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Cytological Analysis of the Histone Component of Hepatocyte Nuclear Chromatin in Rats with Impaired Vagus Innervation of the Liver

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The supramolecular arrangement of rat hepatocyte nuclear chromatin is studied by differentiated staining of the histone component after bilateral subdiaphragmal vagotomy in intact and phenobarbital-induced animals. The spectrum of hepatocyte nuclei staining is not altered in denervated liver; however, pronounced shifts in the quantitative ratio of different types of staining occur 1-2 weeks after denervation. One month after the operation, the hepatocyte population is almost the same as in the control regarding the staining pattern and quantitative ratio of its variants. In the phenobarbital group, the spectrum of hepatocyte staining does not change compared with intact control and vagotomized animals at all terms after surgery. This may be due to the restructuring of the nucleohistone—chromatin complex or lack of such restructuring.

Key Words: *chromatin; histones, hepatocyte; vagotomy*

Bilateral subdiaphragmal vagotomy changes the functional activity of hepatocytes. The ultrastructural basis of the initial morphofunctional changes in various cytoplasmic organelles of denervated liver is known [2], while the status of the cell nuclei during the neurodystrophic process is little studied. Changes in the supramolecular structure of hepatocyte nuclei chromatin in a denervated organ are virtually unknown.

Our purpose was to investigate the supramolecular structure of chromatin, which is maintained mainly by histones, by light microscopy using specific staining with ammonium silver (AS).

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MATERIALS AND METHODS

Outbred male albino rats weighing 160 to 180 g were subjected to bilateral subdiaphragmal vagotomy. The animals were divided into 5 groups: 1) intact rats; 2) laparotomized; 3) treated with phenobarbital (PB); 4) vagotomized; and 5) vagotomized and treated with PB at various terms after surgery. Group 4 animals were examined on days 7, 14, and 30 after vagotomy.

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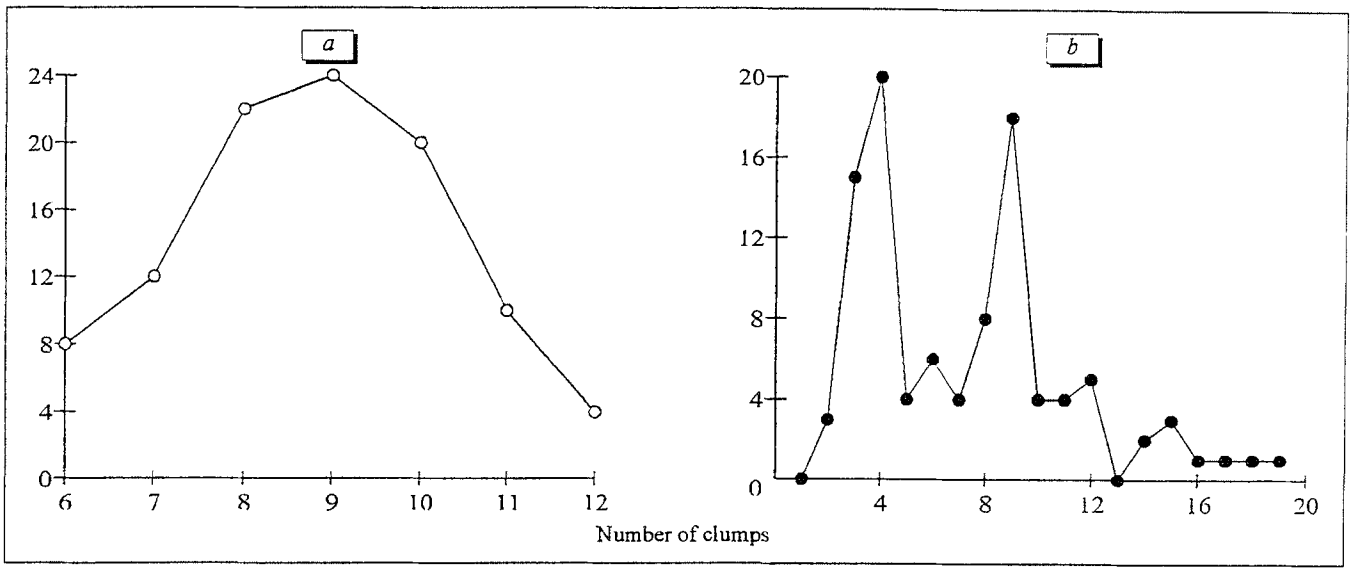

Fig. 1. Distribution of hepatocyte nuclei with medium-sized (*a*) and large (*b*) clumps in intact animals. Ordinate: % of the number of cells estimated.

Aqueous solution of PB was injected intraperitoneally in a dose of 60 mg/kg four times at one-day intervals.

The nucleohistone complex was assessed by AS staining of formalin-fixed preparations [6]. This method is the most sparing, since it involves no procedures destroying the deoxyribonucleoprotein complex (DNP). AS colored the cell nuclei yellow-brown, the yellow color being due to histones rich in lysine and the brown, sometimes black, to arginine-rich histones [1].

Liver tissue prints were examined. The preparations were fixed in 10% formalin buffered with sodium acetate (pH 7.4) for 15 min at room temperature, rinsed 5 times in distilled water and placed in AS solution for 5-20 sec, dried with filter paper, and immersed in 3% formalin for 2-3 min for developing. They were then washed in distilled water, passed through alcohols and xylol, and embedded in Canadian balm. The results were statistically processed using Wilcoxon's test [4].

RESULTS

Analysis of AS-stained liver tissue prints included the assessment of the staining of hepatocyte nuclei in control animals.

Visual assessment of preparations showed dark-brown formation shaped as irregular clumps and smaller grains against the yellow background of the nucleus. The number and size of clumps in the nuclei

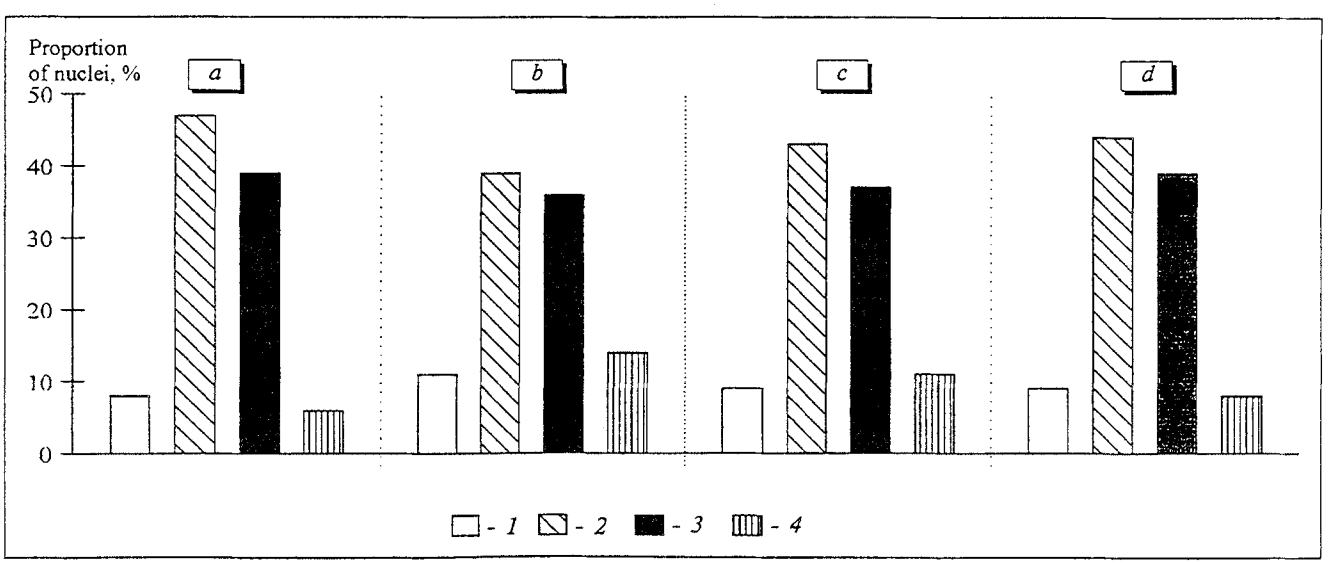

Fig. 2. Ratio of the number of nuclei with different variants of ammonium silver staining in hepatocytes of control (*a*) and vagotomized rats on days 7 (*b*), 14 (*c*), and 30 (*d*) postoperation. *1*) nuclei without large clumps; *2*, *3*, and *4*) nuclei with 3, 9, and 16 large clumps, respectively.

varied. Medium-sized clumps were seen in all hepatocyte nuclei, and their number was approximately the same, whereas the number of large clumps proved to be a better variable index of the nucleus staining. Numerous small dust-like brown grains were seen against the yellow background of the nucleus, diffusely distributed between the clumps in the whole nucleus, and therefore it was impossible to estimate their number visually.

In order to objectively assess chromatin staining, large and medium-sized clumps were counted separately per 50 nuclei for each 5 experimental animals, and the curves for hepatocyte distribution were plotted. The histogram for hepatocyte nuclei population in intact animals for medium-sized clumps is represented by a single-mode distribution close to the norm (Fig. 1, *a*), this allowed us to regard it as uniform with respect to this parameter. On the other hand, the distribution by the number of large clumps is multimodal (Fig. 1, *b*); 4 discrete classes corresponding to a certain type of AS staining can be distinguished. Type I nuclei contained no large dark brown clumps, type II nuclei contained 3 large clumps, type III nuclei contained 9 clumps, and type IV nuclei contained 16 clumps.

The mean percent ratio of all types of hepatocyte nuclei staining in control animals is shown in Fig. 2, *a*. Hence, the hepatocyte population of intact animals is heterogeneous, being represented by 4 patterns of AS staining (Table 1).

Study of AS staining of hepatocyte nuclei in vagotomized rats showed the same 4 patterns as in the control, but the quantitative ratio between these patterns changed significantly in 1 week, and 2 weeks after surgery these changes were less pronounced. The proportion of the nuclei with a greater number of large clumps increased at this period (14% in 1 week and 11% in 2 weeks), as does the share of nuclei without large clumps (11% in 1 week and 9% in 2 weeks). The percentage of types II and III nuclei decreased accordingly.

By the end of the first month after the operation, the hepatocyte population of denervated animals was approximating the control regarding the type of nuclear staining and their quantitative ratio (Fig. 2, *d*).

Our results indicate that the spectrum of AS staining of hepatocyte nuclei does not change in denervated liver, but there are shifts in the quantitative ratio of different types of staining, which are more pronounced early after surgery. An increase in class IV nuclei with a greater content of the brown component at this period indicates a greater number of reactive guanidine groups of histones H3 and H4 participating in the formation of stained complex [1].

TABLE 1. Patterns of the Hepatocyte Nuclei Staining with Different Numbers of Large and Medium-Sized Clumps

Type	Mean number of		Mean % in the control
	medium-sized clumps	large clumps	
I	9	0	8
II	9	3	47
III	9	9	39
IV	9	16	6

This may result from histone modifications, which may alter their binding to DNA and eventually decrease the shielding effect of histones and increase the amount of potentially active chromatin [3]. The data on AS staining of histones permit us to hypothesize changes in the spectrum of potentially active genes. Alteration of isoenzyme spectra of proteins after denervation of organs may be considered as a confirmation of this hypothesis [5].

Study of PB effect on AS staining of hepatocyte nuclei failed to reveal significant changes in the ratio of nuclei with different patterns of AS staining in the controls. PB induction in various periods after vagotomy caused no changes in the quantitative ratio of the staining variants. A peculiar feature of the nuclei in PB-treated animals was the presence of one or several clearly seen nucleoli undetectable by this method in animals administered no PB. Such a response to PB may indicate that there is no profound restructuring of the nucleohistone complex manifesting by changes in the stained complex structure. Presumably, the number of positively charged histone groups participating in DNA binding does not decrease, and the degree of histone binding to DNA does not change. This may indicate that PB-activated genes (potentially active) are more available and require no profound rearrangement of the nucleohistone complex and arrest of the shielding effect of histones for functioning. Hence, it can be hypothesized that the reaction of the nuclear system on xenobiotic PB is evolutionary determined due to the detoxifying function of the liver. This viewpoint helps us explain the appearance of nucleoli under the effect of PB, because this agent realizes its effect by increasing the production of cytochrome P-450 molecules, which involves an increase in the number of ribosomes in order to increase protein production.

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Specific Binding of Triiodothyronine to the Nucleoplasm

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The study of the binding of thyroid hormones to the nucleoplasm demonstrates the presence of triiodothyronine-binding molecules characterized by high affinity and limited binding capacity. The binding of thyroid analogs correlates with their thyromimetic activity. Sucrose gradient ultracentrifugation of triiodothyronine-labeled nuclear extract reveals the presence of triiodothyronine receptors, ribonucleoprotein particles with a sedimentation coefficient about 30S.

Key Words: *thyroid hormones; triiodothyronine; nucleoplasm; specific binding; ribonucleoprotein particles*

It is now well established that the effect of hormones on certain processes is realized through interaction with hormone-binding proteins which mediate the hormone signal transduction [8,9]. Classical studies postulated the existence of a single type of thyroid receptors associated with chromatin proteins acting at the level of DNA and inducing gene transcription [4,6]. However, there are numerous reports on the effects of thyroid hormones on the posttranscription processes [5,7]. These effects apparently involve hormone-binding molecules.

The aim of the present study was to reveal new hormone-binding sites in the nucleus distinct from the common (chromatin-associated) triiodothyronine (T_3) receptors by their compartmentalization.

MATERIALS AND METHODS

The nuclei were isolated from hepatocytes as described previously [11]. The nucleoplasm was isolated as follows: purified nuclei were suspended in the medium containing 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.01 M $MgCl_2$, and 5 mM β-mercaptoethanol, incubated in an ice bath for 10 min, and centrifuged. The procedure was repeated twice. The supernatants were pooled, clarified by centrifugation, and used to study T_3 binding to the nucleoplasm *in vitro*. To this end a 0.15 M NaCl extract of the nuclei (100-150 μg protein) was incubated with varied concentrations of labeled T_3 (0.125-5 nM) in the presence or absence of a 200-fold excess of cold T_3 for determination of nonspecific and total binding of the hormone. The incubation medium contained 10 mM Tris-HCl, pH 8.7, 5 mM β-mercaptoethanol, 1.5 mM EDTA, and 10% glycerin. Incubation was carried out at 0-4°C for 16-18 h. Free hormone was separated from the hormone-protein complex by filtration through Millipore membrane filters (HAWP, 0.45 μ). Nuclear ribonucleoprotein (RNP) particles were isolated as described elsewhere [2]; the animals were preliminary injected with ^{3}H-orotic acid. For evaluation of the binding of thyroid hormones to the nuclear RNP particles *in vivo* the animals were in-

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