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


P. S. Gromov



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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-


Laboratory of Molecular Biology, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.)


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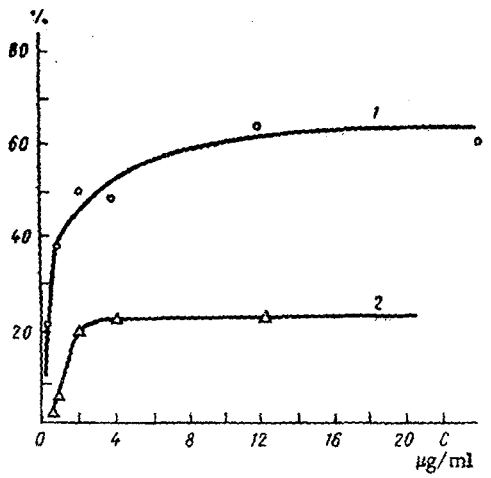


Fig. 1

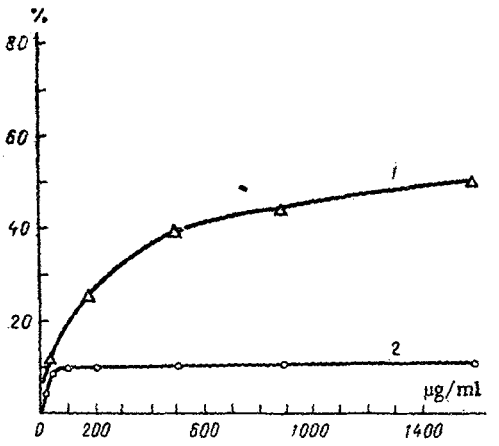


Fig. 2

Fig. 1. Effect of heparin on passage of protein (1) and DNA (2) into supernatant after centrifugation of DNP samples with heparin. Characteristics of original DNP preparation: DNA concentration ($C_{DNA}$) 153 μg/ml, protein concentration ($C_{prot}$) 238 μg/ml. Abscissa, $C_{hep}$ (in μg/ml); ordinate, outflow of protein and DNA (in %).

Fig. 2. Quantity of dissociated protein present in supernatant as a function of heparin concentration in DNP preparation after different times of centrifugation. Characteristics of original DNP: $C_{DNA}$ = 140 μg/ml, $C_{prot}$ = 182 μg/ml. 1) Centrifugation for 6 h, 2) for 18 h. Abscissa, $C_{hep}$ (in μg/ml); ordinate, quantity of dissociated protein in supernatant (in %).

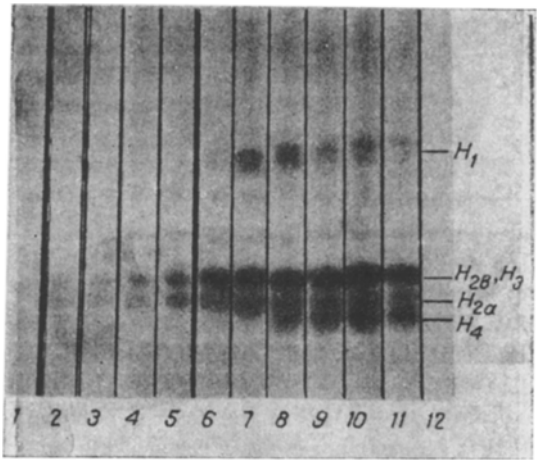


Fig. 3. Electrophoresis of proteins remaining in supernatant after centrifugation of DNP preparation with heparin in different concentrations. Characteristics of original DNP: $C_{DNA}$ = 500 μg/ml, $D_{prot}$ = 740 μg/ml. Heparin/protein ratio in centrifuged samples: 1) 1:25, 2) 1:10, 3) 1:8, 4) 1:5, 5) 1:4, 6) 1:2, 7) 1:1, 8) 2:1, 9) 4:1, 10) 8:1, 11) 10:1, 12) DNP preparation centrifuged without heparin.

atin most widely used at the present time). At the same time, different histones are known to form bonds of different strength with DNA. It was therefore decided also to investigate the possibility of selective dissociation of individual histone fractions from DNA under the influence of heparin.

## EXPERIMENTAL METHOD

Preparations of chromatin (DNP) were isolated from freshly frozen calf thymus tissue [15]. To 8 ml DNP solution an equal volume of solvent (0.7 mM Na-phosphate buffer, pH 7.0), containing different amounts of heparin (from Spofa, Czechoslovakia) was added. After incubation for 30 min at 4°C the samples were centrifuged at 150,000g (Beckman L-2 65B centrifuge, Ti-50 rotor, 50,000 rpm) for 6 or 18 h. The supernatants were investigated for their protein and DNA content. Protein was determined by Lowry's method [9] and DNA by Spirin's method [1]. Electrophoresis of the proteins was carried out in 10% polyacrylamide gel [14]. The samples were dialyzed against a solution consisting of 0.01 M phosphate buffer, 1% 2-mercaptoethanol, 5 M urea, 0.005% bromphenol blue, and 1% sodium dodecylsulfate, at 37°C for 12 h. The electrode buffer was 0.1 M phosphate buffer containing 0.1% sodium dodecyl sulfate, pH 7.1. After the end of electrophoresis the gels were fixed in 12.5% TCA, then washed with distilled water, and stained in a 0.25% solution of Coomassie Blue R-250, 7% $CH_3COOH$, and 20% methanol. The gels were then washed in a mixture of 7% $CH_3COOH$ and 20% methanol.

## EXPERIMENTAL RESULTS

The results of the experiments to study sedimentation of DNP solutions mixed with heparin are given in Figs. 1 and 2. It is clear from Fig. 1 that with an increase in the heparin concentration in the DNP solution the quantity of protein and DNA remaining in the supernatant increased up to a certain level and then remained unchanged despite a considerable increase in the heparin content. The ratio between the protein and DNA concentrations in the supernatant in the region of the plateau was found to vary from one preparation to another between 4 and 5, whereas in the original preparation it varied between 1.4 and 1.6. These results confirm the view that protein dissociates from DNA under the influence of heparin. This conclusion was confirmed even more convincingly by analysis of the protein and DNA content in the top two-thirds of the supernatant. The very high protein/DNA ratio [18-20] obtained in this case suggests the presence of dissociated protein and contradicts the possible alternative explanation that a degraded fraction of DNP, rich in protein, passes into the supernatant under the influence of heparin. The presence of a DNA concentration gradient relative to height of the centrifuge tube is evidence that the DNA, as its protein is removed by heparin, can no longer be sedimented under these conditions of centrifugation. An increase in the centrifugation time to 18 h led to total sedimentation of the DNA, but under these circumstances considerable cosedimentation of complexes of dissociated protein with heparin also took place (Fig. 2).

The following questions arise: Does selective dissociation of proteins take place under the influence of heparin, and what is the order of their dissociation? In this connection an electrophoretic analysis was made of the protein fractions contained in the top half of the supernatant and obtained after centrifugation of a mixture of DNP with heparin for 6 h at 150,000g. The DNA content in the samples analyzed did not exceed 5% of its original concentration in the DNP solutions. An attempt to identify the histone fractions dissociated from DNP under the influence of heparin [11] in 0.9 N $CH_3COOH$ (a method giving good resolution of all five histone fractions) proved to be unsuccessful, for the histone-heparin complex does not break down at pH 2.8. Accordingly the order of dissocation of the proteins was analyzed in Na-phosphate buffer, pH 7.1, by the method described by Weber and Osborn [14]. During electrophoresis of the supernatant fractions with low heparin concentrations – down to a heparin/protein ratio of 1:2 – bands corresponding to the $H_{2b}$, $H_3$, and $H_{2a}$ fractions were found. It must be pointed out that electrophoretic analysis of histones under these conditions does not enable resolution of bands corresponding to the $H_{2b}$ and $H_3$ fractions. With a further increase in the heparin concentration in the system, when the heparin/protein ratio was 1:1, histone $H_1$ appeared in the supernatant. Fraction $H_4$, as before, was not present in the supernatant. The band corresponding to histone $H_4$ appeared only when the heparin/protein ratio was 1:4 (Fig. 3). A marked decrease in the intensity of the $H_1$ band in gel columns Nos. 10 and 11, it should be noted, was due to impairment of the staining of this fraction as a result of the presence of large quantities of heparin in this zone.

It can thus be concluded that at neutral pH values under the influence of heparin, dissociation of histones takes place in DNP solutions of low ionic strength (0.7 mM Na-phosphate buffer) in the following order: $H_{2a}$, $H_1$, $H_4$. The problem of the arrangement of fractions $H_{2b}$ and $H_3$ in this sequence could not be resolved because of inability to distinguish electrophoretically between the bands corresponding to these two histones. The following alternative are formally possible: ($H_{2a}$, $H_{2b}$, $H_3$), $H_1$, $H_4$; ($H_{2a}$, $H_3$), $H_1$, $H_{2b}$, $H_4$; ($H_{2a}$, $H_{2b}$), $H_1$, ($H_3$, $H_4$). Numerous investigations of dissociation of histones from DNA in salt solutions have shown the following order of removal of histones from DNP as the ionic strength of the medium increases [7, 8, 10]; $H_1$, ($H_{2a}$, $H_{2b}$), ($H_3$, $H_4$). This sequence of dissociation also is observed during acid titration of DNP [10], i.e., moderately lysine-rich histones ($H_{2a}$, $H_{2b}$) and arginine-rich histones each dissociate in their own group. It is accordingly natural

to suppose that histone dissociate under the influence of heparin in the following order: ($H_{2a}$, $H_{2b}$), $H_1$, ($H_3$, $H_4$). A similar character of the order of dissociation was found previously by Kleiman and Huang [7] during an increase in the salt concentration in medium containing 5-6 M urea, but the reasons for this phenomenon have not been established. The fact that histone $H_1$ dissociates first under the influence of low-molecular-weight electrolytes, and that during the action of the natural polyanion heparin on DNP this order is changed could be biologically important, although its functional significance is not yet clear. Be that as it may, in the light of the data described above, activation of DNA and RNA synthesis in cells, nuclei, and chromatin of eukaryotes by low concentrations of heparin must be attributed not to dissociation of histone $H_1$, as was naturally considered previously, but to dissociation of moderately lysine-rich histones – $H_{2a}$ and possibly $H_{2b}$. The mechanism responsible for this phenomenon requires further analysis.

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