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STRUCTURE OF INTERPHASE CHROMATIN OF PERIPHERAL BLOOD CELLS IN CHILDREN WITH ACUTE LYMPHATIC LEUKEMIA AND THEIR HEALTHY PARENTS

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It has been shown by thermal denaturation of cellular deoxyribonucleoproteins (DNP) [6], in the writers' modification [2], followed by recording of changes in chromatin structure by cytofluorometry with acridine labeling [5], that binding of acridine orange (AO) with chromatin DNA of human peripheral blood lymphocytes between temperature of 20 and 100°C has clearly defined regular features and is represented by a curve with maxima in particular temperature intervals [2]. Analysis by computer showed that healthy persons with identical individual characteristics (number and location of the maxima) of their melting profiles of lymphocyte chromatin are distributed into groups. In 40% of cases (independent of sex) six maxima were obtained at particular temperatures (the modal class), whereas in 60% of cases various types of deviations (sex-dependent) were observed, and which, by their character, could be divided into a number of subgroups: a control group of five women and seven men, and not less than five identical cases in each subgroup.

Specific differences in cell chromatin melting profiles have been discovered in patients with various types of inborn and acquired [3, 4] chromosomal anomalies, and they correlate with similar characteristics of the curves in individual subgroups of healthy persons, so that the presence of genetic predisposition to particular diseases can be postulated. Accordingly structural features of cell chromatin were analyzed in children with acute lymphatic leukemia (ALL) and in their healthy parents.

EXPERIMENTAL METHOD

Nuclear chromatin of peripheral blood cells was studied in children with ALL aged from 0 to 12 years (21 children) at different stages of the disease, and in their healthy parents (19 families). Blood was taken in the Children's Hematology Department of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, where the diagnosis was made on the basis of clinical-hematologic and immunologic investigations. Changes in chromatin structure on heating (from 20 to 100°C) were recorded as the amount of luminescent label (AO) bound every 2-3°C. The tests were carried out on cells incubated for 1 h in Eagle's nutrient medium with the addition of 10% autologous serum. The intensity of luminescence of AO,

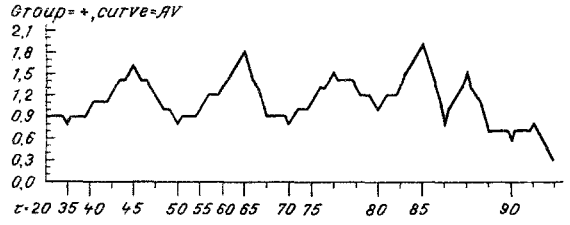

Fig. 1. Melting profile of interphase chromatin of healthy human lymphocytes (modal class), obtained by acridine labeling. Abscissa, temperature (in °C); ordinate, mean data of fluorescence at 530 nm (in relative units).

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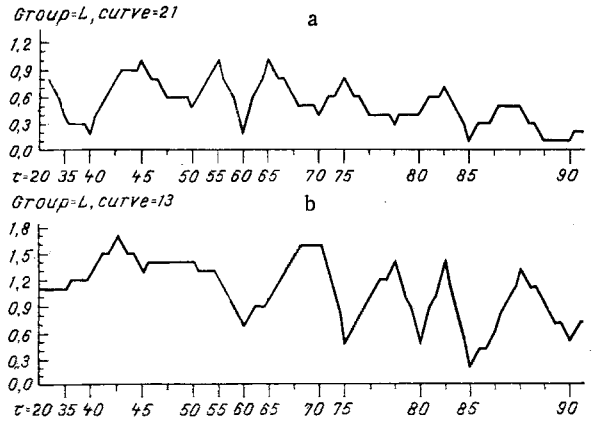

Fig. 2

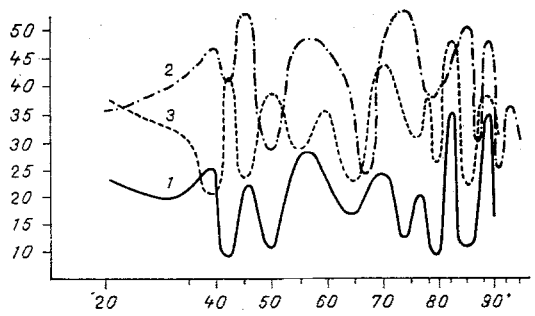

Fig. 3

Fig. 2. Melting profile of interphase chromatin of peripheral blood cells from children with ALL. a and b) Separate cases. Remainder of legend as to Fig. 1.

Fig. 3. Interphase chromatin melting profiles of peripheral blood cells of a patient with ALL (1) and his healthy parents: father (2) and mother (3). Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO bound with chromatin of human peripheral blood cells: $F_{550}T°C/F_{530}20°C$.

bound with DNA of the cell chromatin, was measured on an MSP-0.5 microscope-photometer (Opton, West Germany). Luminescence was induced by light with a wavelength (λ) of 365 nm and recorded at λ = 530 nm. The apparatus, details of the experiments, and processes of isolation and culture of the peripheral blood cells were described previously [4]. Thermal denaturation of chromatin in the cell was done by the method in [6], in the writers' modification. Melting curves of the cell chromatin were compared at all points of the curve relative to the mean intensity of fluorescence and the parallel nature of its change. The significance of differences was estimated by a version of two-factor dispersion analysis [1]. Melting profiles of chromatin were analyzed by computer (Sperry Univac 90-30-B) using a specially written program [2]. Chromatin melting profiles of healthy human leukocytes served as the control.

EXPERIMENTAL RESULTS

The curve of untreated children with ALL (85% of cases), analyzed in the acute period of the disease, had six maxima, of which the first four peaks (unlike in the control) were located in temperature intervals which differed individually for each patient. A general rule also observed for all patients: absence of a maximum at 85°C and the appearance of a maximum at 82°C (Fig. 1).

The results are experimental evidence of a change in the supramolecular packing of the cell chromatin of patients with ALL. No statistically significant differences could be found in details of chromatin structure in cells at different stages of the disease (the acute period and remission).

Analysis of interphase chromatin melting profiles of peripheral blood lymphocytes from healthy mothers of children with ALL showed that the curves differed from those of the modal class, and had an individual, "unclassifiable" character, similar in some respects to those of the affected children. A general rule (95% of cases) also was discovered: absence of a maximum at 85°C and its appearance at 82°C (found in the control group of women in 2% of cases, Fig. 2).

Analysis of the interphase chromatin melting profiles of peripheral blood lymphocytes of healthy fathers of children with ALL showed that, in 80% of cases, there was no maximum on the curves at 85°C, but it appeared at 82°C, and this deviation occurred most frequently in the control group of men (25% of cases). In 20% of cases the curves were indistinguishable from those of healthy individuals belonging to the group of the modal class (Fig. 3).

The similarity of the structural features of interphase chromatin of the children with ALL and those of their healthy mothers thus suggests a connection between the disease in the child and the character of his mother's genotype. It is possible that, if a combination of

certain changes in parental genotypes is present, the degree of risk of giving birth to children genetically predisposed to the development of this disease is increased.

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