highest in the medulla (15-18% of water-soluble proteins), somewhat lower in the thalamus, corpora quadrigemina, cerebral peduncles, and hippocampus (5-10% of water-soluble proteins). The lowest concentration of NSP 10-40-4 was discovered in the cerebral cortex (frontal, occipital) and cerebellum (1-4% of water-soluble proteins).

It can thus be concluded from the results that the content of NSP 10-40-4 differs in different parts of the human brain. On the basis of the results it is impossible to identify the localization of the protein in any particular cell type. It is evidently specific for individual types of neurons. Since diffuse fluorescence of the neuropil was observed in all sections examined, the possibility of its localization in glial cells also cannot be ruled out. Moreover, immunoenzyme assay revealed that its concentration is highest in structures containing predominantly glial cells.

A final conclusion on the cellular localization of NSP 10-40-4 can be drawn after a study of its content in neuronal and glial tumors, and also a study of its appearance in the course of ontogeny.

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# EFFECT OF HEPARIN ON DNA


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The widespread use of heparin in medicine has necessitated active research into its action on various biological objects [1, 2, 7].

The writers have studied the effect of heparin on DNA. To analyze DNA disturbances by mutagenic factors and the effectiveness of cell repair systems under normal and pathological conditions, centrifugation of cell lysates in alkaline density gradients is widely used. Since liberation of DNA from cells and unwinding of its strands in alkali is a long process (up to 24 h [10]), to speed it up in order to prevent degradation of the DNA in alkali, the use of heparin has been suggested [15]. However, our data show that heparin modifies the sedimentation characteristics of DNA. The causes of this phenomenon are analyzed in the investigation described below by centrifugation of DNA in alkaline and neutral sucrose gradients and by viscosimetry.

## EXPERIMENTAL METHOD

DNA was isolated as described previously [3] from pig lymphocytes (obtained by the method in [13]) and calf thymocytes after preliminary isolation of chromatin [5]. Sedimentation analysis was carried out in a 5-20% sucrose density gradient in 0.1 M NaOH + 0.01 M

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TABLE 1. Effect of Heparin on Sedimentation of DNA in Chromatin and of Deproteinated DNA in a Density Gradient (M ± m)

| Preparation | Type of gradient | Heparin/DNA ratio | | | | | $\frac{S_{100}}{S_0}$ |
|---|---|---|---|---|---|---|---|
| | | 0 | | 1 | 10 | 100 | |
| | | M ($\cdot 10^{-6}$), daltons | $S_{20}$, w | $S_{20}$, w | $S_{20}$, w | $S_{20}$, w | |
| Chromatin | Alkaline | 4,29±0,84 | 24,85±1,76 | 26,77±1,53 | 28,75±2,75 | 36,67±0,38 | 1,48 |
| DNA | » | 1,56±0,43 | 15,75±1,76 | 16,11±2,51 | 24,03±1,01 | 31,00±1,10 | 1,97 |
| DNA | Neutral | 3,20±0,23 | 15,71±0,97 | 18,13±1,12 | 21,79±0,48 | 27,76±1,22 | 1,77 |
| DNA | » | 1,77±0,12 | 12,08±0,33 | 12,95±0,36 | 13,96±1,04 | 22,15±1,30 | 1,83 |

Legend. $S_{100}$ and $S_0$ stand for sedimentation coefficiencts with heparin/DNA ratios of 100 and 0 respectively. M) Molecular weight.

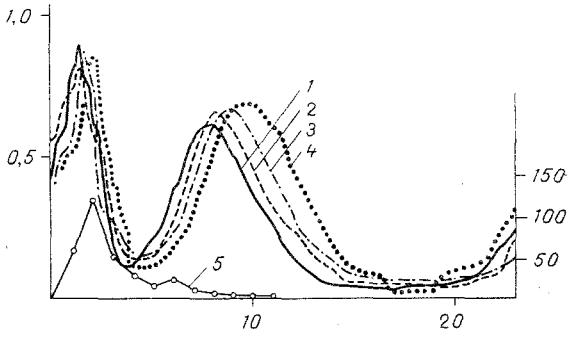


Fig. 1. Distribution profiles of DNA and protein in alkaline sucrose density gradient after centrifugation of chromatin–heparin mixtures. Abscissa, no. of fractions. Direction of sedimentation from left to right; ordinate: on left, optical density 254 nm; on right, protein concentration in fractions (in μg/ml). Heparin/DNA ratio: 1) 0, 2) 1, 3) 10, 4) 100; 5) protein content in fractions.

EDTA (pH 12.5) or in 0.9 M NaCl + 0.001 M EDTA + 0.01 M Tris-HCl (pH 7.5). The sample was centrifuged (20°C, 18 h) at 16,000 rpm (chromatin) or 18,000 rpm (DNA) in an L5-65 centrifuge with SW-27 rotor (Beckman, USA). The gradients were fractionated on an ISCO instrument (USA). Sedimentation coefficients were determined by the method in [14]. Viscosity was measured on a rotary viscosimeter [4] under Newtonian flow conditions of the solutions. Methods of determining concentrations of DNA, heparin, and protein were described previously [6, 9, 12].

## EXPERIMENTAL RESULTS

Distribution profiles of DNA and protein in an alkaline sucrose density gradient after centrifugation of chromatin–heparin mixtures, preincubated for 18 h in 0.15 M NaCl + 0.01 M Tris-HCl (pH 7.0), with heparin/chromatin DNA ratios of 0.1, 10, and 100, are given in Fig. 1. An increase in the heparin/DNA ratio in the mixture was accompanied by a shift of the maximum in the DNA distribution along the density gradient toward the bottom of the centrifuge tube. The sedimentation coefficient of DNA with a heparin/chromatin DNA ratio of 100:1 was increased by 1.5 times (Table 1), but in another study [15] heparin/DNA ratios of 308 and 623 were used. With such high excesses of heparin over DNA, very overestimated values for the molecular weight of DNA in the cell lysates may be obtained.

The sedimentation coefficient of deproteinized DNA increased even more than that of chromatin DNA (Table 1). Since completeness of unwinding of DNA strands is of great importance for sedimentation in an alkaline density gradient, the effect of heparin on denaturation of DNA at alkaline pH values was examined (Fig. 2). At pH 11.2-12.0 hyperchromism of DNA was found to be lower in the presence of heparin, whereas at pH > 12.0 it was higher. Measurements were made 1 h after transfer of the DNA–heparin mixtures to medium with the cor-

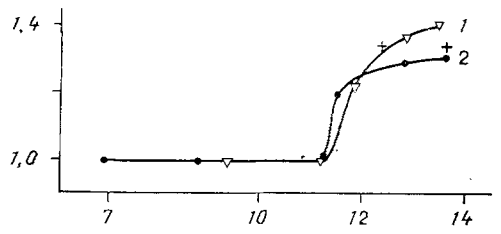


Fig. 2

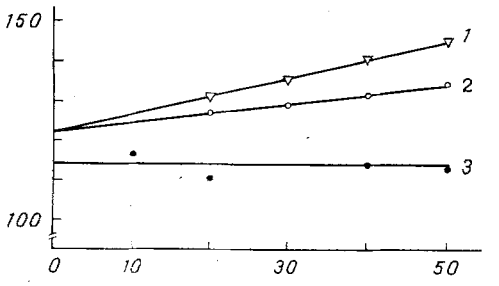


Fig. 3

Fig. 2. Alkaline denaturation of DNA. Abscissa, pH of solutions; ordinate, hyperchromism $(A^{pHc}_{260\ nm})/(A^{pH\ 7.0}_{260\ nm})$ in 0.15 M NaCl + 0.1 M $Na_2EDTA$. Curves 1 and 2 obtained 1 h after preparation of solutions ($C_{DNA}$ = 20 μg/ml). +) Hyperchromism of DNA without heparin after 24 h. Heparin/DNA ratio: 1) 100, 2) 0.

Fig. 3. Dependence of characteristic viscosity of DNA–heparin mixtures on concentration. Absissa, DNA concentration (in μg/ml) in 0.15 M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0); ordinate, characteristic viscosity (in dl/g). Heparin/DNA ratio: 1) 0; 2) 250; 3) 100.

responding pH. The optical density of DNA solutions without heparin was increased after 24 h, at pH 12.4 and 13.6, but in the presence of heparin it was unchanged. Consequently, heparin accelerates unwinding of DNA strands at high pH values, but stabilizes the secondary structure of DNA at pH < 12.0. The increase in optical density at pH 12.0-13.5 in the presence of heparin suggests that denaturation of DNA is incomplete, at least at pH < 13.5.

It can accordingly be concluded that the increase in sedimentation coefficient of DNA in an alkaline density gradient (pH 12.5) with an increase in the quantity of heparin mixed with DNA is due to a decrease in the degree of unwinding of DNA strands (although the rate of unwinding is increased, as was shown in [15]). However, the sedimentation coefficient of DNA was found to be increased by heparin in a neutral density gradient also, in which the DNA is in the double helical form (Table 1). This can be explained on the grounds that heparin causes aggregation or compactization of DNA. Since we were unable to find heparin in fractions in which DNA was located in the density gradient, the structural changes in DNA mentioned above evidently persist after removal of the heparin, i.e., they are irreversible. It can be tentatively suggested that structural changes induced by heparin in DNA are due to intra- or intermolecular aggregation of DNA on account of residual protein.

It will be clear from Fig. 3 that in medium with physiological ionic strength the characteristic viscosity ($[\eta]_o$) of DNA was unchanged even in the presence of a 250-fold excess of heparin. With higher heparin/DNA ratios $[\eta]_o$ decreased, evidence of a decrease in the hydrodynamic volume of the DNA molecules. This phenomenon is compatible with heparin-induced compactization of DNA on account of intramolecular contacts between DNA regions bound with residual protein.

Although a 250-fold excess of heparin still had no effect on $[\eta]_o$ of DNA, even with a heparin/DNA ratio of 10 an increase in the sedimentation coefficient was observed (Table 1). No changes in the sedimentation profile of DNA from cell lysates in an alkaline density gradient were found in [15], even when the heparin/DNA ratio was 623. In the investigation cited the heparin was obtained from Sigma (USA), whereas in our investigations the heparin used for the rheologic measurements was obtained from Spofa (Czechoslovakia), and that used in the experiments whose results are given in Fig. 1 and in Table 1 was obtained from Polfa (Poland). However, the disagreements were not due to the use of different heparin preparations, but evidently arose for the following reasons.

The chromatin and DNA sedimentation experiments were carried out with a DNA concentration ($C_{DNA}$) of over 400 μg/ml, the rheologic measurements with $C_{DNA}$ of 10-50 μg/ml, whereas in [15] $C_{DNA}$ in the cell lystates was about 1.3 μg/ml. The higher the DNA concentration in solution, the greater the total hydrodynamic volume occupied by DNA molecules, and the lower the heparin concentrations that must cause compactization of DNA, and this was indeed observed when the three groups of experiments were compared.

The higher the DNA concentration, the more the reduced viscosity of the DNA solution decreased in the presence of an identical heparin/DNA ratio (Fig. 3). The characteristic viscosity of a DNA–heparin mixture in 0.15 M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0), with a heparin/DNA ratio of 250, was 1.24 times higher when dependence of viscosity on concentration was determined in the region of 10-40 μg/ml, using a mixture with $C_{DNA}$ = 40 μg/ml than when using a mixture with $C_{DNA}$ = 600 μg/ml and with the same heparin/DNA ratio. This is direct proof of the increased compactization of DNA by heparin in a more concentrated DNA solution in the presence of the same excess of heparin, and of the irreversible character of this compactization. The compactization effect may be partly lost through mechanical action on dilution of the solution.

One of us (S.M.B. [5]) showed previously that heparin causes dissociation of histones of nucleosome nuclei in medium of physiological ionic strength, and this was confirmed later [11]. This dissociation is naturally accompanied by decompactization of chromatin at the nucleosome level. Decompactization of chromatin also has been demonstrated in the absence of demonstrable dissociation of proteins in the presence of low heparin/DNA ratios [5]. The present investigation proves the compacting effect of heparin on DNA. Taken together these effects evidently lie at the basis of formation of the structures found after treatment of metaphase chromosomes with heparin [8], which are DNA loops branching out from the so-called "chromosome scaffold," formed by protein firmly bound with DNA.

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