Results of Long-term Genetic Monitoring of Animal Populations Chronically Irradiated in the Radiocontaminated Areas

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Introduction

The artificial geochemical land where all organisms lived and will live under the conditions of increased level of radiation was set up due to the Chernobyl disaster in 1986. An urgent necessity for studying the various biological effects of chronic influence of low intensity radiation on both individual organisms and populations arose.

Combined cytogenetic and radioecological investigations in wild populations of terrestrial small mammals (bank vole = *Clethrionomys glareolus, Schreber* and yellow-necked mouse = *Apodemus flavicollus, Melchior*) and in laboratory mice have been carried out by our laboratory since 1986. Our test organisms have contacted closely with low intensity radiation in the radiocontaminated areas of Belarus and absorbed low whole-body dose.

We study the following problems: dynamics of radionuclide concentration in wild populations of small rodents; dynamics of mutation process in somatic and germ cells over many generations as well as embryonal lethality; dynamics of population density, age and sex structure of mammalian populations. The large part of results obtained are presented here.

Materials and methods

Wild populations of small mammals (rodents)

Bank voles and yellow-necked mouse were collected in summer-autumn period from four forest populations in sites chosen for long-term monitoring with limited people activities and differing ^{137}Cs contamination density of soil:

1. - Priluksky reserve (near Minsk, 330 km north-west from the Chernobyl power station, 8 kBq/m^2);
2. - Berezinsky Biosphere reserve (Vitebsk region, 400 km NNW, 18 kBq/m^2);
3. - the suburbs of Majsk village (Bragin district of Gomel region, 60 km N, 90 kBq/m^2);
4. - the suburbs of Babchin village (Khoiniki district of Gomel region, 40 km NNW, 1526 kBq/m^2).

According to the official data of the Belarus Meteorological Centre, the density of soil radiocontamination for ^{90}Sr ranges up to about 70 kBq/m^2 in sites 3 and 4.

The γ-radiation dosage rates were measured at height of 3-5 cm from the ground surface by the dosimeters SRP-68-01T and DBG-06T (in 1991-1996) manufactured in the former Soviet Union.

The total γ-activity of the whole-body animals in 1986-1987 was analysed using 32 crystal spectrometry of gamma-gamma coincidences (scintillation NaI(Tl) detector, 150 x 100 mm^2) at the Institute of Physics of Belarus Academy of Sciences. The radiometric analyses of laboratory mice and wild animals have been carried out since 1988 using γ-spectrometer ADCAM-300 (ORTEC, detector GEM-30185) at the Belarus Meteorological Centre and the Institute of Radiobiology (Belarus Academy of Sciences). The soil samples selected in 1989 were analysed using the same spectrometer.

The content of ^{90}Sr in bank vole populations in sites 3 and 4 were estimated by radiochemical analysis in the Belarus State University (Minsk).

The radiation load in animals due to the external γ-irradiation was estimated in term of the value of radiation dose rate and due to the internal γ-activity by the radionuclide concentration in the rodents body.

Evaluation of mutation process levels in bone marrow cells of bank vole was carried out by standard metaphase test [1]; structural (chromosome aberrations) and genomic (polyploid cells) mutations were analysed. The levels of germ cells mutability in bank vole were estimated by the frequency of abnormal sperm head (ASH) in males [28]. Embryonal lethality was analysed according to D. Anderson [2].

Bank vole have a short lifespan and every year 2-3 new generations appear in the populations. It is thus believed that there were approximately 20-22 generations of these rodents for 1986-1996.

Laboratory population of mice

The males of hybrid mice (CBAxC57Bl/6$_j$)F$_1$ were kept in the radiocontaminated settlements A and B under the conditions of low intensity of external and internal (radiocontaminated food of local production has been used) irradiation during 4 months in 1989. The radiocontamination densities of soil for ^{137}Cs in settlement A (village Lomachi, Khoiniki district of Gomel region) and B (Bragin city of Gomel region) were 2351 and 825 kBq/m^2, respectively. The average

gamma dosage rates in cages with animals were 43.71 x 10^{-12} and 8.24 x 10^{-12} A/kg (or 610 and 115 μR/h) in A and B, respectively. The control group of animals was kept in vivarium of Institute of Genetics and Cytology (γ-radiation dosage rate was about 0.86 x 10^{-12} A/kg or 12 μR/h).

The radiometric analyses of whole-body animals were carried out using γ-spectrometer ADCAM-300 (ORTEC, detector GEM-30185). The absorbed dose due to the external γ-irradiation was estimated using thermoluminecent dosimeters placed in the cages of animals.

The frequency of chromosome aberrations, polyploid cells in bone marrow as well as the frequency of reciprocal translocations and other cytogenetic injuries in spermatopcytes of the first meiotic division and abnormal sperm head (ASH) were studied by the conventional methods [1, 28].

Dynamics of radiation loads on wild populations of mammals in the radiocontaminated areas

Over the period under consideration the radiation dosage rate was greatly reduced in all sites (Table 1). The most fast reduction of dose rate was recorded in site 4 in May - August, 1986.

It was revealed that over the first five months following the catastrophe (i.e. over the average lifespan of animals under investigation) the absorbed whole-body dose from the external gamma-irradiation in the rodents inhabiting sites 1 and 4 (with contrasting levels of radiocontamination) made up 0,1 and 15 cGy, respectively; and the absorbed dose in animals in site 4 over the five months of 1987 ran to 1,23 cGy [9]. So, daily average dose from the external γ-component in the individuals from the highly contaminated site 4 hardly exceeded 1 mGy even in the first time after the accident. As a whole, it may be said that the animal populations under investigation were exposed to low-dose irradiation from the external and internal components. The research of Cristaldi et al. [6] corroborates this conclusion.

The mean radionuclide concentration in animal populations in all areas under investigation (Table 2, 3) were positively correlated with mean ground deposition of the trapping sites (Spearman, r=1.00, $P<0.05$ for data obtained in 1989). ^{137}Cs and ^{134}Cs made the main contribution to the total γ-activity of soil and animals samples. Besides two caesiums, we recorded other γ-emitters such as ^{106}Ru, ^{144}Ce and ^{241}Am (since 1991 for site 4). ^{90}Sr mean concentration estimated in 1991 in bank vole populations from sites 3 and 4 made up to 114 and 298 Bq/kg, respectively.

In rodents with fast generation succession we annually detected radionuclide concentrations in the completely renewed populations. Dynamics of radionuclide content in consecutive generations of bank vole (and yellow-necked mouse) over the period of 1986-1996 (and 1986-1989) was characterised by three phases: an increase, a maximum (peak) and a decrease [9, 12, 14]. The peaks of radionuclide accumulation in the populations in the areas with different radiocontamination density fell not at the first but in the next years (1987-1989) following the Chernobyl disaster, i.e. the peaks were observed in subsequent animal generations (Table 2, 3). Thus in populations of two mammalian species we observed shifts of the maximum radionuclide content within 1-3 years. The revealed regularity of the time shift in the maximum of population average radionuclide concentrations in animals as against the maximum of their fallout in 1986 seems to be the result of increase in biological accessibility of radioisotopes to plants and thus to the whole biota. The peculiarity of the radionuclide content dynamics in small mammal populations after the Chernobyl accident is in agreement with the known data on the same retardation in the concentration maximum of ^{90}Sr and ^{137}Cs from the global fallout in hoofed animals [27] and are corroborated by similar results obtained for fish from the Baltic Sea [8] and for various groups of people and animals in Norway [26].

Table 1. Gamma radiation dose rate 10^{-12} A/kg (μR/h or R x 10^{-6} / h in brackets) on the ground surface in four sites in 1986-1996

Site	1986	1987	1988	1989	1991	1992	1996
1	1.79 (25)	0.86 (12)	0.86 (12)	0.86 (12)	0.86 (12)	-	0.86 (12)
2	-	-	-	-	0.86 (12)	0.86 (12)	0.72 (10)
3	42.99 (600)	4.66 (65)	4.66 (65)	4.66 (65)	2.29 (32)	-	1.93 (27)
4	1218.05*-177.69 (17000*-2480)	46.57 (650)	46.57 (650)	43.71 (610)	10.75 (150)	-	11.68 (163)

*This level was measured in May 1986, the others were obtained as the average data over the periods of capture. In 1986 the first catchings were initiated in August (about 120 days after the disaster).

Table 2. Dynamics of radionuclide concentrations in wild populations of bank vole in 1986-1996

Site	Year	Number of animals	Total gamma-activity (Bq/kg)			
			Minimum	Maximum	Mean	U-test
1	1986	27	41	525	187	
	1987	46	38	926	274	*
	1988	24	5	750	245	n.s.(1)
	1989	75	5	429	118	*
	1991	15	5	625	140	n.s.
	1996	30	4	20	6	**
	1986-1996	217	4	926	160	
2	1991	20	5	1524	565	
	1996	40	4	108	25	*
	1991-1996	60	4	1524	205	
3	1986	34	38	78070	9293	
	1988	91	111	215196	23623	**
	1989	142	1237	41501	10591	**
	1991	53	757	25293	5587	**
	1996	18	85	344	162	**
	1986-1996	338	38	215196	12629	
4	1986	42	67	78070	17202	
	1987	65	3885	145410	26503	**
	1988	174	58	950100	81966	**
	1989	176	3636	463741	44407	**
	1990	13	4724	22016	13272	**
	1991	129	654	55132	11191	**
	1996	49	148	4528	1204	**
	1986-1996	648	58	950100	40429	

(1) nonsignificant;

* Ð< 0.05; ** Ð< 0.01 in comparison with data obtained in every site in 1986.

Reduction in radionuclide concentration in bank vole populations under investigation was recorded in 1989. However, a significant decrease in radionuclide content as compared with the initial increase in 1986 has been observed only beginning from 1990 (10 generations) in population of bank vole from the highly radiocontaminated site 4. Since both the radiation dose rate and values of the population mean

Table 3. Dynamics of radionuclide concentrations in wild populations of yellow-necked mouse in 1986-1989

Site	Year	Number of animals	Total gamma-activity (Bq/kg)			
			Minimum	Maximum	Mean	U-test
1	1986	27	42	348	237	
	1987	17	63	365	279	n.s.
	1988	21	5	309	59	**
	1989	27	5	265	78	**
	1986-1989	92	5	365	157	
3	1986	5	820	11111	4196	
	1988	7	1146	48799	11174	n.s.
	1989	52	441	7235	2677	n.s.
	1986-1989	64	441	48799	3725	
4	1986	11	1539	46990	11493	
	1987	27	1894	38480	8411	n.s.
	1988	14	1382	36472	9391	n.s.
	1989	24	1371	61204	15559	xx
	1986-1989	76	1371	61204	11295	

n.s. nonsignificant;

** P<0.01 in comparison with data obtained in every site in 1986 and

õõ in comparison with data obtained in site 4 in 1987, 1988.

body burden considerably decreased in all sites by 1991, it can be stated that there was a significant reduction in radiation load starting from the 12th generation of animals.

Genetic injuries in mammals

A. Dynamics of mutation processes in somatic cells of consecutive generations of bank vole

The mean frequency of aberrant cells in bank vole population inhabiting site 1 (with the least soil radiocontamination density, 8 kBq/m^2) in 1986 didn't differ from the historical pre-Chernobyl control that made up 0.41 % in site 2 [29], and had the tendency to increase the next years (Table 4). It became significantly greater in 1991, i.e. in the subsequent generations of animals.

In populations from more contaminated sites 2-4 (18-1526 kBq/m^2) there were observed significant 2.8-6.4 fold increased levels of aberrant cells in bank vole over all period under consideration as against the results for rodents in site 1 and the pre-accident data (Table 4). The tendency towards increase in the chromosome mutation frequency was also noted in these sites. In 1991 the level of aberrant cells in population in site 3 was significant highly (X^2-test, $P<0.05$) than the data on the previous years (1986, 1988).

The analysis of frequencies of chromosome aberrations and aberrant cells in highly radiocontaminated sites 3 and 4 did not reveal significant differences between these two populations in each year studied. However, the average frequency of aberrant cells during whole period 1986-1991 was significantly higher in site 3 than in site 4 (X^2-test, $P<0.05$).

In populations of animals living in the highly contaminated sites 3 and 4, the aberrations of chromosome type (paired fragments, Robertsonian translocations, pericentric inversions) formed the great part in total yield of aberrations in contrast to the populations in sites 1 and 2 with less ground deposition [13; 14]. It should be emphasised that in one of voles captured in site 3 in 1991, more than 50% of analysed metaphases contained pericentric inversion. We believe that this stable aberration had emerged in bone marrow stem cell producing the clone of changed cells.

Additionally, the cells with 2 and 3 aberrations were found in animals from sites 3 and 4 while no cells with above 1 aberration were observed in populations in sites 1 and 2.

Unexpectedly high cytogenetic effects were recorded in bone marrow cells of bank vole by the test of genomic mutations [13] as against the test of chromosome aberrations (Table 4). It turned out that in 1986 the frequencies of polyploid cells were increased in animals at all sites in comparison with the pre-Chernobyl data (0.04 % according to Yeliseeva et al. [29]. The average frequency of polyploid cells during the whole period 1986-1991 significantly correlated with the soil contamination and the γ-radiation dose rate (Spearman, $r=1$, $P<0.05$). The level of the genomic mutations rose significantly in populations under investigation from year to year (X^2-test, $P<0.01$) with one exception: population from site 2 in 1992. The frequency of polyploid cells reached 9-12 % in animals from sites 3 and 4 in 1991 and was 200-300 times higher than the pre-accident

Table 4. Dynamics of the aberrant and polyploid cell frequencies (%) in bone marrow of bank vole in 1986-1991

Site	Year	Number of animals	Number of cells scored	Aberrant cells	Polyploid cells
1	1986	10	997	0.40	0.50**
	1988	3	310	0.65	0
	1991	6	741	1.12*	3.51** xx
	1986-1991	19	2048	0.69	1.51
2	1991	20	2164	1.11**	4.25**
	1992	17	1995	1.22**	1.65** xx
	1991-1992	37	4159	1.17	3.01
3	1986	18	2011	1.71**	1.19**
	1988	21	2380	1.75**	8.87** xx
	1991	16	1824	2.54**	9.27**
	1986-1991	55	6215	1.96	6.50
4	1986	16	1743	1.27**	0.23**
	1987	36	3973	1.14**	7.50** xx
	1988	27	2883	1.77**	5.86** xx
	1991	30	4166	1.86**	12.31** xx
	1986-1991	109	12765	1.53	7.71

* $P<0.05$; ** $P<0.01$ in comparison with data on site 2 in 1981-1983 [29];
xx $P< 0.01$ in comparison with data obtained in every previous year (X^2-test).

level.

The fact of annual significant rise in the genomic mutation frequency in consecutive generations of animals living in areas with different ground deposition deserves particular attention.

The hypothesis on the adaptive character of cellular polyploidy at high levels of radiation is being extended [4, 16, 23]. According to this hypothesis polyploidy is considered as a mechanism for concealment (hibernation) of genetic damages in cell: polyploid formation process plays a protective role saving cells from unbalanced genome. In bone marrow, at the same time, polypotent stem cells as well as precursor-cells are target-cells in forming abnormal myelopoiesis and acute forms of leukaemia [30], which are characterised by increased frequencies of polyploidy and other forms of aneuploidy in many cases. Since there are more questions than answers in hemopoiesis process, it can be assumed that, at some ranges of the mutation effects, polyploidization of bone marrow cells is of an adaptive character and, at other ranges, it goes to pre- and pathologic state. It should be noted that there are data on a rise in the frequency of infant leukaemia in children exposed to in utero radiation whose parents live in Greece with different radiocontamination density due to the Chernobyl fallout [18].

Thus, it can be only supposed that increased frequencies (by 2-3 orders) of bone marrow polyploid cells recorded by us exceed the limits of normal response of bank vole and are close to the pre-pathological state of hemopoiesis processes.

So, the study on dynamics of the mutation process in bank vole populations inhabiting regions with different soil radiocontamination density in 1986-1991 has shown that increased levels of chromosome aberrations and genomic mutations emerging in somatic cells de novo in every generation were observed in bone marrow cells of animals within many generations (from 1 to 14) after the accident.

Considering that cytogenetic effects in proliferating cells of bone marrow reflect the dose received within one cell division cycle, increased frequencies of chromosome aberrations and genomic mutations recorded by us in 1986-1992 are considered to have occurred at very low levels of absorbed doses. Thus, according to calculations of Cristaldi et al. [6], the absorbed doses from the external γ and internal γ and β irradiation from ^{134}Cs and ^{137}Cs in bank vole populations in three regions of Sweden with different soil contamination levels (22, 90 and 145 kBq/m^2 for ^{137}Cs) made up 8.8 x 10^{-6}; 26.8 x 10^{-6} and 39.4 x 10^{-6} Gy per day in 1989, respectively. The levels of soil contamination in our sites 2 and 3 were almost equal to those of some Swedish sites. This suggests that the radiation load in animals in sites 2 and 3 were the same order of magnitude and it can be assumed that the values of the doses absorbed by the investigated populations of animals within the cell division cycle are of the order of a few tens or hundreds of mGy per day.

We believe that significantly greater mean frequency of aberrant cells as well as existence of the individual with high content of stable aberration can indicate stronger structural injuries of chromosomes in population from site 3 than from site 4. Since animals in site 3 had lower range of dose loads than that in animals from site 4, we believe that data obtained resulted from abnormal dose relationship of structural injuries of chromosomes at low doses.

B. Dynamics of mutation process in germ cells of consecutive generations of bank vole

Table 5. Dynamics of abnormal sperm head (ASH) frequency in males of wild small mammals

Site	Year	Bank vole			Yellow-necked mouse		
		Number of animals	Number of cells	ASH (%)	Number of animals	Number of cells	ASH (%)
1	1989	13	19500	0.149	10	10000	0.140
	1991	7	10500	0.076	-	-	-
	1996	13	65000	0.085*	-	-	-
	1989-1996	33	95000	0.097	-	-	-
2	1991	22	33000	0.106	-	-	-
	1996	43	215000	0.091	-	-	-
	1991-1996	65	248000	0.093	-	-	-
3	1989	8	12000	0.175	23	23000	0.222^{x}
	1991	26	39000	0.126	-	-	-
	1996	6	30000	0.123	-	-	-
	1989-1996	40	81000	0.132	-	-	-
4	1989	34	51000	0.208	12	12000	0.300^{xx}
	1991	39	58500	0.214^{xx}	-	-	-
	1996	7	35000	0.109**	-	-	-
	1989-1996	80	144500	0.186	-	-	-

^{x} P<0.05; ^{xx} P< 0.01 in comparison with data on site 1 and within every year;

* P<0.05; ** P<0.01 in comparison with data obtained in every site in 1989 (X^2-test).

The frequencies of abnormal sperm head (ASH) in both species of small mammals (bank vole and yellow-necked mouse) in 1989 were higher in sites 3 and 4 in comparison with the data on site 1 (Table 5). The increased frequency of abnormal sperm remained in bank vole population in site 4 in 1991. Thus, it can be stated that many consecutive generations of animals in site 4 with high radiocontamination density had the same range of mutability in germ cells. The significant decrease in the frequency of ASH (X^2-test, $P<0.05$ and $P<0.01$) was observed in bank vole populations in 1996 (in sites 1 and 4 respectively).

Unfortunately, we have no control data (with absolutely clear region) or pre-accident ones on the levels of ASH in bank vole populations. And thus, we believe that the frequency of abnormal sperm in highly contaminated sites 3 and 4 in 1996 didn't reach the pre-accident level.

Comparative studies of the average frequencies of ASH in bank vole populations during whole period 1989-1996 demonstrated no significant differences between the least contaminated sites 1 and 2 (0.097 and 0.093 % ASH, respectively). Significant increase (X^2-test, $P<0.01$) in the average frequency of ASH were observed in population from more contaminated site 3 (0.132 %). And in males from the most contaminated site 4, the level of abnormal sperm (0.186 %) were significant higher ($P<0.01$) than in animals from all other sites under investigation (1-3). Thus, we can observe the following peculiarity: the higher the contamination density of the site, the higher frequency of ASH. But the frequency of ASH didn't statistically correlate with the soil contamination or the content of incorporated radionuclides. At the time, the average frequency of ASH significantly correlated with the average gamma radiation dose rate during 1989-1996 (Spearman, $r=1.0$, $P<0.05$). We believe that the lack of correlation between the level of abnormal sperms and some radiation factors can indicate complex repair processes in germ cells of chronically irradiated animals. This our speculation is in agreement with the data obtained in germ cells of laboratory mice that is discussed below.

C. Embryonal lethality in wild populations of bank vole

The study on embryonal lethality was initiated only in 1988. During 1988-1996 we have caught a few pregnant females in site 1. Thus we have no data on embryonal lethality in the site with the least ground deposition. In bank vole populations from sites 2-4 a tendency towards increase in the frequency of embryonal lethality during the whole period under investigations was observed (Table 6). The average frequency of lethality throughout this period was significantly high only in site 4 as compared with the average data on site 2 (Table 6). But the average frequency of lethality didn't correlate with the soil radiocontamination, gamma radiation dose rate or average level of incorporated radionuclides (Spearman, $r=0.0$, $P>0.05$). In site 4 the frequency of embryonal lethality positively correlated with population density (Spearman, $r=1.00$, $P<0.05$).

D. Cytogenetic injuries in somatic and germ cells of laboratory mice after protracted internal and external irradiation at low doses

It was revealed that animals kept in the radiocontaminated regions during 133 days accumulated high concentrations of γ-emitting radionuclides and took whole-body radiation loads in the range of low doses (Table 6). Mutagenic effects of long-term irradiation by tests of chromosome aberrations and polyploid cells were observed in mice bone marrow (Table 7). Significantly increased levels of these two types of mutations were recorded in animals kept in the highly contaminated region A (Table 7). There were 56% of chromosome type aberrations from all number of aberrations recorded in

Table 6. Dynamics of embryonal lethality in populations of bank vole

Site	Year	Number of females	Pre-implantation loss (%)	Dead implants (%)	Total embryonal lethality (%)
2	1991	12	0	5.36	5.36
	1992	19	2.20	1.12	3.30
	1996	7	12.50	0	12.50
	1991-1996	38	3.74	2.27	5.88
3	1988	4	0	0	0
	1989	30	5.80	0.77	6.52
	1991	14	8.45	1.54	9.86
	1996	3	20.00	8.33	26.67
	1988-1996	51	7.32	1.33	8.54
4	1988	14	3.17	1.64	4.76
	1989	40	4.48	1.56	5.97
	1991	21	9.90	4.40	13.86
	1996	11	13.73	9.09	21.57
	1988-1996	86	6.73	3.19	9.62 *

* $P<0.05$ in comparison with data on site 2 (X^2-test).

Table 7. Frequency of aberrant and polyploid cells in bone marrow of laboratory mice (males) after long-term internal and external irradiation in radiocontaminated regions of Belarus in 1989

Region	^{137}Cs deposition (kBq/m^2)	γ-activity of animals (Bq/kg)	Whole-body dose from external γ-irradiation (cGy)	Number of animals	Number of cells	Aberrant cells (%)	Polyploid cells (%)
Control	0	6	0.03	3	646	0.31	0
B	825	853	0.43	4	480	0.63	0.83
A	2351	1103	1.71	27	2970	1.16*	1.11*

* P<0.05 in comparison with control data (X^2-test).

Table 8. Abnormal sperm head (ASH) in laboratory mice

Region	Number of animals	Number of cells scored	ASH (%)
Control	24	38208	0.464
B	33	49985	0.456
A	52	79010	0.582**

** P<0.01 in comparison with control data (X^2-test).

Table 9. Frequencies of cytogenetic injuries in spermatocytes of laboratory mice (males)

Region	Number of animals	Number of cells scored	Reciprocal translocations (%)	Fragments (%)	Sex univalents (%)	Autosome univalents (%)	Polyploid cells (%)
Control	21	7114	0.06	0.33	13.27	5.56	5.74
B	21	10615	0.18*	0.56*	11.77	2.92	10.76**
A	21	5257	0	0.23	14.23	3.87	9.53**

* P<0.05; ** P<0.01 in comparison with control data (X^2-test).

bone marrow cells of mice from group A, while the animals from other two groups had no cells with these type aberrations [10].

The level of ASH (Table 8) was revealed to increase in mice from group A when absorbed dose from the external γ-irradiation was 1.71 cGy and the content of incorporated radionuclides was 1103 Bq/kg [10].

Different type of cytogenetic injuries (structural and genomic) were observed in spermatocytes of males studied (Table 9) [11]. The animals of all group were characterised by high individual variability for each parameter tested.

Reciprocal translocations among structural chromosome injuries are of particular interest. It should be noted that radiation-induced reciprocal translocations emerge with low frequency [1, 5, 20, 21, 22, 25].

Translocations, consisting of two bivalents (quadrivalents) mainly in the form of chains and in rare cases in the form of rings, occurred in the animals analysed by us. One male from group B was noteworthy for complicated configuration of 4 bivalents (octovalent) in the form of chain. On the whole, the mice from this group are characterised by the significantly increased level of reciprocal translocations (Table 9). The obtained level of reciprocal translocations in group B under long-term combined irradiation with low doses (0.43 cGy from external γ-irradiation and 853 Bq/kg of incorporated radionuclides) exceed the effects expected during higher dose extrapolation by single or long-term ^{137}Cs input [20, 22], as well as under long-term external γ+β-irradiation [5, 21].

At the same time no reciprocal translocations were revealed in animals from the more contaminated station with higher radiation loads (radionuclide concentration - 1103 Bq/kg and the dose taken from external γ-irradiation -1.71 cGy). Similar data were obtained on analysing another type of structural chromosome damages - fragments (Table 9). Significant increase in chromosome fragment frequency was recorded in animals from group B as against the control and reduction in that parameter in group A.

Thus, the increased frequencies of structural chromosome injuries were observed in immature germ cells (spermatocytes) only at lower radiation load (group B). Absence of increased mutability, evaluated by chromosome aberration test, on increasing the absorbed dose seems to be the consequence of induction of repair system of the “adaptive response” type. There are abundant data on different inducible repair systems (adaptive response, SOS-repair, etc.) in literature at the present. These repair systems can be induced with low doses of acute and chronic irradiation. Discrepancy between dose-effect curves in the chromosome aberration frequency in somatic (bone marrow) and immature germ cells can be accounted for by some reasons including dose differences in inducing repair systems of germ and somatic cells.

It is known that an increase in radiation above the background level induces two contrary processes in living matter - injury and repair. There is a number of models [24, 19] of abnormal dose dependence of

cytogenic injury yield at low levels of absorbed doses. According to one of them [19], repair processes can prevail over injury induction at some levels of low doses. As a result, reduction in the number of injuries even below the spontaneous level can be observed at a certain site of dose-effect curve. However, the number of induced injuries, which make a greater contribution in comparison with repair to the dose-effect curve, increases with a rise in the dose. We think that abnormal dose dependence of chromosome aberration yield in germ cells of the laboratory mice is in agreement with the above-mentioned model of low dose radiation effect. The frequency of chromosome aberrations, as well as reciprocal translocations can be expected to start rising in animal germ cells at higher radiation loads than at those we studied.

The analysis of sex and autosomal univalents (nonconjugated homologous chromosomes) did not exhibit radiation-related effects what is in agreement with the literature data [5, 20, 21].

Genomic injuries in the animals tested (Table 9) were represented by tetra-, hexa-, octo- and higher level ploids. The high frequency of polyploid cells is typical for germ cells of mammal males and, in particular, for mice [17]. Besides, the dependence of the yield of the polyploid germ cell level in mice on the value of absorbed doses is known in the literature [15, 17]. On exposing spermatocytes to X-irradiation with 200 R dose, the frequency of polyploid cells increased by a factor of 15 as against the control [17]. We have revealed that, at much lower absorbed doses but at the combined effects of external and internal irradiation, the levels of polyploid cells in animals from the experimental groups are twice as much as in the control. Furthermore, the tendency towards increasing the ploidy degree was observed in males at the radiocontaminated stations. The cells whose ploidy is above 8n were revealed only in these animals.

Thus, the increased levels of various cytogenetic injuries were detected in somatic and germ cells of laboratory mice exposed to long-term combined external and internal irradiation of low absorbed doses.

Conclusions

Increased levels of the mutation process both in somatic and in germ cells of animals are recorded in natural populations of small mammals exposed to chronic low-dose irradiation during succession of many generations (1-22) following the Chernobyl accident. Since the radiation load on bank vole populations was reduced by 1991, it can be stated that hereditary apparatus of somatic and germ cells of succeeding animal generations (12-22) have higher sensitivity to radiation in comparison with previous ones (1-10) that lived before 1991 and took much higher radiation loads. In other words, there was no genetic adaptation to the mutagenic effect of low level irradiation for the whole investigation period in wild populations of bank vole.

Our results obtained on the somatic and germ cells of small mammals also indicate that the genomic test (examination of polyploidy) has greater sensitivity to evaluate genetic effects of the increased radiation background as compared with the test using chromosome aberrations. The dose-response patterns for genomic mutations in rodent somatic cells differ at low doses from those for chromosome aberrations.

Our investigations show higher sensitivity of both somatic and germ cells of animals to chronic combined external and internal effect of radionuclides in comparison with other irradiation conditions. All cytogenetic effects obtained in wild and laboratory animals at chronic low dose radiation exceeded the expected ones based on extrapolation from the results obtained in the range of higher doses by single or long-term irradiation. Abnormal patterns of dose-response are revealed for different type mutations at low doses of long-term radionuclide influence.

There are data on high rates of genic mutations in animal and human populations in the areas radiocontaminated by the Chernobyl fallout. So, the frequency of germline mutations at human minisatellite loci in children born on heavily polluted areas of Belarus was found to be twice as high as in the control group [7]. In two species of voles (Microtus arvalis and M. rossiaemeridionalis), collected near the Chernobyl nuclear power plant, the range of genic mutations was hundreds of times greater than those typically found in vertebrates [3].

Taking into account increased sensitivity of cells of different organs and tissues of animals from chronically irradiated populations to induction of all types of mutations - genic [3, 7], genomic (polyploid cells) and structural (chromosome aberrations), it is necessary to emphasise that the areas with a wide range of radiocontamination density due to the Chernobyl fallout (8-2351 kBq/m^2 in our tests) are zones with high genetic risk for animals and man.

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