# ANALYSIS OF THE MODIFIED STRUCTURAL STATE OF THE CHROMATIN IN DOWN'S SYNDROME

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The relationship between optical density ($\lambda$ = 260 nm) of the nuclear chromatin of normal human lymphocytes and lymphocytes from patients with Down's syndrome and temperature was studied. Lymphocyte nuclei from healthy donors were characterized by the presence of a low-temperature (about 70°C) absorption maximum which was absent in nuclei of lymphocytes from patients with Down's syndrome. Analysis of this relationship for particular regions of the nucleus showed the existence of at least two types of regions – those with a low-temperature absorption maximum and those without. For Down's syndrome, the content of regions of the latter type in the lymphocyte nuclei was found to be greater than normal.

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

One of the factors determining the functional activity of chromatin is its structural state. Previous investigations [2, 3] have shown that the structural organization of the lymphocyte chromatin in Down's syndrome is abnormal. Accordingly, the important question arises whether the chromatin of the whole cell nucleus is changed in this pathological entity or whether it is changed only in certain regions.

To study this problem a comparative investigation was made of the dependence of the optical density at 260 nm on temperature for chromatin from lymphocytes of healthy human subjects and patients with Down's syndrome for the cell nucleus as a whole and, for the first time, for individual regions of the nucleus.

## EXPERIMENTAL METHOD

Peripheral blood lymphocytes from healthy human blood donors and patients with Down's syndrome were used as the test object. Lymphocytes were sedimented from serum on quartz slides at 37°C and fixed in a mixture (1:1) of alcohol and acetone. The specimens were transferred into 0.14 M NaCl, pH 7.0, after preliminary taking through 100%, 60%, and 30% alcohols. The specimens stayed in each of the alcohol solutions for 5 min and in the medium with NaCl for 30 min before adhesion of the preparation.

The slide was placed in a thermostatically controlled system of the writers' own design, which was combined with the scanning stage of the microscope.* The use of such an arrangement enabled changes in optical parameters taking place in individual regions of the cell nucleus during exposure to different temperatures to be monitored and it did away with the problems connected with the need for preventing renaturation.

Measurements were made on a microscope-photometer (Opton Model 05, West Germany). An Ultrafluar ×100/1.25 Glyz objective was used, together with an Ultrafluar 10/0.2 condenser. The diameter of the probe was 0.8 $\mu$ and the scanning step 0.5 $\mu$.

The results of the measurements were recorded on punched tape and processed by computer. Distributions of optical density over the area of the nucleus were obtained at each temperature, together with the integral optical density and histograms of distribution of the numbers of probes within the optical density intervals.

*The system will be described in a forthcoming publication.

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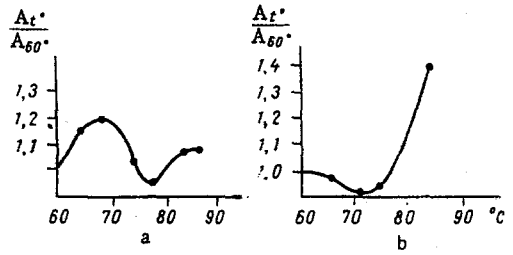


Fig. 1

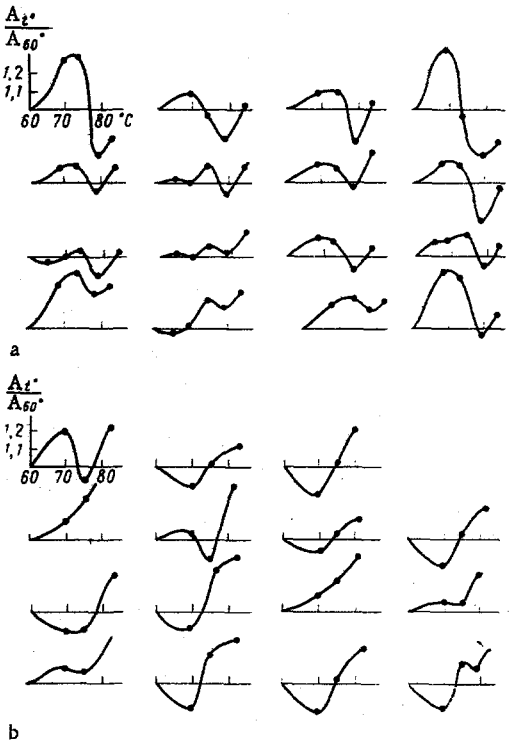


Fig. 2

Fig. 1. Integral optical density of human lymphocytes from normal donors (a) and from patients with Down's syndrome (b) at 260 nm as a function of temperature.

Fig. 2. Changes in absorption at 260 nm of individual regions of lymphocyte nuclei as a function of temperature: a) lymphocytes from healthy donors; b) lymphocytes from patients with Down's syndrome.

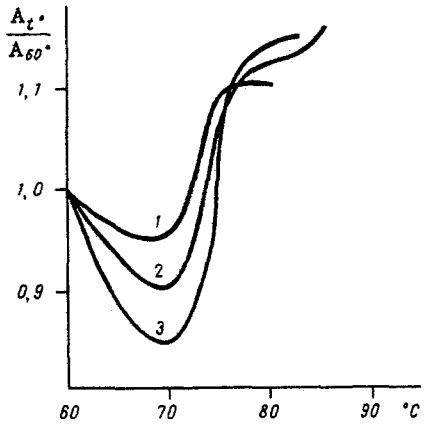


Fig. 3. Optical density as a function of temperature for regions of the nucleus with absence of growth of absorption at 70°C. 1, 2, 3) Quantity of chromatin with low-temperature absorption maximum in these regions is 30, 45, and 55%, respectively.

TABLE 1. Number of Regions Showing Increase of Absorption to 70°C (in % of total number of regions)

| Cells from healthy subjects | Cells from patients with Down's syndrome |
|---|---|
| 75 | 25 |
| 71 | 40 |
| 62 | 13 |
| 78 | 33 |
| 76 | 20 |

## EXPERIMENTAL RESULTS

Curves showing the integral optical density at 260 nm of chromatin from normal human lymphocytes and lymphocytes of patients with Down's syndrome as a function of temperature are given in Fig. 1. A maximum of absorption at about 70°C (low-temperature peak), with a subsequent rise in the region of melting temperatures of free DNA, can be seen on these curves for healthy human lymphocytes. The value of the maximum relative to the optical density at 60°C was $A_{70°} = 1.21 \pm 0.07$. The corresponding curve for Down's syndrome showed no increase in absorption to 70°C ($A_{70°} = 0.94 \pm 0.06$; $P < 0.001$).

To study structural changes in chromatin in individual regions of the cell nucleus, a picture of the nucleus at each temperature, characterized by values of the optical densities in points, was split up into regions with 16 points (probes) in each (with sides measuring 4 × 4).

A typical picture of the relationship between the optical density of the chromatin and temperature in the regions of cells from healthy subjects and patients with Down's syndrome is shown in Fig. 2. Clearly in individual regions of the cell nuclei from patients with Down's syndrome an increase in absorption was observed up to 70°C, as is characteristic of healthy human lymphocytes. Meanwhile, in some regions of the nucleus of lymphocytes from healthy donors the low-temperature peak was absent. The relative percentages of these regions are given in Table 1. Incidentally, no change was found in the size of the nucleus within the temperature range from 60 to 70°C.

In regions of cell nuclei from healthy donors with no maximum at 70°C, the fall in absorption at that temperature characteristic of cells from patients was not found in many cases. This can be explained by the presence of chromatin characterized by a low-temperature structural transition in these regions. Analysis of regions of the nucleus smaller (four probes) than those indicated above (16 probes) in fact showed that some regions with absence of the maximum can be divided into zones of which some were characterized by an increase in absorption to 70°C and others by a decrease. Accordingly, values of optical density were calculated in these regions of the nucleus and plotted as functions of temperature. The possibility that these regions may differ in their content of chromatin for which the dependence of optical density on temperature was the same as that in the rest of the nucleus was taken into consideration (Fig. 3). With an increase in the fraction of such chromatin, a minimum appeared on the calculated curve and it assumed a similar shape to the curve obtained for the patients' lymphocytes.

The distribution of points with maximal optical density for each cell among the regions of the nucleus also was analyzed. The results showed that the number of these points expressed per unit area was on average 4 times greater for regions with absence of a low-temperature maximum compared with regions with an increase of absorption to 70°C.

A characteristic feature of the relationship obtained between integral optical density of the nuclear chromatin from healthy donors' lymphocytes on temperature, revealed by these experiments, was the presence of a low-temperature transition at about 70°C. A similar low-temperature transition has been demonstrated in other investigations using different methods of analysis of the effect of temperature on the chromatin of cell nuclei [1, 4-6]. The problem of the mechanism of the structural transition observed has not yet been finally solved by direct methods and lies outside the scope of discussion in this paper.

No increase in absorption to 70°C could be seen on the curve of total optical density as a function of temperature for the nuclei of lymphocytes from patients with Down's syndrome. However, the study of the depen-

dence of optical density of chromatin in individual regions of the nucleus as a function of temperature showed that a sharp line can be drawn between the regions occupied by two different types of chromatin, characterized by the presence or absence of a low-temperature absorption maximum both in healthy subjects and in patients with Down's syndrome. The difference lies in an increase in the quantity of chromatin with absence of a low-temperature absorption maximum in Down's syndrome.

The increased concentration of points with maximal optical density in these regions suggests that the presence or absence of the low-temperature maximum is connected with concentration of the chromatin.

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# ACTION OF THE HEPARIN POLYANION ON CHROMATIN PREPARATIONS OBTAINED IN SOLUTIONS OF LOW IONIC STRENGTH


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DNP obtained in solutions of low ionic strength (0.7 mM Na-phosphate buffer, pH 7.0) dissociates under the influence of heparin. The three histone fractions dissociate in the following order: $H_{2a}$, $H_1$, $H_4$. The following order of dissociation is suggested: ($H_{2a}$, $H_{2b}$), $H_1$, ($H_3$, $H_4$). Activation of DNA and RNA synthesis in the cells, nuclei, and chromatin of eukaryotes under the influence of small doses of heparin must be attributed not to dissociation of histone $H_1$, but to dissociation of moderately lysine-rich histones – $H_{2a}$ and possibly $H_{2b}$.



KEY WORDS: heparin; DNA; histone; dissociation; chromatin.


Numerous investigations of the effect of natural polyanions on the structural organization and template properties of chromatin have shown that they cause decondensation of chromatin in cell nuclei [3-5], accompanied by marked activation of DNA and RNA synthesis on the template [6, 13]. One of the natural polyanions that has been most widely studied from these aspects is the mucopolysaccharide heparin, found in cell nuclei [3-6, 13]. The view is currently widely held that activation of DNA and RNA synthesis by heparin (as also by other polyanions) takes place as a result of dissociation of histones from DNA. However, this logical explanation has not been finally proved. There is only indirect evidence of possible dissociation of histones from DNA under the influence of heparin, obtained by the study of thermal denaturation of chromatin preparations [2, 12]. Electron-microscopic investigations of nuclei treated with heparin have shown the presence of amorphous globules, which Arnold et al. [3] have interpreted as chromatin repacked under the influence of heparin, whereas other workers regard them as large aggregates of heparin–protein complexes not bound with DNA [4].

The object of this investigation was to discover whether heparin can induce dissociation of proteins from DNA in chromatin preparations isolated in solutions of low ionic strength (the type of model systems of chrom-


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