10. R. F. Burk, K. Nishiki, R. A. Lawrence, et al., J. Biol. Chem., 253, 43 (1978).
11. I. V. Fridovich (J. Fridowich), in: Molecular Mechanisms of Oxygen Activation, New York (1974), p. 453.
12. S. Hosoda and W. Nakamura, Biochim. Biophys. Acta, 222, 53 (1970).

COMPUTER CLASSIFICATION OF MELTING PROFILES OF INTERPHASE CHROMATIN FROM HUMAN LYMPHOCYTES

K. N. Fedorova, I. É. Yudina, and E. B. Voronov

UDC 612.112.94.014.24.001.33

It was shown previously by the method of luminescence fluorometry with acridine orange labeling, in the writers' suggested [1] modification of the method of thermal denaturation of cell DNP [4], that the structure of the interphase chromatin in cells of patients with Down's syndrome has specific differences from that of identical healthy human cells [2, 3]. In melting profiles or temperature-dependent structural transitions of interphase chromatin of healthy human lymphocytes polymorphism was found.

The object of the present investigation was to study the structure of the chromatin complex of healthy human lymphocytes in interphase.

EXPERIMENTAL METHOD

The structure of the interphase chromatin of peripheral blood lymphocytes of 164 healthy persons (98 men and 66 women) aged from 20 to 45 years was studied by comparative analysis of the melting curves or temperature-dependent structural transitions in intracellular DNP. Readings were taken at intervals of 1-2°C. Changes in the structure of chromatin during heating were recorded as the quantity of luminescent label (acridine orange, AO) bound. The investigations were conducted on short-term cell cultures, i.e., on cells incubated for 1 h in Eagle's nutrient medium with the addition of 10% autologous serum.

The intensity of luminescence of AO bound with DNA of lymphocyte chromatin was measured on the MSP-0.5 microscope-photometer (from Opton). Luminescence was excited by light with λ = 365 nm and recorded at λ = 530 nm with the aid of an appropriate interference filter. To determine the degree of orderliness of DNA in the cell, or to determine the coefficient $\alpha = F_{640}/F_{530}$, parallel recordings were made of changes in the intensity of luminescence in the region λ = 640 nm. However, since the shape of the curve for α was practically independent of F_{640}, which was only 10-15% above the background level, data are given in this paper only for the change in F_{530}. The apparatus, details of the experiments, and method of isolation and culture of the lymphocytes were described previously [1].

The data on the intensity of fluorescence (F_{530}) of the intracellular DNP—AO complex within the temperature range 20-100°C were analyzed on a Sperry Univac 90-30-B computer, by means of a specially written program (by E. B. Voronov, All-Union Computer Center). The initial data for this program were numerical values of F_{530} of the DNP—AO complex at 24 points corresponding to temperatures of 20, 35, 40, 42, 45, 47, 50, 55, 60, 65, 76, 70, 75, 77, 78, 80, 82, 85, 87, 88, 89, 90, 92, and 95°C. Each curve was analyzed for the presence of a maximum in the vicinity of points corresponding to 45, 65, 78, 88, and 92°C. These neighborhoods were as follows: [42, 47], [60, 67], [77, 78], [85, 85], [90, 92]. Characteristics of the presence or otherwise of a maximum were established on the recorded curve for each of these neighborhoods: M) maximum present; N) no maximum present; V) indefinite (absence of data). For this purpose, the presence of a maximum was first analyzed at each point of the neighborhood separately. The analysis was carried out as follows: values of the curve at the point of analysis and two neighboring points were examined. If the value of the curve at the point

KEY WORDS: lymphocytes; chromatin; thermal denaturation; fluorescence microscopy.

Institute of Medical Genetics, Academy of Medical Sciences of the USSR. All-Union Computer Center, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 4, pp. 453-455, April, 1981. Original article submitted July 2, 1980.

 0007-4888/81/9104-0490$07.50

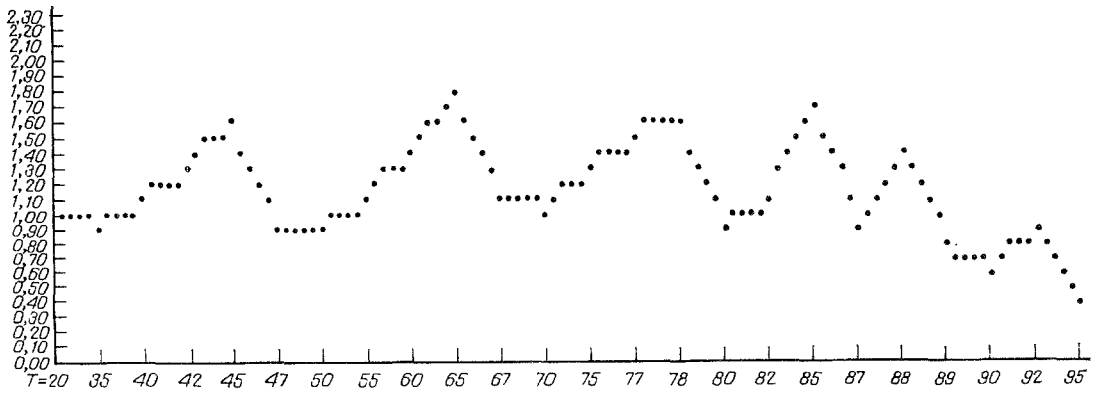

Fig. 1. Melting profile of interphase chromatin of lymphocytes of most healthy persons. Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO bound with chromatin of human lymphocytes at that temperature to intensity of fluorescence of bound AO at 20°C.

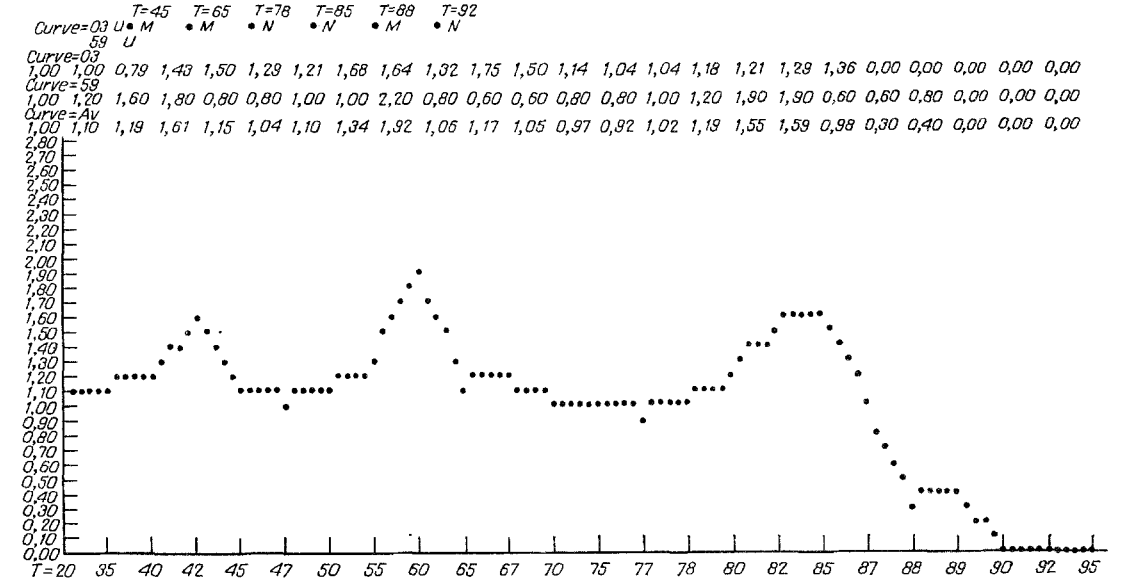

Fig. 2. Melting profile of DNA of lymphocytes from women with the most frequent type of deviations from the standard variant of normal — fusion of two neighboring maxima (78 and 85°C). Legend as to Fig. 1.

of analysis was greater than or equal to values at neighboring points, the presence of a maximum (not strict: [±2°]) was noted. In all other cases it was considered that no maximum was present. This operation was carried out for all points of the neighborhood. Next, all the curves were classified, i.e., divided into classes or groups of curves with coincident characteristics of the presence of maxima M (or N) in six neighborhoods.

EXPERIMENTAL RESULTS

Analysis of the data (F_{530} of the DNP—AO complex within the temperature range 20-100°C) by computer showed that in about 40% of cases, irrespective of sex of the subject, the melting profiles or temperature-dependent structural transitions of healthy human interphase chromatin have a complex, but regularly repeating pattern, consisting of a curve with six maxima at certain temperatures: 45, 65 (±3), 78 (±1), 85, 88, and 94 (±2)°C, i.e., this parameter has a clearly defined "similarity" in different subjects. A similar sort of general pattern of melting profiles of intracellular DNA was found in the most numerous group of healthy subjects (in 50% of women tested and in 40% of men), and this was described as the classical or standard variant of normal (Fig. 1).

In the remaining 60% of cases the types of abnormalities observed were varied but they were regularly repeated in different individuals: 1) absence of one maximum (or sometimes of

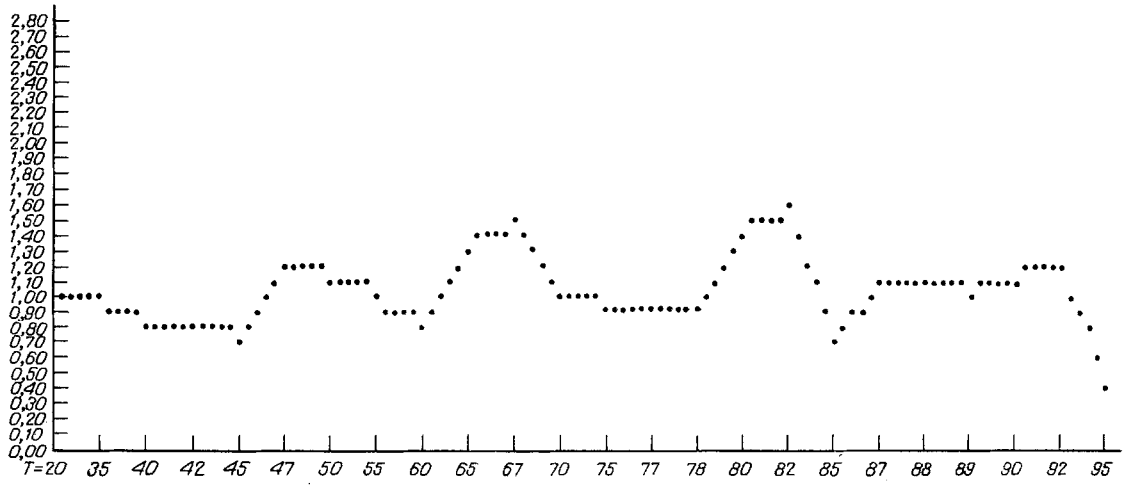

Fig. 3. Melting profile of DNA of lymphocytes from men with most frequent type of deviations — absence of maximum at 85°C and the appearance of a maximum at 82°C. Legend as to Fig. 1.

two); 2) the merging of two neighboring maxima into one; 3) a shift of one maximum to the left or right by more than 3°C.

The character of the abnormalities depended on the subject's sex in most cases. The commonest type of abnormality in women was the merging of two neighboring maxima into one: 78 and 85°C in nine cases, 85 and 88°C in eight cases, 88 and 92°C in nine cases (Fig. 2). An abnormality less frequently found in the group of women was absence of one maximum: 42°C in two cases, 85°C in three cases, 92°C in two cases. Unclassifiable or nonstandard melting profiles were found in two cases. In the entire control group consisting of women, five subgroups could be distinguished on the basis of matching characteristics for the presence of maxima on the DNP melting curve, with at least five identical cases in each subgroup.

In the male control group seven subgroups were distinguished. The main group consisted of cases in which all six maxima were found at particular temperatures — about 35-40%. The commonest type of abnormality was absence of the maximum at 85°C and the appearance of a maximum at 82°C, in about 25% of cases (Fig. 3).

In isolated cases abnormalities characteristic of the female group were found, i.e., merging of maxima (about 2%). Compared with the control group consisting of women, there were more unclassifiable, strictly individual types of abnormalities (about 17%).

Thus for the first time a computer has been used in an attempt to classify individual cases of structure of the interphase chromatin on the basis of melting profiles or temperature-dependent structural transitions of DNP of healthy human lymphocytes.

LITERATURE CITED

1. K. N. Fedorova and D. M. Spitkovskii, Byull. Éksp. Biol. Med., No. 6, 672 (1976).
2. K. N. Fedorova and V. N. Shchepilov, Byull. Éksp. Biol. Med., No. 5, 603 (1980).
3. K. N. Fedorova, J. Ment. Defic. Res., 21, 85 (1977).
4. R. Rigler, D. Killanber, and Z. Bolund, Exp. Cell Res., 55, 215 (1969).