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# CHANGES IN LYMPHOCYTE CHROMATIN IN DOWN'S SYNDROME REVEALED BY THE THERMAL DENATURATION METHOD

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A luminescence-microscopic study using acridine orange with a short-term culture of human cells showed that DNA melting profiles of the chromatin of intact lymphocytes of healthy donors are curves with maxima (F 530) in the temperature regions of 45, 65, 78, 85, 88, and 92°C ($P < 0.01$). The melting profiles of lymphocytes of patients with Down's syndrome are curves with maxima in the temperature regions of 65, 85, 88, and 92°C ($P < 0.01$). The absence of a decrease in the intensity of fluorescence between 78 and 85°C is evidently due to the greater degree of condensation of certain regions of the chromatin complex of the trisomic cells. The possible mechanisms of the structural changes in the interphase chromatin of human lymphocytes under the influence of temperature are discussed.

KEY WORDS: Down's syndrome; lymphocytes; interphase chromatin.

By luminescence microscopy with acridine orange the writers previously showed changes in the structure and function of chromatin of interphase nuclei of peripheral blood lymphocytes from patients with Down's syndrome [5]. It was postulated that changes in the functional activity of the cell chromatin were connected with an increased degree of its condensation. Differences in the degree of condensation of the chromatin of normal and trisomic cells, whatever the changes inducing them, must ultimately, it is considered, be determined by changes in the relations between DNA and protein. This change must be expressed as a change in the degree of dissociation of the protein of the chromatin complex. Unfortunately, no methods are yet available for determining the quantity of dissociated protein directly in interphase nuclei. However, the number of phosphate groups of DNA accessible to binding with test agents can be determined. Acridine orange [7] was used as the test agent, and the temperature factor [6, 8] as the procedure leading to dissociation of the protein component of the cell chromatin in a medium of physiological ionic strength.

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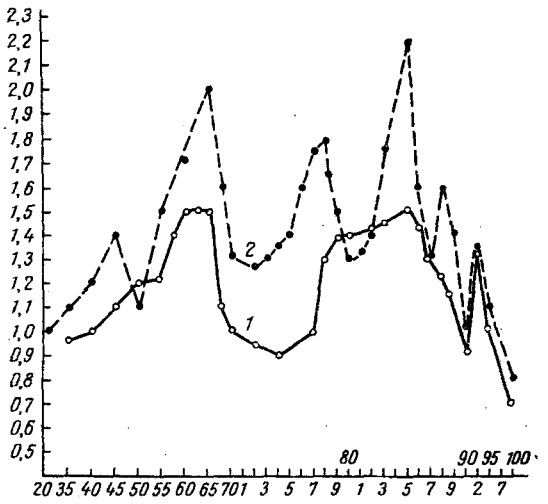


Fig. 1. Melting curves of DNA of nuclear chromatin of human peripheral blood lymphocytes obtained by luminescence microscopy with acridine orange (F 530). Ordinate, ratio of intensity of fluorescence of acridine orange at the given temperature and intensity of fluorescence at 20°C (F 530 T°)/(F 530 20°); abscissa, temperature (in °C): 1) lymphocytes of patients with Down's syndrome; 2) lymphocytes of healthy donors.

Opinions in the literature are not entirely unanimous [2, 7] on the temperature when DNA of cell chromatin begins to melt, probably for two main reasons: 1) purely mechanical aspects of fixation of the cells at a particular temperature, for the ability of DNA in the composition of the cells to undergo rapid renaturation is well known [1]; 2) differences in the physiological states of the chromatin of cells studied even under the same experimental conditions.

The object of the present investigation was to study temperature-dependent changes in the binding of acridine orange with DNA of the chromatin of peripheral blood lymphocytes from human donors and patients with Down's syndrome.

## EXPERIMENTAL METHOD

The nuclear chromatin of the peripheral blood lymphocytes of 20 patients with Down's syndrome aged from 16 to 20 years and of 26 healthy donors aged from 18 to 30 years was studied. The methods of taking the blood, preparing the specimens for microspectrofluorometric analysis, the parameters of the apparatus and the limits of the optical regions within which the measurements were made, were described previously [5]. Temperature denaturation of the chromatin in the cells was carried out by Ringertz's method [8] with the following modifications: 1) Formalin was removed from the fixing solutions because it causes changes in the DNP complex that are difficult to control. 2) The resistance of the cell chromatin to temperature was investigated between 25 and 100°C at intervals of 2 and 2.5°C and from 70 to 90°C at intervals of 1°C (±0.1°). 3) To prevent renaturation, the slides with the fixed cells after heating in standard salt solution (SSC) for 20 min at the appropriate temperature were transferred to the fixative (acetone:ethanol in the ratio 1:1), previously cooled to between −5 and −7°C. After this, the temperature of the fixing mixture did not exceed 0°C.

As the control tests showed, heating the preparation for 20 min was sufficient to cause completion of the corresponding temperature-dependent changes in the cell chromatin at all temperatures within the range studied.

## EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the binding of acridine orange by DNA of the lymphocyte chromatin in response to the action of heat is a complex picture. For lymphocytes of healthy donors an increase in the intensity of fluorescence of the dye by 2-2.5 times was observed in the region of temperatures of 45, 65, 78, 85, 88, and 92°C ($P < 0.01$). Starting from 94°C, the values of F 530 fell.

In investigations on intact animal and human cells some workers [3, 4] observed two maxima on the curve of this parameter and the optical density of the substrate as a function of temperature: in the region of DNA melting temperatures (about 85°C) and at 55-60°C. In the present experiments six maxima were found, evidently because of the higher resolution of the curve when the parameters were recorded every 1-2°C. What is the nature of the observed maxima?

It is difficult at the moment to give an unequivocal explanation of the phenomena discovered. However, there is experimental evidence to show that physicochemical modifications both of the isolated nucleoprotein and of the chromatin complex of intact cells under the influence of temperature may be due to two main factors: 1) dissociation of the protein or labilization of its bond with DNA [9], leading to a reduction in thermal stability and to an increase in the dye-binding properties of the cell chromatin (in that case it must be remembered that any deproteinization must in principle change the structure of the chromatin complex, although this consideration unfortunately still remains highly theoretical); 2) different regions of DNA in chromatin differ in their degree of thermostability [4]. At the present level of knowledge it is difficult to estimate the contribution of each of these components to the structural modifications of the DNP in cells at different temperatures.

As regards structural changes in the cell chromatin at temperatures close to the melting temperature, the evidence in the literature of the absence of DNA renaturation at 65°C [1] suggests that within this range of temperatures the changes in DNP discovered are due mainly to processes of dissociation of the protein (or labilization of its bond with DNA). This conclusion, however, in no way rules out the possibility of denaturation of certain of the most thermolabile regions of DNA, a situation probably connected with a decrease in intensity of luminescence after each maximum. The ascending regions of the curve thus reflect an increase in the quantity of acridine orange bound with DNA. This phenomenon can be interpreted broadly as a process of chromatin decondensation. After each maximum there is a decrease in the quantity of dye bound with DNA. These phenomena correspond in general to the structural changes in chromatin leading to its denser packing, although the changes may be different in nature within the different temperature intervals. The sharp decrease in the value of F 530 after 94°C suggests that this temperature can be taken as the beginning of cooperative transition of DNA into the coiled state.

As Fig. 1 shows, in short-term cultures of trisomic cells (obtained from patients with Down's syndrome) the intensity of luminescence of the dye in 17 of 20 cases rose regularly by 1.5-2 times at temperatures of 65, 85, 88, and 92°C ($P < 0.01$), i.e., four maxima were obtained. In cells from patients with regular trisomy the curve rose smoothly in the region of temperature from 78 to 85°C, with one indistinct maximum in the region of 85°C. The relative sizes of all the peaks in all cases were much smaller than for the control preparations.

These experimental results point unequivocally to the lower intensity of the processes leading to an increase in the binding of acridine orange depending on the temperature. This phenomenon must be interpreted as reduced ability of the nuclear chromatin of these individuals to undergo decondensation; it definitely confirms the view expressed previously that the degree of condensation of the nuclear chromatin is increased in patients with Down's syndrome.

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