FREQUENCY OF CHROMOSOMAL ABERRATIONS IN BONE MARROW CELLS OF MONKEYS AT VARIOUS TIMES AFTER IRRADIATION

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The number of chromosomal aberrations was significantly higher than in the control in bone marrow cells of monkeys 3 months and 5 years after acute γ-ray irradiation in doses of 550-650 R. The principal type of structural rearrangements of chromosomes are acentric transpositions. The number of chromosomal transpositions in the anaphases and telophases was greater in monkeys examined 6, 8, and 12 years after irradiation, but the difference was not statistically significant. The late somatic effect of radiation is independent of the monkey's sex.

Cytogenetic effects of irradiation are found soon after the procedure and they are expressed as an increase in the number of structural changes in the chromosomes, death of the cells, and various other disturbances [2, 5, 6]. If the irradiated cells remain viable, a certain percentage of them will carry a modified nuclear structure [1, 7, 9]. The question of the quantitative principles governing late cytogenetic changes after irradiation has received little study [8].

The object of the present investigation was to analyze the frequency of chromosomal aberrations in bone marrow cells of monkeys at various times after a severe form of radiation sickness (LD_{70-90}).

EXPERIMENTAL METHOD

Monkeys previously used in radiobiological experiments to study the clinical features of radiation sickness were used in the investigation. The animals received whole-body irradiation with Co^{60} γ-rays in a single dose of 550-650 R. Monkeys of the species Macaca mula (11 males, 7 females), surviving acute radiation sickness, were investigated cytogenetically at various times after irradiation (3 months, 5, 6, 8, and 12 years). The experimental results for each time of investigation were added together because of the similarity of the late effect in the monkeys irradiated in doses of 550-650 R. The natural level of mutation was studied in 12 control monkeys.

Chromosomal aberrations were investigated in bone marrow cells obtained by puncture from the long bones. The material was fixed and treated by the method described previously [4]. In each animal 300 anaphases and 300 telophases were analyzed, and in 3 monkeys, 400, 450, and 500 cells respectively were studied. The after-effects of radiation were assessed from the number and character of the chromosomal aberrations. During analysis of the material, single and paired fragments, chromatid and chromosomal bridges were taken into account during analysis of the material (adhesion bridges and deletions of chromosomes were not included in the analysis). This paper gives the results of analysis of 6050 cells of irradiated monkeys and 3850 cells of control animals. The numerical results, describing the frequency of structural injuries to chromosomes, were analyzed by the statistical methods described by Romanovskii (1947) in his book.

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TABLE 1. Dynamics of Structural Injuries to Chromosomes in Bone Marrow of Irradiated Monkeys

Group No.	Sex of animal	Number of animals	Time after irradiation	Number of cells analyzed	Number of aberrations per 100 cells	Types of aberrations per 100 cells		Percentage of cells with aberrations	Percentage of cells with aberrations		
						acentric fragments	bridges		with one	with two	with three
1	Males and females (3-5, 9-11 years)	9 and 3	Not irradiated	3 040	0,59±0,08	0,59±0,08	—	99,44	0,53	0,03	—
2			"	810	0,99±0,04	0,99±0,04	—	99,99	0,01	—	—
3	Males	2	3 months	600	19,55±4,13	18,90±3,65	0,65±0,46	87,15	7,85	3,30	1,70
4	"	3	5 years	900	4,09±0,71	3,76±0,63	0,34±0,27	96,90	2,29	0,60	0,20
5	"	3	8 "	900	1,79±0,29	1,58±0,23	0,22±0,08	98,40	1,59	0,10	—
6	"	3	12 "	1 050	2,90±0,73	2,70±0,80	0,20±0,00	97,26	2,58	0,16	—
7	Females	4	6 "	1 800	3,08±0,85	2,84±0,79	0,24±0,15	97,00	2,2	0,44	—
8	"	3	8 "	900	3,09±0,48	2,64±0,65	0,45±0,23	98,00	2,69	0,20	—

EXPERIMENTAL RESULTS

The spontaneous level of chromosomal aberrations in the bone marrow cells of the monkeys was determined for two age groups (3-5 and 9-11 years), corresponding approximately to the ages of the irradiated animals. As Table 1 shows, with an increase in the monkeys' age, the number of spontaneous chromosomal aberrations increased, but the increase was not statistically significant ($P < 0.01$). No significant differences likewise were found in the spontaneous mutagenic effect in monkeys of different sexes.

Table 1 also gives the mean data for several groups of irradiated monkeys depending on the animal's sex and the time after irradiation. For instance, for two males examined 3 months after radiation sickness, the number of chromosomal aberrations was significantly higher than the control values ($P < 0.01$). In another group of males, examined 5 years after radiation sickness, the number of chromosomal aberrations was also significantly higher than the control level ($P = 0.01$). However, 8 and 12 years after irradiation, although the number of aberrations was higher than the spontaneous level in the males examined, the difference was no longer statistically significant ($P > 0.01$). No significant difference likewise was found in the number of chromosomal fragmentations in irradiated females examined 6 and 8 years after irradiation and the spontaneous level of aberrations ($P > 0.01$).

With an increase in the time after irradiation the number of chromosomal aberrations in dividing cells at the anaphase–telophase stage thus fell to the control level.

As Table 1 shows, 3 months after irradiation the aberrant cells had one or more (2-3) anomalies. Later, however, the number of aberrations in the cells was reduced, and by the end of the experiment, i.e., 8 and 12 years after irradiation, as a rule the aberrant cells had only one anomaly.

The principal types of chromosomal aberrations in the bone marrow cells of the irradiated monkeys are illustrated in Fig. 1. Analysis of the results for types of structural chromosomal aberrations in the late stages after radiation sickness showed that the number of single fragments was greater than the number of pairs, but in no case was the difference statistically significant. Chromosomal aberrations of the bridge type were extremely rare in the irradiated monkeys, whereas in the control none whatever were found during the investigation of 12 monkeys.

The results on the whole indicate that in monkeys, regardless of the period after irradiation (except the group of monkeys examined 12 years after irradiation; $P > 0.01$) and of the sex of the animal, the most common type of chromosomal aberration is the presence of acentric fragments. Statistical analysis of the material showed that in the animals examined 3 months after irradiation, and also in those examined 8 years after irradiation (Table 1), the difference between the number of acentric fragments and the number of bridges is close to statistically significant ($P = 0.03$). Statistically significant differences ($P = 0.01$) were observed 5 and 6 years after irradiation.

To summarize the results of this analysis of the incidence of chromosomal aberrations in hematopoietic cells at the anaphase stage, the increased cytogenetic effect in monkeys is well marked during the first 5 years after irradiation. The number of chromosomal anomalies

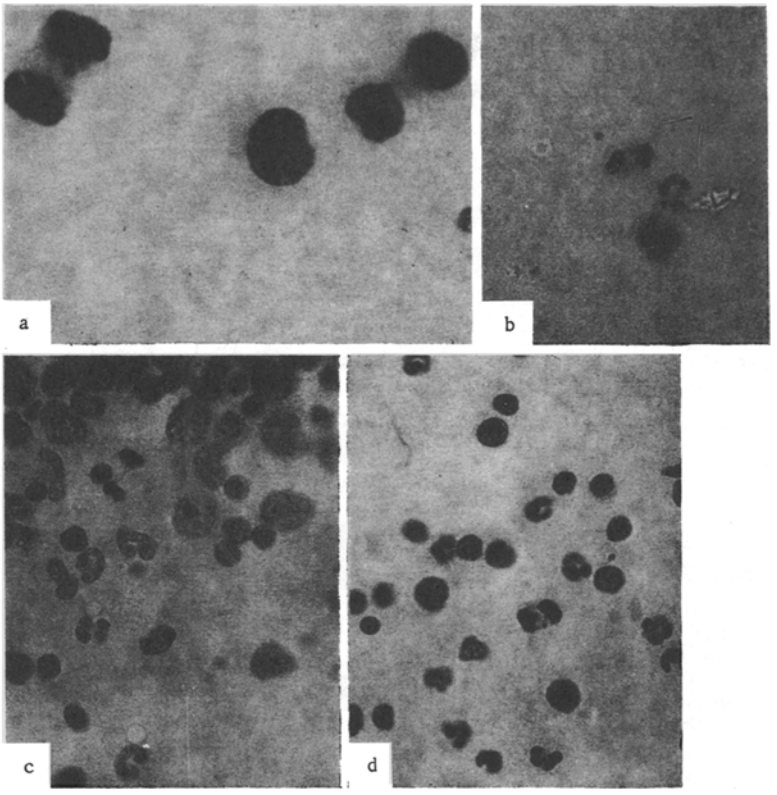

Fig. 1. Types of chromosomal aberrations in bone marrow cells of monkeys irradiated in a dose of 550-650 R: a) paired fragment (6 years after irradiation); b) two fragments (3 months after irradiation); c) chromosomal bridge (5 years after irradiation).

in bone marrow anaphases 6 years and more after irradiation was not significantly higher than the control. These results also show that there is no difference between the late effects of irradiation in animals of different sexes. Evidently the radiosensitivity of organisms of different sexes is the same [3], and the post-irradiation processes in somatic cells follow a similar course in organisms of both sexes.

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