predetermines survivability or death of the animal.

After irradiation end by 2 G dose increased Fe^{3+} -TF pool was also detected in dogs, whereas for 0.5 G dose in blood it was decreased more than by 50% of the initial value. Figure 9 shows dynamics of Fe^{3+} -TF pool change in blood of three dogs irradiated by 0.5 G dose. As follows from the data obtained, the lower the dose and, consequently, irradiation time is, the clearer the phase type of post-irradiation changes of Fe^{3+} -TF pool in blood is. For example, duration of dogs' irradiation by 7.64 and 2.0 G doses was 13.5 and 3.5 h, respectively. In this connection, after irradiation end by these doses blood was sampled from dogs at different stages of post-irradiation period. If the end of irradiation by 2 G dose coincides with the beginning of inhibition of the most

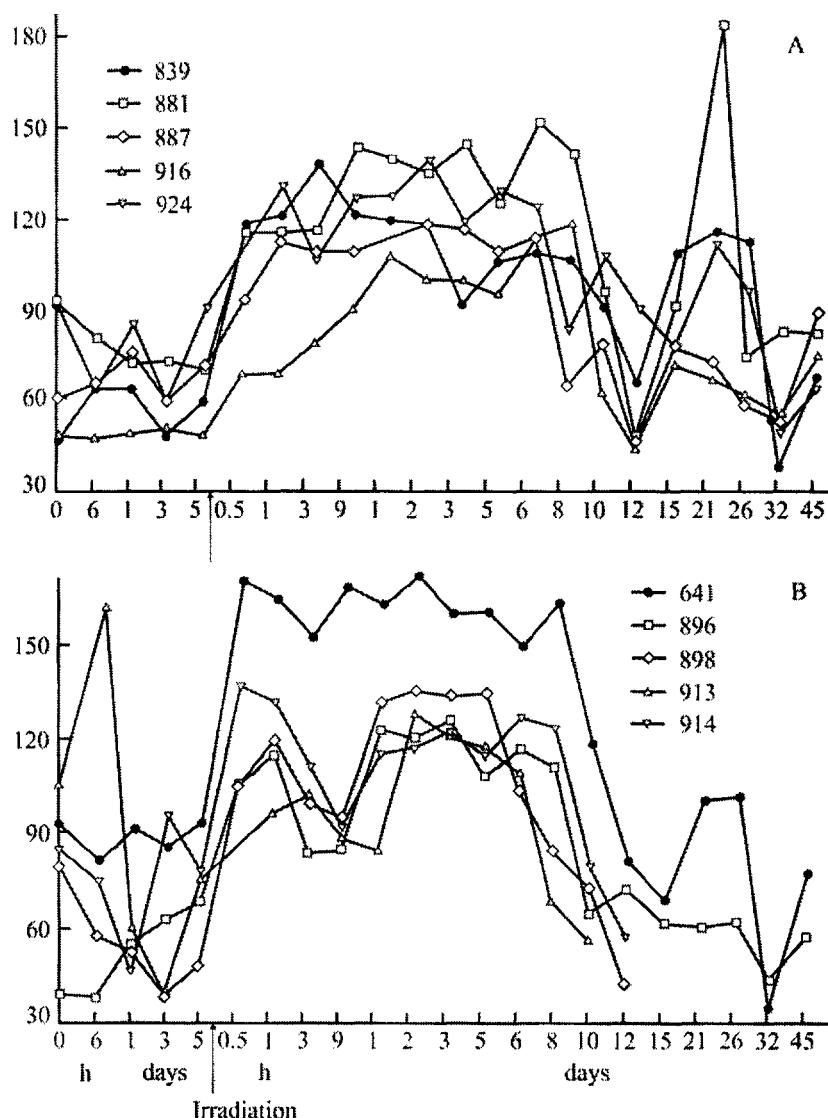


Figure 8. Time changes of Fe^{3+} -TF pool in blood of dogs, protected by indometafen (A) and unprotected (B), after total protract irradiation by 7.64 G dose of 0.96 cG/min intensity extended to 13 h 30 min. Indometafen in dose 30 mg/kg was administrated 5 days before irradiation (A).

Ordinate axis: ESR signal amplitude of Fe^{3+} -TF in blood. Each curve relates to one dog. Numbers of dogs are shown.

Abscissa axis: time after irradiation.

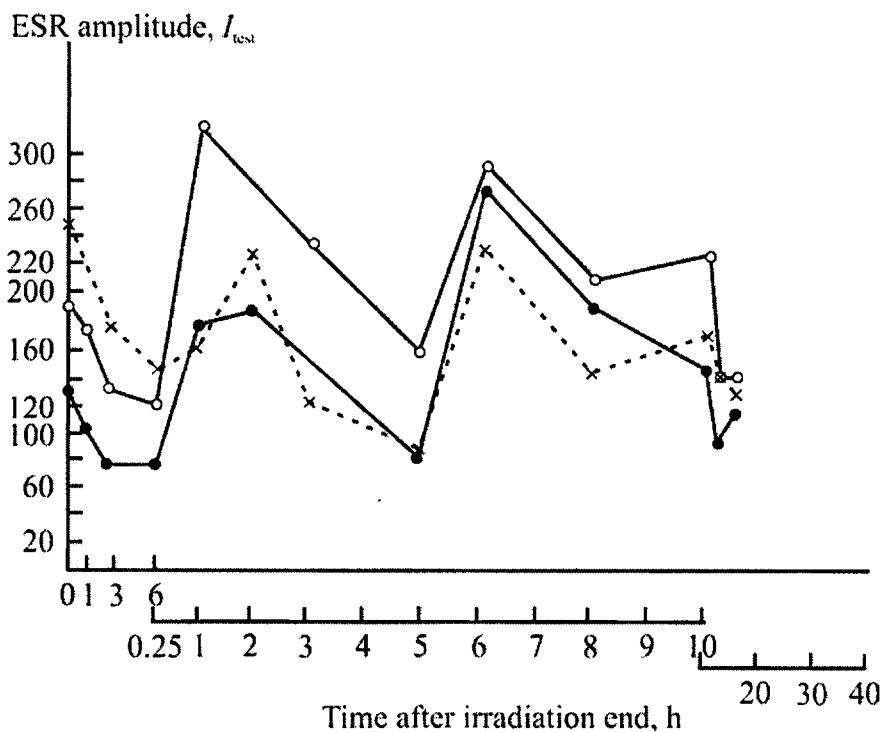


Figure 9. Time changes of Fe^{3+} -TF pool in blood of dogs after total protract irradiation by 0.5 G dose of 0.96 cG/min intensity. Irradiation lasted 50 – 55 min. Data are shown for three dogs with initial blood values typical of apparently healthy animals.

Ordinate axis: ESR signal amplitude of Fe^{3+} -TF in blood of a dog.
Abscissa axis: time after irradiation end.

important molecular-cell processes (the stage of radiation damage intensification), the end time of dog irradiation by 7.64 G dose was the time of compensatory-adaptive reaction initiation. The data obtained show that the phase dependence of post-irradiation changes of Fe^{3+} -TF pool in blood is the most clear in dogs irradiated by 0.5 G dose.

Superoxide dismutase activity in blood of dogs also increases on days 1 – 2 and 5 – 6 after irradiation, the second increase being much higher compared with the first one, which is stipulated by reduction of proteins' biosynthesis rate in these periods. Thus the blood system of dogs responds to irradiation by increasing antioxidant activity and the highest values of superoxide dismutase

activity are observed, when Fe^{3+} -TF and Cu^{2+} -CP pools in blood reach their maximums, namely, on days 1 – 2 and 5 – 6 of the post-irradiation period.

The effect of radioprotectors on dynamics of changes in Fe^{3+} -transferrin pool in blood of irradiated animals

The above-shown data testify about nonspecific and universal type of compensatory-adaptive reactions of molecular-cell systems in animal organs. They are also phase-dependent and independent of irradiation dose with time. Hence, in the periods of its maximum, intensity of these reactions linearly increases with irradiation dose up to lethal level. Under lethal irradiation doses, molecular-cell systems of organs and the total organism are in extreme ‘physiological and biochemical stress’ state, long preservation of which may cause compensatory-adaptive reaction break-down and death of animal. Protection of irradiated organism requires elimination of this critical state. It was found that radioprotectors effective in survivability tests [15 - 17, 31 – 33] prevent development of hyperforms of response reactions in molecular-cell systems of animal organs irradiated by lethal doses. Figure 10 shows that dogs protected by indraline in the course of irradiation by doses exceeding 1 G display no further increase of Fe^{3+} -TF pool in blood both on days 1 – 2 and 5 – 6 of the post-irradiation period. It is assumed that application of radioprotectors preserves pathways of iron ion utilization by cells for purposes of iron-containing protein synthesis, primarily, ribonucleotide reductase, hemoglobin, CET component of mitochondria and endoplasmic reticulum. This is testified by previously obtained data [15, 17, 33, 34] which show that animals protected by indraline and indometafen display: 1) weakened inhibiting effect of radiation on protein and DNA biosynthesis in the early post-irradiation period; 2) intense induction of dNTP synthesis for the purpose of DNA damage repair and replication synthesis in hematopoietic organs; 3) development of earlier and intense proliferative adaptive processes versus those in unprotected animals.

It will be observed that radioprotectors not only prevent development of hyperforms of response reactions in molecular-cell systems of animal organs (on days 1 – 2 and 5 – 6 of the post-irradiation period), but also induce Fe^{3+} -TF pool increase in the critical period of radiation disease development, namely, in the period of hematopoietic regeneration. For example, on 10 – 20 day radioprotector indometafen induced a significant increase of Fe^{3+} -TF pool in blood of many experimental dogs (Figure 2B and C; Figure 8A). It has been

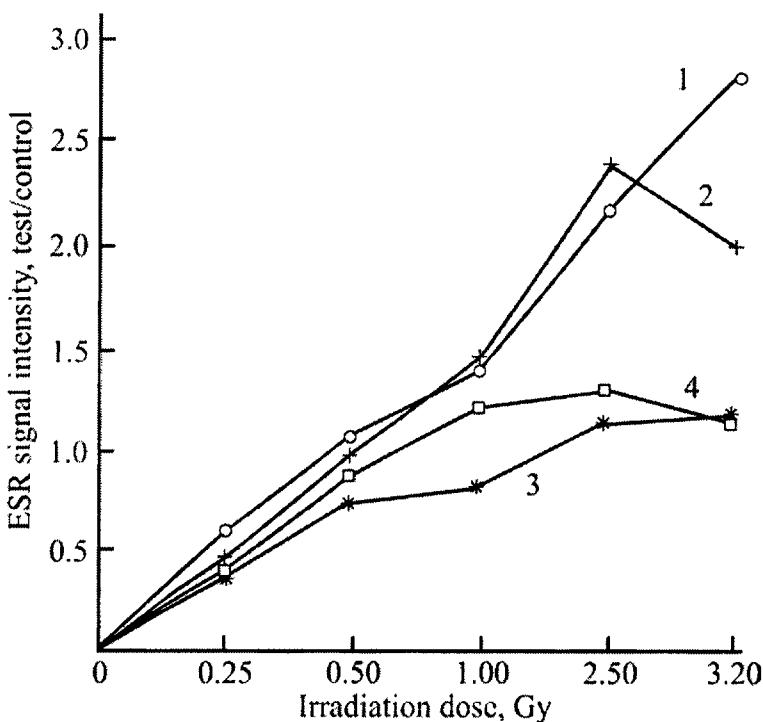


Figure 10. Dose dependencies of Fe^{3+} -TF pool in blood of dogs in periods of maximal intensity of the compensatory-adaptive reaction on the 2nd (1, 3) and the 6th (2, 4) days after irradiation.

1, 2 – irradiated unprotected dogs;

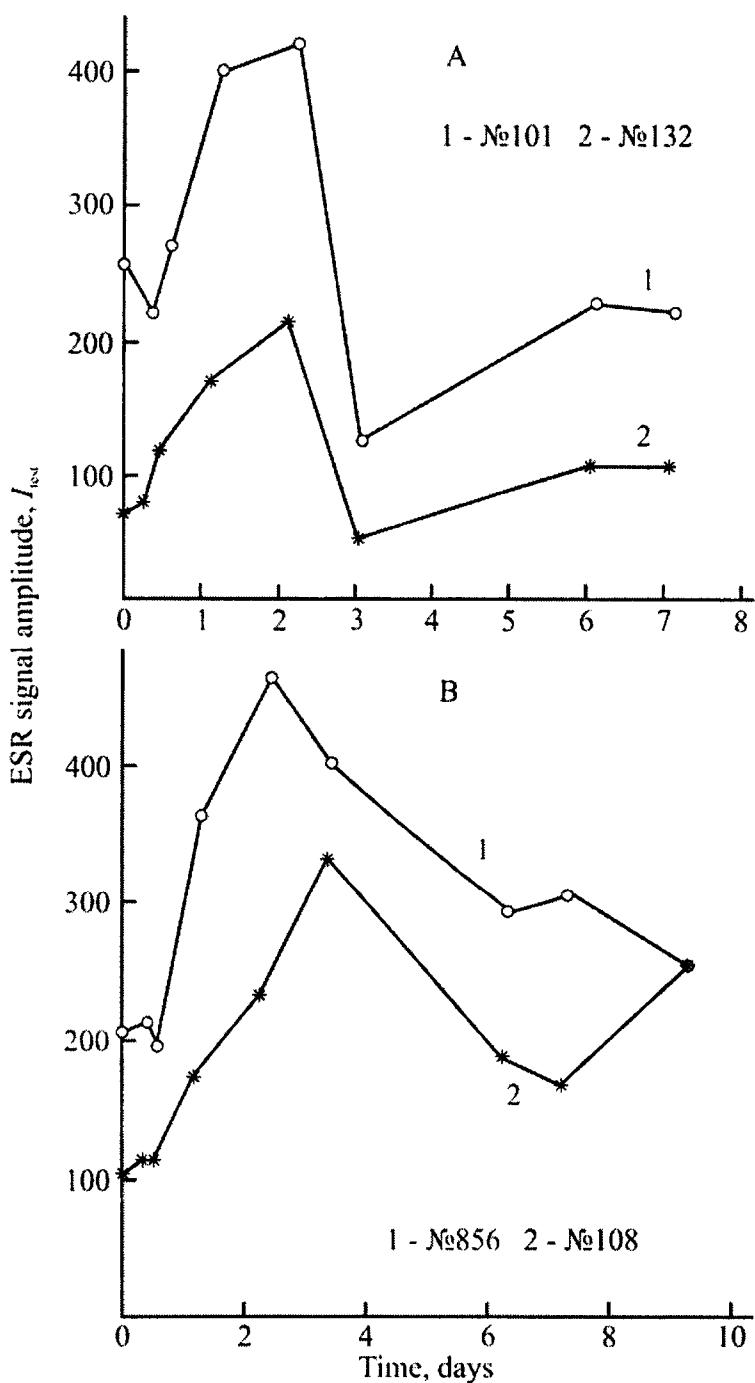
3, 4 – irradiated dogs protected by 15 mg/kg indraline dose, injected intramuscularly 15 min before irradiation. This dose value is optimal for radioprotection [16].

currently reported that iron-transport protein transferrin is the important participant of hematopoiesis and erythropoiesis [1, 2, 24], therefore, the increase of Fe^{3+} -TF pool observed in blood testifies about hematopoiesis activation in this period. Anti-radiation effect of indraline on the hematopoietic system of various animals was studies [16, 32]. It was shown that this action affected all stem cells. Indraline is capable of decreasing radiation damage of bone marrow and spleen stem cells, decrease post-irradiation level of chromosomal aberrations in bone marrow cells in mice and dogs, and cause earlier and intense proliferative, adaptive processes of the cell population in bone marrow and spleen. According to data obtained [32], reduction of the blood cellular

composition in dogs irradiated by 3.84 G dose and protected by indraline may begin by 15th – 20th day of the post-irradiation period.

Antioxidant α_2 -tocopherol effect on Fe^{3+} -TF pool in blood plasma of dogs

The blood system of dogs responds to irradiation by increasing antioxidant activity of blood and organs, which is the integral part of the protective mechanism (see above). Shown in this Section are investigation results of antioxidant α_2 -tocopherol effect on Fe^{3+} -TF pool in blood of dogs and mice. In all dogs with one-shot oral administration of 20 mg α_2 -tocopherol, a emergent increase of Fe^{3+} -TF and Cu^{2+} -CP pools in blood was observed (Figure 11). A day after antioxidant (AO) administration, Fe^{3+} -TF pool in dog No. 132 increased 2.5 times above the initial value and in the rest three dogs (No. 101, 108 and 856) by 1.5 – 1.7 times. It is of importance that in dog No. 132 with the lowest initial Fe^{3+} -TF pool its increase responding to AO administration was maximal. Dogs No. 108 and 856 were daily given 20 mg of α_2 -tocopherol during 6 days. Maximal Fe^{3+} -TF pools in blood of these dogs were observed on the 2nd or 3rd day after AO administration. Such increase of the pool is associated with α_2 -tocopherol-induced activation of protein biosynthesis and apotransferrin, in particular. Previously, the ionol-dependent activation of protein biosynthesis in liver at this AO administration to both intact animals and the ones after partial hepatectomy operation was determined [35]. Significant increase of Fe^{3+} -TF pool in blood indicates α_2 -tocopherol ability to stimulate synthesis of iron-containing proteins, including iron-containing ribonucleotide reductase. Ribonucleotide reductase activity of tissues in organs increased with radioprotectors administration to animals: indraline, indometafen and antioxidant α_2 -tocopherol (Figure 12). The value of Fe^{3+} -TF pool reduced to the initial level 7 days after AO intake. At multiple daily intake of α_2 -tocopherol Fe^{3+} -TF pool in blood of dogs (Figure 11B) increased during initial three days, reaching its maximum. Fe^{3+} -TF pool increased above the initial level was preserved during all observation periods, foregoing ribonucleotide reductase activity intensification in tissues of organs (Figure 12A). Increased ribonucleotide reductase activity results in increase of dNTP and, consequently, DNA synthesis volume (Figure 13). Moreover, antioxidant-stimulated increase of Fe^{3+} -TF pool in blood and organs affects the ratio of monocytes and macrophages, increasing the immune statute of hematopoietic organs [9]. The



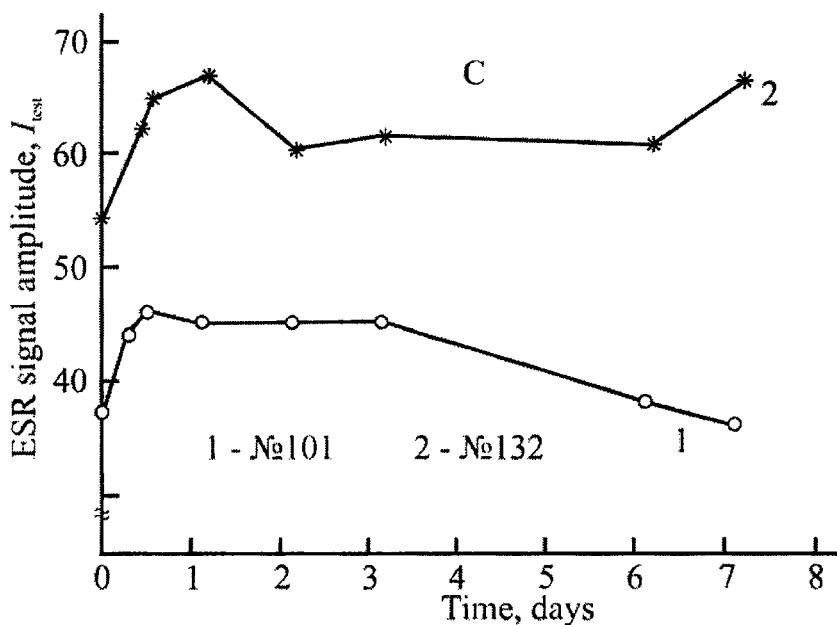


Figure 11. Changes of Fe^{3+} -transferrin (A and B) and Cu^{2+} -ceruloplasmin (C) pools in blood plasma of dogs after one-shot oral intake of 20 mg α_2 -tocopherol (A and C) and daily intake of 20 mg during 6 days (B). Dog numbers in the experiment are 101, 132, 108 and 856.
 Ordinate axis: ESR signal amplitude of Fe^{3+} -TF.
 Abscissa axis: time after one-shot (A and C) or initial (B) intake of antioxidant.

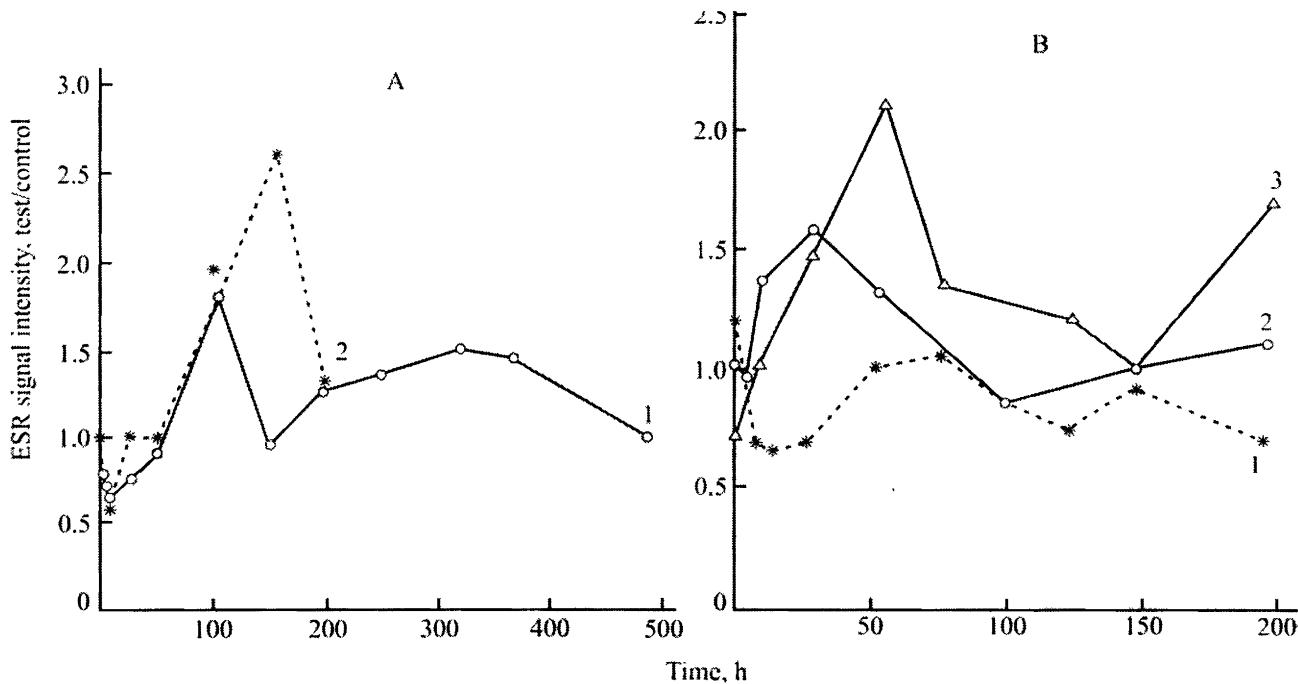


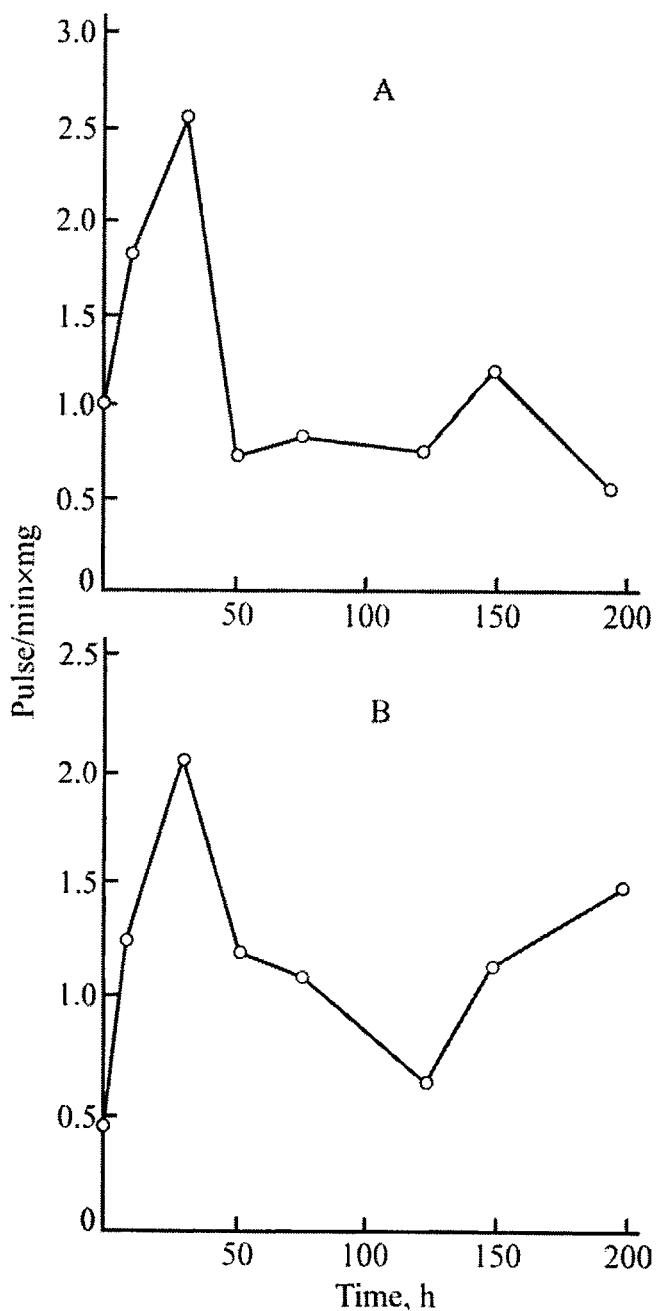
Figure 12. Dynamics of changes in Fe^{3+} -TF pool (A1) in blood and ribonucleotide activity of spleen (A2, B1, B2 and B3) in mice.

A – after intragastric injection of 30 mg/kg indometafen; B – after total irradiation by 6.5 G dose (1), after irradiation by 6.5 G dose with 100 mg/kg indraline injected 15 min before irradiation (2), and after one-shot administration of 17 mg/kg α_2 -tocopherol (3) (0.25 mg per mice).

Ordinate axis: ESR signal intensity of Fe^{3+} -TF (A1) and active ribonucleotide reductase (A1, B1, B2 and B3), ($\frac{I_{\text{test}}}{I_{\text{control}}}$ ratio). For control, intact animals were taken. Blood and spleen in control

and test mice were sampled in all test periods, simultaneously.

Abscissa axis: time after irradiation (B1 and B2) or after administration (A1, A2 and B3).



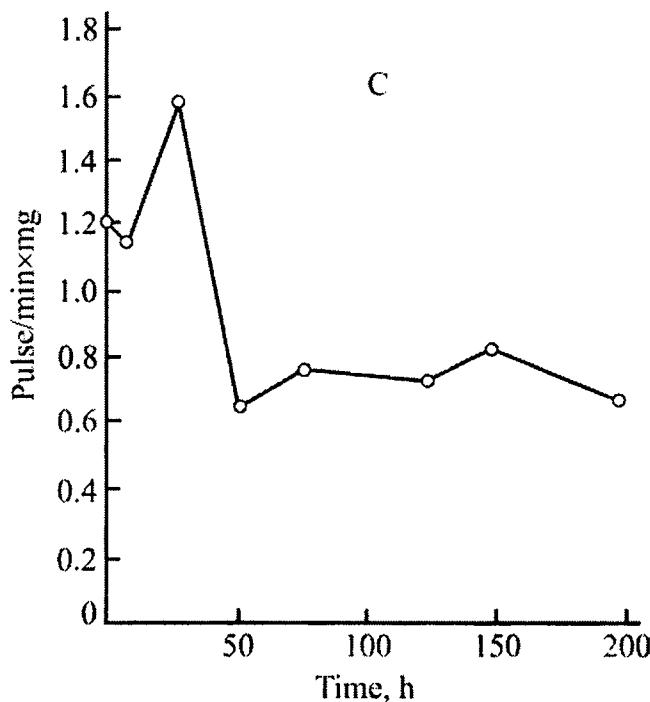


Figure 13. Dynamic of changes in DNA synthesis rate in liver (A), spleen (B) and thymus (C) in mice during four-shot intake of 0.25 mg α_2 -tocopherol per mouse daily.

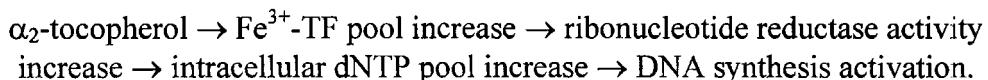
Abscissa axis: time after initial antioxidant intake.

Ordinate axis: the ratio of DNA synthesis rate in experiment and control group.

DNA synthesis rate was determined by the rate of radioactive label inclusion, pulse/min·mg. For the control, intact mice were taken. Organs of control and test groups were sampled in all test periods, simultaneously.

organism resistance increase with the help of antioxidants proceeds with transfer of a complex of radiosensitive molecular-cell reactions to higher stability state. Among these reactions, biosynthesis of deoxyribonucleotides, DNA and proteins are of the key importance.

The data obtained allow a conclusion that α_2 -tocopherol induces the following sequence of reactions in cells of animal organs:



CONCLUSION

It is observed that the cell response of total organism to irradiation or specimen impact may be estimated by changes of a series of metabolic parameters, including peripheral blood marker ($\text{Fe}^{3+}\text{-TF}$), highly sensitive and quickly responding to the impact. The role of this iron-transport protein is specifically important, because it controls the rate-limiting, iron-dependent stage in DNA synthesis. The increase of functionality of this transport protein and ribonucleotide activity in hematopoietic organs associated with increased power of the whole protein-synthesizing system of cells in response to a damaging impact are the key events in development of compensatory-adaptive reactions of cell systems in the organism. These response reactions are characterized by the phase dependence with the most clearly expressed maximums on the 2nd, 6th and 10 – 17th days. Dynamics of these reactions is independent of irradiation dose that indicates their nonspecific and universal type. Therefore, intensity of these reactions linearly depends on the irradiation dose up to lethal values, if apparently healthy animals were selected for tests with respect to their primary biochemical and radiospectroscopic blood values. However, at low dose irradiation of animals with changed initial blood values typical of ‘suppressed’ or ‘activated’ state a sharp increase of the response reaction intensity was observed, i.e. intensity of the organism response was inadequate to the irradiation dose and corresponded in value to response induced by irradiation by sublethal or even lethal dose. In these cases, one may suggest a ‘spurious’ irradiation dose and, therefore, selection of a ‘threshold’ permissible dose set with respect to other markers of radiation damage loses its significance. This is of special importance for estimation of remote

consequences of low radiation dose impact on people living in radionuclide-polluted zones.

REFERENCES

1. Crichton R.R., *Adv. Prot. Chem.*, 1990, vol. **40**, pp. 281 - 363.
2. De Jong G., van Dijk J.P., and van Eijk H.G., *Clin. Chim. Acta*, 1990, vol. **190**(1-2), pp. 1 – 40.
3. Thelander L. and Reichard P., *Ann. Rev. Biochem.*, 1979, vol. **48**, pp. 133 – 158.
4. Chitambar Ch.R., Matthaeus W.G., Antholine W.E., Graff K., and O'Brien W.J., *Blood*, 1988, vol. **72**(6), pp. 1930 – 1936.
5. Chitambar Ch.R. and Zivkovic Z., *Blood*, 1987, vol. **69**(1), pp. 144 – 149.
6. McClarty G.A., Chan A.K.M., Engstrom Y., Wright J.A., and Thelander L., *Biochemistry*, 1987, vol. **26**, pp. 8004 – 8011.
7. Klausner R.D., *Clin. Res.*, 1988, vol. **36**, pp. 494 - 500.
8. Neckers L.M., *Pathobiology*, 1991, vol. **59**, pp. 11 - 18.
9. Testa U., Camagna A., Giannella E., Pelosi-Testa E., Petrini M., Samoggia P., Montesoro E., Bottero L., Sposi N., Salvo G., Malivio F., Isacchi G., Mastroberardino G., and Peschle C., ‘Normal and neoplastic blood cells: from genes to therapy’, *Annal. NY Acad. Sci.*, Ed. Peschle C., 1987, vol. **511**, pp. 131 - 137.
10. Frieden E., *Metal Ions In Biological Systems*, vol. **13** ‘Copper proteins’, Ed. Sigel H., Basel: Marcel Dekker, 1981, No. 4, pp. 117 - 142.
11. Mitrokhin Yu.I., Gutnikova M.N., Boikov P.Ya., Chirkov G.P., Shevchenko N.A., Sidorenko L.I., Efremova O.I., Sizova S.T., Mitrofanova M.A., and Todorov I.N., *Biokhimia*, 1987, vol. **52**(6), pp. 969 - 977. (Rus)
12. Pulatova M.K., Rikhireva G.T., and Kuropteva Z.V., *Electron Spin Resonance In Molecular Radiobiology*, Ed. L.Kh. Eidus, Moscow, Energoatomizdat, 1989, 229 p. (Rus)
13. Nartikova V.F. and Paskina T.S., *Voprosy Med. Khim.*, 1979, vol. **25**(4), pp. 94 - 99. (Rus)
14. Vartanyan L.S. and Gurevich S.M., *Voprosy Med. Khim.*, 1982, vol. **28**(5), pp. 23 - 26. (Rus)
15. Pulatova M.K., Sharygin V.L., and Shlyakova T.G., *Radiatsionnaya Biologiya. Radioekologiya*, 2003, vol. **43**(1), pp. 29 - 43. (Rus)

16. Il'in L.A., Rudny N.M., Suvorov N.N., Chernov G.A., Antipov V.V., Vasin M.V., Davydov B.I., and Mikhailov P.P., *Radioprotectors. Radioprotector Properties, Pharmacology, Action Mechanisms And Clinical Studies Of Indraline*, Moscow, Izd. GNTs - Institut Biofiziki MZ RF, 1994, 435 p. (Rus)
17. Pulatova M.K., Sharygin V.L., and Todorov I.N., *Biochem. Biophys. Acta*, 1999, vol. 1453, pp. 321 – 329.
18. *Genetic Consequences Of Nucleotide Pool Imbalance*, Ed. F.S. De Serres, NY, L.: Plenum Press, 1985, 512 p.
19. Cory J.G. and Carter G.L., *Advances In Enzyme Regulation*, Ed. G.V. Weber, NY; L.: Pergamon Press, 1986, vol. 24, pp. 385 - 401.
20. Snyder R.D., *Mutat. Res.*, 1984, vol. 131(3-4), pp. 163 – 172.
21. Snyder R.D., *Biochem. Pharmacol.*, 1984, vol. 33(3), pp. 1515 – 1518.
22. Elledge S.J. and Davies R.W., *Mol. Cell. Biology*, 1989, vol. 9(11), pp. 4932 – 4940.
23. Walker G.C., *Ann. Rev. Biochem.*, 1985, vol. 54, pp. 425 – 457.
24. *The Biochemistry And Physiology Of Iron*, Eds. P. Saltman and J. Hegenauer, Amsterdam: Elsevier Biomedical, 1982, 485 p.
25. Grigoriev A.Yu., *Individual Radiosensitivity*, Moscow, Energoatomizdat, 1991, 81 p. (Rus)
26. Gutteridge J.M.C., *FEBS Lett.*, 1983, vol. 157(1), pp. 37 – 44.
27. Antonenko S.G., Berdinskikh N.K., Shishko E.B., and Okolot E.N., *Voprosy Onkologii*, 1985, vol. 31(5), pp. 48 – 54. (Rus)
28. Muksimova K.N. and Mushkacheva G.S., *Cellular and Molecular Grounds Of Hematopoiesis Under Long-Term Radiation Impact*, Moscow, Energoatomizdat, 1990, 159 p. (Rus)
29. Lebedev V.G., Vorotnikova T.V., and Deshevoi Yu.B., *Patologicheskaya Fiziologiya I Eksperimentalnaya Terapia*, 1994, No. 3, pp. 20 - 22. (Rus)
30. Lebedev V.G., Moroz B.B., Vorotnikova T.V., and Deshevoi Yu.B., *Radiatsionnaya Biologia. Radioekologiya*, 1999, vol. 39(5), pp. 528 – 533. (Rus)
31. Chernov G.A., Shlyakova T.G., Sharygin V.L., Sharf V.G., Todorov I.N., Mitrokhin Yu.I., Khristianovich D.S., Rozantseva T.V., and Pulatova M.K., *Izvestiya RAN. Ser. Biol.*, 1994, No. 1, pp. 20 - 37. (Rus)
32. Vasin M.V., Antipov V.V., Chernov G.A., L'vova T.S., Koroleva L.V., Semenova L.A., Lyutykh V.P., Komarova S.N., Gaidamakin N.A., and Vilkina G.A., *Radiatsionnaya Biologia. Radioekologiya*, 1996, vol. 36(2), pp. 168 - 189. (Rus)

33. Chernov G.A., Sharygin V.L., Pulatova M.K., Sharf V.G., Borovkov M.V., Shlyakova T.G., Khristianovia D.S., Mitrokhin Yu.I., and Todorov I.N., *Izvestiya RAN. Ser. Biol.*, 1996, No. 3, pp. 282 - 291. (Rus)
34. Pulatova M.K. and Sharygin V.L., 'Free radicals in biology and environment', *NATO, ASI Series, Ser. A, Life Science*, Ed. F. Minisci, Kluwer Academic Publishers, Dordrecht, 1997, 3. High Technology - vol. 27, pp. 305 - 315.
35. Pulatova M.K., Todorov I.N., Kosaganova N.Yu., Mitrokhin Yu.I., and Efremova O.I., *Khim.-Farm. Zh.*, 1985, vol. 19(1), pp. 21 - 26. (Rus)

The main principles of radiation biology

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ABSTRACT

The problem of “energetic paradox”, i.e. low expenditures of ionizing radiation energy in the realization of the biological effect of irradiation has been a major focus of interest throughout the history of radiation biology. The postulation of the *hit principle* made a large contribution to the quantitative radiation biology. This principle follows from the physical properties of ionizing radiation: the discreteness, quantization, and probabilistic distribution of energy in space. Hits, i. e., the acts of interaction of the absorbed energy with the elements of the substance do not depend on each other and obey the Poisson distribution. Another well-known principle, *the target principle*, is based on the understanding that the structure of the elements of a living system and their functions are heterogeneous, nonequivalent, and these elements respond differently to one and the same hit. It is generally accepted that the unique DNA macromolecule is a critical structure, a target of radiation. Biological membranes, with their barrier--matrix, energetic, and regulatory functions, which underlie all living processes, can also be considered as a sensitive critical target structure. The third principle, *the principle of amplification of primary radiation lesions* in critical target structures, is based on the radiation post-effect, the well-documented fact in radiation biology. The fourth principle is *the principle of repair of target damage* (regulation of cell homeostasis), a systemic response of the cell to damage, which involves the mechanisms of protection and repair of damage to DNA and biological membranes.

Advances in molecular biology and radiation biophysics made in two last decades gave a strong impetus for the development of the principles that follow from the analysis of radiobiological effects developing with time.

Radiobiological principles reflect the peculiar features of the physical and biological action of ionizing radiation.

Keywords: principles of hits, target, amplification of lesions of target structures, systemic response, recovery, repair, protection; DNA, BM, ROS, NO, peroxynitrite, prooxidants, lipid radiotoxins, antioxidants, chain processes of lipid peroxidation, genotoxic and multitoxic effects, endogenous background radioresistance, advances in radiation biophysics and molecular biology

Even at the early stage of the development of radiation biology as a science, it has been established that ionizing radiations, as opposite to nonionizing radiations, possess an extremely high biological efficiency, which leads to the damage to molecules and the death of the cell or the whole organism. In the context of traditional heat energetics, the small magnitude of the ionizing radiation dose capable of producing the biological effect looks puzzled.

Thus, the calculations show that the minimum dose of external single whole-body irradiation causing the human death (*minimum absolutely lethal dose*) is 7 Gy. When recalculated per body mass of a human weighing 70 kg, this dose is 490 J/man or 117.6 cal of heat energy. Such a negligibly small thermal energy, being uniformly distributed throughout the human body, would “heat” it by as few as approximately two thousandths of a degree.

This well-known energetic paradox makes us focus our attention on the peculiar “energodynamics” of ionizing radiation for open systems, i. e., the mechanisms of absorption, distribution, and transfer of ionizing radiation energy as well as the type of the response of a living system to radiation. The main principles of radiation biology were laid in the 20s of the last century. A significant contribution to the theoretical radiation biology was made by the investigations of the quantitative dependence of the biological effects on radiation dose and the postulation of the principles of *hits* and *heterogeneity*, which were developed by Li [1], N.V. Timofeev - Resovskii and his school [2, 3], and others. As it is known, the first principle follows from the physical properties of ionizing radiation: discreteness, quantization of the interaction of radiation with the substance, and the stochastic distribution of *energy* in space.

Let us recall the starting propositions of the fundamental principles of radiation biology.

I. HIT PRINCIPLE

- Ionizing radiations possess a very low volume density compared with, e.g. heat emitted by an energetically equivalent dose; i. e., they transfer energy in a discrete form, entering the object in “concentrated portions”.
- The photons of X-ray and γ -radiations, accelerated electrons and heavy charged particles posses a very high discrete energy whose magnitude is significantly greater than the energy of any chemical bond.
- In the first (physical) stage of the action of ionizing radiation on a biological system (10^{-16} - 10^{-13} s), the absorption, redistribution, and degradation of energy take place.
- The energy of photons and charged particles absorbed by a living system is completely expended (directly or indirectly) for the excitation and ionization of atoms and molecules.
- The probability that the energy will be transferred to a molecule does not depend on its chemical structure but is determined by the total electron density, which is approximately equal for different cell elements.

Another principle, the *target principle*, is based on the understanding that the structure of the elements of a living system and their functions are heterogeneous, nonequivalent, and these elements respond differently to one and the same hit. To put it figuratively, whereas radiation is “equally indifferent” to substrates being irradiated (it does not “choose” them), the injuries of individual elements of a biological object are of different significance for the fate of the cell.

II. TARGET PRINCIPLE

--Because high energy is discretely absorbed in the cell, and the action of radiation is not selective, ionizing radiation can affect any molecule and any biological system. In biological objects, there are no structures that preferentially

absorb radiation energy: both proteins and nucleic acids, lipids and carbohydrates, water molecules and various low-molecular organic substances undergo excitation and ionization.

--However, changes occurring in heterogeneous cell elements that absorb equal radiation energy cause, after some time (which is different for each element of the living system), injuries different in magnitude and biological importance.

--Characteristic dose - effect dependences reflect the quantized mode of interaction of the radiation with the substance and the presence in the cell of highly sensitive critical volumes, targets.

--The sequence of further physicochemical, chemical, and biological stages of radiation-induced changes in molecules and their diverse responses to one and the same hit have been described.

Starting from the hit principle (discreteness) and the target principle (heterogeneity), which formed the basis of quantitative radiation biology, a **target theory** was developed. Depending on the interpretation of "radiosensitivity" and biological significance of radiobiological effects, this theory considers as cell targets highly sensitive volumes (critical structures), such as DNA [3, 4] and biological membranes (BM) [5 - 10] whose damage may be responsible for the lethal outcome. Whereas there was no doubt that DNA is a critical radiobiological target whose damage leads to the "genetic" cell death, the idea that BM may also be a target raised strong objections.

The fundamental discoveries in radiation biophysics in the 1950 - 1960s opened a new stage in the theoretical radiation biology, which was devoted to the primary mechanisms of interaction of ionizing radiation with biological systems. The discoveries of the oxygen effect, the indirect effect of radiation, and the chemical means of protection against radiation damage made it possible to initiate studies on the biophysical nature of "hit events" and the lesions of the elements of the heterogeneous living system. Attempts were made to assess the significance of the lesions of the critical structures of the irradiated cell. These discoveries led researchers to abandon the conception of the instantaneousness and irreversibility of primary lesions and recognize the idea of the modification of radiation damage to cell and the organism. An important achievement at this stage was the finding that activated (due to radiolysis) water and oxyradicals play a role in the development of radiobiological effects. Numerous investigations were performed, which indicated that ionization and excitation not only cause lesions of organic molecules, but also these lesions themselves are able to transfer the radiation energy, i. e., to exhibit a new destructive activity toward various cell elements. Thus, there emerged the idea of **amplification of**

radiation damage and the dependence of the radiobiological effect on the time elapsed after irradiation.

These discoveries in radiation biophysics were erroneously interpreted by some radiobiologists as indicating that "the definition of a target becomes diffused and loses its real meaning". However, at that time there was still no reason to consider nongenetic structures as a real radiobiological target.

In 1970 Dertinger and Young [4] suggested that BM, like DNA, is a structure that undergoes strong changes after one or several energy absorption events. However, the authors were able to observe *only the degradation of DNA* after irradiation but not of *membranes* or any other cell components. This enabled the authors to state that *at this stage of research* no experimental evidence was available in favor of the idea that in the cell there are other targets comparable in significance with DNA [4, p. 235]. Since there was some uncertainty concerning the fatal role of damage to BM for the cell, the biological membrane has long been assigned the modest role of a *candidate for target*.

New experimental evidence for the radiobiological significance of BM, its radiation-induced degradation, and DNA degradation was obtained owing to impressive advances in molecular biology, in particular, the discovery of the role of oxyradicals in the damage to DNA and BM.

These data experimentally confirmed and substantially advanced the conception of B.N. Tarusov of the chain mechanism of radiation damage [5, 6] and the role of toxic lipid peroxidation products (LPP) (lipid radiotoxins (LRT)) in the development of radiation-induced reactions of amplification of damage [11, 12].

Let us consider this problem in more detail since it will be touched upon in the discussion of two other energodynamics principles relating to the radiation events in the cell that develop with time.

Among the facts that confirmed the possibility of amplification of radiation effects was the discovery of the cytogenetic effect of LRT and genotoxic oxidative DNA damage caused by the indirect effect of ionizing radiation and other physical and chemical agents [13 - 17]. Thus, in 1966 in collaboration with the laboratory of N.V. Luchnik, we found for the first time that radiation-induced lipid oxidation products LRT induce a cytogenetic effect [13]. Later it was shown that LPP are able to destroy the microstructure of nucleic acids [14]. Interesting results were obtained in the laboratory of E.B. Burlakova in radiobiological studies of the role of lipids and LPP in the biosynthesis and functional activity of DNA [15]. By using the present-day methods of molecular biology, a basic research of the effect of highly labile

short-lived oxyradicals and oxyproducts on the DNA macromolecule was performed [16].

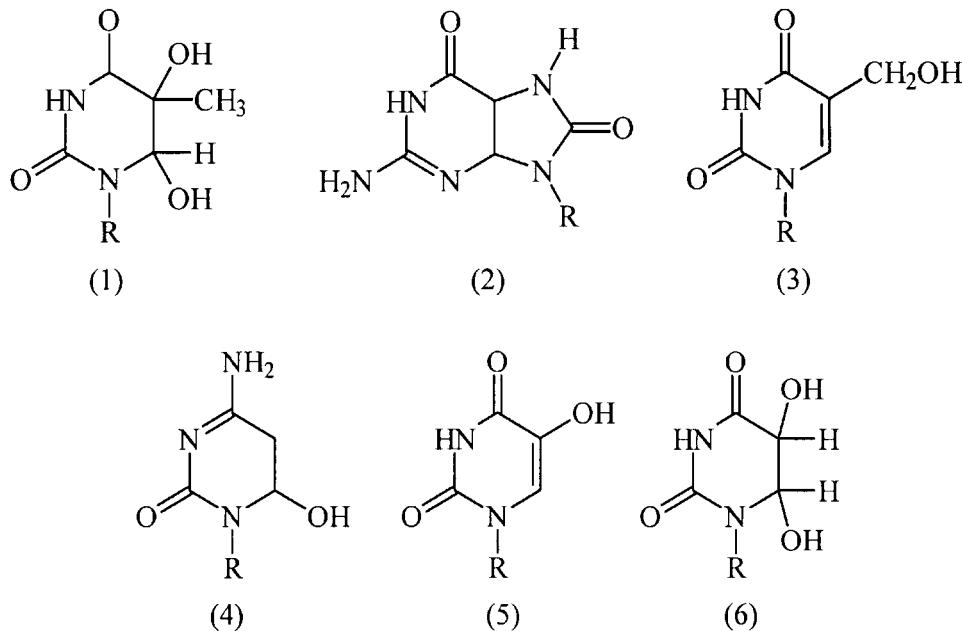


Figure 1. Oxidized DNA bases

Key: 1. Thymine glycol; 2. 8-oxo-dG; 3. 5-hydroxymethyluracil; 4. 6-hydroxy-5,6-dihydrocytosine; 5-hydroxyuracil; 6. Uracil glycol

Due to methodical improvements in gas and thin-layer chromatography, selective ion mass spectrometry, EPR, chemiluminescence and the use of radioactive labels and radical inhibitors, it became possible to perform highly sensitive and specific identification of DNA lesions [16 - 23]. The analysis of nuclear DNA with the use of novel methods showed that many endogenous lesions of the macromolecule in the organism are induced by the effect of reactive oxygen species (ROS), LPP, and the nitric oxide system on DNA. It was found that endogenous oxidative DNA lesions (breaks of bases and strands by oxyradicals) are genotoxic and induce mutations upon replication of lesions [16]. It was shown that oxidative lesions are an important link in the etiology of not only radiation-induced diseases [12 - 17] but also many other diseases, including cancer [16, 19], aging [19], apoptosis [21], and stress [22].

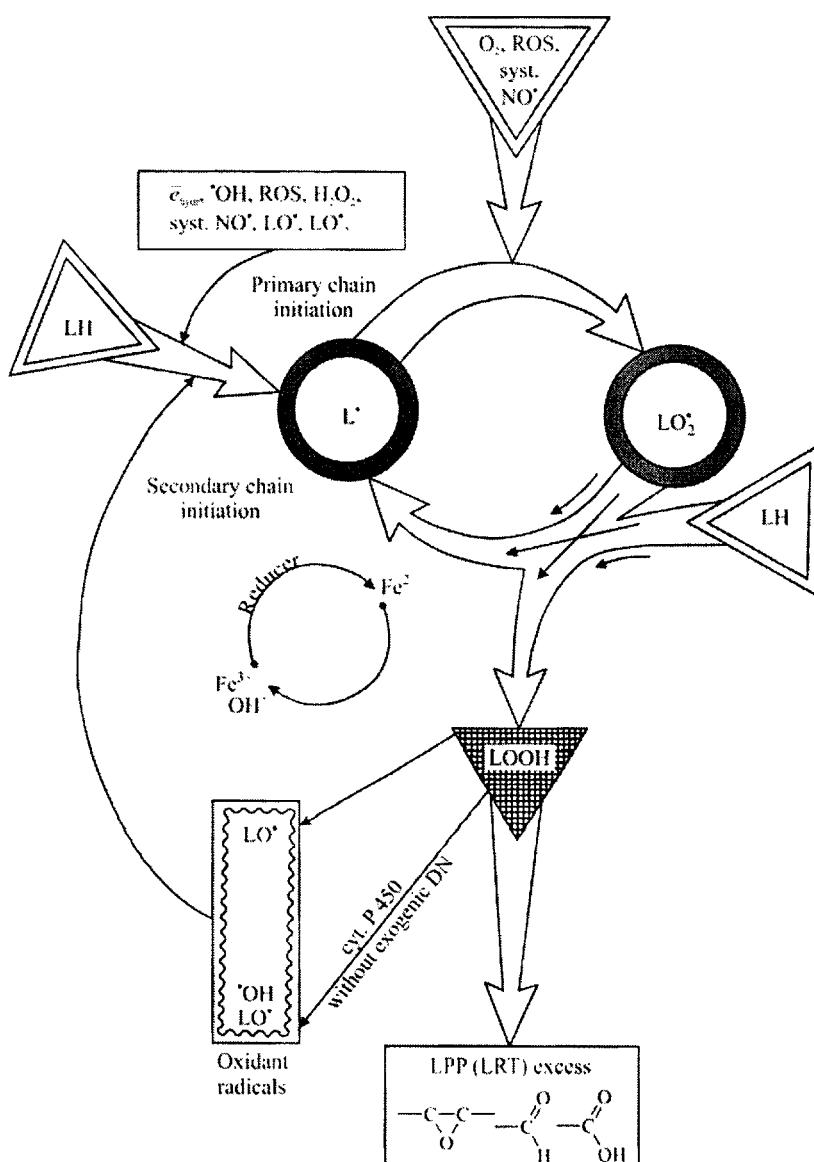


Figure 2. Chain free-radical lipid peroxidation processes in the cell [28].

Of a variety of oxy adducts that cause the cytotoxic, genotoxic, and mutagenic effects, 8-oxo-deoxyguanosine (8-oxo-dG) and cytosine and uracil adducts have been studied most comprehensively [16 - 18, 23] (Figure 1).

The radiation-induced oxidation of DNA is a well documented fact; however, the study of this very important phenomenon is at the initial stage, whereas the mechanisms of oxidative degradation of BM and the radiation-induced toxic effect of LPP have been studied extensively [6, 12, 24, 25].

It is known that BM are most prone to oxidative degradation since unsaturated regions of fatty acids (e. g., linolenic, arachidonic, and other acids) of phospholipids are extremely sensitive to oxidation [26]. A high content of polyunsaturated fatty acids in phospholipids determines a high ability of BM for chain oxidation reactions and the formation of new oxidation initiators possessing the oxidizing activity.

The chain oxidation of lipids initiated by ionizing radiation plays an important role in the pathology and death of cell. This reaction contributes to the accumulation of a large excess of toxic oxidation products, LPP, due to their repeated reproduction (Figure 2).

The term *chain reaction* was proposed in 1913 by M. Bodenstein who discovered an unusual photochemical reaction, the transformation of tens of thousands of chlorine molecules upon absorption of only one light photon. A sequence of oxidation reactions can repeat many times like the links of one chain (like a stone falling from the mountain and causing a land-slide), but these reactions proceeds without absorption of energy quantum. Thus, a chain reaction is a catalytic cyclic reaction of self-acceleration (amplification) in which free radicals act as a catalyst [24].

The chain reaction is initiated by reactive radicals, e. g., OH[·], which penetrate into the lipid layer of BM [28 - 30]. Irradiation induces an activation of the interaction between reactive radicals and polyunsaturated fatty acids LH and the formation of lipid radicals L[·] (Figure 2). Lipid radicals react with molecular oxygen dissolved in the medium (the reaction with ROS and peroxynitrite proceeds more effectively) to form prooxidants, free lipid radicals: alkoxyl LO[·] or peroxy LO₂[·]. These radicals in turn interact with adjacent molecules of polyunsaturated phospholipids of BM yielding lipid hydroperoxide LOOH and, again, the lipid radical L[·] (stage of “elongation” and “development” of the chain). In norm the hydroperoxide LOOH and LPP formed by it are contained in the cell at a stationary level, which is no higher than 1 μM (Figure 2). By the action of ionizing radiation, this level increases, and due to the repeated accumulation, an excess of LPP: hydroperoxides, epoxides, aldehydes and

ketones is formed (Figure 2), which produce a toxic effect on the cell. It was found previously in our laboratory that excessive LPP (LRT) are able to induce a wide spectrum of damaging effects on cell structures. They produce the genotoxic effect on chromosomes and DNA and induce a delay of cell division and cell death [31 - 33]. It was also found that, in cells of the roots of *Crepis capillaris* seedlings, LRT exhibit a mutagenic activity, inducing chromosomal aberrations at all stages of the nucleus cycle [33]. There is evidence that radiation can produce an indirect effect through LRT radicals [34], similar to what was reported on the mediating effect of water radicals.

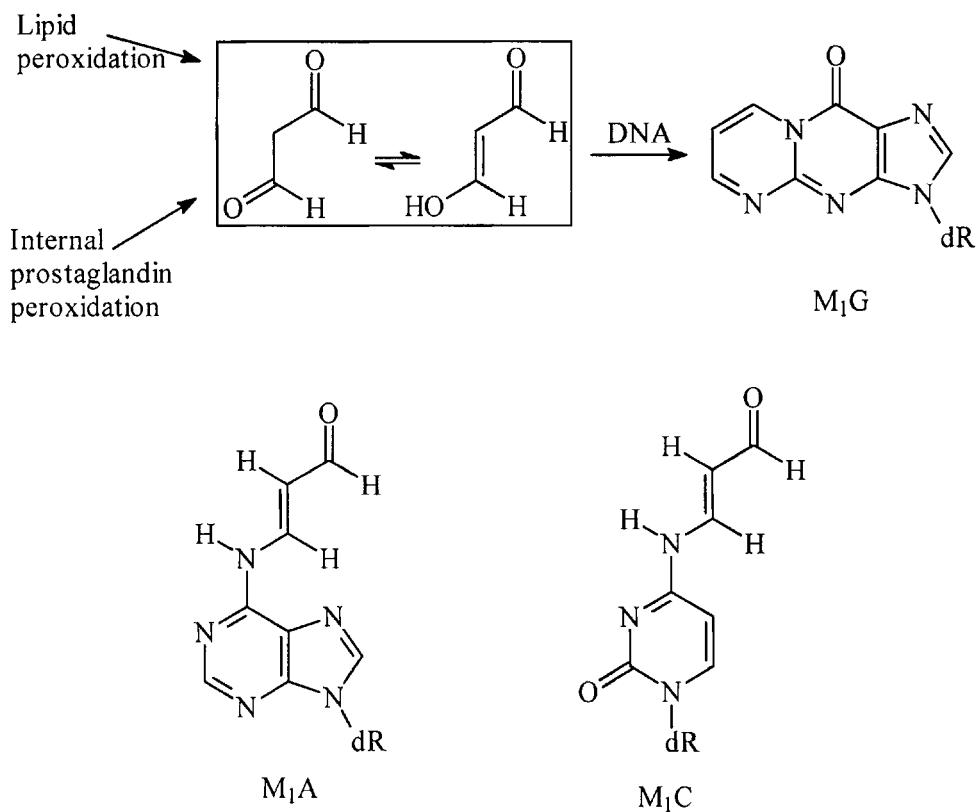


Figure 3. Formation of malondialdehyde (MDA) and its effect on DNA bases.

Yet it remained unclear until recently what is the structure of individual LTR: aldehydes, epoxides, and other oxidants (LPP) involved in the mechanisms

of the indirect effect of radiation on particular regions of DNA, i. e., what is the mechanism by which the radiation lesion of the target is amplified with time.

Of the end LPP, the following compounds have been identified: malondialdehyde (MDA) [35 - 41], acrolein and crotonaldehyde [42, 43], and the epoxide 2,3-epoxy-4-hydroxynonenal (HNE) [44 - 50]. There is experimental evidence that these oxidants produce not only the toxic effect. What is particularly important, they exhibit, to a greater or lesser extent, the genotoxic, mutagenic, and carcinogenic effects in cells, tissues, and organs of mammals (including humans), in single cells, and in other biological objects. This has been established at least for aldehyde, propano, and etheno adducts [35 - 53]. The structural formulas for some oxidation products of DNA bases in the genome are given in Figures 3 - 5 [16].

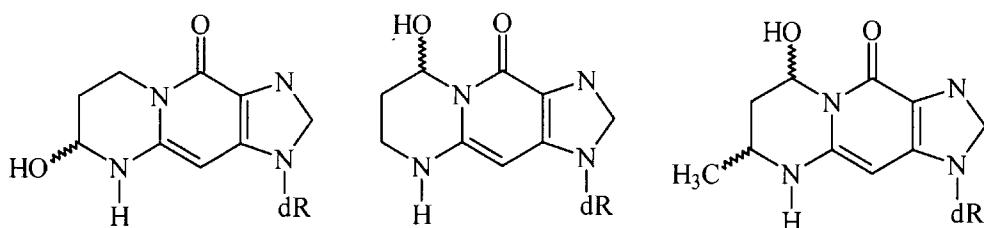


Figure 4. Propano adducts.

It should be noted that the effect of LPP is not confined to the oxidative degradation of BM and DNA. Other cell components (radiation targets) can be directly attacked by various water- and fat-soluble prooxidants. The content of some prooxidants in the intact cell and some of their characteristics are given in the table.

Electrons liberated by the action of ionizing radiation during the primary ionization process are of particular interest from the biological viewpoint. These electrons cause the polarization of water molecules and are stabilized to a state of hydrated electrons $\text{e}^{-}_{\text{hydr}}$, which are able to diffuse over large molecular distances and effectively interact with molecular oxygen and other molecules.

It is also known that the accumulation of OH^{\cdot} and H_2O_2 results not only from the radiation-induced four-electron non-enzymatic reduction of oxygen in the cell but also from water radiolysis. The indirect effect of radiation through water radicals has long been known [7, 54, 55]. However, only in recent years it became possible to gain a better insight into the role of the cyto- and genotoxic

effects of OH[•] and other reactive products in the mechanism of amplification of radiation damage.

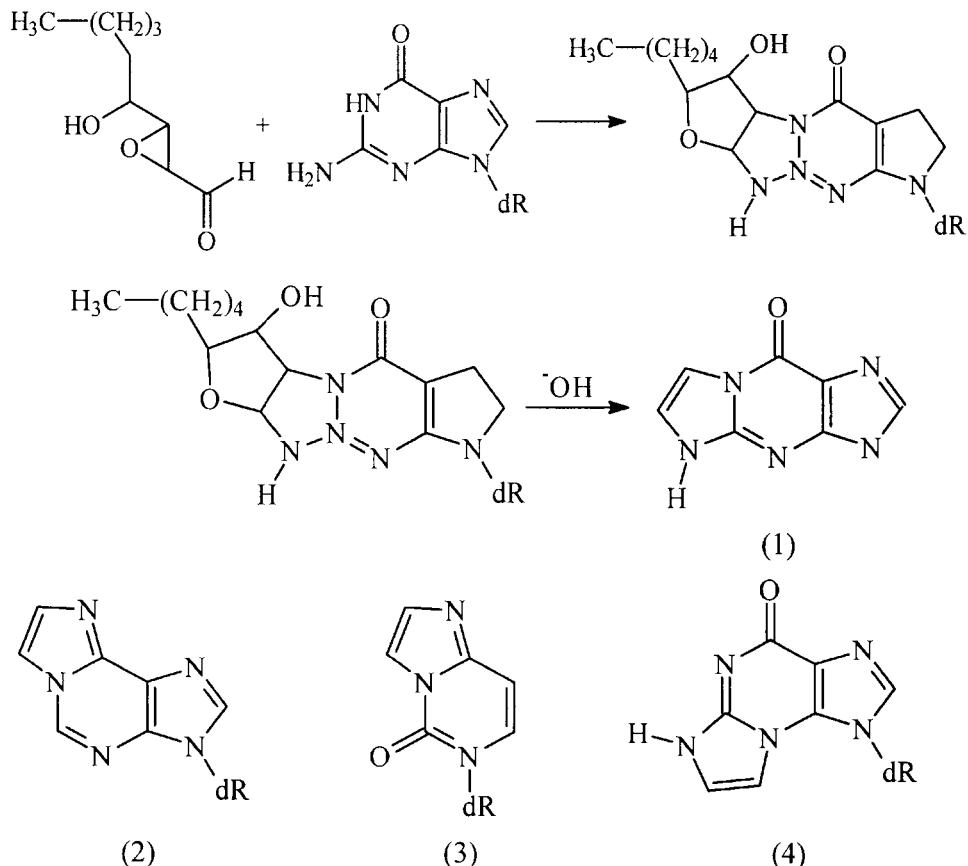


Figure 5. Etheno adducts formed by the action of epoxide EHN (2,3-epoxy-4-hydroxynonenal) on DNA bases.

Key: 1. 1,N²-etheno-dG; 2. Etheno-dA; 3. Etheno-dC; 4. N²,3-etheno-dG

The hydroxide radical is an extremely reactive oxidant, which is able to destroy virtually any adjacent molecule in the cell [7, 23, 30]. When attacking thiol protein molecules, OH[•] denatures them and inactivates enzymes. In nucleic acids, the hydroxide radical reacts with bases to form destruction products in the

genome. It also destroys carbohydrate bridges between nucleotides and induces breaks of DNA and RNA strands. Thereby, OH[•] is able to induce mutations and cell death [30]. When interacting with the other target, BM, the hydroxide radical penetrates into the lipid layer and initiates chain lipoperoxidation reactions (Figure 2), which leads to the disturbance of functions of BM and cell death. However, it should be noted that OH[•] has a short lifetime (table), and it manages to diffuse for no more than one-two molecule diameters to interact with cell components. Therefore, the probability that it would directly interact with the nuclear DNA is low. Hydrogen peroxide is a diffusing "latent" form of the hydroxide radical. It has a longer lifetime and is contained in the cell in relatively large amounts (table). When reacting with a metal ion, the H₂O₂ molecule "conveys" OH[•] to chromatin and membrane structures [23, 30]. The contact of water and lipid radicals is facilitated in DNA - lipid complexes, which was comprehensively studied in 1962 - 1974 [57]. It is highly probable that the DNA - membrane complex (3×10^9 Da) is a unified radiobiological target whose radiation damage can lead to the inactivation and death of the cell [56 - 58].

In the last 10 - 15 years, one more important discovery has been made in molecular biology. It was established that the simple chemical compound nitric monoxide, which is formed in the organism enzymatically, acts as a universal regulator of metabolism [59 - 61]. Presumably, NO is one of the ancient universal regulators of intra-and intercellular signaling systems. This compound is assigned to the "wrong side" of metabolism related to non-enzymatic oxidative processes [62]. Owing to the use of molecular biology methods, ever increasing evidence for the regulatory role of NO in oxidative processes and in the mechanism of gene expression and protein biosynthesis is accumulating.

Small dimensions and the absence of a charge provide a high permeability of diatomic gaseous molecules of NO through plasmatic and intracellular membranes. Nitric monoxide molecules easily diffuse in biological media and are relatively long-lived (table). Since NO has one electron with the unpaired spin (structure formula N = O), it is highly reactive. In particular, it is able both to activate and inhibit chain free radical reactions [30].

Table

Content and some characteristics of oxidants in the cell

Name	Symbol	Content in cell (in norm), M	Half-life, s (at 37°C)	Properties	Reactivity
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MOLECULAR OXYGEN

Oxygen in the main, triplet state	${}^3\Sigma gO_2$	[10^{-6}]	$> 10^2$	Capable of diffusing across biomembranes	Weak oxidant
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REACTIVE OXYGEN SPECIES (ROS)

Superoxide anion radical of oxygen	O_2^-	[10^{-11}]	10^{-6}	Signal-triggering functions in the cytoplasm; does not penetrate across biomembranes	0
Hydroperoxy radical (perhydroxide)	HO_2^-	[10^{-11}]	10^{-8}	Capable for diffusion and oxidative modification	1
Singlet oxygen	1O_2	[10^{-11}]	10^{-6}	Capable for diffusion and pronounced oxidative modification	10^1
Hydrogen peroxide	H_2O_2	[10^{-8}]	$10-10^2$	Signal-triggering functions; diffuses for long distances	> 1
Hydroxide radical	OH^-	[$<10^{-11}$]	10^{-9}	Diffuses for short distances; a very strong oxidative modification	10^7

ACTIVE NITRIC COMPOUNDS

Nitrogen monooxide radical	NO^-	[10^{-6}]	0.1-6.4	Signal-triggering functions; diffuses for long distances	Not measured
Peroxy-	ONO_2^-	[$<10^{-6}$]	1-2	Diffuses for long	10^2

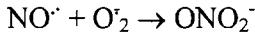
nitrite				distances; a very strong oxidative modification	
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LIPID PEROXIDATION PRODUCTS

Alcoxyl radical	LO [·]	[10 ⁻⁶]	10 ⁻⁶	Initiation of chain peroxidation reaction	10 ⁴
Peroxyl radical	LO [·] ₂	[10 ⁻⁶]	10 ⁻²	Initiation of chain peroxidation reaction	1
Molecular LPP	LOOH, LO ₂ , R-LO	[10 ⁻⁶]	10 ⁻²⁻¹	A high capacity for diffusion and oxidative modification	10 ²

It was found that, in tissues of γ -irradiated animals, the activation of NO-synthase takes place, which results in the accumulation of excessive nitric oxide [63, 64]. It was shown that this process is initiated by ROS, probably through the activation of the transcription factor, the NFkB protein. The diffusing molecular messenger nitric oxide plays an important role not only in the radiation-induced pathology but also in the pathologies of other geneses [62]. It was found, e. g., that prolonged generation of NO initiates apoptosis [65]. Together with other oxyradicals, nitric oxide and its derivatives are the key factors responsible for inflammation, infections, carcinogenesis [66], and the development of stress and adaptive responses of the organism [67].

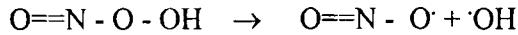
It was also shown that the cytotoxic and cytogenetic effects are due to the formation of the extremely reactive oxidant, peroxy nitrite (table), which results from the interaction of nitric oxide with the superoxide anion radical [68 - 70]:



or the protonated form of peroxy nitrite (in structural presentation):



Peroxy nitrite can decompose to form a hydroxide radical [30]:



The protonated peroxynitrite, like hydrogen peroxide, acts as a diffusing “latent” form of the hydroxide radical [71]. As it is evident, peroxynitrite is a link between two highly reactive low-molecular-weight systems, NO and ROS.

It was found that, when diffusing in the cell and penetrating across membranes by means of anion carriers, peroxynitrite and its protonated form exhibit a unique spectrum of chemical reactivity. It induces breaks of chains and oxidation of DNA bases, the nitration of guanine and proteins, the oxidation of lipids of biological membranes, etc. [66, 72]. Many of these reactions can give rise to the cytogenetic effects and mutagenesis [66].

These data indicate that DNA and BM are the targets not only for the direct attack by ionizing radiation energy quanta but also for the indirect oxidative action of radiation [73] since the targets in irradiated cells are destroyed by multitoxic and mutagenic prooxidants OH[·], H₂O₂, ONO₂[·], NO, and lipid radiotoxins.

Before going to the consideration of *the post-effect inherent in ionizing radiations*, with subsequent progressive degradation of critical structures, let us dwell in short on one more principle of radiation biology *the principle of amplification of primary radiation lesions*.

III. PRINCIPLE OF AMPLIFICATION OF PRIMARY RADIATION LESIONS IN CRITICAL TARGET STRUCTURES

- Radiation-induced primary lesions of DNA (single- and double-strand breaks, breaks and/or release of bases) and BM (initial stage of oxidation of polyunsaturated lipids) are only potentially dangerous for the fate of the cell.
- The real danger of these disturbances is related to the accumulation of large amounts of the products of radiation-induced lesions of critical structures.
- If direct primary radiation lesions are localized in the structural domain of a gene (mutations), the expression of this gene would result in the synthesis of hundreds and thousands of molecules of mutated proteins, which would disturb the functioning of the cell or induce changes incompatible with life. The next stage of the amplification of primary DNA lesions may be related to their transmission (inheritance) to the descendants of maternal cells, i. e., an increase in the number of maternal cells carrying lesions.
- The ionization process, the formation of excessive amounts of ROS, NO[·], peroxynitrite, and primary LPP, the formation of toxic oxidative adducts of

DNA and BM at the physicochemical and chemical stages of radiation energy transfer and the factual universality of the oxygen effect, all these processes point to an extremely important role of oxygen in the transformation of energy upon radiation-induced cell damage.

- The development of the oxidative process in BM and chain oxidation reactions represent a mechanism of amplification of primary lesions, which culminates in the irreversible oxidative degradation of cell membrane structures.
- Irreversible degradation of both DNA and BM may lead to consequences fatal for the cell.

It should be emphasized once again that the very important biological role of cell membranes in energy, their barrier, matrix, and regulatory functions in the cell, a high capacity for radiation-induced oxidation and formation of excessive multitoxic and mutagenic oxyradicals and LPP, all suggests that, like DNA, BM is a critical structure whose damage may be fatal for the cell.

Oxidative processes in an intact cell are under strict and diverse control of enzymatic and nonenzymatic systems (Figure 6). Therefore, the rate of oxidative reactions is slow, and the content of natural prooxidants (primary products of water radiolysis, ROS, the NO system, and LPP) is maintained at a low stationary level (table). The important role of oxidative processes for the normal vital activity of the cell is an experimentally established fact. Studies along this line are currently being continued [30, 74 - 78]. There is also convincing evidence that oxidative degradation, which arises from the accumulation of excessive oxidation products and causes a variety of pathological states (referred to as the radiation toxic effect [79], radiation stress [80], the lipoperoxidation syndrome [81], oxidative stress [77], etc.), is a result of the general nonspecific response of cells and the organism to the external influence. In cells and tissues, this process leads to changes in the relationships between the stationary levels of antioxidants (AO) and prooxidants (PO). In radiation biology, this relationship received the name endogenous background radiation (EBR) [82]. The specificity of disturbances of oxidative processes in various diseases is determined by the nature and magnitude of changes in each component, AO and PO. As it was mentioned above, the radiation-induced damage is characterized by the radiation post-effect of the mechanisms by which the amplification of degradation of critical structures with time takes place. A closer look at lipoperoxidation in BM shows that, although this process develops in the lipid phase of the cell, many

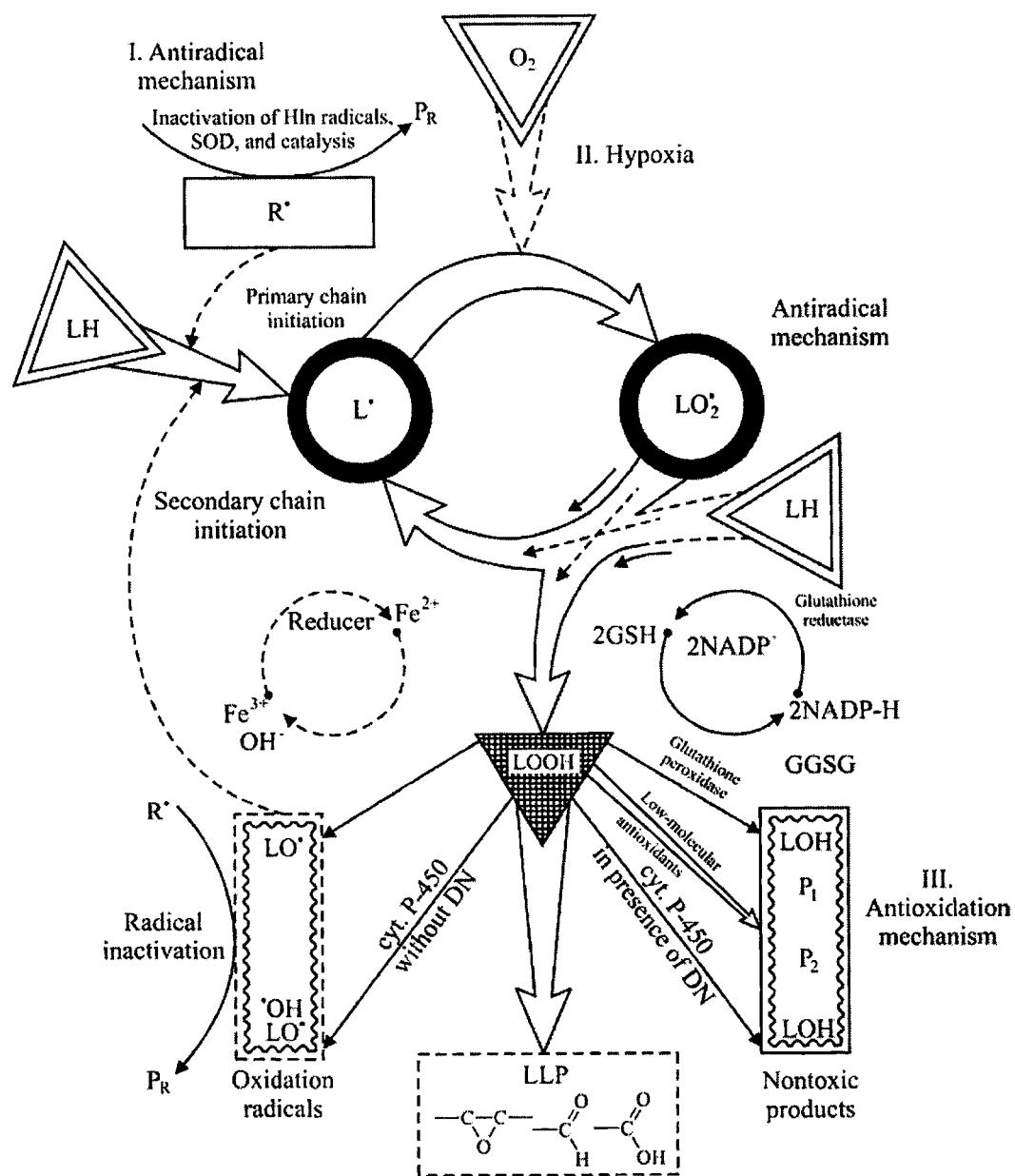


Figure 6. Mechanisms of protection of BM from oxidative degradation [28].

stages of this complex system of oxidation reactions proceed in the aqueous medium as well [25, 30]. Therefore, some endogenous protective systems in the cell are localized in BM lipids and others in the aqueous phase.

Figure 6 gives a schematic representation of the mechanism for the prevention or reduction of the oxidative degradation of BM through the normalization of the level of oxidation products in the cell. It is evident from the figure that enzymatic and non-enzymatic intracellular protection and recovery from accumulation of excessive LPP are multistage processes.

At the stages of the formation of primary radicals in water and lipids, antiradical protective resources reduce the formation of free radicals by destroying them or preventing their formation in the cell (the so-called antiradical mechanism of protection; Figure 6 I) [25, 30, 83, 84].

At the stage of the formation of oxyradicals, protective mechanisms begin to operate that decrease the oxygen delivery from the blood to the cell (e.g., formation of met- and carboxyhemoglobin, vasoconstriction [84]) and regulate the level of ROS (e.g., activation of superoxide dismutase [85]) and enzymes controlling the NO⁻ level [62, 86, 87]. At this stage, a hypoxic mechanism of protection is realized (Figure 6, II).

The next stage of lipoperoxidation (LP), the formation of LPP, is controlled by a variety of enzymes (catalase, peroxidase, cytochrome c-450 in the presence of hydrogen donors (HD), etc.) and low-molecular antioxidants (Figure 6 III).

Below is given a list of the most active low-molecular antioxidants that comprise the so-called antioxidant buffer [25, 74, 76, 77, 83, 84, 88]:

- thiols: reduced glutathione, cysteine;
- biogenic amines: serotonin, histamine, catecholamines, corticosteroids;
- peptides: carnosine, anserine;
- vitamins: cell cytosol ascorbic acid, tocopherol, β-carotene, and other carotenoids localized in the lipids of biological membranes; and
- other antioxidants: phospholipids, ubiquinone, urates, bilirubin, phenols, microelements, ions of metals of variable valence.

It is noteworthy that many of these biologically active compounds are used in radiation biology as radioprotectors, substances protecting cells and organisms from ionizing radiation [83, 84].

These components of EBR serve to realize the antioxidant protection mechanisms, which lead to the destruction of LPP and (or) prevention of

formation of their excessive amounts and the restoration of their stationary level (usually up to 1 μM).

Endogenous protection of BM against oxidative degradation is a process inherent in the intact cell; during radiation damage it experiences peculiar changes (Figure 7) [83].

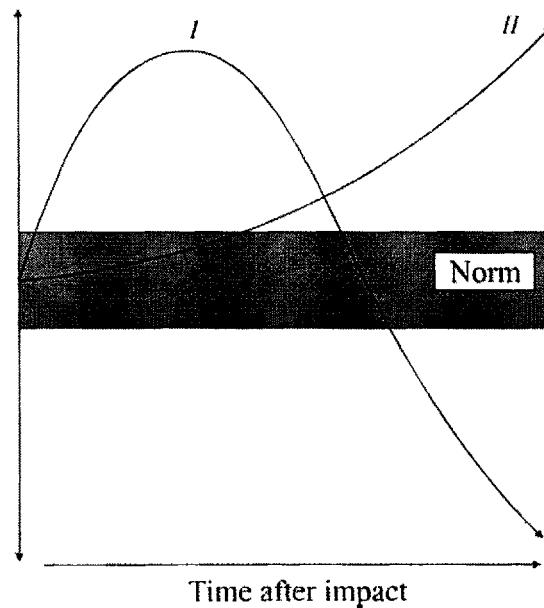


Figure 7. Scheme showing the relationship between the capacity of the antioxidant buffer (level of antioxidants) (*I*) and the intensity of lipid peroxidation (level of prooxidants) (*II*) at different stages of the response of the irradiated cell. On the abscissa is the time after irradiation; on the ordinate are the levels of anti- and prooxidants.

The capacity of the antioxidant buffer changes in response to irradiation and other extreme influences and goes through the following stages: mobilization, normalization, and exhaustion. At the stage of exhaustion of AO, the intensity of LP increases, and an irreversible unbalance between antioxidant (Figure 7 *I*) and oxidative processes (Figure 7 *II*) may arise at some dose.

If the reserves of the protective antioxidant activity are exhausted and the cell cannot cope with the progressive toxic effect, which can induce, e. g., a considerable disturbance of the genetic apparatus, the mechanisms of cell suicide

begin to operate. One of the programs of cell death is the oxide-dependent apoptosis. The cell "sacrifices itself" to preserve the protective resources of other intact (or little affected) cells and the organism as a whole [76].

Another important versatile mechanism of the systemic response to irradiation is the post-radiation repair of the genetic apparatus of the cell. This problem has been intensively explored since the second half of the last century. In the early 1950s, N.V. Luchnik studied thoroughly the incidence of structural mutations in cells dividing at different times after irradiation and showed that the greater is the interval between the treatment and the response, the less pronounced is the biological effect. The author explained this regularity by the ability of some cells to recover from the primary damage. Evidence was obtained that there is a correlation between the radioresistance of chromosomes and the slope of the dose - effect curves [89].

In 1957 V.I. Korogodin confirmed the phenomenon of the post-radiation recovery in experiments on yeast cells [90]. He showed that the ability of cells to form macrocolonies is partially restored if irradiated yeast cells are seeded onto nutrient medium not immediately after irradiation but after keeping them for some time in water. A principally new finding was the hitherto unknown ability of cells to spontaneously recover from radiation-induced and other DNA-dependent lesions that cause lethal and genetic effects. The idea of recovery by itself was inconsistent with the then popular conception of irreversibility of radiation damage to the target. After the discovery of the post-irradiation repair phenomenon, V.I. Korogodin put forward a conception of *potential lesions* in the irradiated cell, which actually was the advancement of the principles of energodynamics in terms of the time - effect relationship. According to this conception, the absorption of ionizing radiation energy may give rise to potential lesions of cell structures [91]. When developing with time, these lesions "can lead to the manifestation of the response only after having gone through the realization phase" [91, p. 387]. Within the time interval between a potential lesion and its realization, recovery processes can take place, which obey statistical rules. Later it was found that the cell is able to repair DNA breaks induced by ionizing radiation [92]. It was shown that there is a relationship between the extent of DNA repair and cell radiosensitivity [93 - 95]. It became obvious that the repair of radiation damage proceeds by a variety of ways at all levels of the organization of living matter: molecular, cellular, and whole-organism levels [96].

Concurrently, studies of the physical and molecular biological mechanisms of post-radiation repair were performed in some laboratories.

Evidence was obtained that cells of mutant bacteria, protozoa, plants, and mammals repair lethal and sublethal alterations and mutations [96 - 101]. In multicellular organisms, cell repair in different organs and tissues has its specificity. Thus, the rate of cell repair is the highest in actively proliferating tissues, due to stem cells that still retain their viability [101]. It was shown that there is a quantitative relationship between the relative biological efficiency and linear energy loss for various types of ionizing radiation and between relative biological efficiency and changes in the genetic apparatus (lethal effect for chromosomes and mutations of individual genes) [102]. Studies on the mechanisms of post-radiation recovery led to important conclusions about the key role of oxygen in these processes [16, 17, 96, 103, 104] and the modifying effect of some radioprotectors and radiosensitizers [104].

Thus, it became apparent that it is necessary to invoke new approaches to studying the biophysical mechanisms of radiation effects. In particular, it is necessary to take into account *the systemic response of the cell to irradiation* and to study, along with the amplification of destructive processes, the control and repair systems built into the cell.

The advances of molecular biology in studies of enzyme systems involved in DNA damage repair, again, played a crucial role in understanding the post-radiation repair processes. A variety of repair enzymes were studied of which some enzymes enhance primary DNA radiation lesions (e. g., exo- and endonucleases, excision enzymes) and others eliminate them (polymerases, ligases, phosphatases). In addition, some hormones and growth factors affecting chromosomes and their fragments were studied [105, 106]. The assessment of the relationship between the activities of the enzymes of the first and second groups provided important information on the mechanisms of amplification of damage and the repair of the DNA target. The good coordination of diverse DNA repair processes that proceed at different rates and are observed in cells irradiated even with very high, absolutely lethal doses, and of systems controlling the amplification of post-radiation degradation of polynucleotide chains made possible a detailed study of the mechanisms of reproductive and interphase cell death, apoptosis, necrotic cell death, metabolic (radiation-induced) genome instability, i. e., the main participants of the systemic response of the cell to ionizing radiation [105 - 119].

Turning back to radiobiological models, one can see that the conceptions of the mechanisms of radiation-induced damage underwent evolution. The inclusion of the temporal factor significantly transformed the conception of the mechanism of action of ionizing radiation on the cell. Whereas at the very

beginning of radiation biology as a science preference was given to static models, which considered cell death as a result of a mere destruction of the subcellular target, the modern radiation biology uses a dynamic, systemic approach, which in the whole is consistent with the concepts of "dynamic" biochemistry and biophysics.

Based on the quantitative estimation of cell repair effects, stochastic models were suggested [91, 113, 120, 121], which substantially revised the concepts of energodynamics of ionizing radiations. They are based on the ideas that stochastic and diffusely located ionization and excitation acts inevitably lead to cell death only in rare and hardly probable cases. This "first-order stochasticity" should be superimposed with the stochasticity of higher orders, which is determined by the dynamic instability of living processes that either enhance or eliminate the primary radiation damage. A mathematical apparatus was developed, which made it possible to formally assess the probability of the transition of damage from one level to another. This transition is related to *the principles of amplification and repair of radiation lesions*. Thus, Huge and Keller obtained a family of dose - effect curves that were in good agreement with curves registered in experiments on cells. This was a strong argument in favor of the applicability of dynamic models for the explanation of the phenomena of radiation biophysics. The stochastic theory introduced, e. g., the definition of *disperse initial damage* to not only the genetic apparatus but also the elements of other cell structures. The mechanisms of repair that determine the final radiobiological process are formed owing to the nonspecific *compensatory capacity of the object*. Therefore, the stochastic theory is more "biological" in its nature, and its mathematical apparatus is rather intricate [115]. It seems likely that further development of the probability models is associated with the elaboration of a mathematical apparatus based on Markov processes [122].

The importance of the stochastic conceptions for radiation biology lies in the fact that they give a deep insight into the role of the systemic cell response to the lesions of cell structures. L.Kh. Eidus, who has been engaged in this problem for many years, drew a very important conclusion about the role of the systemic cell response to external influences: "The phenomenon of DNA repair enables one to understand the role of nonspecific cell response to external influences. Presumably, the main *goal* of this adaptive response, which has developed during evolution, is to maintain the integrity of its main property, the genome. The nature devised a very simple way to *accomplish* this goal: the effect on the

plasma membrane, the most vulnerable cell structure, which can be avoided by none of the chemical and physical damaging agents.” [116, p. 676].

In addition to what was said, we give below a brief interpretation of the principle of systemic cell response.

IV. PRINCIPLE OF SYSTEMIC CELL RESPONSE TO THE LESION OF RADIATION TARGETS

(Regulation of cell homeostasis)

- Irradiation of cell, as well as any complex biological systems, activates the functions of homeostasis autoregulation. Their role is to mobilize the compensatory mechanisms that serve to prevent lesions or activate the repair of damaged structures and disturbed dynamic equilibrium of the irradiated system.
These compensatory processes are of particular importance for the maintenance of the structure and functions of critical target structures.
- The control over the accumulation of excessive amounts of toxic products: OH, H₂O₂, as well as ROS, NO, ONO₂⁻, and LPP in BM is accomplished by a complex many-stage system of protective resources of the cell (water- and fat-soluble products). This system involves enzymatic and nonenzymatic “antiradical”, “hypoxic”, and “antioxidant” mechanisms whose activities depend on the dose and the time elapsed after irradiation. Variations in the relationship between the contents of anti- and prooxidants in the cell characterize the level of endogenous background resistance and the extent of cell damage (radiation toxic effect, oxidative stress, etc.).
- In addition, the cell contains numerous DNA repair systems, which depend on the type of damage. Some of these systems are activated by DNA damage itself, and hence their activity depends on the value of the absorbed dose. The repair of DNA damage with the participation of repair enzymes is preceded by the transformation of chromatin or the displacement of chromosomal loci in the cell nucleus.

It is known that the interest of radiobiologists in recent years has focused on the biological effect of low doses and small dose rates of ionizing radiations [116 - 121, 123 - 127]. Assumptions were put forward that, at some minor

disturbances, the systemic cell responses not only decrease but can either be absent at all or manifest themselves in a different way (e. g., the phenomenon of hormesis). Based on the data on different rates of damaging and repair processes, conclusions were drawn that the biological effect depends nonmonotonously on the dose of ionizing radiation [123]. Below we present the main views of E.B. Burlakova on this problem.

The biological effect of low-dose and low-intensity radiations manifests itself in narrow ranges of doses and dose rates not only quantitatively but also qualitatively.

- Systems of inducible repair and the normalization of functions that change after irradiation of cells with low doses and at small dose rates are not “switched on” or operate with low effectiveness. The lower is the intensity of irradiation, the later the systems of inducible repair are actuated.
- Irradiation with low doses and at low dose rates can increase the sensitivity to various environmental factors, which forms the basis for the synergistic effect.
- Irradiation of biological objects with low doses and at low dose rates can induce a delayed instability of the genome.

It is evident that these ideas significantly contribute to the understanding of the problem of the systemic response of living objects to ionizing radiations of different intensity.

In recent years, special emphasis in studies of the systemic post-radiation effects was placed on the molecular mechanisms and pathogenic significance of radiation-induced genome instability. It was found that the state of genome instability can be transmitted to the survived progeny of irradiated cells by epigenetic mechanisms, and this state exists until the cells return to the initial level of the systemic response to DNA damage. This question was considered in detail in recently published reviews [117 - 119, 126, 127].

Altogether, the advances in quantitative radiation biology, radiation biophysics, and molecular biology and the analysis of the order of physicochemical processes in the irradiated cell may serve as the basis for a general theory, which would unravel the mechanisms of action of ionizing radiation on the living cell. The theory has not been completely developed. However, it is clear that this theory should take into account the intricate probabilistic nature of the manifestations of radiobiological effects, the possibility of modification of radiation damage, and the main stages of the

development of few primary molecular lesions into visible final biological effects.

The logic of the present-day research requires a step-by-step investigation of the mechanisms of action of ionizing radiation. For this, it is necessary to use all available data obtained on systems of different complexity. By comparing these data with processes occurring in the irradiated cell, it will be possible to reconstruct a real successive picture of radiation damage.

REFERENCES

1. Li D.E., *Effect of Radiation on Living Cells*, Moscow, Gosatomizdat, 1963, 288 p. (Rus)
2. Timofeev-Resovskii N.V., *Primary Mechanisms of the Biological Effect of Ionizing Radiations*, Moscow, AN SSSR, 1963, pp. 162 - 164. (Rus)
3. Timofeev-Resovskii N.V., Ivanov V.I., and Korogodin V.I., *The Application of the Hit Principle in Radiation Biology*, Moscow, Atomizdat, 1973, 228 p. (Rus)
4. Dertinger G. and Jung Ch., *Molecular Radiation Biology*, Moscow, Atomizdat, 1973, 248 p. (Rus)
5. Tarusov B.N., *The Role of Peroxides and Oxygen in the Initial Stages of the Radiobiological Effect*, Moscow, Gosatomizdat, 1960, pp. 60 - 65. (Rus)
6. Tarusov B.N., *Primary Processes of Radiation Damage*, Moscow, Gosatomizdat, 1962, 96 p. (Rus)
7. Bak Z. and Alexander P., *Fundamentals of Radiation Biology*, Moscow, Inostr. Lit., 1963, 500 p. (Rus)
8. Alper T., 'Cell death and its modification: the role of primary lesion in membranes and DNA', *Biophysical aspects of radiation qualities*, Vienna, JAEA, 1971, pp. 171 - 194.
9. Okada Sh., *Radiation biochemistry of the cell*, Moscow, Mir, 1974, 408 p. (Rus)
10. Savich A.V., 'Radiation - chemical transformations and the radiosensitivity of macromolecules', *Radiation Damage*, Ed. Yu.B. Kudryashov, Moscow, Izd. MGU, 1987, pp. 73 - 83. (Rus)

11. Kudryashov Yu.B. 'On the nature and mode of accumulation of the hemolytic factor arising in the liver of rats irradiated with X-rays', *Dokl. AN SSSR*, 1956, vol. **109**(3), pp. 515 - 521.
12. Kudryashov Yu.B. 'The role of lipid radiotoxins in the radiation-induced toxic effect', *Radiotoxins, their nature and role in the biological effect of high-energy radiation*, Moscow, Atomizdat, 1966, pp. 105 - 117. (Rus)
13. Labzina N.G., Kudryashov Yu.B., and Luchnik N.V., 'Cytogenetic action of lipid radiotoxins', In: *Radiotoxins, their nature and role in the biological effect of high-energy radiation*, Moscow, Atomizdat, 1966, pp. 176 - 181. (Rus)
14. Vilyuman Yu.N., 'Effect of radiation and lipid radiotoxins on the microstructure of nucleic acids', *Thesis of Cand. Sci. (Biology) Dissertation*, Moscow, 1969.
15. Alesenko A.V., 'The role of lipids and peroxidation products in the biosynthesis and functional activity of DNA', *Biochemistry of lipids and their role in the metabolism*, Moscow, Nauka, 1981, pp. 3 - 16. (Rus)
16. Marnett L.J., 'Oxyradicals and DNA damage', *Carcinogenesis*, Oxford, 2000, vol. **XXI**(3), pp. 361 - 370.
17. Ward J.F., Limoli C.L., Calabro - Jones P. et al., 'Radiation versus chemical damage to DNA', Eds. Cerutti P.A., Nygaard O.F., Simic M.G., *Anticarcinogenesis and Radiation Protection*. N. Y.: Plenum, 1987, pp. 321 - 327.
18. Lindahl T., 'Instability and decay of the primary structure of DNA', *Nature*, 1993, vol. **362**, pp. 709 - 715.
19. Ames B.N., 'Endogenous DNA damage as related to cancer and aging', *Mutat. Res.*, 1989, vol. **214**, pp. 41 - 46.
20. Dizdaroglu M. and Gajewski E., 'Selected -ion mass spectrometry: assay of oxidative DNA damage', *Methods Enzymol.*, 1990, vol. **186**, pp. 530 - 544.
21. Johnson T.M., Yu Z.X., Ferrans V.J. et al., 'Reactive oxygen species are downstream mediators of p53-dependent apoptosis', *Proc. Natl. Acad. Sci.*, 1996, vol. **93**, pp. 11848 - 11852.
22. Floyd R.A., 'Measurement of oxidative stress in vivo', *Proceedings of the First Oxygen Society Meeting*, Ed. Davies K.A.J., N.Y., Pergamon, 1994, P. 79 - 84.
23. Floyd R.A., Watson J.J., Wong P.K. et al., 'Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanism of formation', *Free Radical Res. Commun.*, 1986, vol. **1**, pp. 163 - 172.

24. Emanuel N.M., 'The role of free radicals in radiobiological processes and some new potentialities in the development of drugs against radiation damage', *Primary mechanisms of biological effect of ionizing radiations*, Moscow, AN SSSR, 1963, pp. 73 - 84. (Rus)
25. Burlakova E.B. and Khrapova N.G., 'Peroxidation of lipids and the nature of antioxidants', *Uspekhi Khimii*, 1985, vol. **54**(9), pp. 1540 - 1558.
26. Porter N.A., 'Mechanisms for the autoxidation of polyunsaturated lipids', *Acc. Chem. Res.*, 1986, vol. **19**, pp. 262 - 268.
27. Purmal A.P., *Chain reactions, vol. 1, Physical Chemistry*, Moscow, Advanced natural science; IS-SEP; Nauka; Flinta, 2000, pp. 116 - 122. (Rus)
28. Kudryashov Yu.B., 'Radiation damage to critical systems', *Radiation Damage*, Ed. Kudryashov Yu.B. Moscow, Izd. MGU, 1987. pp. 5 - 72. (Rus)
29. Vladimirov Yu.A., Azizova O.A., Deev A.I. et al., 'Free radicals in living systems', *Itogi Nauki i Tekhniki. Biofizika*, 1992, vol. **29**, pp. 3 - 250.
30. Vladimirov Yu.A., 'Free radicals in biological systems', *Soros Obrazovatel'nyi Zhurnal*, ISSEP, 2000, vol. **6**(12), pp. 13 - 19.
31. Kudryashov Yu.B., Gasanov G.I., Goncharenko E.N. et al., 'A natural radiomimetic and its effect on various biological objects and systems', *Zhurn. Obshchey Biologii*, 1964, vol. **XXV**(1), pp. 3 - 21.
32. Goncharenko E.N., Baltbarzdys Z.Ya., Graevskaya E.E. et al., 'On the chemical nature of the lipid radiotoxin', *Radiobiologiya*, 1968, vol. **8**(4), pp. 497 - 505. (Rus)
33. Graevskaya E.E., 'A study of the role of toxic substances of lipid nature in radiation damage to cel', *Thesis Cand. Sci. (Biology) Dissertation*, Moscow, 1970.
34. Kudryashov Yu.B., Baltbarzdys Z.Ya., and Le Dak Leu, 'On the possible indirect effect of ionizing radiation in lipid solutions', *Dokl. AN SSSR*, 1964. vol. **156**(1), pp. 191 - 193. (Rus)
35. Schauenstein E. and Ersterbauer H., 'Formation and properties of reactive aldehydes', *Submol. Biol. Cancer Ciba Fnd.*, 1978, vol. **67**, pp. 225 - 241.
36. Yau T.M., 'Mutagenicity and cytotoxicity of malondialdehyde in mammalian cells', *Mech. Ageing Dev.*, 1979, vol. **11**, pp. 137 - 144.
37. Basu A.K. and Marnett L.J., 'Unequivocal demonstration that malondialdehyde is a mutagen', *Carcinogenesis*, 1983, vol. **4**, pp. 331 - 333.

38. Spalding J.W., 'Toxicology and carcinogenesis studies of malondialdehyde sodium salt (3-hydroxy-2-propenal, sodium salt) in F344/N rats and B6C3F1 mice. NTP', *Tech. Rep.*, 1988, vol. **331**, pp. 5 - 13.
39. Ersterbauer H., Schaur R.J., and Zollner H., 'Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes', *Free Radic. Biol. Med.*, 1991, vol. **11**, pp. 81 - 128.
40. Chaudhary A.K., Nokubo M., Reddy G.R. *et al.*, 'Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver', *Science*, 1994, vol. **265**, pp. 1580 - 1582.
41. Marnett L.J., 'Lipid peroxidation - DNA damage by malondialdehyde', *Mutat. Res.*, 1999, vol. **424**, pp. 83 - 95.
42. Nath R.G. and Chung F.-L., 'Detection of 1,N²-propanodeoxyguanosine adducts in rodent and human liver DNA by ³²P-post-labeling', *Proc. Am. Assoc. Cancer Res.*, 1993, vol. **34**, p. 137.
43. Nath R.G. and Chung F.-L., 'Detection of exocyclic 1,N²-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans', *Proc. Natl. Acad. Sci. USA*, 1994, vol. **91**, pp. 7491 - 7495.
44. Sodium R.S. and Chung F.-L., '1,N²-Ethenodeoxyguanosine as a potential marker for DNA adduct formation by trans-4-hydroxy-2-nonenal', *Cancer Res.*, 1988, vol. **48**, pp. 320 - 323.
45. Cheng K.C., Preston B.D., Cahill D.S. *et al.*, 'The vinyl chloride DNA derivative N²,3-ethenoguanine produces GA transitions in Escherichia coli', *Proc. Natl. Acad. Sci. USA*, 1991, vol. **88**, pp. 9974 - 9978.
46. Basu A.K., Wood M.L., Niedernhofer L.J. *et al.*, 'Mutagenic and genotoxic effects of three vinyl chloride-induced DNA lesions: 1,N⁶-ethenoadenine, 3,N⁴-ethenocytosine and 4-amino-5-(imidazol-2-yl)imidazole', *Biochemistry*, 1993, vol. **32**, pp. 12793 - 12801.
47. Moriya M., Zhang W., Johnson F *et al.*, 'Mutagenic potency of exocyclic DNA adducts: marked differences between Escherichia coli and simian kidney cells', *Proc. Natl. Acad. Sci. USA*, 1994, vol. **91**, pp. 11899 - 11903.
48. Pandya G.A. and Moriya M., '1N⁶-ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells', *Biochemistry*, 1996, vol. **35**, pp. 11487 - 11492.
49. Chung F.L., Chen H.J.C., and Nath R.G., 'Lipid peroxidation as a potential endogenous source for the formation of exocyclic DNA adducts', *Carcinogenesis*, 1996, vol. **17**, pp. 2105 - 2111.

50. Langouet S., Chen A.N., Muller M. *et al.*, 'Misincorporation of nucleotides opposite five-membered exocyclic ring guanine derivatives by Escherichia coli polymerases in vitro and in vivo: 1,N²-ethenoguanine, 5,6,7,9-tetrahydro-9-oxoimidazo(1,2-a)purine and 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo(1,2-a)purine', *Biochemistry*, 1998, vol. 37, pp. 5184 - 5193.
51. Saparbaev M., Kleibl K., and Laval J., 'Escherichia coli, *Saccharomyces cerevisiae*, rat and human 3-methyladenine DNA glycosylases repair 1,N⁶-ethenoadenine when present in DNA', *Nucleic Acids Res.*, 1995, vol. 23, pp. 3750 - 3755.
52. Fink S.P., Reddy G.R., and Marnett L.J., 'Mutagenicity in Escherichia coli of the major DNA adduct derived from the endogenous mutagen, malondialdehyde', *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, pp. 8652 - 8657.
53. Johnson K.A., Mierzwa M.L., Fink S.P., and Marnett L.J., 'MutS recognition of exocyclic DNA adducts that are endogenous products of lipid oxidation', *J. Biol. Chem.*, 1999, vol. 274, pp. 27112 - 27118.
54. Ebert M., 'Formation of hydrogen peroxide under different irradiation conditions', *Voprosy Radiobiologii*, Moscow, Inostr. Lit., 1956, pp. 56 - 68.
55. Deil V., 'Effect of ionizing radiations on enzymes in vitro', *Ionizing Radiations and Cell Metabolism*, Moscow, Inostr. Lit., 1958, pp. 43 - 57. (Rus)
56. Polivoda B.I., Kornev V.V., and Popov G.A., *Biophysical Aspects of Radiation Damage to Biomembranes*, Moscow, Energoatomizdat, 1990. 160 p.
57. Struchkov V.A. and Strazhevskaya N.B., 'DNA-linked lipids: composition and possible functions', *Biokhimiya*, 1993, vol. 58, pp. 1154 - 1176. (Rus)
58. Kudryashova N.Yu., 'Effect of ionizing radiation on DNA - membrane complexes', *Radiation Damage*, Moscow, Izd. MGU, 1987, pp. 93 - 97. (Rus)
59. Vanin A.F., 'Nitrogen oxide in biology: history, status and perspectives of research', *Biokhimiya*, 1998, vol. 63(7), pp. 867 - 869. (Rus)
60. Nedospasov A.A., 'Biogenic NO in competitive relations', *Biokhimiya*, 1998, vol. 63(7), pp. 881 - 901. (Rus)
61. Severina I.S., 'Soluble guanylate cyclase in the molecular mechanism of the physiological effects of nitrogen oxide', *Biokhimiya*, 1998, vol. 63(7), pp. 939 - 951. (Rus)

62. Men'shchikova N.B., Zenkov N.K., and Reutov V.P., 'Nitrogen oxide and NO-synthases in the organisms of mammals in different functional states', *Biokhimiya*, 2000, vol. **65**(4), pp. 485 - 503. (Rus)
63. Voevodskaya N.V. and Vanin A.F., 'Gamma irradiation potentiation of L-arginine-dependent NO formation in mice', *Bioch. and Biophys. Commun.*, 1992, vol. **186**(3), pp. 1423 - 1428.
64. Mikoyan V.D., Voevodskaya N.B., Kubrina L.N. *et al.*, 'Exogenous ferrum and γ -irradiation induce the synthesis of NO-synthase in mouse liver', *Biokhimiya*, 1994, vol. **59**(5), pp. 732 - 738. (Rus)
65. Brune B., Sunday K., and A. von Kneton, 'Apoptic cell death and nitrogen oxide: mechanisms of activation and antagonistic signaling pathways', *Biokhimiya*, 1998, vol. **63**(7), pp. 966 - 974.
66. Maeda Kh. and Akaiki T., Nitrogen oxide and oxygen radicals in infection, inflammation and cancer, *Biokhimiya*, 1998, vol. **63**(7), pp. 1007 - 1018.
67. Malyshev I.Yu. and Manukhina E.B., 'Stress, adaptation, and nitrogen oxide', *Biokhimiya*, 1998, vol. **63**(7), pp. 992 - 1006. (Rus)
68. Koppenol W.H., Moreno J.J., and Pryor W.A., 'Peroxynitrite: a cloaked oxidant from superoxide and nitric oxide', *Chem. Res. Toxicol.*, 1992, vol. **5**, pp. 834 - 842.
69. Kissner R., Nauser T., Bugnon P. *et al.*, 'Formation of properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique and pulse radiolysis', *Chem. Res. Toxicol.*, 1997, vol. **10**, pp. 1285 - 1292.
70. Richeson C.E., Mulder P., Bowry V.W. *et al.*, 'The complex chemistry of peroxynitrite decomposition: new insights', *J. Am. Chem. Soc.*, 1998, vol. **120**, pp. 7211 - 7219.
71. Beckman J.S., Beckman T.W., Chen J. *et al.*, 'Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide', *Proc. Natl. Acad. Sci. USA*, 1990, vol. **87**, pp. 1620 - 1624.
72. Radi R., 'Peroxynitrite reactions and diffusion in biology', *Chem. Res. Toxicol.*, 1998, vol. **11**, pp. 720 - 721.
73. Polivoda B.I. and Konev V.V., 'Correlation of membrane and genetic effects of lipid peroxidation', *Radiobiologiya*, 1986, vol. **26**(6), pp. 803 - 805.
74. Vladimirov Yu.A., 'Biological membranes and unprogrammed cell death', *Sorosovskii Obrazovatel'nyi Zhurnal. ISSEP*, 2000, vol. **6**(9(58)), pp. 2 - 9. (Rus)

75. Skulachev V.P., *Energetics of Biological Membranes*, Moscow, Nauka, 1989, p. 564. (Rus)
76. Skulachev V.P., 'Oxygen in a living cell: good and evil', *Sorosovskii Obrazovatel'nyi Zhurnal. ISSEP*, 1996, vol. 2(3 (4)), pp. 4 - 10. (Rus)
77. Kulinskii V.I., 'Reactive oxygen species and oxidative modification', *Sorosovskii Obrazovatel'nyi Zhurnal. ISSEP*, 1999, vol. 5(1 (38)), pp. 2 - 7. (Rus)
78. Merzlyak M.N., 'Activated oxygen and oxidative processes in plant cell membranes', *Itogi Nauki*, 1989, vol. 113(1), pp. 107 - 122. (Rus)
79. Kudryashov Yu.B. 'Role of lipid radiotoxins in the radiation-induced toxic effect', *Radiotoxins, their nature and role in the biological effect of high-energy radiation*, Moscow, Atomizdat, 1966, pp. 105 - 118. (Rus)
80. Kudryashov Yu.B. and Gorcharenko E.N., 'Stress in response to ionizing radiation', *Nuclear Encyclopedia*, Moscow, Izd. Blagotvoritel'nogo fonda Yaroshinskoi, 1996, pp. 327 - 330. (Rus)
81. Dubur G.Ya. and Velena A.Kh., *Biomembranes. Structure. Functions. Medicinal Aspects*, Riga, 1981, pp. 257 - 277. (Rus)
82. Goncharenko E.N. and Kudryashov Yu.B., *The hypothesis of the endogenous background radioresistance*, Moscow, Izd. MGU, 1980, 170 p. (Rus)
83. Kudryashov Yu.B., 'Chemical protection against radiation damage', *Sorosovskii Obrazovatel'nyi Zhurnal. ISSEP*, 2000, vol. 6(6 (55)), pp. 21 - 26.
84. Goncharenko E.N. and Kudryashov Yu.B., *Chemical protection against radiation damage*, Izd. MGU, 1985, 249 p. (Rus)
85. Vartanyan L.S., Gurevich S.N., Kozachenko A.I. et al., 'Changes in the rate of formation of superoxide radicals and the activity of superoxide dismutase and glutathion peroxidase in subcellular organelles of mouse liver exposed to low-intensity low-dose radiation', *Biokhimiya*, 2000, vol. 65(4), pp. 522 - 527. (Rus)
86. Gilyano N.Ya., Bondarev G.N., Bikeneeva E.G. et al., 'A study of the radioprotective effect of the inhibitor of NO synthase L-name in a Chinese hamster cell culture', *Trudy 4-go s'ezda po radiatsionnym issledovaniyam (Proc. 4th Meeting on Radiation Research)*, Moscow, 20-24 November 2001. (Rus)
87. Ryabchenko N.I., Konoplyannikov A.G., Ivannik B.P. et al., 'Application of modifiers of nitric oxide production for the protection of the organism against radiation damage and stress influences', *Trudy 4-go s'ezda po*

- radiatsionnym issledovaniyam (Proc. 4th Meeting on Radiation Research)*, Moscow, 20-24 November 2001. (Rus)
- 88. Boldyrev A.A., *Carnosine*. Moscow, Izd. MGU, 1998, p. 320. (Rus)
 - 89. Luchnik N.V., ‘Molecular bases of mutational changes’, *Doctor’s Dissertation Thesis (Biology)*, Moscow, 1966, 300 p. (Rus)
 - 90. Korogodin V.I., *Biofizika*, 1957, vol. 2(5), pp. 178 - 186. (Rus)
 - 91. Korogodin V.I., *Problems of post-radiation recovery*, Moscow, Atomizdat, 1966, 392 p. (Rus)
 - 92. McGrath R.A. and Williams R.W., *Nature*, 1966, vol. 212(5051), pp. 537 - 544.
 - 93. Zhestyannikov V.D., *Recovery and cell radioresistance*, Leningrad, Nauka, 1968, 222 p. (Rus)
 - 94. Zhestyannikov V.D., Zakharov I.A., and Kozhina T.N., ‘Recovery and radioresistance’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 150 - 153. (Rus)
 - 95. Zhestyannikov V.D., *DNA repair and its biological significance*, Leningrad, Nauka, 1979, 285 p. (Rus)
 - 96. Eidus L.Kh., ‘Physical repair of biological macromolecules’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 12 - 24. (Rus)
 - 97. Valdstein V.A., ‘Mechanisms of DNA repair’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 25 - 71. (Rus)
 - 98. Paribok V.P., ‘Repair in the case of mutation injuries’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 72 - 104. (Rus)
 - 99. Luchnik N.V., ‘Recovery in the case of radiation-induced damage to chromosomes’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 154 - 189. (Rus)
 - 100. Malinovskii O.V., ‘Quantitative regularities of post-radiation repair of cells’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 191 - 227. (Rus)
 - 101. Strelin G.S. and Yarmonenko S.P., ‘Recovery processes in the irradiated organism’, *Current problems of radiation biology. Post-radiation repair*, Moscow, Atomizdat, 1970, pp. 105 - 153. (Rus)
 - 102. Kozubek S., Krasavin E.A., Soska I. et al., ‘Induction of SOS response in *Escherichia coli* by heavy ions’, *Mut. Res.*, 1989, vol. 215, pp. 49 - 53.

103. Eidus L.Kh. and Korystov Yu.N., *Oxygen in radiation biology*, Moscow, Energoatomizdat, 1984. 176 p. (Moscow)
104. Petin V.G. and Komarov V.P., *A quantitative description of radiosensitivity modification*, Moscow, Energoatomizdat, 1989, 192 p. (Rus)
105. Gaziev A.I., *Mechanisms of radiation-induced damage and DNA repair*, Pushchino, 1972. (Rus)
106. Gaziev A.I., 'Enzymes in the post-radiation metabolism of DNA', *Current problems of radiation biology. Radiation biochemistry*, Moscow, Atomizdat, 1975, pp. 41 - 82. (Rus)
107. Mazurik V.K., 'Some problems of radiation biochemistry of DNA', *Current problems of radiation biology. Radiation biochemistry*, Moscow, Atomizdat, 1975, pp. 7 - 40. (Rus)
108. Komar V.E. and Khanson K.P., *Information molecules in the case of radiation damage to cells*, Moscow, Atomizdat, 1980, 175 p. (Rus)
109. Umanskii S.R., *Advances in present-day biology*, 1982, vol. 93(1), pp. 139 - 148.
110. Sakai Kosudo and Okado Shigofumi, *Radiation Res.*, 1984, vol. 98(3), pp. 479 - 490.
111. Pelevina I.I., Saenko A.S., Gotlieb V.Ya. et al., *Survival of irradiated mammalian cells and DNA repair*, Moscow, Energoatomizdat, 1985, 120 p. (Rus)
112. Shevchenko V.A. and Pomerantseva M.D., *Genetic consequences of the effect of ionizing radiations*, Moscow, Nauka, 1985, 279 p. (Rus)
113. Korogodin V.I., *Cascade mutagenesis. Soobshch. OIYaI, Reprint*. 19-92-81. Dubna, 1992. 15 p. (Rus)
114. Khanson K.P., 'Apoptosis: the current status of the problem', *Izv. RAN, Ser. Biol.*, 1998, No. 2, pp. 134 - 141. (Rus)
115. Yarmonenko S.P., *Radiation biology of human and animals: Textbook*, Moscow, Vysshaya shkola, 1988, 424 p. (Rus)
116. Eidus L.Kh., 'On the mechanism of induction of DNA damage repair in cells exposed to ionizing radiation', *Radiats. Biologiya. Radioekologiya*, 2000, vol. 40(6), pp. 674 - 677. (Rus)
117. Mazurik V.K. and Mikhailov V.F., 'On some molecular mechanisms of the main radiobiological consequences of the effect of ionizing radiations on mammalian organisms', *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(1), pp. 91 - 98. (Rus)

118. Baverstock K. 'Radiation-induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer', *Mutat. Res.*, 2000, vol. **454**(1-2), pp. 89 - 109.
119. Little J.B., 'Radiation-induced genomic instability', *Int. J. Radiat.*, 1998, vol. **74**(6), pp. 89 - 109.
120. Hug O. and Keller A., *Stochastic radiation biology*, Moscow, Atomizdat, 1969, 183 p. (Rus)
121. Kapultsevich Yu.G., *Quantitative regularities of radiation-induced damage to cells and radiosensitivity*, Moscow, 1978, 11 p. (Rus)
122. Zelentsov B.P., 'Mathematical models based on the process of reproduction and death of objects', *Sorosovskii Obrazovatel'nyi Zhurn. ISSEP*. 2001, vol. 7(6 (67)), pp. 92 - 97.
123. Burlakova E.B., 'New aspects of regularities in the action of low doses of low-level irradiation', In: *Low doses of radiation: are they dangerous?*, Ed. E.B. Burlakova, N.Y.: Nova Science Publishers Hungtington, 2000. Ch. 1, pp. 1 - 14.
124. Pelevina I.I., Afanas'ev G.F. et al., 'Radiation-induced adaptive response in children and the effect of external and internal factors on this phenomenon', In: *Low doses of radiation: are they dangerous?*, Ed. E.B. Burlakova, N. Y.: Nova Science Publishers, 2000. Ch. 1. pp. 141 - 153.
125. Shmakova N.L., Abu Zeid O., Fadeeva T.A. et al., 'The dose dependence of cytogenetic lesions and the adaptive response of mammalian cells to low-dose ionizing radiations', *Radiats. Biologiya. Radioekologiya*, 2000, vol. **40**(4), pp. 405 - 409. (Rus)
126. Bezlepkin V.G., Vasil'eva G.V., Lomaeva M.G. et al., 'A study of genome instability by the method of DNA fingerprints of the progeny of male mice exposed to low doses of chronic γ -radiation', *Radiats. Biologiya. Radioekologiya*, 2000, vol. **40**(5), pp. 506 - 512. (Rus)
127. Mazurik V.K. and Mikhailov V.F., 'Radiation-induced genome instability: phenomenon, molecular mechanisms, pathogenetic significance', *Radiats. Biologiya. Radioekologiya*, 2001, vol. **41**(3), pp. 272 - 289. (Rus)

Modern problems of antiradiation chemical defense of organisms

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ABSTRACT

Preparations for antiradiation chemical defense were classified and characterized. The preparations were divided into three groups: radioprotectors, adaptogens, and sorbents. Radioprotectors were in turn subdivided into myelo-, entero-, and cerebroprotectors. Adaptogens act as stimulators of radioresistance and are promising as antiradiation chemical protecting agents upon low-intensity irradiation. Natural antiradiation preparations influence the regulatory systems of the irradiated organism, mobilize the endogenous background radioresistance (EBR), the immunity, and the total nonspecific response of the organism (TNRO). Natural (extracted from cells, plants, and animal tissues) antiradiation preparations are low- or nontoxic and can be used as food substances. Sorbents, the means of protection against internal irradiation, include preparations that prevent the incorporation of ^{131}I by the thyroid gland and the preparations that prevent the absorption of radionuclides (^{137}Cs , ^{90}Sr , ^{239}Pu , ^{241}Am) from the intestine. The main features distinguishing the groups of antiradiation substances of different mechanisms of action are given.

Keywords: antiradiation chemical defense, radioprotectors, adaptogens, immunomodulators, sorbents, endogenous background radioresistance (EBR), total nonspecific response of the organism (TNRO), lipid peroxidation, antioxidative activity, mobilization of protective resources of the organism

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The effect of antiradiation chemical defense was first detected in studies of the indirect action of ionizing radiation and the role of free radicals and peroxides in aqueous solutions in primary radiation-induced physicochemical processes. There emerged the concepts of *protecting agents* and *protection* against radiation [1 - 3]. When studying the mechanisms of indirect effect of radiation on enzyme solutions, W. Dale showed that thiourea and colloidal sulfur reduce the extent of radiolysis of enzymes. He suggested that the protective effect results from the competition for free radicals of water arising during its irradiation. Subsequently, the radioprotective action of cysteine, glutathione, tryptophan, and other substances in bacteriophages was revealed [4]. Shortly thereafter, it was shown that laboratory animals can also be protected against low-lethal-dose irradiation by injection of the solutions of cysteine [5], culfocyanide, and tocopherol [6].

I. RADIOPROTECTORS

In early 1950s Zenon Bacq and coworkers reported that the preparations of two classes of chemical compounds: aminoalkyl thiols and indolylalkylamines (cysteamine, serotonin, tryptamine, noradrenaline, and others) produce the antiradiation protecting effect on animals [7--9]. The preparations studied were effective only when injected prior to irradiation; therefore, they were called *radioprotectors*, and antiradiation defense was named *radioprophylactic* protection.

To date many thousands of radioprotectors capable of reducing the degree of acute radiation damage upon prophylactic application are known.

Until recently advances and failures in the search for radioprotecting agents were associated with the synthesis of radioprotectors that for the most part belong to sulfur-containing compounds and indolylalkylamines. Their action was mainly explained by the direct involvement in various radiation-induced (e. g., scavenging of radicals, hypoxic and antioxidant effects) and repair processes and the formation of endogenous background radioresistance (EBR) [10 - 11]. It is primarily the investigations of the mechanisms of radioprophylaxis that enabled the radiobiologists of different schools to gain insight into the biophysical and biochemical regularities of primary radiation damage processes [12 - 14].

To produce the protecting effect, radioprotectors are injected to animals in high nonphysiological toxic doses and are therefore often unsuitable for humans. Thus, a concept of a limit or threshold in the antiradiation activity of radioprotectors appeared. The factor of dose decrease (FDD) for highly effective preparations is usually no higher than 1.5 - 1.8. A promising approach to overcoming this limit is the development of formulas that include a combination of two or several radioprotectors, which enables one to reduce the toxicity of one preparation by means of another [12, 15].

Since the discovery of the first classical radioprotectors, a half of the century has passed; however, despite intensive quest and investigations, only a few among many thousands of preparations used in clinic have no toxic side effects on the exposed organism [16]. A search for novel harmless radioprotectors is being continued. This is justified at least by the fact that this enables one to extend the area of application of preparations in laboratory studies owing to the possibility to use a great variety of agents and methods of administering drugs at different exposure regimes [17 - 23].

One of the urgent problems in current studies of antiradiation agents is the reduction of the severity of the radiation syndrome in the organism exposed to high lethal doses.

I.1. Myeloprotectors

It is known that classical radioprotectors are most effective in the range of doses of a single total external irradiation of mammals that affect the bone marrow (1 - 10 Gy). These preparations are classed with myeloprotectors of short-term and prolonged action [18, 19].

I.2. Enteroprotectors

In the last few years, attempts were made to synthesize enteroprotectors (derivatives of thiazole, triazole, thiadiazine, heteroalkane, prostaglandins, and others) effective in the radiation dose range 10 - 20 Gy. These preparations increase the pool of enterocytes, decrease oxygen uptake by intestinal epithelial cells, and "convert" the intestinal form of radiation damage to the bone marrow form [18 - 20].

I.3. Cerebroprotectors

Cerebroprotectors are preparations (metal-containing complexes, blockers of glutamatergic mediation, amides of aromatic carboxylic acids, and others) that reduce the severity of the cerebral radiation-induced syndrome (exposure to doses above 80 Gy) to the intestinal syndrome [18, 19]. At present, the problems of pharmacological antiradiation defense are being extended, the range of radiation doses being examined widened (from lethal and superlethal to sublethal and low doses), the limits of dose rates decreased from high to low values, and the duration of radiation exposure increased from acute short-time to chronic. In view of this, new systems of classification of radioprotectors have been developed, which take into account modern approaches to the problem of radiation protection [19 - 23].) A common feature of these classifications is the division of protectors according to the type of exposure: protectors against acute external irradiation (radioprotectors), against chronic external irradiation (biological preparations, stimulators of radioresistance, adaptogens), and against internal radiation from incorporated radionuclides (sorbents, antidots).

A search for means of protection against chronic low-intensity irradiation has become a particularly pressing problem relatively recently, after the Chernobyl nuclear power station accident. Traditional radioprotectors, which have a rather short time of action and are highly toxic, appeared to be unsuitable in the case of chronic irradiation. The assessment of chronic radiation damage by itself and, consequently, the means of protection against it has some specificity. Thus, the *radioecological syndrome* is stochastic, substantially protracted in time, and can differ qualitatively from the manifestations of the acute radiation disease [24]. Evidence continues to accumulate that the dependence of biological effects on the dose of low-intensity radiation is not only linear or square linear but also shows up as nonmonotonous curves [25].

The present-day concepts of the mechanisms of action of low-intensity radiation and the features of biological effects produced in the organism by external and internal irradiation posed new goals in the search for, and assessment of ways and means of antiradiation defense. Among these are the prevention of the incorporation of radionuclides into the organism (use of special agricultural technologies and a reduction of the migration of radionuclides in the gastrointestinal tract), the protection of cells and tissues (use of the methods of substituting prophylaxis and therapy), the removal of incorporated radionuclides from the body (using a special diet and the addition into the ration of various

plant and mineral enterosorbents). Of great importance is the use of preparations and food substances counteracting the enhancement of lipid peroxidation (LP) processes in biological membranes [26]. A particularly pressing problem is the development of methods for the rehabilitation of persons exposed as a result of Chernobyl nuclear power station accident [27].

Particular emphasis in the present-day classification of antiradiation agents was given to medicinal preparations and food additives that enhance the resistance of the organism to radiation and other unfavorable environmental factors [18 - 23].

Clearly, the FDD used upon acute irradiation cannot be applied for assessing the radioresistance stimulators in the case of low-intensity irradiation. Therefore, it became necessary to invoke integrated indicators such as EBR, which takes into account the LP processes, and the total nonspecific response of the organism (TNRO) to chronic irradiation and stress factors [10, 28, 29]. The concept of EBR is based on the idea that in the organism there are systems of protection against, and sensitization to irradiation, which provide a general mechanism of the formation of radioresistance [10]. The endogenous protective resources of the organism involve biogenic amines, thiols, and other antioxidants and antiradical systems. Molecular oxygen, reactive oxygen species, the products of peroxidation of unsaturated lipids (in biological membranes and cell organoids), and probably other peroxidation products (peroxides of DNA, RNA, pyrimidine bases, thiyl peroxyradicals) belong to endogenous radiosensitizers. The relationship between the content and activity of endogenous radioprotectors and those of endogenous radiosensitizers determines the resistance of biological objects and systems to ionizing radiation [11]. The administration of antiradiation preparations enhances the EBR of the organism by mobilizing its protection resources and suppressing the activity of radiosensitizers.

The realization of EBR occurs through the triggering of the system of cell regulation (cyclonucleotides and the inositide cycle) and the activation of the system controlling the peroxidation processes (antiradical, hypoxic, and antioxidant mechanisms of regulation) [10, 22, 23]. The formation and realization of enhanced EBR are multistage processes, which develop successively with time and gradually involve the most important neurohormonal and hemopoietic regulatory systems. The protection of the irradiated organism also leads to a reduction of the TNRO and the severity of damage and the enhancement of post-irradiation repair processes [11, 24].

II. ADAPTOGENS

Based on the EBR concept, it was suggested that adaptogens, preparations of natural origin (PNO) and antiradiation medicinal drugs (AMD), including drugs modulating TNRO and the immune system, that have long been used in folk medicine can serve as protecting means against chronic irradiation [10, 22, 30, 31].

Earlier most studies concerned with the use of these preparations were conducted under conditions least favorable for the manifestation of their antiradiation activity: the activity of radioprotectors was estimated using a traditional scheme, i. e., upon acute irradiation. The efficiency of radioprotectors under these experimental conditions was low (FDD usually was no higher than 1.2). However, as opposite to classical radioprotectors, the activity of PNO can increase as the radiation dose rate decreases and show up against the background of technogenic chemical pollution. PNO exhibiting the properties of adaptogens are usually harmless and can be continuously taken as food additives. Many PNO produce the antiradiation effect prior to and after irradiation and have a broad spectrum of biological activity.

Below we present, with some modifications and additions, the classification of adaptogens (PNO and some AMD), which is based on our previous studies [22]. These preparations are of interest in view of their possible use in studies of their applicability upon chronic exposures to ionizing radiation under unfavorable environmental conditions. It would be also expedient to use the available data on their application upon single short-term acute irradiation, with due regard for the current ideas on the effect of these drugs upon chronic irradiation. For detailed references to the available literature on PNO and AMD, see [22].

II.1. Zoopreparations

II.1.1. Trehphons.

Trehphons (trophic factors, biostimulators) are extracts from animal cells and tissues. As early as 1922 it was found that trephon extracted from lymphocytes stimulates the hemopoiesis (Karrel effect) [32]. In 1956 Ellinger showed that extracts from the thymus and spleen stimulate the proliferation and

differentiation of cells of hemopoietic organs and the regeneration of the bone marrow and can be used in the treatment of radiation disease [33].

Protein extracts from tissues of animals distinguishing for a high resistance to unfavorable environmental factors, including the extreme action of ionizing radiation, possess a treatment-and-prophylactic RPE. Among them is the preparation of Turdyev, a protein stimulator of hemopoiesis (PSH) isolated from the tissues of the Horsfield' terrapin [34]. It was shown that the PSH increases the EBR by suppressing LP processes and restoring the microsurroundings of proteins of mitochondrial membranes and the energetic system of the liver of exposed animals. Some of the most effective PSH preparations produce the RPE that is high even for adaptogens (FDD up to 1.35).

II.1.2. Zootoxins.

Zootoxins are the venoms of snakes (cobra, blunt-nosed viper, and others) and some insects (bees, spiders, and scorpions) [35, 36]. At doses causing hormesis, they are used to increase the EBR and induce the RPE ($FDD \leq 1.2$).

The activity of the preparations is based on peptide fractions, e. g., butoxin from the mottled scorpion venom and melittin from bee venom. The scorpion and Kirghizian 'black death' spider venoms outperform all known zootoxins in many respects. Thus, they have a treatment-and-prophylactic RPE upon acute and chronic irradiation. A single microinjection of the preparation (10 - 100 $\mu\text{g}/\text{kg}$, intraperitoneally) produces a prolonged effect (from 1 h to 3 days) and a broad spectrum of therapeutic action (therapeutic index up to 20). At radioprotective doses, these preparations are nontoxic. The increase in EBR occurs through the release of biogenic amines from the bound state and an increase in the concentration of gamma-aminobutyric acid.

II.1.3. Hormonal preparations.

Estrogens (estradiols, estriols, and estrones) occupy an important place among zoopreparations. They produce a prolonged (over a period of 1 - 3 weeks) RPE through long-term hyperestrogenism, which can be attributed to enhanced EBR. Such a steady homeostasis is explained by a long residence of the estrogen in the fat tissue followed by its slow entry into the blood [12]. The enhancement of EBR is realized via reversible inhibition of the proliferative activity of the bone marrow (protection against tissue damage) and the stimulation of the synthesis of the nucleic acids and proteins that govern the restoration of hemopoiesis. One of the most extensively studied preparations

diethylstilbestrol induces an increase in radioresistance 2 days after the injection, which persists over a period of 1 - 2 weeks (FDD 1.15 - 1.2 at LD_{60/30}). The increase in EBR in turn contributes to the activation of the reticuloendothelial system and subsequent increase in the resistance of the organism to toxemia and bacteremia [18].

II.2. Phytopreparations

In 1957 Brekhman developed a conception of the biological action of phytoadaptogens (adaptogens of plant origin), which regulate the homeostasis by exerting the stimulating and tonic effects on humans and improving their manual and intellectual working capacity [37].

Later it was shown that rats and mice fed or injected with the preparations of ginseng, eleuterococ and Chinese magnolia vine exhibited TNRO and RPE (13 - 30% protection at LD_{100/30}) [38]. The great majority of phytoadaptogens show a higher antiradiation efficiency as the dose rate and dose value decrease and have an optimum RPE upon chronic low-dose irradiation (the so-called bell-shaped effect in plots). However, this conclusion has been drawn only very recently because the protective capacity of the preparations has long been assessed by classic methods using only two criteria: FDD and mean life duration (MLD) of exposed animals.

Of great interest among studies of natural phytopreparations, are those concerned with the utilization of the experience of ancient folk medicine. Chinese radiobiologists made a large contribution to this area of research.

Many phytoadaptogens have the treatment-and-prophylactic RPE, influencing the neurohumoral regulation of the organism and increasing EBR. The most popular PNO of folk medicine are conveniently divided into four groups: alkaloids, polysaccharides, bioflavonoids, and multicomponent mixtures (crude extracts, potions).

II.2.1. Phytoalkaloids.

The most effective preparations from the relatively small group of alkaloids [22] possess as a rule only the *radioprophylactic* action. The preparation Kushen contains alkaloids matrine, oxymatrine, oxysophokarpin, and sophoridine. A related preparation Kushensu contains oxymatrine and oxysophokarpin. Both mixtures are used in clinic in chemotherapy as an antileukopenic factor. It was found in experiments on animals that the

administration of these preparations leads to a steady increase in radioresistance (during 10 days after the injection). The most pronounced RPE was observed after multiple injections of the preparations prior to irradiation. Interestingly, a preparation from another species of the Japanese pagoda tree *Sophora subprostata* contains alkaloids producing a toxic effect on lung carcinoma cells.

Hydrolyzates from the Criciferae family: cabbage *Brassica oleracea*, swede *Brassica napus*, white mustard *Brassica juncea*, garden radish *Rorippa montana* and *Raphanus sativus* also have a radioprotective leukostimulating action. It was shown that sinapin and sinapinic acid contained in these plants are active alkaloids and increase EBR. The preparation Linkiau [39] is an alkaloid-containing infusion from the seeds of *Forsythiae suspensa*. The injection of the preparation three days prior to irradiation reduces the severity of the radiation-induced hemopoietic syndrome and protects 30% of animals against lethal irradiation.

A group of phytopreparations, alkaloid extracts from *Herbas thermopsis*, *Rhisoma coridalys humosae*, *Semen iridis*, *Oleum carcumae aromaticae*, *Radix codonopsis pilosulae*, *Herbas artemisiae annuae*, *Filium camelliae sinensis*, *Venenum bufonis* have a common feature, the ability to induce and maintain for a long time the enhanced general biological resistance to unfavorable environmental factors and ionizing radiation.

II.2.2. Phytopolysaccharides [22]

A. Phytopolysaccharides of radioprotective action. This group includes polysaccharide extracts from woody edible fungi *Auricularia auricula Judae*, lichens *Paramella tinctorum Desper* (preparation Shikhua), *Lycopodium clavatum* and from plants *Lobaria pulmonaria Hollm*, *Bupleurum chinense D.S.*, and others.

Of interest is the preparation Shikhua. Enhanced EBR by the action of this drug is formed over a period of many hours. Thus, taking the preparation 8 h prior to irradiation with lethal doses results in a 40% survival of animals, whereas the administration of the medicine 18 h prior to exposure leads to a survival of as high as 55%.

Another preparation, a polysaccharide extract from *Lobaria pulmonaria Hollm* has an even higher radioprotective activity. When administered 1 h prior to lethal-dose irradiation, it protects 60 - 80% of mice from death. Other radioprotective phytopolysaccharide preparations are less effective; however, their RPE persists for several hours and even days. All preparations are little toxic. An important feature of these preparations is the ability to

enhance the radioresistance over a long period of time, which enables their application during long-term irradiation. The enhancement of EBR is provided by an increase in the antiradical and antioxidative activity of components and is realized via the stimulation of hemopoiesis, immunity, and moderate tissue hypoxia.

- B. *Phytopolysaccharides of radiotherapeutic action.* Polysaccharides extracted from tricholomoid woody edible fungi and other plants, such as *Armillaria meilia* (Bull. ex Fr.) Karst, common globe thistle *Hericium crinaceum* (Bull. ex. Fr.) Pers, gametochet fungus *Polyporus bellatus*, laminaria *Tallus laminariae*, and medicinal grass *Fructificatia ganodermae*, produce the RPE when applied after irradiation. Polysaccharide preparations from these plants are able to decrease the severity the hemopoietic radiation syndrome (FDD 1.15 - 1.2).
- C. *Phytopolysaccharides of treatment-and-prophylactic action.* The most effective among phytopolysaccharides are extracts from edible fungus *Tremella fuciformis* Berk (preparation no. 201 [39] and the preparation "Dzhiner"). They are equally effective as radioprotectors and medicinal drugs and, when applied for treatment and prophylaxis, increase the survival of lethally irradiated mice, rats, dogs, and monkeys up to 50 - 90%. The extracts can be used in combination with other preparations, e. g., estrogens, increasing the RPE. "Dzhiner" was successfully used in clinic in 226 cancer patients to stimulate leukopoiesis in radio- and chemotherapy.

II.2.3. Bioflavonoids.

Flavonoids, a class of chemical compounds with a marked antiradiation biological activity, have been extensively studied during the past few years. Thus, it was found that genistein-8-C-glycosyl, an isoflavone from the buds of European yellow lupin, inhibits LP and the oxidation of SH-groups of membrane proteins and slows down the inactivation of cytochrome P-450 and its conversion to an inactive form of cytochrome C-420. Among isoflavonoids, genistein is the most effective in binding H₂O₂ and inhibiting the oxidative lesion of cell DNA induced by hydrogen peroxide. The preparation exhibits the antiradiation activity, when injected before and after irradiation at doses inducing the bone marrow syndrome.

Another flavonoid quercetin is the most effective radioprotector, which acts as a "scavenger" of radicals formed in the reaction of peroxyxinitrite ONOO⁻.

II.3. Polycomponent Mixtures

This group includes crude infusions, extracts, phyto- and zoopreparations, sea products, and complexes of vitamins [22, 23]. The antiradiation activity of some preparations (crude infusions and poorly purified extracts from plant and animal tissues and cells) may be due to the effect of not one but several components of a biologically active substance the interactions between which often mutually complement and enhance each other and are equilibrated by the nature of endogenic substances. Indeed, we know many cases when the activity of a preparation decreases with increasing grade of its purity. These multicomponent mixtures are difficult to identify, and preparations with stable activity are difficult to prepare from these mixtures.

II.3.1. Multicomponent phytoadaptogens.

“Dze-Gui” is an infusion from *Angelica sinensis* Diels, which is used in Chinese folk medicine to normalize the immunity and hemopoiesis. The preparation is nontoxic, it possesses a moderate action of an adaptogen and is used in clinic to normalize the level of leukocytes in radio- and chemotherapy of cancer patients.

Infusions from blade-kelp (*Laminaria*), Phaeophyceae, diatoms (Diatomeae), and red algae (Rhodophyceae) have weak antioxidative and antiradiation effects on lethally exposed rats and mice (FDD 1.1 - 1.15) [42]. However, it is known that diatomites and alginates isolated from algae are enterosorbents decreasing the incorporation of radionuclides in the gastrointestinal tract [18, 20].

The preparation “Kao” is an extract from the rhizome of the knotweed *Radix polygonum multiflorum*, which is highly resistant to unfavorable environmental factors. It was shown that the RPE of “Kao” is twofold higher than that of the extract from ginseng. “Kao” is able to enhance the EBR by increasing the level of endogenous thiols and to stimulate hemopoiesis.

As early as in 1960s, the researchers of the Institute of Biophysics of the USSR Ministry of Health obtained convincing evidence that the preparations of highly homo- and heterologous RNA and DNA polymerases have the antiradiation effect on the irradiated organism [43]. Later it was found in the laboratories of V.K. Mazurik, E.S. Chertkov, L.B. Shagalov, and V.I. Legeza [19] that some polynucleotides and nucleosides, such as polyribonate, phosphaden, ridostin, and riboxin, have the RPE. Thus, riboxin has a prophylactic protecting effect on animals irradiated in a wide range of acute and

chronic radiation doses and decreases the yield of chromosomal aberrations. An extract from *Rhodiola rosea*, when used for the protection against ionizing radiation, reduces the yield of chromosomal aberrations and the frequency of micronuclei [44]. The authors related the antiradiation effect of this natural adaptogen not only to the systemic effect, the TNRO, but also to the DNA repair in cells.

The mixture «ECCL» from the extracts of the medicinal plant greater celandine *Chelidonium majus* and choline lactate has a treatment-and-prophylactic RPE: it stimulates hemopoiesis, restores the bone marrow cellularity, and normalizes radiation-induced disturbances in the liver. In addition, it exhibits the antioxidative activity, has an antiworming action and a high antiradiation effect (according to the data of the authors, the FDD can be as high as 1.5) [22]. The mixture is recommended in China as an antiradiation drug in accidents and is used in clinic for the radiotherapy of cancer patients.

Since 1992 a search for agents capable of replenishing the exhausted reserves of antioxidative systems in the irradiated organism has been performed at the Institute of Clinical Radiology (Research Center of Radiation Medicine, Ministry of Health of Ukraine), which contribute to the normalization of LP processes [45]. A large group of adaptogens, vitamins and food substances have been tested. Some of these agents were recommended for treating persons exposed in the Chernobyl nuclear power station accident. Among these preparations are phytoproducts "Flora-7" (medicinal flower pollen, a pot marigold tincture), "Melisan-3" (medicinal flower pollen, liquorice, and propolis extract), "Flaminar" (a polyextract from mountain ash berries), and "Vetoron" (a complex of vitamins A, E, C).

The treatment-and-prophylactic application of the above-cited multicomponent food phytoadaptogens invariably increases the EBR, normalizes LP processes, and replenishes the reserves of the antioxidative system (through both mobilization and exogenous supply). A lipocarotenoid preparation isolated from the extract of mycelium of *Basidiomycetes* fungus, a producer of carotenoids, enhances the antioxidative activity of blood and leukopoiesis and normalizes the mass of spleen, testis, and thymus [46].

The antiradiation effect of carotene has long been known in radiobiology. In the last years, there has been an increasing interest in this substance. Thus, a food additive consisting of a mixture of vitamins C, E, and β-carotene has been successfully used for the prophylactic and therapeutic protection of the organism against chronic irradiation and stress [47]. A water--dispersion mixture of β-carotene with chloroamphenicol and methyluracil (preparation "Karley") has a

highly anti-inflammatory and regenerative action and is used in the intracavitary radiation therapy [48].

Multicomponent mixtures possessing a strong antioxidative action and effectively protecting the organism against chronic irradiation are being developed at the Minsk Medical Institute and Institute of Radiobiology (National Academy of Sciences of Belarus). Thus, on the basis of carotene, the novel preparation "AK β ", a multicomponent antioxidant antiradiation mixture of vitamins A, E, and C at adequately chosen doses was developed, which protects the organism upon chronic and acute irradiation [49].

II.3.2. Multicomponent zooadaptogens.

About 100 preparations from sea products: mollusks, starfish, sea-cucumbers, brittle stars, common shrimps, and fish, used in Chinese folk medicine, were studied at the Shanghai Institute of Military Medicine in Yu Chidzhi. Ten of these preparations were found to have the treatment-and-prophylactic antiradiation action (FDD was no higher than 1.15 - 1.2). It was shown that the most effective extracts (from mollusks, starfish, and sea cucumbers) are able to increase the EBR in irradiated rats by accumulating serotonin in the bone marrow and enhancing the activity of cAMP. They also were found to normalize the content of blood lymphocytes.

Of some interest are the hydrolyzates from the meat of Black Sea, White Sea, and Far East mussels *Mytilus galloprovincialis*, *Mytilus edulis*, *Grenomytilis grayanus*. The first results of studies initiated in our laboratory in 1974 showed that the acidic hydrolyzate from mussel (preparation My - Hy-A) has a treatment-and prophylactic antiradiation effect (FDD 1.25 - 1.30) [50]. It was also found that preparations from mussel meat can be used as food additives to protect the body against low-intensity chronic irradiation and act as adaptogens under unfavorable environmental factors and in the therapy of diseases of various etiology [30, 51, 52].

The preparations are balanced mixtures of microelements, amino acids, amines, carnosine, taurine, melanoids, and saturated and unsaturated fatty acids [30, 51]. It is known that some components of My - Hy-A, e. g., microelements, metal salts, [18, 30, 53], taurine [30], carnosine [54, 55], and melanoids [56 - 58], by themselves produce the RPE. However, the natural balance of these and other components of the preparation imparts to My - Hy-A peculiar properties of an adaptogen sorbent and an antiradiation agent [59]. The mechanism of the treatment-and-prophylactic antiradiation action of My - Hy-A is related to its ability to enhance EBR, which results in antioxidative and hemoregulating

effects. In addition, the preparation enhances the withdrawal of ^{45}Ca , ^{90}Sr , and ^{137}Cs radionuclides from the irradiated organism [30, 51, 59 - 61]. In the last years, several mussel preparations, analogues of My - Hy-A, have been developed, which possess a broad spectrum of antiradiation and antistress action. The preparations have found application during the execution of the program of the Moscow State University on the rehabilitation of Chernobyl accident recovery workers [62 - 63].

II.3.3. Multicomponent phyto - zoo mixtures [22].

The preparation "Shen Bay Ning" (extracts of phospholipids and alkaloids from plants and animal organs) is recommended by the Chinese folk medicine as an antioxidative hemoregulating and tonic agent. In radiobiological experiments, the preparation protects dogs against gamma-neutron radiation.

Another antioxidative preparation, a mixture of the ethanol extract from *Sophora japonica* and pantocrin, induces the RPE (FDD 1.25) when applied for prophylaxis (5 - 15 min before irradiation) and subsequent treatment (2--4 days after exposure).

It is seen from the data presented above that the common feature of these adaptogens is the ability to stimulate an increase in EBR by activating antioxidative and repair processes and mobilizing (or replenishing) antiradiation and general biological protection resources of the organism [22, 23]. When normalizing (or decreasing) the TNRO to irradiation, each of these preparations also produces its own specific effect on the organism, which is aimed at curing concrete, not obligatorily radiation-induced illnesses accompanying the TNRO to whose treatment and prophylaxis the efforts of many generations of folk physicians have been directed. The curative properties of folk medicinal drugs have been described in detail in numerous monographs and manuals.

II.4. Immunomodulators [18, 22]

In previous sections we described the natural preparations of antiradiation action, which stimulate the antioxidative and reparative reserves of the organism. However, these processes often proceed simultaneously with the mobilization of another very important mechanism of protection of the organism, the immune system. The studies of biogenic immunomodulators as antiradiation agents were initiated more than 40 years ago in connection with studies of endotoxin lipopolysaccharides, which stimulate the functioning of the

reticuloendothelial system, hemopoiesis, and the formation of endogenous biologically active substances [64].

As adaptogens, immunomodulators rank below many classical synthetic radioprotectors in FDD. However, they are favorably distinguished from these radioprotectors by a low (or even the absence of) toxicity, the ability to maintain enhanced radioresistance of the organism over a longer time, the capacity to potentiate the RPE of aminothiols and hypoxia, and a more marked antiradiation effect during chronic irradiation [65].

II.4.1. Antiradiation preparations stimulating the immunity.

The well-known polysaccharide immunomodulators heparin and glucan stimulate the immunity and radioresistance of animals. Heparin induces the postradiation recovery of the thymus, the proliferation of colony-forming units, and an increase in the activity of T and B lymphocytes and macrophages. Glucan activates the hemopoiesis, the formation of macrophages and the accumulation of antioxidants, the most important components of EBR in the organism [22]. Other antiradiation immunomodulators, such as prodigiosan, pyrogeal, levan, and zymozan have also been extensively studied [18].

A novel antiradiation preparation of prolonged action indomethafan merits special attention. Its ability to enhance the immune reactivity of the organism, in particular, the synthesis of antibodies and phagocytosis plays a role in the mechanism of the antiradiation effect of this radioprotector. In addition, the preparation reduces the magnitude of the infection syndrome [66].

Among the drugs used in the therapy of acute and chronic radiation diseases, biogenic immunomodulatory peptides thymalin, thymogen, myclopid, tactivin, thymoptin [19, 21] and synthetic peptides, the analogues of the biologically active fragment of the cell differentiation factor HL-60, deserve special attention [67].

The immunity and hyperradioresistance can also be stimulated by preliminary irradiation of animals with low radiation doses [66, 67] and the injection of corpuscular microbial preparations and extracts from various plant and animal organisms, yeast cells, fungi, and bacteria and a variety of vaccines [19, 21, 22, 67]. Thus, vaccines from *Escherichia coli*, the enteric fever vaccine, and several antiviral vaccines cause hyperradioresistance, which is based on the rearrangement of the immune system induced by tissue destruction and the acquired antigen pool [68, 69].

Of great interest are antiradiation immune preparations isolated from spirulina microalgae. Thus, the product of destruction of the biomass of this

microalgae "Platensis" has a complex composition of biologically active substances (vitamins, β -carotene, B₁ - B₁₂, E, C, biotin, pantothenic acid, inositol; microelements; essential amino acids, polyunsaturated fatty acids, phycocyanin, chlorophyll, and several enzymes). The preparation is nontoxic; it has a pronounced immunostimulating and a radioprotective effects [70]. Another preparation from spirulina "Splat" is currently used for correcting the immune insufficiency in workers engaged in atomic industry [71].

II.4.2. Cytokines.

In the last few years there has been an increasing interest among radiobiologists in cytokines, polypeptides regulating the growth, differentiation, and the functional activity and radioresistance of cells. The RPE of cytokines is due to their hemo- and immunostimulating activity and the ability to restore the bone marrow cellularity. There is evidence that the antiradiation effect of cytokines is related to their ability to increase the EBR [22, 72, 73]. An important feature of cytokines is their ability to maintain radioresistance over a long period of time, up to several days.

Interleukins: lymphokines, monokines, the colony-stimulating and tumor necrosis factors, interferons, etc. have the antiradiation effect [18, 74]. Of interest is the recently reported observation that some cytokines possessing the RPE, including interleukin-1, are potent stimulators of the biosynthesis of nitric monoxide (NO), and it is the overproduction of nitric monoxide that is responsible for their pharmacological effects [75]. Interestingly, the endogenous NO, even in physiological concentrations, fulfills several functions pertinent to the realization of EBR and acts as an acceptor of superoxide ion, a regulator of the regional blood flow and a factor of immunity [75].

It is believed that one of interleukins, interleukin-1- β may be used in case of emergency in human irradiation accidents. When administered in effective doses, this genetically engineered adaptogen of the natural stimulator of hemopoiesis is satisfactorily endured by humans and is currently used in clinic treatment courses [76]. L.M. Rozhdstvenskii has supposed with good reason that the application of cytokines, primarily for urgent antiradiation help (the least studied field of radiation medicine), is very promising in antiradiation chemical defense.

These data do not embrace all lines of the search for the agents of antiradiation chemical protection of the organism. This holds true particularly for novel natural preparations [77] and means of protection against internal

irradiation that hinder the entry of radionuclides into the organism and promote their withdrawal from the body [16, 19, 21].

III. SORBENTS

Measures aimed at withdrawing radionuclides from the body consist mainly in the termination of internal irradiation at some stage using sorbents. However, only the iodine method is a truly prophylactic agent of urgent radiation protection.

III.1. Prophylaxis with iodine preparations

The potassium iodide preparation is a water - alcohol infusion of stable iodine (Lugol solution). An optimum protective dose of potassium iodide for an adult human is 0.125 g (1 tablet daily) over a period of 7 - 10 days or 3 - 5 drops of the Lugol solution per glass of water three times a day [16]. This dosage results in the saturation of the thyroid gland with iodine, and the thyroid loses the ability to adsorb new portions of ^{131}I so that radioactive iodine entering the blood is rapidly withdrawn from the body [21].

III.2. Preparations for adsorption and withdrawal of radionuclides from the body

It is known that organic sorbents are not always suitable for removing radionuclides since they are digested in the body. Radionuclides can be sorbed using natural minerals and inorganic nonsoluble compounds in the form of powders. Owing to the structure of the crystal lattice (clathrates) they are able to selectively and irreversibly bind radionuclides and withdraw them from the blood and gastrointestinal tract. As a result, the level of radionuclides in the body can decrease severalfold.

III.2.1. Adsorption and removal of cesium.

It is known that pectins, plant fibers isolated from apples, common beet, and citrus fruits, are active enterosorbents of various radionuclides and normalize the intestinal microflora [75]. The use of pectin-containing

preparations, first of all "Pektopal", in the Gomel region demonstrated a high capacity of this preparation for the decorporation of ^{137}Cs . The organosilica sorbent "OK-M", the coal sorbent "KAU-2", and others were exceeded in effectiveness by pectins [76].

At present, much attention is being given to the protection of agricultural animals against ^{137}Cs using a large group of effective enterosorbents ferrocyanides. Among them, the preparation "Bifezh" (biological ferrocyanide "Ferrocin" applied in a special way onto wood sawdust) merits special attention [78, 79]. Having a peculiar crystal lattice, ferricyanides selectively sorb cesium to form an insoluble complex with it, which passes as "transient goods" through all parts of the gastrointestinal tract of animals and is rapidly withdrawn from the body [79].

III.2.2. Adsorption and decorporation of strontium.

It is recommended to remove isotopes of strontium (and barium) from the body using calcium alginate or its combination with hyaluronic acid (preparation Algisorb), the highly purified crystalline microcellulose "MKTs", activated carbon with additives (K, Mg, Al) "Adsorbar", barium sulfate "Polysurmin", marinide, cryopreparations of chaga fungus, etc. [19, 21, 80, 81].

A challenging problem of modern radiation medicine is the development of complex treatment-and-prophylactic preparations based on sorbents. Recently, a nontoxic complex preparation "Sorboces" based on a carbon fibrous enterosorbent with high macro-, meso-, and microporosity was synthesized, which simultaneously adsorbs and decorporates ^{90}Sr and ^{137}Cs [82].

III.2.3. Adsorption and decorporation of transuranium radionuclides.

The development of complex sorbents is a promising line of research in the State Research Center of the Institute of Biophysics (Ministry of Health of the Russian Federation). Thus, it was found in experimental studies that highly esterified pectins, when used as food additives, considerably decrease the content of transuranium radionuclides ^{238}Pu , ^{239}Pu , ^{241}Am , and ^{137}Cs after their single and chronic entry into the body. The coefficient of resorption and the extent of the accumulation of these radionuclides in the skeleton and liver decrease on the average twofold (in the case of cesium even threefold). Pectins considerably reduce the content of transuranium radionuclides in the walls of the gastrointestinal tract, thereby lowering the irradiation dose and normalizing the intestinal microflora [83]. In other studies it was shown that plutonium encapsulated into liposomes is highly effectively decoporated by "Pentacin"

(complexons of polyaminopolyacetic acid) [84, 85] and "Trimefacin" and "Phosphicin" (complexons of the derivatives of phosphonic acid) [21].

Since the Chernobyl accident, many years have passed. However, the development of novel effective agents for the prophylaxis of internal irradiation and withdrawal of its sources from the body as well as for the prophylaxis and treatment of radiation-induced disorders remains one of the most urgent problems in radiobiology, radioecology, and medicine.

REFERENCES

1. Dale W.M., *Biochem. J.*, 1940, vol. **34**, pp. 1367 - 1377.
2. Dale W.M., *Biochem. J.*, 1942, vol. **36**, pp. 80 - 92.
3. Dale W.M., Gray L., and Meredith W., *Phil. Trans.*, 1949, vol. **242A**, pp. 3 - 9.
4. Latarjet R. and Ephrati E., *C. R. Soc. Biol.*, 1948, vol. **142**, p. 497.
5. Patt H., Tyree E., Straube R., and Smith D., *Science*, 1949, vol. **110**(2852), pp. 213 - 214.
6. Herve A. and Bacq Z.M., *C. R. Soc. Biol.*, 1949, vol. **143**(881), p. 1158.
7. Bacq Z.M., Herve A., Lecomte I. et al., *Arch. Intern. Physiol. Biochem.*, 1951, vol. **59**(4), pp. 442 - 446.
8. Bacq Z.M. and Herve A., *Bull. Acad. Roy. Med. Belg.*, VI series, 1952, vol. **17**, pp. 13 - 23.
9. Bacq Z.M., *Schweiz. Med. Wschr.*, 1952, Bd. **82**, S. 1018.
10. Goncharenko E.N. and Kudryashov Yu.B., *A hypothesis about the endogenous background resistance*, Moscow, Izd. MGU, 1980, 176 p. (Rus)
11. Goncharenko E.N. Kudryashov Yu.B. Chemical protection against radiation damage. Moscow, Izd. MGU, 1985. 248 p. (Rus)
12. Vladimirov V.G., Poddubskii G.A., and Razorenov G.I., *Radioprotective formulas. Optimization of the composition and mechanism of action*, Leningrad, Izd. LGU, 1988, 186 p. (Rus)
13. Tarusov B.N., *Primary processes of radiation damage*, Moscow, Gosatomizdat, 1962, 96 p. (Rus)
14. Emanuel N.M., *Primary mechanisms of biological action of ionizing radiations*, Moscow, AN SSSR, 1963, pp. 73 - 84. (Rus)
15. Grachev S.A. Sverdlov A.G., Nikanorova N.G., and Timoshenko S.I., *Radiats. Biologiya. Radioekologiya*, 1999, vol. **39**(2-3), pp. 260 - 262. (Rus)

16. Vladimirov V.G., Goncharov S.F., Legeza V.I., and Avetisov G.M., *Radiobiological aspects of the medicine of fatal accidents*, Moscow, VTsMK "Zashchita", 1997, 220 p. (Rus)
17. Vasin M.V., Ushakov I.B., Semeneva L.A., and Kovtun V.Yu., *Radiats. Biologiya. Radioekologiya*, 2001, vol. 41(3), pp. 307 - 309. (Rus)
18. Vladimirov V.G., *Voenno-Meditsinskii Zhurnal*, 1978, No. 6, pp. 39 - 43. (Rus)
19. Legaza V.I. and Vladimirov V.G., *Radiats. Biologiya. Radioekologiya*, 1998, vol. 38(3), pp. 416 - 425. (Rus)
20. Rasina L.N. and Chupakhin O.N., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 225 - 228. (Rus)
21. Vasin M.V., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 213 - 224. (Rus)
22. Goncharenko E.N. and Kydryashov Yu.B., *Uspekhi Sovr. Biol.*, 1991, vol. 3(2), pp. 302 - 316. (Rus)
23. Tarakhtii E.A., Kshnyasev I.A., Yushkov B.G. et al., *Radiats. Biologiya. Radioekologiya*, 2000, vol. 40(6), pp. 668 - 673. (Rus)
24. Kudryashov Yu.B., *Radiats. Biologiya. Radioekologiya*, 1977, vol. 37(4), pp. 673 - 675. (Rus)
25. Burlakova E.B., Goloshchapov A.N., Gorbunova N.V. et al., *Consequences of the Chernobyl accident: human health*, Moscow, Center of the ecological policy of Russia, 1996, pp. 149 - 182. (Rus)
26. Bortkevich L.G., *Generally accessible methods for the protection of population living in regions contaminated with radionuclides. Nuclear Encyclopedia*, Moscow, Phylanthropic Foundation of Yaroshinskaya, 1996, 306 p. (Rus)
27. Zubovskii G.A. and Tararukhina O.B., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 296 - 298. (Rus)
28. Kydryashov Yu.B. and Goncharenko E.N., *Stress under the action of ionizing radiation; Nuclear Encyclopedia*, Moscow, Phylanthropic Foundation of Yaroshinskaya, 1996, pp. 327 - 330. (Rus)
29. Kydryashov Yu.B., *A search for and study of the mechanisms of action of new natural and synthetic antiradiation agents*, Moscow - Perm; MGU, PGU, 1989, pp. 6 - 21. (Rus)
30. Kydryashov Yu.B. and Goncharenko E.N., *Theses of the 1st All-Union Radiobiological Meeting*, vol. 1, Pushchino, 1989, p. 10. (Rus)

31. Karrel A., *J. Exp. Med.*, 1922, vol. **36**, pp. 365 - 372.
32. Ellinger F., *Proc. Soc. Exp. Biol. Med.*, 1956, vol. **92**(4), pp. 670.
33. Turdyev A.A. and Usmanov R.B., *Informatsionnyi Bulletin Po Radiobiologii AN SSSR*, Moscow, 1984, Iss. 28, p. 35. (Rus)
34. Khalikov S.K., 'Biochemical mechanisms of treatment and prophylactic action of venoms of snakes of Middle Asia upon radiation-induced damage', *Thesis of Doctor Dissertation*, Tashkent, Izd. Biokhimii AN UzbSSR, 1978, 39 p. (Rus)
35. Vernigorova L.A. and Lebedev V.G., *Radiobiologiya*, 1986, vol. **26**(4), pp. 532 - 540. (Rus)
36. Brekhman I.I., *Ginseng*, Leningrad, Medgiz, 1957, 182 p. (Rus)
37. Brekhman I.I., *Eleuterococ*, Leningrad, Nauka, 1968, 185 p. (Rus)
38. Ou-Yung Yan and Zhou Lianlang, *Abstr. Internat. Conf. Biol. Effects: Large-dose Ioniz. and Nonioniz. Radiat.*, Hangzhou, China, vol. **1**, 1988, p. 84.
39. Zavodnik L.B., *Radiats. Biologiya. Radioekologiya*, 2003, vol. **43**(4), pp. 432 - 438. (Rus)
40. Wang Bingjt, Huang Shafet, Cheng Lurong *et al.*, *Abstr. Internat. Conf. Biol. Effects: Large-dose Ioniz. and Nonioniz. Radiat.*, Hangzhou, China; vol. **1**, 1988, pp. 86.
41. Ju Zhijie, Gui Zhengde, Mao Wenxian *et al.*, *Abstr. Internat. Conf. Biol. Effects: Large-dose Ioniz. and Nonioniz. Radiat.*, Hangzhou, China, vol. **1**, 1988, p. 83.
42. Fedorova T.A., Tereshchenko O.A., and Mazurik V.K., *Nucleic acids and proteins in the organism upon radiation damage*, Moscow, Meditsina, 1972, 408 p. (Rus)
43. Mikhailov V.F., Salikhova R.A., Ushenkova L.N., Raeva N.F., Aleksandrova I.V., and Mazurik V.K., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, p. 98. (Rus)
44. Ovsyannikova L.M., Kvita G.I., and Kovalenko A.I., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 116 - 117. (Rus)
45. Konoplya E.F., Kapich A.N., and Vereshchako G.G., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 39 - 40. (Rus)
46. Ryabchenko I.I., Krikunova L.I., and Guseva L.I., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, p. 104. (Rus)

47. Ryabchenko I.I. and Konoplyannikova A.G., *Problems of antiradiation chemical defense. Materials of the conference*, Moscow, RAN, 1998, p. 104. (Rus)
48. Rutkovskaya Zh.A., Morozkina T.S., and Konoplya E.F., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, p. 7 - 8. (Rus)
49. Goncharenko E.N., Deev L.I., Karagodin V.P., and Kudryashov Yu.B., *Radiobiologiya*, 1974, vol. 14(6), pp. 752 - 759. (Rus)
50. Goncharenko E.N., Deev L.I., Kudryashov Yu.B., Parkhomenko I.M. et al., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 308 - 313. (Rus)
51. Platonov A.G., Akhalaya M.Ya., Deev L.I., and Kudryashov Yu.B., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 317 - 321. (Rus)
52. Gudkov I.N., Kitsno V.E., Grisyuk S.N., and Tkachenko G.M., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 353 - 357. (Rus)
53. Kudryashov Yu.B., Deev L.I., Goncharenko E.N., and Baizhumanov A.A., *Radiats. Biologiya. Radioekologiya*, 1999, vol. 39(2-3), pp. 270 - 275. (Rus)
54. Sutkovoi D.A., Guk A.P., Glushenko I.V., and Todor I.N., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 140 - 141. (Rus)
55. Izomest'eva O.S., Dubovik B.V., Zhavaronkov L.P., and Pavlova L.N., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, p. 89. (Rus)
56. Druzhina N.A., Sidorik E.P., Burlaka A.P. et al., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 86 - 87. (Rus)
57. Mosse I.B., Plotnikova S.I., Kostrova L.N., and Lyakh I.P., *Theses of the reports of the scientific session on the problem "Modification of the effect of ionizing radiation"*, Pushchino, 1992. Iss. 2, Radiobiological Society, pp. 46 - 47. (Rus)
58. Kudryashov Yu.B., Goncharenko E.N., Parkhomenko I.M., Deev L.I. et al., *Application of the preparation My-Hy-A for increasing the total resistance of the organism and the antiradiation effect: Chemical composition. Mechanisms of action. Application in ChNPS in 1986 - 1987. Instruction for the application of the preparation My-Hy-A. Recipes of vegetable meals and drunks with My-Hy-A additions. Priority for the preparations. Act of introduction*, Moscow – Kiev, MGU-KGU, 1987, 32 p. (Rus)

59. Goncharenko E.N., Deev L.I., Kudryashov Yu.B., and Parkhomenko I.M., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 70 - 71. (Rus)
60. Parkhomenko I.M., Kossova G.V., Baikhodzhaeva B.U., Khlebnikov V.I., and Kudryashov Yu.B., *A search for and study of the mechanisms of action of new natural and synthetic antiradiation agents*, Moscow - Perm'; MGU, PGU, 1989, pp. 93 - 108. (Rus)
61. Kudryashov Yu.B. and Perov Yu.F., *A preparation from tissues of sea hydrobionts, the method for its isolation, the treatment-and prophylactic application for reducing the severity of the radiation disease*. Patent of Russia. Moscow, RU No. 94035039. 1994. 30.09. (Rus)
62. Kudryashov Yu.B., Rubin A.B., Perov Yu.F. et al., *Biologisch Aktives Pharmazeutisches Preparat. Europ. Patent Berlin*; MPK No. 43093396. 1993. B. 17.03. / Publ. Patent Blatt, 22.09. 1994. 47.01-1100. Berlin.
63. Smith W.W., Aderman I.M., and Gillespie R.E., *Amer. J. Physiol.*, 1957, No. 191, pp. 124 - 128.
64. Kuna P., *Chemical protection*, Moscow, Medgiz, 1989, 191 p. (Rus)
65. Mikhailov P.P., Mal'tsev V.N., Shlyakova G.G. et al., *Radiats. Biologiya. Radioekologiya*, 2003, vol. 43(4), pp. 431 - 438. (Rus)
66. Goncharenko E.N., Deev L.I., Kostanyan I.A. et al., *Radiats. Biologiya. Radioekologiya*, 2002, vol. 42(2), pp. 164 - 168. (Rus)
67. Tymenev R.S., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 106 - 107. (Rus)
68. Konoplyannikov A.G. and Konoplyannikova O.A., *Radiats. Biologiya. Radioekologiya*, 2002, vol. 42(4), pp. 395 - 398. (Rus)
69. Bubnova O.M., Baum R.F., Ashmarov V.V., and Znamenskaya E.V., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 81 - 82. (Rus)
70. Oradovskaya I.V., Khmel'nitskii V.P., Oprishchenko M.A., and Ivanov V.V., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 74 - 75. (Rus)
71. Weiss I.E., *Proc. Internat. Conf. Biol. Effects of Large-dose Ion. and Nonioniz. Radiat.*, Hangzhou. China, 1988, p. 167.
72. Davydovskii A.G. and Murzenok P.P., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, p. 129. (Rus)
73. Lebedev V.G., Moroz B.B., Deshevoi Yu.B. et al., *Radiats. Biologiya. Radioekologiya*, 2002, vol. 42(1), pp. 60 - 64. (Rus)

74. Krasil'nikov I.I., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, p. 53 - 54. (Rus)
75. Rozhdestvenskii L.M., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 43 - 44. (Rus)
76. Koterov A.N., Pushkareva N.B., and Nikol'skii A.V., *Radiats. Biologiya. Radioekologiya*, 2003, vol. **43**(6), pp. 647 - 653. (Rus)
77. Ivanov A.A., Kalistratova V.S., and Nisimov P.G., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 87 - 88. (Rus)
78. Bandazhevskii Yu.I., Fomchenko N.E., and Lyakhova I.K., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 80. (Rus)
79. Ivannikov A.T., Il'in L.A., Popov B.A. et al., *Radiats. Biologiya. Radioekologiya*, 2002, vol. **42**(5), pp. 515 - 519. (Rus)
80. Rasina L.N., *Radiats. Biologiya. Radioekologiya*, 2002, vol. **42**(4), pp. 399 - 403. (Rus)
81. Vasil'ev A.V., Ratnikov A.N., Aleksakhin R.M., and Krasnova E.G., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 76 - 77. (Rus)
82. Korzun V.N., Saglo V.I., Litvinov V.F., and Kovalenko V.N., *Problems of antiradiation defense. Materials of the conference*, Moscow, RAN, 1998, pp. 94 - 95. (Rus)
83. Il'in L.A., Ivannikov A.T., Altukhova G.A. et al., *Radiobiologiya*, 1989, vol. **29**(2), pp. 197 - 201. (Rus)
84. Zhorova E.S., Il'in L.A., Ivannikov A.T. et al., *Radiats. Biologiya. Radioekologiya*, 2002, vol. **42**(5), pp. 520 - 525. (Rus)

Regularities of somatic mutagenesis in long dates after low dose ionizing radiation effect on human organism

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ABSTRACT

Frequency analysis of somatic cell with gene mutations by glycophorin A (GPA) and T-cell receptor (TCR) loci was performed on liquidators of the Chernobyl Accident irradiated in doses up to 0.25 Gy and control persons. The effectiveness of damaged cell apoptotic elimination in these persons was studied and then this index was compared with mutant cell frequency in TCR-locus. The following regularities of long date somatic mutagenesis after low dose irradiation were determined:

- the average frequency if PCR-mutant lymphocytes increases and the quantity of mutant hemopoietic stem cells is preserved at the control level. It was judged about by the quantity of GPA (NO)-variant erythrocytes;
- the absence of monotonic dependence between TCR-mutant cell frequency and the irradiation dose;
- preservation of stable effect (increased frequency of TCR-mutant lymphocytes) during 9 – 17 years after irradiation impact;
- the effect manifestation in part of irradiated persons.

The investigation results of damaged cell apoptotic death testify about importance of this process as the mechanism for genetic stability support at the

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level of cell population. However, in most cases, increased frequencies of TCR-mutant cells in tested liquidators are not associated with this process efficiency decrease.

Keywords: gene mutations, GPA-locus, TCR-locus, apoptosis, low doses, flow cytofluorometry

The study of genetic effects of low dose irradiation impact is the urgent problem of radiobiology, widely discussed by specialists. Of the greatest importance in this discussion is assessment of possible change in genetic material of human somatic cells in long dates after irradiation. Some data, mostly obtained *in vitro*, indicate a possibility of genome instability occurrence in descendants of irradiated cells [1 – 3]. There are reasons to assume that this phenomenon also manifests in somatic human cells *in vivo* [4, 5]. The study of this problem is of special interest, both theoretical and applied, because changes in genetic material of somatic cells represent the main reason for malignant transformation, which causes carcinogenic effects of the irradiation impact [6, 7]. The overwhelming majority of human genome instability studies *in vivo* were performed with the help of structural mutation cytogenetic analysis. Gene mutations are studied much worse, despite the fact that they allow determination and quantitative assessment of small changes in genetic material. As shown by studies on genome instability in irradiated cell cultures [2, 3, 8], these changes dominate in the long dates after irradiation impact.

As shown before, mutant cell frequency by T-cell receptor locus increased in liquidators of the Chernobyl Accident 9 – 11 years after irradiation by doses up to 0.25 Gy compared with the control, e.g. non-irradiated people [9]. This work is devoted to further study of regularities and mechanisms of radiation mutagenesis in various somatic cells in long dates after irradiation. In particular, basing on literary data on different efficiency of DNA damage repair and the level of apoptotic death in high and low differentiated human cells [10, 11], one may assume that the quantity of radiation-induced changes of genetic material in these cells is also different. Here this suggestion is checked by comparative analysis of gene mutations by TCR- and GPA-loci which, as commonly supposed, occur in end-stage lymphocytes and hemopoietic stem cells, respectively. Moreover, of special interest was the stability assessment of previously observed changes by TCR-locus. According to common ideas on the radiation mutagenesis, the increased frequency of mutant cells in long dates after irradiation may be induced by high mutation frequency due to genome

instability occurred in descendants of irradiated cells and the elimination mechanism break for cells with damaged DNA. Actuality of the second explanation is currently studied. For this purpose, first, the efficiency of damaged cell elimination is studied using apoptosis in liquidators of the Chernobyl Accident and control group. Secondly, this parameter was compared with TCR-mutant cell frequency in the same patients.

MATERIALS AND METHODS

People were examined, who participated in liquidation of the Chernobyl Accident in 1986 – 1988 and irradiated by doses up to 0.25 Gy (according to data of Shelter radiation monitoring service). The average irradiation dose equaled 0.13 Gy. The test target was peripheral blood samples of the mentioned patients. In accident liquidators the frequency of cells with mutations by GPA- and TCR-loci and apoptotic death of lymphocytes, induced by γ -irradiation *in vitro* in various doses, were studied. In some patients the mentioned parameters were determined simultaneously. Blood samples from people of the same age having no registered contact with genotoxic impacts, including ionizing radiations, were taken for control.

Gene mutation determination by TCR-locus

The TCR-method is based on the use of monoclonal antibodies, labeled by different fluorochromes, to CD3- and CD4-antigens. It is common knowledge that T-cell receptor and CD3-antigene complex is expressed on the surface of T-lymphocytes. TCR-genes are functionally hemizygous. Therefore, products of one allele only are present on the surface of lymphocytes. Mutation in functioning allele causes the absence of CD3 complex expression on T-lymphocyte surface. Mutant cell frequency is determined with the help of flow cytometry as the relation between numbers of CD3-CD4+ and CD3+CD4+ phenotype cells. In this work, modified technique by Kyoizumi S. *et al.* [12] was used. See also [13].

In the period of 1995 – 2003 (e.g. 9 – 17 years after the irradiation impact) 184 liquidators of the Chernobyl Accident and 126 persons of similar age from the control group were examined using the TCR-technique.

Gene mutation determination by GPA-locus

Only blood samples from donors heterozygous by this locus (about 50% of the population) are suitable for the analysis. Both allele forms of glycophorin A (M and N) are represented on the surface of heterozygous donor erythrocytes. At the present time, methods for obtaining antibodies to the mentioned glycophorin A forms and their further detecting with the help of fluorescent dyes are developed. It is commonly assumed that mutation in genes, coding glycophorin A, causes loss of corresponding glycoprotein on the surface of erythrocytes, if the appropriate mutation has happened in low-differentiated erythrocyte precursor cells prior to glycophorin expression. If N-allele mutates, only glycophorin M will be observed on the cell surface and vice versa. The flow cytometry analysis allows identification of erythrocytes with lost ability to bind one of antibodies in the appropriate gene allele due to mutation. This is the way to assess frequency of variant/mutant cells. The so-called BR-6 method was used in the current study. It allows determination of NO-variant erythrocyte frequency as a consequence of glycophorin A (M-form) expression loss. Refer to [13, 14] for detail description of the technique.

In the period of 1994 – 2003 the frequency of GPA (NO)-variant erythrocytes was detected in 71 liquidators of the Chernobyl Accident and 33 controls of similar age.

Determination of lymphocytes apoptotic death after irradiation impact in vitro

The method is based on the occurrence of fragmented DNA in cells subject to apoptotic death. This DNA is extracted from the cells by HCl solution. Then DNA remaining in the cells is dyed by propidium iodide and its amount per cell is determined. Apoptotic death of cells is assessed by the part of cells with hypodiploid DNA content [15].

In this work, lymphocytes extracted by centrifuging were cultivated in RPMI-1640 complete medium (Paneko, Russia) during 24 hours and then exposed to γ -irradiation from ^{60}Co at different doses (0.5 – 5 Gy) with 0.5 Gy/min dose rate. After that they were cultivated more 24 h, fixed in 70% ethanol, treated by 0.6M HCl, and dyed by propidium iodide (Sigma, USA) at 50 $\mu\text{g/ml}$ final concentration. Using flow cytometry DNA concentration per cell was analyzed and the part of cells without apoptotic death signs was determined.

The linear regression equation

$$\ln(P) = a + bD$$

(where D is the dose, Gy; P is the part of cells without apoptosis signs at irradiation by the appropriate dose; a and b are regression coefficients determined by the least-square method) was calculated by the plot of this parameter dependence on the irradiation dose. Coefficient b characterizes the ability of irradiation to cause apoptosis of lymphocytes 24 h after incubation in a substrate. Linear regression parameters were calculated for each of 38 examined liquidator and 24 persons from the control group.

Statistics

Statistical analysis was implemented by Origin software (MicroCal Software, USA). Studied indices of somatic mutagenesis in groups of examined people were compared by Student and Mann-Whitney t -criteria. TCR-mutant lymphocyte frequency and apoptotic cell death were compared by Fischer criterion. Differences were accepted statistically valuable at $p < 0.05$.

RESULTS AND DISCUSSION

The frequency of cells with mutations by TCR-locus is determined in liquidators of the Chernobyl Accident 9 – 17 years after irradiation impact. The average frequency (\pm SD) of TCR-mutant cells equaled: $(4.8 \pm 2.9) \times 10^{-4}$ in the liquidator group and $(3.8 \pm 1.4) \times 10^{-4}$ in the control group (Figure 1). Calculations implemented by Mann-Whitney and Student criteria testify about statistically valuable increase of TCR-mutant cell frequency in the liquidator group compared with the control group ($p < 0.01$). Among 184 liquidators, in 31 persons (16.8%) the increased TCR-mutant cell frequency was detected. It exceeded 95% confidence interval established for current control group, i.e. was above 6.6×10^{-4} . If people possessing increased frequencies are excluded from the liquidator and control groups, the average TCR-mutant cell frequency equals $(3.8 \pm 1.2) \times 10^{-4}$ and $(3.6 \pm 1.3) \times 10^{-4}$, respectively ($p > 0.05$ by any criterion). Consequently, the increase of TCR-mutant cell frequency in general liquidator

group is explained by the presence of patients with high values of this parameter.

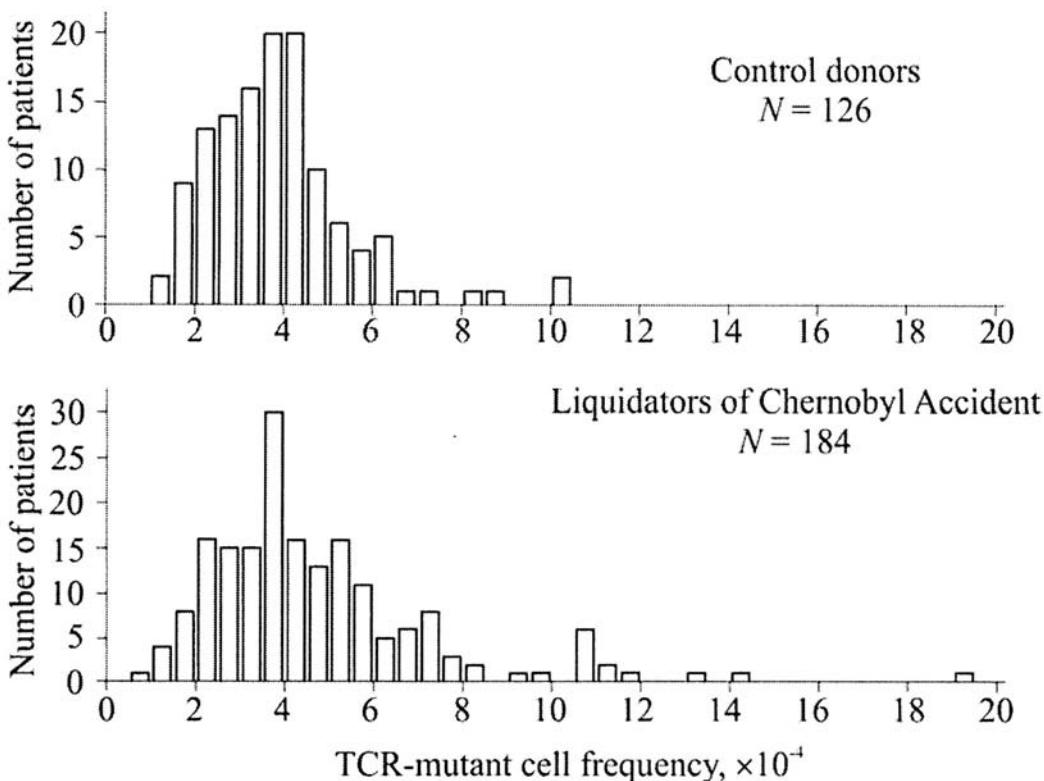


Figure 1. TCR-mutant cell frequency distribution in liquidators and in control

Further on, stability of results obtained in time was analyzed using two approaches. Firstly, TCR-mutant cell frequency in liquidator subgroups examined 9 – 11, 12 – 14 and 15 – 17 years after irradiation impact was compared with the control. It is found that the average frequency of mutant cells in these subgroups is 1.4, 1.3 and 1.4 times higher, respectively, than in control groups of similar aged people examined in the same periods, i.e. in 1995 – 1997, 1998 – 2000 and 2001 – 2003. The number of patients with increased TCR-mutant cell frequencies was almost the same for liquidator subgroups, examined in different period after irradiation: 16.8, 15.3 and 21.6%, respectively.

Secondly, possible change of individual TCR-mutant cell frequency was analyzed by the results of multiple examinations of the same people during 9

years. It is found that the most of liquidators possess unchanged or slightly increased frequency of mutant cells (Figure 2).

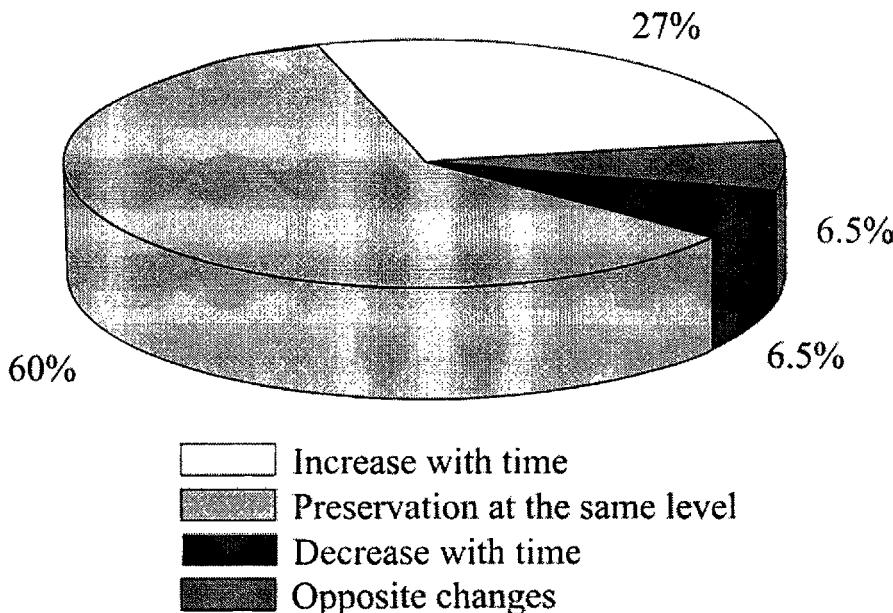


Figure 2. Multiple study results of TCR-mutant cell frequency in liquidators: distribution of examined patients by type of changes in studied parameter of somatic mutagenesis

Therefore, analysis of possible changes in TCR-mutant cell frequency, implemented by two methods, in liquidators of the Chernobyl Accident with time after the impact shows stability of the studied parameter of somatic mutagenesis during 9 – 17 years after irradiation.

The absence of monotonic dependence of TCR-mutant cell frequency on the irradiation dose of liquidators was also found (Figure 3).

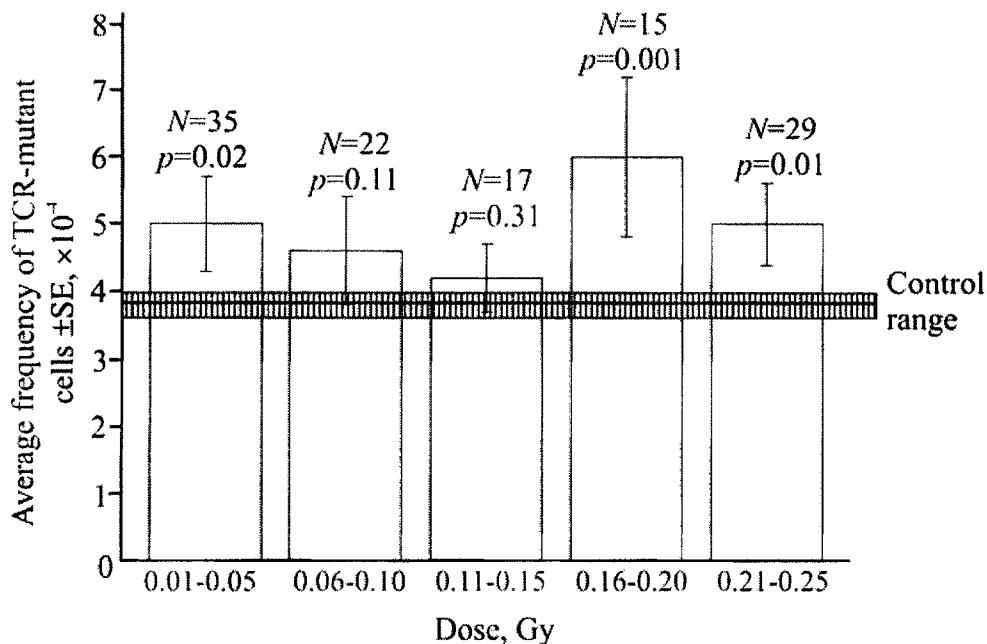


Figure 3. The average frequency of TCR-mutant cells in liquidator subgroups irradiated by different doses

From the authors' point of view, the results of TCR-examination of liquidators (many years after the irradiation impact) may be unambiguously interpreted. Taking into account:

- 1) the features of TCR-mutation formation, detected with the help of the method used, in lymphocyte population after their differentiation in thymus [16];
- 2) data on quite rapid elimination of cells with radiation-induces TCR-mutations as a consequence of natural regeneration of end-state lymphocyte population [17, 18];
- 3) time since irradiation (9 – 17 years);
- 4) nonconformity of extremely high frequency of mutant cells determined in some liquidators to the irradiation dose;

one may conclude that TCR-mutant cell frequency increase in liquidators was not direct consequence of irradiation impact on lymphocytes. Apparently, the observed TCR-mutant cells were formed *de novo* in long dates after irradiation and were caused by genome instability occurred in irradiated cell descendants.

The average (\pm SE) frequency of mutant cells by GPA-locus in liquidators was statistically similar to the control group: $(21.3 \pm 2.8) \times 10^{-6}$ and $(18.8 \pm 2.7) \times 10^{-6}$, respectively. Similar results were obtained by collectives of scientists in the USA, Russia and Baltic states [19, 20]. The frequency of mutant cells was determined by GPA- and TCR-loci simultaneously in 16 liquidators. The absence of correlation in data of two methods ($r = 0.03$) was also found. It is believed that mutations detected by the GPA-method are formed in stem cells and they are able to preserve in the organism for a long time (over 40 years) [21, 22]. Therefore, one may suggest that the number of mutations induced directly by the irradiation impact during the accident liquidation is apparently low. In this connection, the frequency of mutant cells by GPA-locus equals to the control results. Nonconformity between data of two methods also allows a suggestion that processes increasing TCR-mutant cell frequency happen in long dates after irradiation and do not affect stem cells. Apparently, the observed selective effect of irradiation on different cell populations may be explained (though partly) by different efficiency of systems supporting genetic stability, including DNA damage repair, apoptosis induction, etc., in cells with different differentiation level [10, 11, 23, 24].

There are data on the lymphocyte apoptosis level, radiation-induced *in vitro*, in 38 liquidators of the Chernobyl Accident and 24 control persons (Table 1). The average value of coefficient b , which reflects efficiency of damaged cell apoptotic elimination, in the liquidator group was statistically similar to that of the control group ($p = 0.2$). Then coefficient b was compared with TCR-mutant cell frequency in liquidators. These patients were divided into two groups with respect to coefficient b value. The comparison shows that 5 of 7 liquidators with the lowest b value have increased frequencies of mutant cells (Table 2). Increased frequencies of mutant cells are statistically more frequently observed in people with low cell apoptosis level in response to radiation damage rather than in people with higher apoptosis. Thus, current results reflect general biological regularities and conform to the ideas about importance of apoptosis as the mechanism supporting genetic stability of cell populations. At the same time, it should be noted that only 5 of 12 liquidators with high TCR-mutant cell frequency possess low apoptosis level. Thus, for the most of liquidators the increase of lymphocyte frequency with mentioned gene mutations is not connected to a decrease of damaged cell apoptotic elimination. It may be caused by other reasons, which is again proved by the conclusion about genetic instability induction in these cells.

Table 1

Coefficient *b* values in liquidators and in control

Examined patients	Number of patients	Coefficient $b \times 10^{-2}$ range	Average (\pm SD) coefficient $b \times 10^{-2}$ value	<i>p</i>
Liquidator group	38	0.1 – 23.0	6.9 ± 4.4	0.2
Control group	24	4.1 – 20.0	8.2 ± 3.2	

Table 2

Comparison frequency of TCR-mutant cells and apoptotic death of lymphocytes, radiation-induced *in vitro*, in liquidators

Coefficient $b \times 10^{-2}$ absolute value	Mutant cell frequency ($\times 10^{-4}$)		<i>p</i>
	over 7.0	below 7.0	
over 3.0	7	24	0.02
below 3.0	5	2	

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REFERENCES

1. Grosovsky A.J., Parks K.K., Giver C.R., and Nelson S., *Molecular and Cellular Biology*, 1996, vol. 16(11), pp. 6252 - 6262.
2. Little J.B., *Int. J. Radiat. Biol.*, 1998, vol. 74(6), pp. 663 - 671.
3. Harms- Ringdahl M., *Mutat. Res.*, 1998, vol. 404, pp. 27 - 33.
4. Vorobtsova I.E., 'The effect of parents' irradiation on physiological full value and risks of carcinogenesis in first generation descendants of organisms of different species', *Doctor Thesis on Biology*, 1988, Leningrad, Central Research X-ray and Radiological Institute. (Rus)
5. Vorobtsova I.E., Vorob'eva M.V., Bogomazova A.N. et al., *Radiats. Biologija. Radioekologija*, 1995, vol. 35(5), pp. 630 - 635. (Rus)
6. Hagmar L., Brogger A., Hansteen I.L. et al., *Cancer Research*, 1994, vol. 54, pp. 2919 - 2922.

7. Ullrich R.L. and Ponnaiya B., *Int. J. Radiat. Biol.*, 1998, vol. **74**(6), pp. 747 - 754.
8. Pelevina I.I., Gotlieb V.Ya., Kudryashova O.V. et al., *Radiats. Biologija. Radioekologija*, 1996, vol. **36**(4), pp. 546 - 560. (Rus)
9. Saenko A.S., Zamulaeva I.A., Smirnova S.G. et al., *Radiats. Biologija. Radioekologija*, 1998, vol. **38**(2), pp. 181 - 185. (Rus)
10. Buschfort-Papewalis C., Moritz T., Liedert B., and Thomale J., *Blood*, 2002, vol. **100**(3), pp. 845 - 853.
11. Josefsen D., Myklebust J.H., Lynch D.H. et al., *Exp. Hematology*, 1999, vol. **27**(9), pp. 1451 - 1459.
12. Kyoizumi S., Umeki S., Akiyama M. et al., *Mutat. Res.*, 1992, vol. **265**, pp. 173 - 180.
13. Saenko A.S., Zamulaeva I.A., Smirnova S.G. et al., *Radiats. Biologija. Radioekologija*, 1998, vol. **38**(2), pp. 171 - 180. (Rus)
14. Langlois R.G., Nisbet B.A., Bigbee W.L. et al., *Cytometry*, 1990, vol. **11**, pp. 513 - 521.
15. Orlova N.V., Smirnova S.G., Zamulaeva I.A. et al., *Radiats. Biologija. Radioekologija*, 2001, vol. **41**(4), pp. 366 - 372. (Rus)
16. Kyoizumi S., Akiyama M., Hirai Y. et al., *J. Exp. Med.*, 1990, vol. **171**, pp. 1981 - 1999.
17. Akiyama M., Kusunoki Y., Umeki S. et al., *Radiation Research: A Twentieth-Century Perspective. Vol. II: Congress Proceedings*, Ed. Dewey W.C. et al., Academic Press, 1992, pp. 177 - 182.
18. Umeki S., Kusunoki Y., Endo K. et al., *Proc. Int. Conf. Rad. Effects and Protection*, Mito, Japan, Japan Atomic Energy Research Institute, Tokyo, 1992, pp. 151 - 154.
19. Jones I.M., Galick H., Kato P. et al., *Radiat. Res.*, 2002, vol. **158**(4), pp. 424 - 442.
20. Bigbee W.L., Jensen R.H., Veidebaum T et al., *Radiat. Res.*, 1997, vol. **147**, pp. 215 - 224.
21. Saenko A.S. and Zamulaeva I.A., *Radiats. Biologija. Radioekologija*, 2000, vol. **40**(5), pp. 549 - 553. (Rus)
22. Nakamura N., Umeki S., Hirai Y. et al., *New Horizons In Biological Dosimetry*, 1991, Wiley- Liess, pp. 341 - 350.
23. Kolesnikova A.I., Konoplyannikov A.G., and Hendry J.H., *Radiat. Res.*, 1995, vol. **144**(3), pp. 342 - 345.
24. Myllyperkio M.H. and Vilpo J.A., *Mutat. Res.*, 1999, vol. **425**(1), pp. 169 - 176.