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Cytogenetic Changes in the Liver of Progeny of Irradiated Male Rats

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Radiation / Cytogenetic and nucleic acid changes / Transgenerational transmission

The transgenerational transmission of radiation damage of rat genom was studied on the basis of cytogenetic changes in somatic cells (hepatocytes). It was found, that the irradiation of rat males with dose of 3 Gy of gamma radiation caused latent cytogenetic damage to the liver, which was expressed during the course of an induced proliferation of hepatocytes (by partial hepatectomy) by lower proliferative activity and a high frequency of chromosomal aberrations. In the progeny of irradiated males (in the F_1 generation), the radiation damage to DNA was manifested by similar changes, i.e. by lower proliferation activity and increase in "spontaneus" chromosomal aberration occurence in liver regeneration after partial hepatectomy. Irradiating the progeny of irradiated males (the total radiation load of the progeny being 3 Gy + 3 Gy) caused slighter changes in compared with irradiating the progeny of non-irradiated control males (the total radiation load of the progeny being 0 Gy + 3 Gy), which suggests some kind of adaptive response, which was also found in other experimental systems and parameters. An analogous course of RNA and DNA quantitative changes in the liver of the F_0 and F_1 generations of rats confirms the partial transmission of radiation damage of genom to the progeny.

INTRODUCTION

In association with a continual increase in the environmental radiation load, mainly as a consequence of long-lasting radioactive fall out coming from the testing of nuclear weapons and from accidents of nuclear fittings in different parts of the world, the question of the transmission of radiation-induced genetic damage to the next generations has again becomes very real.

Some papers concerning in vitro studies indicate the mechanism, by which radiation induces the heredity of genome instability at the cellular level. Genome instability can be expressed in the forms of both clonal and non-clonal (arising *de novo*) chromosomal aberrations. After the exposure of murine bone-marrow stem cells to α-particles (^{235}Pu), Kadhim *et al.* observed a high incidence of colonies of primitive haematopoietic precursor cells containing non-clonal chromosomal aberrations[1)]. The high frequency of different, i.e. non-clonal chromosomal aberrations arising *de novo* in the same colony suggests the transmission of chromosomal instability from α-irradiated stem cells to their progeny. The transmission of chromosomal instability in the case of mouse haematopoietic cells also persisted after 10-13 cell divisions[1)] and, in the case of irradiated human dermal fibroblasts, the instability persisted at least during 15–25 passages[2)]. Roy *et al.* studied X-ray-induced delayed cellular changes, such as cell death, giant cell formation and chromosomal aberrations in normal human embryo cultures to explore the relationship between initial radiation damage and delayed effect, which appeared at 14 to 55 population

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doubling numbers after X-irradiation[3]. Their results indicated that the potentiality of genetic instability was determined during the repair process of initial radiation damage and suggested that the mechanism for formation of delayed chromosomal aberrations by radiation might be different from that of direct radiation-induced chromosomal aberrations.

It was also found, that acute whole-body irradiation of mouse males of the F_0 generation with low doses of γ-radiation 6-7 weeks before mating caused a significant decrease in the proliferation of embryonal cells of the F_1 and F_2 generations[4]. The analysis of DNA isolated from the liver and spleen of rats of several generations permanently living in the Chernobyl region indicated an abnormally large quantity of low-molecular-weight DNA fractions. The amount of low-molecular fractions increased not only with the age of the rats but, also with successive generations of the animals[5]. These findings, and especially those of Dubrova *et al.*, regarding an increased instability of repeat-DNA sequences in descendants of unexposed F_1 offspring of irradiated parental mice, suggest that there could be an indirect effect of radiation on the soamatic genome stability, which is transmissible through the germ line of the irradiated parents[6].

In this study, we attempted to demonstrate the possibility of the transgenerational transmission of genome instability from irradiated (3 Gy of γ-radiation) male rats of the F_0 generation to the progeny of the F_1 generation. The genome instability was evaluated based on latent cytogenetic damage and nucleic acid changes in the liver.

Latent damage to the liver caused by various harmful stimuli is ascertainable in the case of inducing the majority of non-proliferating hepatocytes and litoral cells to divide.

The most frequent method for liver cell induction is resection of approximately 70% of the organ mass (partial hepatectomy). Such a great loss of liver parenchyma induces regenerating processes in the liver rest, which are associated with the transition of the cell majority from the G_0 phase into the G_1 phase of the cell cycle, followed by an increase in the DNA-synthetic and mitotic activity[7–9]. The first (and highest) wave of mitotic activity of hepatocytes after induction of proliferation achieves its maximum at the 30th hour after partial hepatectomy, the second and third waves of mitotic activity at the 48th and 72nd hour, respectively[10,11].

Latent liver damage, evoked in adult animals by preceding irradiation, manifests itself during the course of liver regeneration after partial hepatectomy by various biochemical and cytological changes, mainly by the inhibition of DNA-synthesis and mitotic activity and by an increase in the occurrence of chromosomal aberrations [12–14].

MATERIALS AND METHODS

Animals

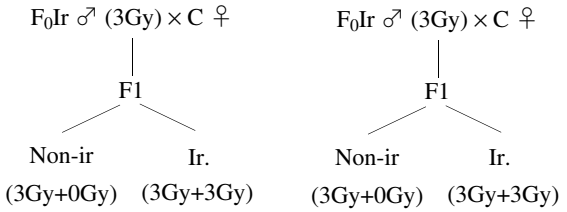
Male Wistar rats were used in the experiment. The age of the rats of the parental generation at the time of irradiation was 6 months; the age of the progeny at the time of the analysis was 3 months. The animals were kept under standard conditions (temperature of 22–24°C, natural light rhythm), while being fed and watered ad libitum. They were housed in cages, with 5–6 in each.

Description of experiment

Males of the parental generation were irradiated with a dose of 3 Gy of gamma radiation (F_0 3 Gy) 25 days before mating with non-irradiated control females (C ♀). The paternal exposure on day 25 before mating corresponds to the post-meiotic spermatids in rats[15].

One half of the offspring of these animals was irradiated with a single whole-body dose of 3 Gy, i.e. their total radiation load was 3 Gy (from irradiation of father) + 3 Gy. The second half of the offspring was not irradiated, i.e. their total radiation load was 3 Gy (from irradiation of father) (diagram – left side). The control group was presented by non-irradiated males (F_0 0 Gy), mated with non-irradiated control females (C ♀) and their progeny (F_1). One half of the offspring was irradiated, i.e. their total load was 0 Gy (after non-irradiated parents) + 3 Gy and the second half of offspring was not irradiated, i.e. with a total of zero radi-

ation load (diagram - right side).



The experiment was performed three times with the number of rats being:

group F_0 Ir♂ (3 Gy) – 9 males,
group F_0 C♂ (0 Gy) – 11 males,
group F_1 (3 Gy + 0 Gy) – 14 males,
group F_1 (3 Gy + 3 Gy) – 15 males,
group F_1 (0 Gy + 0 Gy) – 11 males,
group F_1 (0 Gy + 3 Gy) – 13 males.

Irradiation

Rats were irradiated with a single whole-body dose of 3 Gy by gamma rays from a ^{60}Co source (apparatus Chisostat, Chirana, Czech Republic) at a dose rate of 0.229 Gy.min^{-1}. The males of the parental F_0 generation were irradiated 25 days before mating with intact control females. Their progeny, males of the F_1 generation, were irradiated with the same dose 30 minutes before a partial hepatectomy.

Partial hepatectomy

In the liver of animals in all groups, the cell proliferation was stimulated by partial hepatectomy by a standard procedure, in which the middle and left lateral lobes (amounting to 70–72% of the organ mass) were removed under light ether anaesthesia.

Cytogenetic analysis

The rats were examined at the 30th hour after the operation. A study of the rat-liver regeneration kinetics indicated that the first wave of DNA synthesis, induced by partial hepatectomy in the majority of the liver cells, was completed, and the mitotic activity reached its maximum in the remaining lobes of the regenerating liver at this time[10,11]. Squash preparations were prepared from the tissue of regenerating liver using the Feulgen method. Through examining about 40,000 cells in each experimental group, all of the mitotic figures and chromosomal aberrations (mainly chromosomal bridges) were recorded. Based on these data, the mitotic index (MI) (i.e. the number of mitotic figures per 1,000 registered cells), the ratio of metaphases to prophases (MP) (i.e. the relative proportion of dividing cells in the metaphase and prophase, which reflects the kinetics of cell division) and the frequency of chromosomal aberrations (expressed as % of cells containing the chromosomal aberrations) were calculated.

Determination of nucleic acids

Changes in the nucleic acids were studied in the regenerating liver tissue, which was excised 30 h after partial hepatectomy. Quantitative changes in the nucleic acids were determined by a method of Tsanev and Markov[16]. Tissue samples were homogenized in 5% trichloracetic acid, and then deproteinized and purified by consecutive washing with methanol, chloroform, benzene and ether. The separation of RNA and DNA was carried out by hydrolysis in alkaline and acidic media, respectively. The concentration of DNA and RNA in the purified hydrolysates was determined by spectrophotometric measurements (Hitachi 1031, Japan) at two wavelengths (DNA at 268 and 284 nm, RNA at 260 and 286 nm) and expressed as mg of RNA or DNA per g wet tissue. The content of RNA or DNA was determined by calculating the total weight of an organ.

The concentration and total content of DNA are integral indicators of tissue cellularity and organ cellularity, respectively. The concentration and total content of RNA reflect the relative proportion of cells with different nucleoplasmic ratios and different activities of RNA and protein synthesis.

Statistical analysis

The experimental cytogenetic data were statistically evaluated by a one way-analysis, and the experimental data of the nucleic acids were statistically evaluated by a Peritz´ F-test[17]. The results are given in the tables as the mean ± S.E.M.

RESULTS

Mitotic index (MI)

In the regenerating liver of non-irradiated control males of the parental generation (F_0 0 Gy), MI increased from 0.1‰ (non-demonstrated data for intact liver) to 16.3‰ as a consequence of stimulating cell proliferation by partial hepatectomy (Table 1, Fig. 1). The MI of the progeny of these control non-irradiated parents (gr. F_1 0 Gy + 0 Gy) was higher compared with the parents (18.2‰ vs. 16.3‰). The irradiation of siblings (gr. F_1 0 Gy + 3 Gy) caused a decrease in MI by 10‰, i.e. more than by one half, while irradiation of the males of the parental generation (F_0 3 Gy) caused MI the decrease by only 4‰. In the regenerating liver of an irradiated male progeny (gr. F_1 3 Gy + 0 Gy), the mitotic activity was lower compared to that of the progeny of non-irradiated males (gr. F_1 0 Gy + 0 Gy) (13.4‰ vs. 18.2‰), and approached the level of the values of the irradiated fathers (F_0 3 Gy) (12.4‰).

Table 1. Mitotic index and M/P ratio in the regenerating liver of the F_0 and F_1 generations of rats after irradiation with a dose of 3 Gy gamma radiation.

Parameter	F_0 generation	F_1 generation	
Mitotic index [‰]	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	16.33	18.25	8.25 ***
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	12.37••	13.40•••	10.78*•
M/P ratio	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	0.96	0.91	1.13
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	1.11	0.88	1.79

Statistical significance of difference:
irradiated progeny in comparison with non-irradiated progeny:
$P < 0.05$ = *; $P < 0.005$ = **; $P < 0.001$ = ***
irradiated fathers and their progeny in comparison with non-irradiated fathers and their progeny, respectively: $P < 0.05$ = •; $P < 0.005$ = ••; $P < 0.001$ = •••

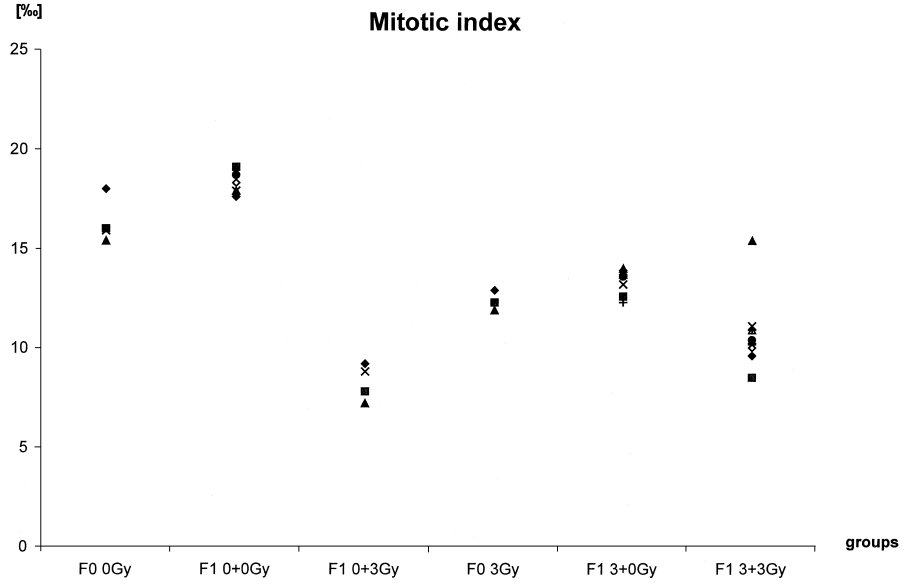


Fig. 1. Mitotic index (individual values) in the regenerating liver of the F_0 and F_1 generations of rats after irradiation with a dose of 3 Gy gamma radiation.
group F_0 Ir♂ (3 Gy) – irradiated parental males
group F_0 C♂ (0 Gy) – non-irradiated parental males
group F_1 (3 Gy + 0 Gy) – non-irradiated progeny of irradiated parental males
group F_1 (3 Gy + 3 Gy) – irradiated progeny of irradiated parental males
group F_1 (0 Gy + 0 Gy) – non-irradiated progeny of non-irradiated parental males
group F_1 (0 Gy + 3 Gy) – irradiated progeny of non-irradiated parental males

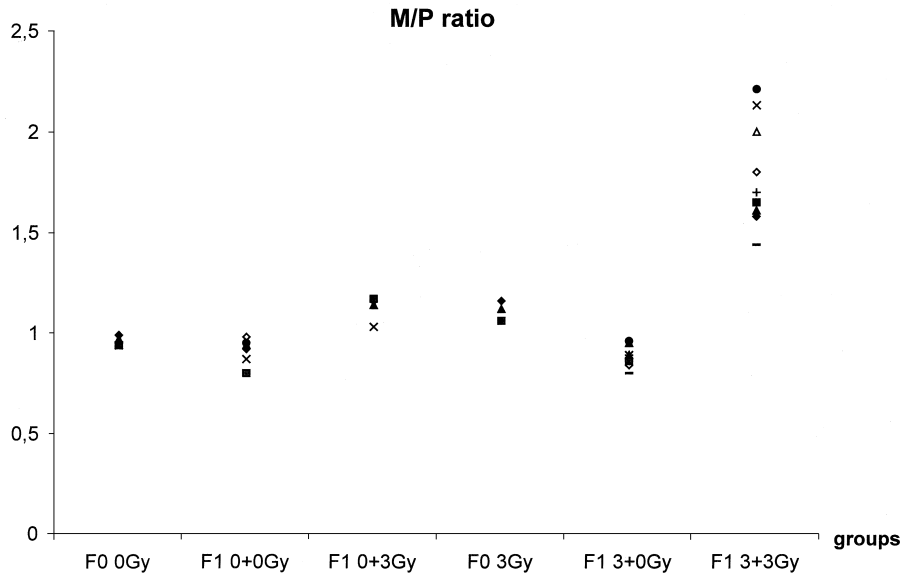


Fig. 2. M/P ratio (individual values) in the regenerating liver of the F_0 and F_1 generations of rats after irradiation with a dose of 3 Gy gamma radiation.
For an explanation see Fig.1.

The irradiation of siblings (gr. F_1 3 Gy + 3 Gy) caused a lower decrease in MI in the progeny of the irradiated males compared with irradiation of the progeny of non-irradiated males (gr. F_1 0 Gy + 3 Gy).

Ratio of the metaphase to the prophase numbers (MP)

In the regenerating liver of non-irradiated control males of the F_0 generation, the MP ratio was near to 1, what indicates similar numbers of the prophases and metaphases among the dividing liver cells (Table 1, Fig. 2). In the non-irradiated progeny of the control and irradiated males (gr. F_1 0 Gy + 0 Gy and F_1 3 Gy + 0 Gy) the MP ratio was slightly decreased. The single irradiation of male rats of the parental generation and the progeny of the control parents (F_0 3 Gy and F_1 0 Gy + 3 Gy) caused an increase in the MP ratio to a value of 1.1. In the irradiated progeny of the irradiated male rats (gr. F_1 3 Gy + 3 Gy), the MP ratio was increased to 1.8. The increase in the MP ratio may have been caused by prolongation of the metaphase duration or by the inhibition of the cell entrance into the prophase in the irradiated animals.

Chromosomal aberrations

Regarding to the high degree of hepatocyte polyploidia in adult rats, more detailed determination of chromosomal aberrations could not be performed[18]. Chromosomal bridges in post-metaphase figures were the only unambiquously detectable chromosomal aberrations.

In the regenerating liver of non-irradiated animals of the F_0 and F_1 generations (F_0 0 Gy and gr. F_1 0 Gy + 0 Gy), no chromosomal aberrations were found. Single irradiation of the males of the parental generation (F_0 3 Gy) resulted in an increase in the number of chromosomal aberrations, mainly chromosomal bridges, to the same extent as that due to irradiation of the progeny of the control males (gr. F_1 0 Gy + 3 Gy), that is by 37% (Table 2, Fig. 3). In the regenerating liver of the progeny of the irradiated males (gr. F_1 3 Gy + 0 Gy), in contrast to the progeny of the control males

Table 2. Frequency of chromosomal aberrations in the regenerating liver of the F_0 and F_1 generations of rats after irradiation with a dose of 3 Gy gamma radiation.

Parameter	F_0 generation	F_1 generation	
Frequency of chromosomal aberrations [%]	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	0	0	36.9 *
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	37.2$^{\bullet}$	16.4$^{\bullet\bullet}$	31.2***$^{\bullet}$

The notes are recorded under table 1.

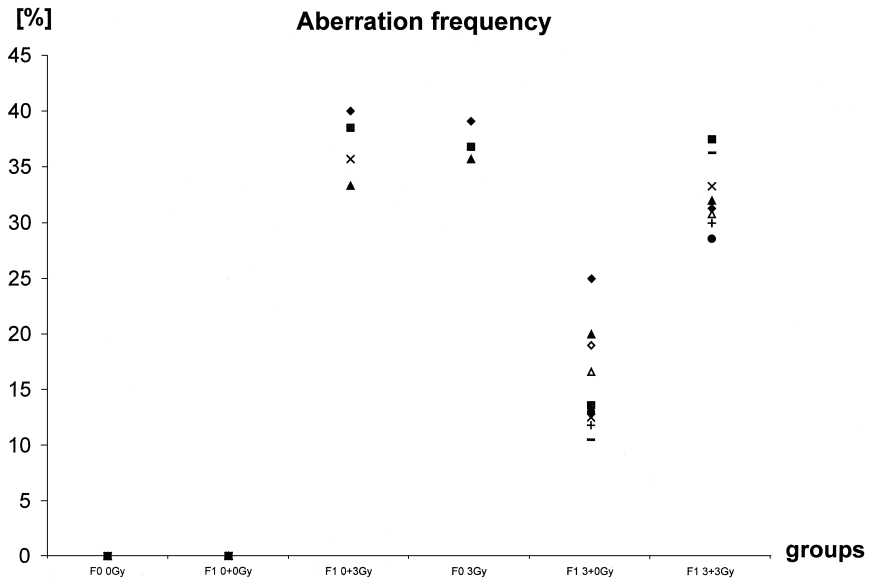


Fig. 3. Frequency of chromosomal aberrations (individual values) in the regenerating liver of the F_0 and F_1 generations of rats after irradiation with a dose of 3 Gy gamma radiation.
For an explanation see Fig. 1.

(gr. F_1 0 Gy + 0 Gy), chromosomal aberrations were noted in 16.4% of the dividing cells, which represents almost one half of the number of chromosomal aberrations recorded in the irradiated fathers (F_0 3 Gy – 37.2%). The irradiation of siblings coming from the same litters (gr. F_1 3 Gy + 3 Gy) in our experiment caused an increase in the chromosomal aberrations from 16.4% to 31.2%. Thus, in the F_1 generation of rats with a total radiation load of 3 Gy + 3 Gy, the total number of chromosomal aberrations was lower (31.2%) compared to that of the F_1 generation with a smaller total radiation load (36.9% in gr. F_1 0 Gy + 3 Gy).

Nucleic acids

The irradiation did not influence the RNA concentration in the regenerating liver of all groups of the F_0 and F_1 generations and the total RNA content was significantly decreased only in the irradiated parental males (gr. F_0 3 Gy) and their non-irradiated progeny (gr. F_1 3 Gy + 0 Gy) (Table 3). In the progeny of the irradiated males (gr. F_1 3 Gy + 0 Gy), the total RNA content was significantly decreased as compared to that of the progeny of the control non-irradiated males (gr. F_1 0 Gy + 0 Gy). After irradiating the progeny of the irradiated males (gr. F_1 3 Gy + 3 Gy), the RNA

Table 3. Concentration and content of RNA and DNA in the regenerating liver of the F_0 and F_1 generations of rats after irradiation with a dose of 3 Gy gamma radiation.

Parameter	F0 generation	F1 generation	
RNA concentration [mg/g]	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	9.92±0.71	10.10±0.96	10.89±1.42
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	9.40±0.29	10.15±0.29	9.87±0.65
RNA content [mg/org.]	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	53.6±3.8	49.41±5.13	45.63±4.32
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	44.89±0.80$^{\bullet}$	35.10±2.43$^{\times\bullet}$	46.44±4.05*
DNA concentration [mg/g]	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	1.26±0.09	1.16±0.06	1.23± 0.05
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	1.14±0.04	1.19±0.05	1.41±0.09
DNA content [mg/org.]	non-ir (0 Gy)	non-ir (0 Gy+0 Gy)	ir (0 Gy+3 Gy)
	6.80±0.47	5.52±0.24	5.04±0.24
	ir (3 Gy)	non-ir (3 Gy+0 Gy)	ir (3 Gy+3 Gy)
	5.51±0.20$^{\bullet}$	4.08±0.21$^{\times\bullet}$	6.71±0.52$^{**\bullet}$

The notes are recorded under table 1.

content was decreased to the same level as in the progeny of the control males (gr. F_1 0 Gy + 3 Gy).

The quantitative changes of DNA in all experimental groups were similar to those of the RNA (Table 3).

DISCUSSION

The presented results show that in the rat liver regenerating after partial hepatectomy, cytogenetic changes were found, which could not be detectable in the intact liver due to its low proliferation activity.

The higher mitotic activity, which was observed in the regenerating liver of the progeny of the non-irradiated control males compared with the parents (gr. F_1 0 Gy + 0 Gy vs. F_0 0 Gy – MI 18.2‰ and 16.3‰, respectively), was associated with their lower age at the time of the examination (3 months old progeny and 7 months old parents) because, in general, the regenerating processes proceed faster in young animals compared with that of older ones. On the other hand, the more profound decrease in MI in the regenerating liver of irradiated siblings of these progeny compared with that of the irradiated males of the parental generation (gr. F_1 0 Gy + 3 Gy vs. F_0 3 Gy – MI 8.2‰ and 12.4‰, respectively) is caused by a significantly higher inhibitional effect of radiation on cell proliferation in the liver of young individuals compared to that of the older ones.

The mitotic activity decrease in the regenerating liver of the progeny of the irradiated males compared with that of the progeny of the non-irradiated males (gr. F_1 3 Gy + 0 Gy vs. F_1 0 Gy + 0 Gy – MI 13.4‰ and 18.2‰, respectively) is remarkable. It indicates the transmission of some genetic damage, leading to the inhibition of cell proliferation and/or G_0/G_1 transit (stimulated by partial hepatectomy) from irradiated males to their progeny. An increased chromosomal aberration frequency in the non-irradiated progeny of the irradiated parental males compared with the non-irradiated progeny of the non-irradiated control males (gr. F_1 3 Gy + 0 Gy vs. F_1 0 Gy + 0 Gy –frequency of aberrations 16.4% and 0%, respectively) confirms an indirect effect of the radiation on the somatic genome stability, which is transmissible through the germ line from the irradiated parents[6,19], and manifests itself by an increase in the chromosomal aberration frequency in the progeny. Luke *et al.*, who investigated a transgenerational transfer of radiation-induced genome instability of haemopoietic cells of mice, came to similar conclusions[15]. They found, that increasing the dose (0.1–4 Gy) of gamma irradiation, applied to the males of the parental generation before mating, also increased the frequency of mutations in bone-marrow cells in the F_1 generation of mice.

Less prominent changes in the mitotic activity and chromosomal aberration frequency in the regenerating liver of the irradiated progeny of the irradiated parental males compared with the irradiated progeny of the non-irradiated control parental males (gr. F_1 3 Gy + 3 Gy vs. F_1 0 Gy + 3 Gy – MI 10.8‰ and 8.2‰ chromosomal aberrations 31.2% and 36.9%, respectively) indicates the posibility of a adaptive response to radiation, which may be a consequence of e.g. a more effective repair of chromatin damage in the progeny of irradiated males. On the contrary, Vorobtsova found, that the sensitivity of chromosomes in the liver and bone marrow cells to genotoxic factors was increased in the progeny of males irradiated with a dose of 4.5 Gy compared with the progeny of non-irradiated males, what rather indicates some kind of hypersensitivity of the irradiated male progeny to the influence of other genotoxic factors[20]. It is possible that this difference in the response of the progeny of the F_1 generation to radiation is associated with different doses and different times between the irradiation and mating of males of the F_0 generation in Vorobtsova´s[20] and our experiments.

Scatter plots of individual values showed that changes in all of the cytogenetic parameters varied over a wide range only in the irradiated progeny of irradiated males (gr. F_1 3 Gy + 3 Gy). As for the chromosomal aberration frequency, a wide range of values was also typical for the non-irradiated progeny of irradiated males (gr. F_1 3 Gy + 0 Gy). Widening of the value ranges in gr. F_1 3 Gy + 3 Gy and gr. F_1 3 Gy + 0 Gy, respectively, suggests an increase in the interindi-

vidual variability in the progeny of irradiated parental males in comparison with the progeny of non-irradiated parental males (gr. F_1 3 Gy + 0 Gy and F_1 3 Gy + 3 Gy vs. gr. F_1 0 Gy + 0 Gy and F_1 0 Gy + 3 Gy), presumably as a result of increased genome instability.

The dramatic radiation-induced changes in the cytogenetic parameters did not show any corresponding reflection in the process of liver regeneration after partial hepatectomy, because the changes in the nucleic acid contents were much slighter. This seeming disproportion results from the fact that cytogenetic indicators may provide information that is distorted in some manner. For example, cells with chromosomal aberrations (e.g. chromosomal bridges) persist in the post-metaphase for a much longer time (as long as they are eliminated) than undamaged cells, which quickly pass through anaphase and telophase. Hence, in a given time period a higher number of aberrant cells occurs that which really occurred. From this point of view, changes in the total DNA content are exceedingly important, because the DNA content is an integral biochemical indicator of organ cellularity reflecting also the result of preceding processe[21,22].

Regardless of this fact, the changes of DNA content and other biochemical parameters showed a course similar to the changes in the cytogenetic parameters, which confirmed the transmission of part of the genome radiation damage from irradiated males of the parental generation to the progeny. However, the quantitative changes of the nucleic acids in irradiated rats of the F_0 and F_1 generations were slighter than the changes in mitotic activity and the chromosomal aberration frequency.

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REFERENCES

1. Kadhim, M. A., Macdonald, D. A., Goodhead, D. T., Lorimore, S. A., Marsden, S. J. and Wright, E. G. (1992) Transmission of chromosomal instability after plutonium α-particle irradiation. Nature **355**: 738–740.
2. Sabatier, L., Dutrillaux, B. and Martin. M. B. (1992) Chromosomal instability. Nature **357**: 548.
3. Roy, K., Kodama, S., Suzuki, K., Watanabe, M. (1999) Delayed Cell Death, Giant Cell Formation and Chromosome Instability Induced by X-irradiation in Human Embryo Cell. J. Radiat. Res. **40**: 311–322.
4. Wiley, L. M., Baulch, J. E., Raabe, O. G. and Straume, T. (1997) Impaired cell proliferation in mice that persists across at least two generations after paternal irradiation. Radiation Research **148**: 145–151.
5. Blagoi, Yu. P., Kornilova, S. V., Leont´ev, V. S., Sorokin, V. A., Gladchenko, G. O., Valayev, V. A., Grigor´ev, D. N., Kapinos, L. Ye., Bondarenko, V. N. and Kolod, V. Ya. (1994) Structural and physicochemical characteristics of DNA of animal tissue exposed to prolonged chronic irradiation in the Chernobyl zone. Biophysics **39**: 641–649.
6. Dubrova, Y. E., Plumb, M., Gutierrez, B., Boulton, E. and Jeffreys, A. J. (2000) Transgenerational mutation by radiation. Nature **405**: 37.
7. Barbason, H., Rassenfosse, C. and Betz, E. H. (1983) Promotion mechanism of phenobarbital and partial hepatectomy in DENA hepatocarcinogenesis cell kinetics effect. Br. J. Cancer **47**: 517–525.
8. Michalopoulos, G. K. (1990) Liver regeneration: molecular mechanisms of growth control. Faseb. J. **4**: 176–187.
9. Kropáčová, K., Mišúrová, E. and Haková, H. (1998) Protective and therapeutic effect of silymarin on the development of latent liver damage, Radiacionnaya biologiya. Radioekologiya **38**: (3) 409–413.
10. Giliano, N.Ya. and Malinovskii O.V. (1984) The kinetic of cell proliferation in regenerating rat liver by using flow cytophotometry. Tsitologiya igenetika **4**: 256–259 (in Russian).
11. Mišúrová, E., Kriváňová, M., Kropáčová, K. and Fabiánová, B. (1987) Regeneration of liver after partial hepatectomy in X-ray irradiated rats. Radiobiol. Radiother. **28**: 59–68.
12. Albert, M. D. and Bucher, N. L. R. (1960) Latent injury and repair in rat liver induced to regenerate at intervals after X-radiation. Cancer Res. **20**: 1514–1523.
13. Kropáčová, K. and Mišúrová E. (1988) Persistence of latent damage in the liver after prolonged irradiation. Radiobiologiya **28**: (1) 44–47 (in Russian).
14. Bucher, N. L. R. (1991) Liver regeneration: an overview. J. Gastroenterol. Hepatol. **6**: 615–624.

15. Luke, G. A., Riches, A. C. and Bryant, P. E. (1997) Genomic instability in haematopoietic cells of F_1 generation mice of irradiated male parents. Mutagenesis **12**: 147–152.
16. Tsanev, R. and Markov, G. (1960) To the question of quantitative spectrophotometrical estimation of nucleic acid. Biokhimiya **25**: 151–159 (in Russian).
17. Harper, J. (1984) Basic program of a robust multiple comparison test for statistical analysis of all differences among group means. Comput. Biol. Med. **14**: 437–445.
18. Fabianová, B., Mišúrová, E. and Kropáčová, K. (1981) Effect of X-irradiation on the nuclear DNA content and size of the nuclei in the regenerating rat liver. Bull. Experiment. Biol. Med. **91**: 490–492.
19. Morgan, W. F., Day, J. P., Kaplan, M. I., McGhee, E. M. and Limoli, C. L. (1996) Genomic instability induced by ionizing radiation. Radiat. Res. **146**: 247–258.
20. Vorobtsova, I. E. (2000) Irradiation of male rats increases the chromosomal sensitivity of progeny to genotoxic agents. Mutagenesis **15**: 33–38.
21. Beran, M., Jonasson, G., Sundius, G., and Tribukait, B. (1971) Quantitative DNA-measurements for determination of bone marrow and spleen cell numbers after irradiation. Acta Radiol. Suppl (Stockh) **310**: 57–67.
22. Fiszer-Szafarz, B. and Szafarz, D. (1984) DNA and protein content as cellular biochemical parameters. Analyt. Biochem. **138**: 255–258.

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