Effect of Epinephrine on the Ultrastructure of Nuclear Chromatin in Dog Hepatocytes

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Electron-microscopic study of the liver nuclear chromatin state is of particular interest under epinephrine treatment because the effect of epinephrine on hepatocytes involves different biochemical processes such as the metabolism of carbohydrates, lipids, and proteins and changes in the nuclear chromatin state [5] and rearrangement.

The aim of the present study was to estimate the areas of nuclei, nucleoplasmic chromatin (NpC), and dense perimembrane chromatin (PmC) in an electron-microscopic examination of dog hepatocytes under high doses of epinephrine.

MATERIALS AND METHODS

The experiments were carried out on 10 male mongrel dogs 3-5 years old weighing 12 kg. The experimental dogs received epinephrine i.v. 100 μg/kg during 5 days . The control animals were injected with 2.0 ml saline every day. On the fifth day the animals were killed with sodium thiopenal i.v. injection.

Pieces of tissue 1 mm^3 were fixed in 2.5 % glutaraldehyde (Serva, Germany), and postfixation was performed with 2% osmium tetroxide. The tissue was then dehydrated in ascending grades of alcohol and acetone and embedded in Epon. Polymerization was performed during 3 days.

The ultrathin sections were obtained with an LKB ultramicrotome (Sweden) and examined under a JEM-7 electron microscope (Japan). For each experimental series 10 hepatocyte nuclei were studied. The area of dense PmC in the hepatocyte nuclei was estimated as described previously [5]. The obtained values were multiplied by 100 (for convenience of calculation) and expressed in conventional units [1].

The results were processed statistically by Student's t test.

RESULTS

Some differences in the state of the hepatocyte nuclear chromatin were reveaed in the control and experimental dogs . The dense PmC in hepatocyte nuclei is attached to the internal layer of karyolemma by large separate masses (Fig. 1, *b*) in control ani-

TABLE 1. Areas (conventional units) of Nuclei, NpC, and Dense PmC in Dog Hepatocytes under High Doses of Epinephrine ($M \pm m$)

Group	Area			Ratio of PmC to nucleus area	PmC percent of nucleus area
	nuclei	NpC	PmC		
Control	25.67±0.69	20.20±0.90	5.46±0.81	0.21	21.3
Treated	24.28±0.85	21.14±1.22	3.15±0.62	0.13*	13.0*

Note. Asterisk – reliable in comparison with control ($p<0.05$).

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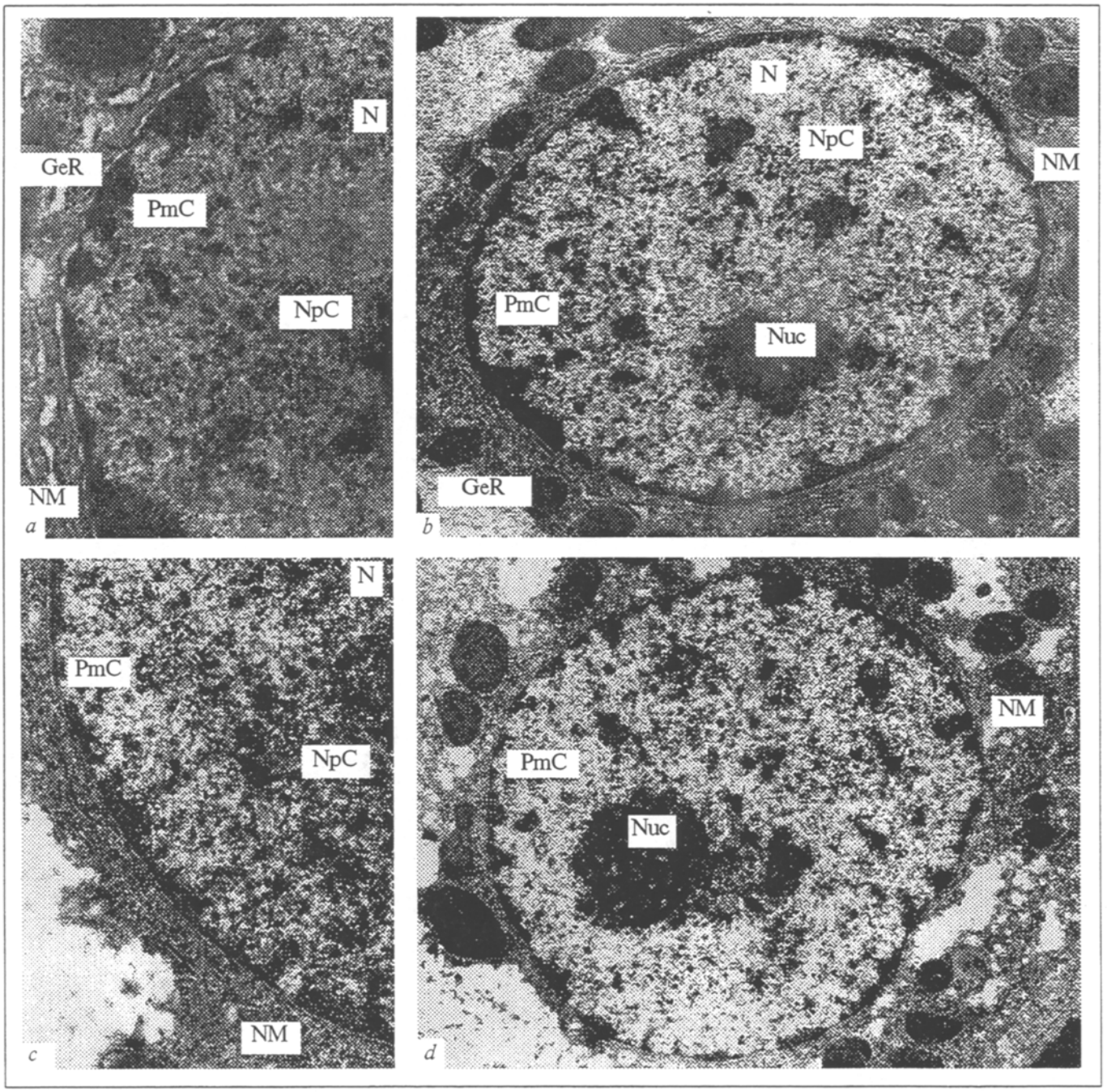

Fig. 1. Ultrathin section of dog hepatocyte nuclei. *a*, *b*) control, *c*, *d*) experiment. *a*, *c*) fragments of nuclei (×16,000), *b*, *d*) nuclei (×37,500). N: nucleus; Nuc: nucleolus; NM: nuclear membrane; NpC: nucleoplasmic chromatin; PmC: perimembrane chromatin; GER: granular endoplasmic reticulum.

mals. Treated dog hepatocytes present a thinner, continuous layer of dense PmC (Fig. 1, *c*, *d*) along the internal nuclear membrane.

The results of the electron-microscopic study are presented in Table 1. We see that the ratio of dense PmC to the nucleus area is 0.21 in the control versus 0.13 in the experiment (1.6-fold decrease, $p<0.05$).

The area of dense PmC sonstitutes 21.3% of the hepatocyte nucleus area in the control animals and 13.0% in the experimental group.

In our previous experiments [5] we found a 2-fold decrease of the ratio of dense PmC to the nucleus area in dog cardiomyocytes under epinephrine treatment .

Thus, epinephrine injection in high doses results in uniform changes of the nuclear chromatin state both in cardiomyocytes and hepatocytes, namely a decrease of the ratio of dense PmC to the nucleus area .

The obtained results may be explained as follows: the epinephrine signal, acting on the target cells

through the secondary messengers cAMP and cGMP [2, 3, 7], gives rise to a cascade of biochemical reactions, which are connected, ultimately with the methylation of DNA cytosine [4, 6] and manifest themselves in chromatin rearrangement.

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An Ultrastructural Study of Colon Cancer Explants in a Long-Term Organ Culture

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The organ culture (OC) is one of the important and at the same time unique methods permitting an *in vitro* study of the laws governing the formation of a human tumor heterogenous cell population. OC of colon cancer (CC) [4-6, 9] as well as of the normal colon mucosa has been described in several reports [2, 3, 7, 8]. The analysis of these makes it clear that organ-specific structures develop in the OC that are characteristic of the normal colon mucosa and malignant neoplasms derived from it. These as a rule have the histological structure of adenocarcinomas. Earlier [1] we have showed the capacity of tumor cells in OC to synthesize carcinoembryonic antigen (CEA), and presented a comprehensive light-optic characterization of the peculiarities of explant growth depending on the period of incubation.

The goal of this work was a detailed electron-microscopic study of the ultrastructure of CC in OC in order to define the potential of OC as a model for the investigation of the histogenesis or, more precisely, the cytogenesis of colon epithelial tumors.

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MATERIALS AND METHODS

Specimens of tumor tissue obtained immediately after operation on the colon were used for the establishment of OC. Histologically, all the tumors belonged to highly to moderately differentiated adenocarcinomas. The tumor tissue was cut into pieces of a size less than or equal to 1 mm^3 and placed on filters with pores of 40 μ diameter (Synpor, Czechoslovakia). The incubation medium consisted of RPMI 1640 supplemented with 20% bovine serum, 10% embryonic extract, 0.06 mg/ml glucose, 0.03 mg/ml ascorbic acid, 5 mg/ml hydrocortisone, 50 μg/ml cantomycin and 50 μg/ml gentamycin. The mincing of the tumor tissue was carried out in incubation medium supplemented with a double quantity of cantomycin and gentamycin under ultraviolet illumination. The OC was grown in a Bruveau incubator at 36.0-36.5°C, in an atmosphere of 100%

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