transport systems for phenylalanine in mammalian cell membranes [10] and detection of the PH-antigen in a tissue directly involved in regulation of the phenylalanine supply to the fetus.

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CHANGES IN NUCLEOID DNA STRUCTURE AND ADHESIVE PROPERTIES OF BLOOD LEUKOCYTES IN ANIMALS SOON AFTER IRRADIATION

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Much attention is currently being paid to the study of the molecular mechanisms of cellular adhesion. The structure of adhesion receptors and their connection with individual segments of chromosomes and concrete regions of DNA coding for receptor molecules or their fragments are being investigated [8, 10, 11]. Meanwhile it has been shown that during irradiation of cells significant disturbances of the supercoiled structure of DNA take place [9], which as we know are associated with expression, repair, and degradation of genetic material [1, 7]. These structural changes can be recorded by two-wave fluorescent analysis of the DNA of blood cell nucleoids [3, 4]. However, relations of the above-mentioned postradiation changes in DNA and functional properties of the leukocytes, especially their adhesive properties, have not been investigated.

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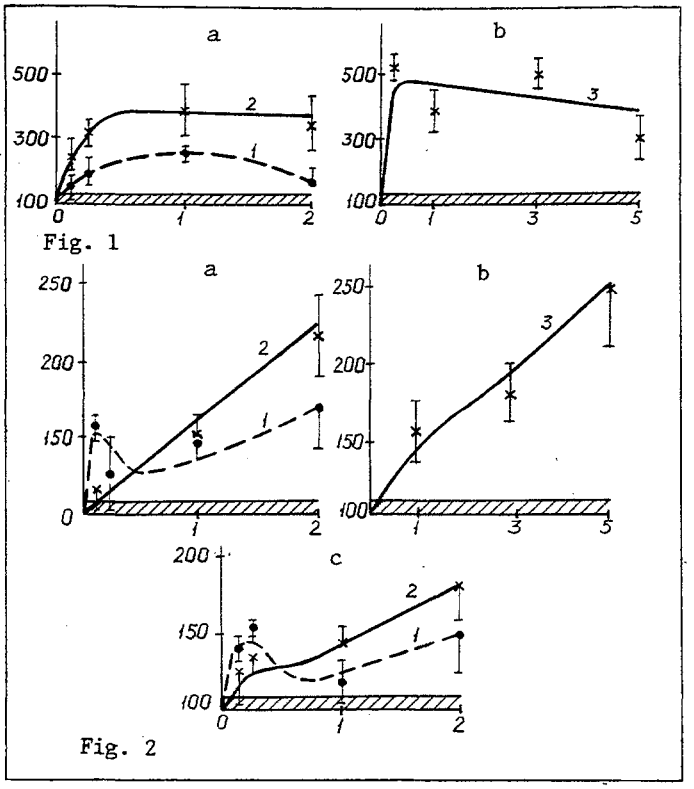

Fig. 1. Dynamics of postradiation changes in fraction of adhesive leukocytes in blood of rats (a) and monkeys (b). Abscissa, time after irradiation (in days); ordinate, fraction of adhesive leukocytes (in per cent of control values). Legend to graph: 1) irradiation of rats in dose of 1.4 Gy; 2) irradiation of rats in dose of 8.4 Gy; 3) irradiation of monkeys in dose of 6.2 Gy.

Fig. 2. Dynamics of postradiation changes in fluorescent index of supercoiled structure of nucleoid DNA of total (a) and nonadhesive (c) peripheral blood leukocytes from rats and total leukocytes (b) from monkeys. Abscissa, time after irradiation (in days); ordinate, value of CRF of leukocyte nucleoid DNA (in percent of control values). Legend to graph the same as to Fig. 1.

The aim of this investigation was to study the correlation between receptor-mediated adhesion and postradiation changes in the cell genome. The aim of the research was to compare the dynamics of changes in adhesive properties and in the degree of supercoiling of DNA of the peripheral blood leukocytes in the early stages after whole-body uniform irradiation of small and large laboratory animals.

EXPERIMENTAL METHOD

The experimental material consisted of whole blood from monkeys (*Macaca nemestrina*) weighing 4-6 kg, irradiated in a dose of 6.2 Gy on the GUBE-4000 apparatus (dose rate 1.2 Gy/min ^{60}Co), corresponding to LD 100/45, or noninbred male albino rats weighing 160-180 g, irradiated on the "Luch-1" apparatus (dose rate 0.45 Gy/min ^{60}Co) in doses of 1.4 Gy (LD 0/30) and 8.4 Gy (LD 100/30). Membrane changes in the total population of blood white cells were assessed on the basis of changes in adhesion of the leukocytes on columns of cotton-viscose wadding for 10 min at 37°C [2]. Values of the parameter measured, consisting of fractions of adhesive leukocytes (FAL) were determined as the ratio of the number of cells adherent to the column with wadding and the total number of cells applied to the column. Analysis of the leukocyte population in the eluate showed that, under the

conditions of incubation used, mainly cells of the granulocytic series were adsorbed on to the column, whereas the eluate was rich (up to 80%) in lymphocytes. Aliquots of the original cell suspension and fractions of leukocytes not adherent to the column were subjected to lysis in order to obtain DNA in the form of nucleoids [9]. After this, the structural state of the DNA was compared in paired samples as described previously [3, 4], by means of the coefficient of relative fluorescence (CRF). Under these circumstances a system of two fluorescent dyes was used: ethidium bromide, the intercalation of which into the polynucleotide depends on the degree of its supercoiling [9, 15], and 4′,6-diamidino-2-phenylindole – an intercalating ligand whose luminescence depends on its specific binding with 3-4 AT pairs along the small groove of the DNA double helix [12, 14]. Thus the value of CRF in relative units reflected the degree of coiling of the polynucleotide per unit length of DNA.

EXPERIMENTAL RESULTS

The value of FAL in the control animals was 10.3 ± 1.2% for rats and 23.5 ± 5.8% for monkeys. Evaluation of the postradiation dynamics of membrane changes showed that 6 h after irradiation in lethal doses and 24 h after exposure to a nonlethal dose of gamma-rays the fraction of adhesive leukocytes in the animals' blood was significantly increased (Fig. 1). After irradiation of the rats in a nonlethal dose the adhesive power of the blood leukocytes returned to the control values within 48 h (Fig. 1a), whereas after lethal irradiation, the parameter did not return to normal even after 7 sec. Our results are in definite agreement with data in the literature on evaluation of the state of the cell surface by means of concanavalin A [13]. These workers also found early (a few hours after nonlethal x-ray irradiation) postradiation changes in the membranes, as well as comparatively rapid return to normal.

Disturbance of the degree of supercoiling of blood leukocyte DNA in the case of lethal irradiation was observed after 24 h and it increased progressively until the end of the experiment. This increase was similar in rats with a "lymphocytic" type of hematopoiesis, and in large laboratory animals, whose blood formula is similar to that in man (Fig. 2a, b). Meanwhile, after nonlethal irradiation of rats, an increase in the value of CRF of total leukocytic DNA took place as early as 3 h after γ-ray irradiation, after which its value fell temporarily, and only then did it rise once again until 48 h after irradiation. These changes were most probably due to lymphocytes, for similar fluctuations of this parameter were observed in the fraction of nonadhesive leukocytes (Fig. 2c), in which, as was pointed out above, cells of the lymphoid series predominate.

Our suggested method of biparametric fluorescent analysis of DNA (with the addition of ethidium bromide and of 4′,6-diamidino-2-pheylindole to the sample), has been used by several other workers to study the supercoiled structure of DNA of somatic cell nucleoids [5, 6]. Results obtained in this way were compared with other methods of DNA analysis (in particular, viscosimetry), and it was shown that with an increase in the value of CRF the results obtained by the two methods can be interpreted as one, and they indicate loosening of the supercoiled organization of chromosomal DNA after irradiation.

The results of the present experiments suggest that early temporary disturbances of DNA supercoiling in response to nonlethal irradiation of rats are most probably due to repair of the genome and are not reflected in changes in the adhesive properties of the white blood cells. Meanwhile the progressive increase in the value of CRF with time after irradiation probably reflects loosening of the structure of DNA of the blood leukocytes in the course of their death, for it depends on the dose of γ-irradiation and it was more marked when the animals were irradiated in doses of LD 100/30 than LD 0/30. Comparison of the dynamics of the membrane changes with changes in DNA structure indicates that during postradiation death of leukocytes disturbance of receptor-mediated adhesion precedes and, indeed, may perhaps initiate destruction of the supercoiling of their genetic material. Thus a comparative study of postradiation disturbances of DNA structure and of leukocyte membranes led to the conclusion that after nonlethal irradiation the early changes in DNA supercoiling are temporary, they are recorded mainly in lymphocytes, and they have no significant effect on the adhesive properties of white blood cells. After lethal γ-irradiation functional changes in leukocyte membranes precede decondensation of the supercoiled structure of DNA, and may perhaps participate in the initiation of this process. Changes which we observed in the adhesive properties and supercoiling of blood leukocyte DNA may be the basis for establishment of the differential diagnosis between radiation lesions of different degrees of severity in the early period after irradiation.

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