different phases of the cell cycle possess differential sensitivity to the formation of chromosomal aberrations [9].

SCE are thus both a more sensitive and a more stable indicator of the action of a mutagen *in vitro* and *in vivo* than chromosomal aberrations.

LITERATURE CITED

1. N. P. Bochkov, S. V. Stukalov, and A. N. Chebotarev, Byull. Éksp. Biol. Med., No. 3, 90 (1982).
2. A. M. Malashenko and N. I. Surkova, Genetika, No. 1, 71 (1974).
3. A. N. Chebotarev and T. G. Selezneva, Genetika, No. 10, 1941 (1979).
4. A. N. Chebotarev, T. G. Selezneva, and V. I. Platonova, Byull. Éksp. Biol. Med., No. 2, 242 (1978).
5. E. Gebhart, Hum. Genet., 58, 235 (1981).
6. S. B. Neal and G. S. Probst, Mutat. Res., 113, 33 (1983).
7. W. Vogel and T. Bauknecht, Nature, 260, 448 (1976).
8. V. S. Zhurkov and K. N. Yakovenko, Mutat. Res., 41, 107 (1976).

RADIOSENSITIVITY OF LYMPHOCYTE CHROMOSOMES IN DOWN'S SYNDOME

A. P. Akif'ev, G. R. Mutovin, I. V. Filyushkin, and E. K. Khandogina

UDC 616.899-07:616.155.32-076.5:575.224.23

KEY WORDS: repair; Down's syndrome; lymphocytes.

Data on variability of individual radiosensitivity of human chromosomes are of considerable theoretical and practical interest. On the one hand, an increased frequency of radiation-induced chromosomal aberrations objectively reflects the defectiveness of the DNA repair system [7, 9], and it enables the efficacy of genetic control of this trait to be studied. On the other hand, even the comparatively small amount of information on changes in spontaneous and induced mutation of chromosomes in some hereditary human diseases is evidence that a tendency toward malignant tumors and to premature aging is causatively linked with DNA repair defects [4]. For example, in xeroderma pigmentosa, which is the most closely studied disease from the standpoint of repair defects, the likelihood of appearance of skin cancer is three orders of magnitude greater than the corresponding figure for a control population consisting of persons of the same age [10].

The discovery of a particular defect in the repair system of genetic material can thus be regarded as a criterion of belonging to an increased risk group with regard to these traits, and even now that is sufficient to justify the need for prophylactic measures and, in the near future, for special treatment also. Investigations carried out previously by the present writers [1, 2, 5] have shown that belonging to a high risk group can be determined sufficiently reliably by the use of lymphocytes cultured *in vitro*, by studying dose dependence on the yield of radiation-induced chromosomal aberrations over a wide range of doses.

The object of this investigation was an experimental analysis of dose—effect curves for the yield of chromosomal aberrations produced by the action of ^{60}Co γ-rays in lymphocytes from patients with various forms of Down's syndrome. The basis for the investigation

Institute of Chemical Physics, Academy of Sciences of the USSR. N. I. Pirogov Second Moscow Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 12, pp. 69-71, December, 1983. Original article submitted December 19, 1982.

0007-4888/83/9612-1736$07.50

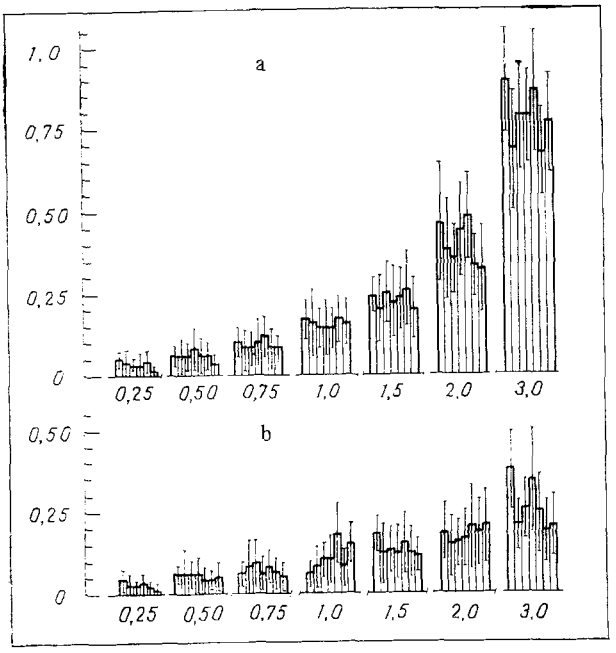

Fig. 1. Yield of radiation-induced exchanges (a) and fragments (b) in lymphocytes of clinically healthy blood donors of different ages, forming the control group. Abscissa, here and in Fig. 2 — dose of irradiation (in Gy); ordinate, here and in Figs. 2 and 3: a) number of exchanges per cell, b) number of fragments per cell.

TABLE 1. Relative Radiosensitivity (r) of Cells of Patients with Down's Syndrome

Donor	Donor's karyotype	r	
		for yield of exchanges	for yield of fragments
I	46, XY, −14, +*t* (14, 21)	2,08±0,06*	0,96±0,05
II	46, XY, −21, +*t* (21, 21)	1,78±0,04*	0,91±0,07
III	47, XY, −21	1,36±0,07*	0,97±0,05
IV	47, XY, −21	1,32±0,10*	1,08±0,07
V	47, XY, −21	1,67±0,05*	0,97±0,04
VI	47, XX, −21 — 35%	∽2,1*	∽1,1
	46, XX, — 65%	∽1,4*	∽0,8

Legend. Asterisk indicates that increase in yield of aberrations is significant.

was provided by the few data in the literature on changes in efficacy of repair processes and a high incidence of malignant tumors among patients with Down's syndrome [7, 8].

EXPERIMENTAL METHOD

Venous blood from six patients with Down's syndrome and seven normal healthy blood donors of different age groups was irradiated 1.5-2 h after collection by γ-rays from a ^{60}Co source within the dose range 0.25-3.0 Gy, at a temperature of 18°C, and with a dose rate of 0.20 Gy/min. Lymphocytes were cultured by a modified Hungerford's method [3]. The culture time (56-66 h) was determined allowing for radiation delay of mitosis. For each fixation point 50-200 metaphase plates were analyzed: The number of exchanges, prefragments, and chromatid aberrations in them was counted. The spontaneous level of chromosomal aberrations was determined for all the healthy donors.

TABLE 2. Coefficients of Dose Dependences of Yield of Radiation-Induced Exchanges and Fragments in Cells of Donors of Control Group (C) and Patients with Down's Syndrome

Donor	Donor's karyotype $Y = \alpha D + \beta D^2$		Yield of fragments $Y = kD$
	α, Gy^{-1}	β, Gy^{-2}	k, Gy^{-1}
C	0,080±0,018	0,054±0,009	0,092±0,007
I	0,09±0,09	0,16±0,05	0,088±0,012
II	0,09±0,05	0,13±0,03	0,084±0,022
III	0,11±0,06	0,08±0,03	0,089±0,016
IV	0,16±0,10	0,05±0,05	0,099±0,019
V	0,19±0,04	0,07±0,02	0,089±0,009

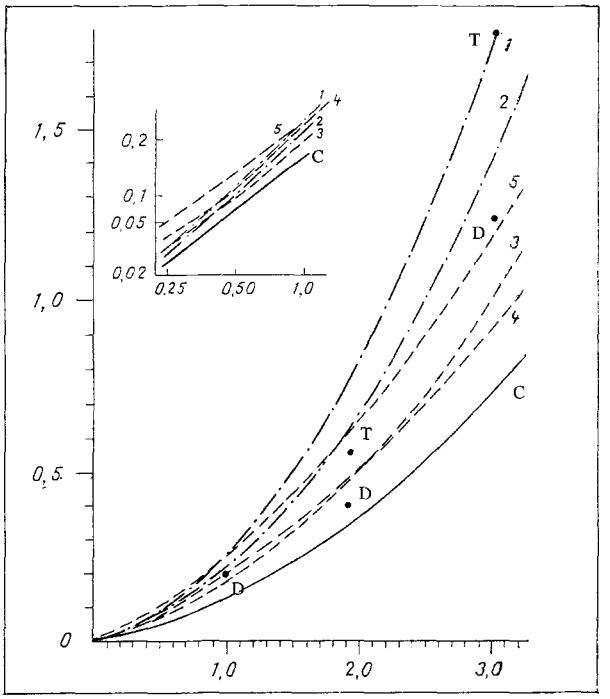

Fig. 2. Dose dependence of yield of radiation-induced exchanges in cells of patients with Down's syndrome. 1-5) Yield of exchanges in cells of donors I, II, III, IV, and V respectively; D and T) yield of exchanges in diploid and trisomic cells of a patient with the mosaic form of Down's syndrome; C) control.

As indicator of relative radiosensitivity we chose a value numerically equal to the ratio of the areas below the dose–effect curve for the test donor and for the control respectively: $r = S_d/S_c$ (the areas below the dose–effect curve were determined by integration of the corresponding dose–effect curves within the relevant range. The value of r chosen in this way characterizes the ratio between average yields of aberrations for the given range of doses.

EXPERIMENTAL RESULTS

No statistically significant differences were found between the yield of aberrations in lymphocytes of clinically healthy blood donors belonging to different age groups (Fig. 1), so that the results could be pooled.

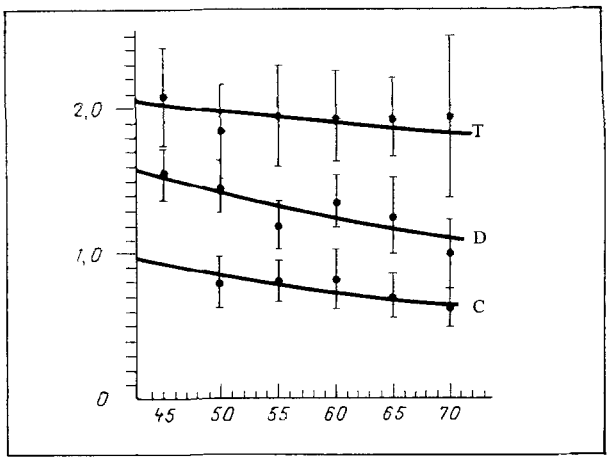

Fig. 3. Dependence of yield of radiation-induced exchanges in diploid (D) and trisomic (T) cells of a patient with the mosaic form of Down's syndrome and in cells of a control donor (C) on culture time. Abscissa, culture time (in h).

Table 1 gives values of relative radiosensitivity of cells of patients with Down's syndrome. The coefficients of linear and quadratic equations approximating the yield of exchanges and of linear equations approximating the yield of fragments in cells of control donors (C) and of patients with various forms of Down's syndrome are given in Table 2.

The spontaneous level of aberrations and the yield of fragments and chromatid aberrations in lymphocytes of all the patients, incidentally, did not differ from the control values, but the yield of exchanges in donors I, II, and V was significantly higher than in the control (Student's t test). Application of this test to donors III and IV gave $t_{0.05} < t < t_{0.01}$, but comparison of the yield of aberrations point by point for individual dose values led to the conclusion that the yield of exchanges in the cells of these donors also was higher than in cells of the control donors.

A similar result for the yield of exchanges in cells of a patient with the translocation form of Down's syndrome was obtained by the writers previously [5]. However, the spontaneous level of aberrations was raised in the lymphocytes of this patient and also of his mother, a phenotypically normal woman with the karyotype 45, XX, t(D; 21); the number of fragments in irradiated cells from this woman also was increased.

The dose—effect curves for the yield of radiation-induced exchanges are illustrated in Fig. 2.

Comparison of the radiosensitivity of trisomic and diploid cells from a patient with the mosaic form of Down's syndrome with the radiosensitivity of cells of patients with the trisomic form and of control donors revealed an increase in the yield of radiation-induced exchanges in the patient's trisomic cells. The yield of fragments and chromatid aberrations and also of spontaneous chromosomal aberrations in the diploid and trisomic cells of this patient did not differ from the corresponding values or control donors.

Investigations of dependence of the observed yield of chromosomal aberrations on the duration of culture in cells of the patients with the mosaic form of Down's syndrome, irradiated in a dose of 3 Gy, showed that the yield of radiation-induced exchanges in trisomic and diploid cells changes very little with a change in culture time, and remains practically constant between 55 and 65 h after irradiation. Similar results were obtained for cells of one of the control donors (Fig. 3).

The results of this investigation thus indicate an increase in the radiosensitivity of chromosomes in the cells of patients with different forms of Down's syndrome. Despite the variability of individual radiosensitivity observed as a whole, it must be accepted that the increased yield of radiation-induced chromosomal aberrations is a sufficiently characteristic feature of the cells of patients with Down's syndrome.

The cellular nature of the repair defect leading to an increase in radiosensitivity of the chromosomes can be revealed most convincingly by comparison of our own data showing the greater fragility of chromosomes in trisomic cells than in diploid cells of the patient with the mosaic form of Down's syndrome and data in the literature [6]. This important conclusion must be taken into account when investigations into the prevention of increased radiosensitivity of aberrant human cells are planned.

Since, as already mentioned above, in Down's syndrome there is an evident increase in the frequency of malignant tumors, it can be concluded that the repair defect relative to the criterion of increased chromosomal mutability under the influence of ionizing radiation reflects a defect (or defects) of DNA repair causatively linked with carcinogenesis.

Elucidation of the connection between defective DNA repair and other symptoms characteristic of Down's syndrome is of great interest. It can be concluded from the results of the present investigation and data in the literature that both trisomic and translocation forms of Down's syndrome undoubtedly imply that these patients belonged to an increased risk group.

LITERATURE CITED

1. A. P. Akif'ev, G. R. Mutovin, E. V. Korotkov, et al., in: The Cytogenetics of Aging [in Russian], Tbilisi (1977), p. 11.
2. L. O. Badalyan, G. R. Mutovin, E. K. Khandogina, et al., in: Artificial Lengthening of the Lifespan of a Species [in Russian], Moscow (1978), p. 31.
3. "A method of analysis of chromosomal aberrations as a biological indicator of the influence of external environmental factors on man," Technical Recommendations [in Russian], Moscow (1978).
4. V. M. Mikhel'son and N. V. Tomilin, in: Human Genetics [in Russian], Vol. 4, Moscow (1979), p. 103.
5. E. K. Khandogina, G. R. Mutovin, I. V. Filyushkin, et al., Med. Radiol., No. 2, 53 (1980).
6. H. J. Evans, in: Chromosome and Cancer, ed. J. German, New York (1974), p. 191.
7. C. C. Huang, A. Bannerjee, J. C. Tan, et al., J. Natl. Cancer Inst., 59, 33 (1977).
8. B. Lambert, R. Hansson, F. H. Bui, et al., Am. J. Hum. Genet., 39, 293 (1976).
9. M. C. Sasaki, Mutat. Res., 29, 433 (1975).
10. R. B. Setlow, Nature, 271, 713 (1978).