# EFFECT OF ETHIDIUM BROMIDE ON VISCOSITY OF CHROMATIN ISOLATED FROM NORMAL AND DENERVATED RAT LIVER

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Much experimental evidence has now been obtained to indicate that correlation exists between the structural organization and functional activity of chromatin [2]. Several histological, biochemical, and physicochemical methods of investigation of the structural organization of chromatin, reflecting its functional state, are now available. One of the most important details determining the transcription activity of chromatin is supercoiling of DNA, closed into circular domains [4]. To detect circular DNA molecules a phenomenon of extremal dependence of viscosity of DNA solutions on concentration of the intercalating agent and, in particular, of ethidium bromide, is used [4, 5]. Supercoiling of chromatin DNA may be substantially influenced by various exogenous and endogenous factors. We know that the nervous system is involved in the regulation of processes connected with realization of genetic information in eukaryotic cells [1]. However, information on the role of nervous regulation in the activity of the genetic apparatus of the cell is highly contradictory. The aim of the present investigation was to study changes in the state of chromatin isolated from the liver of vagotomized rats.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 160-180 g, kept under standard animal house conditions. The animals were divided into four groups: 1) intact, 2) surviving 1 week after the operation of bilateral subdiaphragmatic vagotomy, 3) surviving 2 weeks after the operation, and 4) surviving 4 weeks after the operation. The vagus nerves were ligated under general anesthesia. The rats were decapitated during the morning after preliminary deprivation of food for 18 h. Chromatin preparations were obtained from the liver of the experimental animals by washing a tissue homogenate 5 times to remove components soluble in 0.025M $Na_2EDTA$ + 0.075M NaCl, at pH 8.0. The residue of chromatin, after the last washing, was suspended in 0.15M NaCl + 0.7 mM Na-phosphate buffer, pH 7.0, and the DNA concentration (by the method in [3]) and the RNA concentration were determined. The chromatin preparations were diluted to a concentration of DNA in the suspension of 20 $\mu$g/ml, and then treated with an equal volume of lytic solution (2 M NaCl + 0.2 M $Na_2EDTA$, 4 mM Tris-HCl, 1% Triton X-100, pH 8.0). The viscosity of the chromatin solutions in the final deproteinizing medium, containing 1 M NaCl, 0.1 M $Na_2EDTA$, 2 mM Tris-HCl, and 0.5% Triton X-100, was measured on a rotary viscosimeter, with shear stress of $4.5 \times 10^{-3}$ dyine/cm$^2$. The concentration of ethidium bromide ("Serva") was determined spectrophotometrically from absorption in water ($\varepsilon_{460}$ = 4800 $M^{-1} \cdot cm^{-1}$).

## EXPERIMENTAL RESULTS

The measurements revealed the character of dependence of the relative viscosity of chromatin preparations obtained from the liver of the four groups of experimental animals on concentration of the intercalating agent, namely ethidium bromide. The curves I, II, III, and IV shown in Fig. 1 were obtained from preparations from animals of the intact group (1) and the experimental groups (2, 3, and 4). The presence of extrema on the curves can be attributed to the presence of supercoiled DNA molecules in the chromatin preparations. It is now known that an increase in the viscosity of chromatin is due to a decrease in

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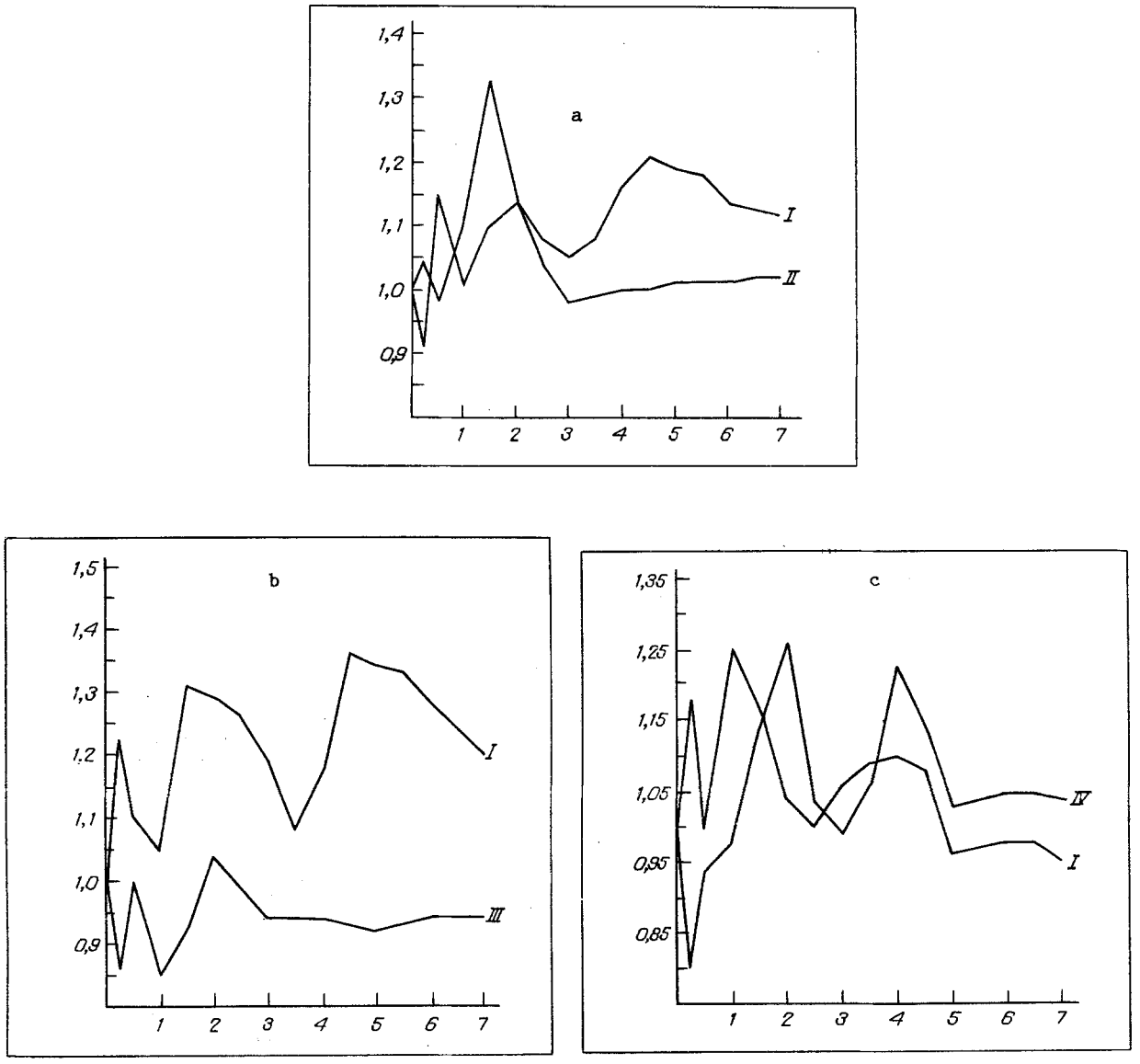


Fig. 1. Effect of ethidium bromide on relative viscosity of solutions of chromatin isolated from rat liver, at different times after denervation: a) 1 week after operation (II), b) 2 weeks after operation (III), c) 4 weeks after operation (IV). (Curve 1 in all figures obtained for chromatin isolated from liver of rats of group 1.) Abscissa, concentration (C) of ethidium bromide (EtBr), in $\mu$g/ml; ordinate, ratio $\eta^{EtBr}_{rel}/\eta^{0}_{rel}$, where $\eta^{EtBr}_{rel}$ denotes relative viscosity of mixture of chromatin with ethidium bromide, and $\eta^{0}_{rel}$ denotes relative viscosity of chromatin solution without ethidium bromide.

the degree of negative supercoiling of circular DNA on account of intercalation of ethidium bromide into the DNA, and that a decrease in viscosity is due to positive supercoiling of the DNA molecules as the concentration of the intercalating agent rises [6, 7].

The experimental data show that in the early stage after denervation (1 and 2 weeks, see Fig. 1: a, b) chromatin isolated from hepatocytes does not appear to contain families of DNA domains with a high degree of supercoiling, as is shown by disappearance of the second extremum on the curves of viscosity as a function of ethidium bromide concentration. This last result cannot be explained on the grounds that the degree of supercoiling in the domains examined was reduced to the level of domains of group 1, for the first maximum on the curves was not increased. It can thus tentatively suggested that domains with a previously high degree of supercoiling in chromatin isolated from parenchymatous liver cells of the rats of groups 2 and 3 are in the relaxed state. This state may be induced by several factors: first, activation of proliferation, when the number of cells with

breaks in DNA molecules is increased, and second, by degradation of a certain proportion of hepatocytes as a result of a disturbance in denervated liver cells to activation of DNA-tropic factors.

The experimental results suggest that on the 30th day after vagotomy (Fig. 1c) there is a tendency for the chromatin to return to its original state. This view is supported by the closeness of the character of dependence of the relative viscosity of the chromatin on concentration of the intercalating agent (this is clearly visible when curves I and IV are compared, see Fig. 1c).

Thus for the first time a difference has been found in the state of isolated chromatin from rat hepatocytes of the control group and in different stages after the operation of bilateral subdiaphragmatic vagotomy. A characteristic feature of these changes is absence of domains with a high degree of supercoiling, present in chromatin isolated from the liver of intact rats. Restoration of the state of rat hepatocyte chromatin on the 30th day after vagotomy suggests that the nervous system can influence activity of topoisomerases, which determine the topologic and, consequently, the transcriptional state of the genome.

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# INTERACTION OF BLOOD SERA FROM PATIENTS WITH AUTOIMMUNE DISEASES WITH EXPRESSED cDNA FRAGMENT OF TOPOISOMERASE I AND WITH MONOCLONAL ANTIBODIES TO THE ENZYME


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Until recently the molecular theory of the origin and development of the autoimmune process was far from being expressed in its final form. For this reason many authorities paid increased attention to the study of idiotype — anti-idiotype interaction in autoimmune processes [4, 6]. One approach to the study of the molecular bases of autoimmune pathology is to undertake the discovery, cloning, and immunochemical investigation of the individual characteristics of genes and of proteins that are products of their expression [8]. In particular, the appearance of positive reactions in autoimmune sera to antigens of nucleoprotein nature has been observed in many investigations [5]. We know that in systemic scleroderma (SSD) a high titer of antibodies to one of the main enzymes of DNA metabolism, namely type I topoisomerase, is found in the patients' sera [3]. Previously, we described the cloning of this enzyme in the expressed vector $\lambda$gt 11, and also the obtaining of monoclonal


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