a further organ-specific differentiation, e.g., in the direction of brush-border and goblet-like enterocytes (7th-9th and subsequent days of OC). It is possible to assume on the basis of the data obtained that in the first 3 days a portion of the tumor cells die, as judged from the observed signs of necrosis and dystrophy, this stage being followed by a gradual adaptation of individual surviving tumor cells to the new conditions. Later, on the 3rd-5th day, an intensive proliferation of ultrastructurally undifferentiated cells can be seen. Perhaps some of these cells are represented by cambial elements (stem cells? committed cells? and/or precursor cells?) which on the 5th-7th day acquire, due to the proceeding differentiation, the ultrastructural features of first immature and then mature brush-border enterocytes. These form gland-like structures characteristic for this type of tumor. As for the second direction of the specific differentiation of tumor cells in OC, the first ultrastructural features of goblet-like enterocytes appear on the 7th day and reach their maximum development on the 9th-12th day. The finding of mixed-type cells also provides evidence in favor of the existence of a monoclonal source of development in the CC cell population.

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Ultrastructure of Nuclear Chomatin in Hepatocytes from Regenerating Guinea Pig Liver: Effect of Vitamin B_{12}

R. I. Klyasheva

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The study of the effect of vitamin B_{12} on chromatin ultrastructure (specifically, the area of dense perimembrane chromatin - PmC) in the nuclei of the regenerating liver is very topical. For this reason we carried out an electron-microscopic study of the area of nuclei, nucleoplasmic chromatin (NpC), and PmC in the regenerating liver of guinea pigs which received high doses of vitamin B_{12}.

Department of Pharmacology, Medical Faculty of the I. N. Ul'yanov State University, Chuvash. (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The experiments were carried out on 10 male guinea pigs aged 6 months and weighing 0.5 kg. The experimental animals underwent a partial hepatectomy (resection of the left external lobe - lobus externus sinister) and received vitamin B_{12} in a dose of 0.8 mg

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TABLE 1. Effect of High-Dose Vitamin B_{12} on Areas (Coventional Units) on Hepatocyte Nucleus, NpC, and Dense PmC in Regenerating Liver of Guinea Pig

Experimental group	Area ($X \pm x_s$)			Ratio of PmC to nucleus area	Ratio of PmC to nucleus area (%)	p
	Nucleus	NpC	PmC			
Control	31.14±0.96	29.25±1.29	1.88±0.04	0.06	6.03	*
Regenerating liver, vitamin B_{12} administered (group I)	14.58±0.41	12.27±0.38	2.29±0.18	0.16	15.70	**
Regenerating liver, no vitamin administered (group II)	12.52±0.45	9.27±0.47	3.24±0.18	0.26	25.87	***

Note. The reliability of the differences (p) between groups is shown by asterisks:* – $0.02<p<0.05$, comparison between control and group I: ** – $p<0.01$, comparison between comtrol and group II; *** – $0.01>p>0.002$, comparison between group I and group II.

per kg body weight (group I) or no vitamin treatment (group II). Tissue specimens were taken 24 hours later. The tissue was fixed in 2.5% glutaraldehyde (Serva, USA) solution, followed by 1% osmium tetroxide solution, dehydrated with alcohols and acetone, embedded in a mixture of Epon resins, and polymerized for 3 days. The sections were cut on an LKB-III ultratome (Sweden) and examined uder a JEM-100 CX electron microscope (Japan), at a magnification of 10,000 in the experiment and 12,000 in the control. Ten nuclei were examined in each sample; the results were expressed in conventional units [1, 8]. The statistical evaluation was performed using the Student method.

RESULTS

In the course of the study of the structural organization of the chromatin of hepatocyte nuclei in the control group we observed the following. PmC ocurred as a thin uniform layer adjacent to the karyolemma (Fig. 1). A nucleolus with clear areas could be seen. In the experimental animals the nuclei of the regenerating liver exhibited considerable changes in chromatin structure. The ratio of dense PmC to the nucleus area was increased and reached 0.16 in group I and 0.26 in group II (0.06 in the control).

The PmC occupied 6.03% of the nucleus area in the control animals, 15,7% in group I, and 25.9% in group II.

The index of reliability of the differences between the control and group I was $0.02<p<0.05$, between the control and group II $p<0.01$, and between group I and group II $0.01>p>0.002$.

Thus, the area of PmC in the regenerating liver was increased in comparison to the control, by 9.7% in group I and by 19.8% in group II.

Against the background of high-dose B_{12} administration (Fig. 2) the PmC in the regenerating liver occupied 15.7% of the nucleus area, while in the absence of vitamin this value was equal to 25.9% (Fig. 3).

Thus, in the nuclei of guinea pig regenerating liver the area of dense PmC was increased by 9.7% in group I and by 19.8% in group II vis-a-vis the control.

Analysis of the results presented above shows that in guinea pigs administration of a high dose of vitamin B_{12} results in a reduction of the PmC area

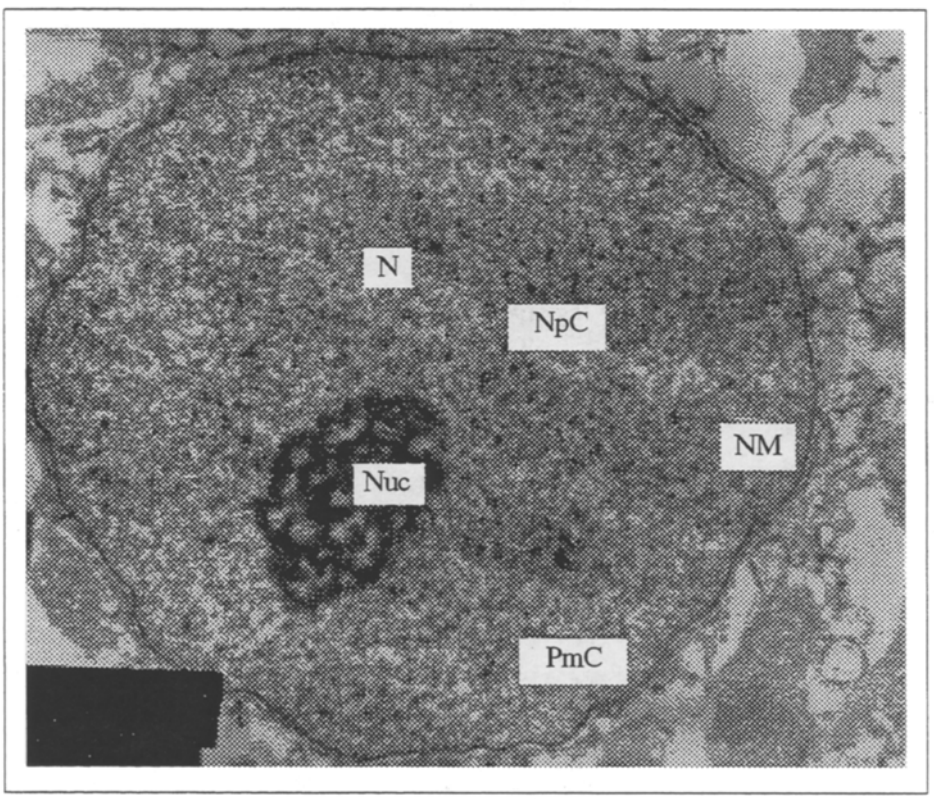

Fig. 1. Ultrathin section of a hepatocyte nucleus (control guinea pig). ×12,000. Here and in Figs. 2 and 3: N: nucleus, Nuc: nucleolus, NM: nuclear membrane, NpC: nucleoplasmic chromatin, PmC: perimembrane chromatin.

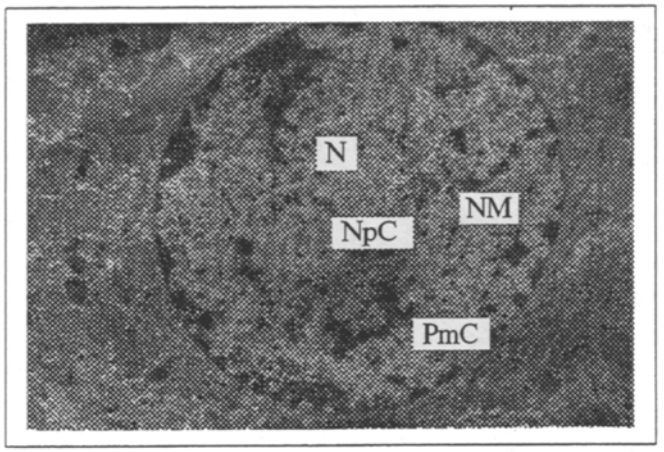

Fig. 2. Ultrathin section of a hepatocyte nucleus from the regenerating liver of a guinea pig treated with vitamin B_{12}. ×9000.

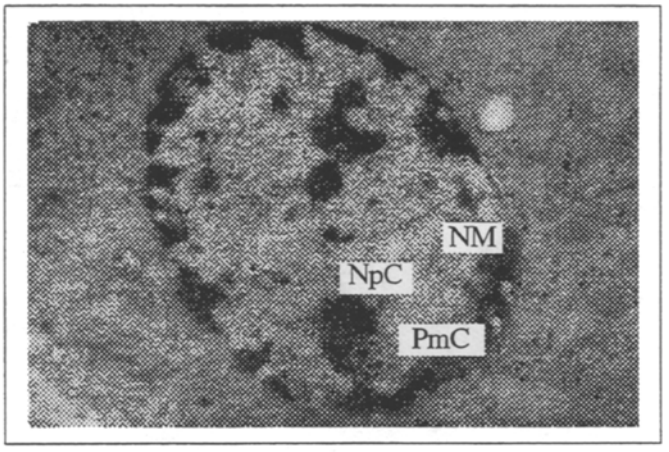

Fig. 3. Ultrathin section of a hepatocyte nucleus from the regenerating liver of a guinea pig not treated with vitamin B_{12}. ×9000.

in the regenerating liver nuclei by 10.1% as compared to the regenerating liver in the absence of vitamin. The results of the electron-microscopic study are presented in Table 1.

The experimental data make it possible to conclude that vitamin B_{12} administered in a high dose promotes decondensation (loosening) of the PmC clumps (Fig. 2).

Earlier we showed that high doses of vitamin B_{12} increase the content of 5-methylcytosine in guinea pig liver DNA [4-7].

The increase of the 5-methylcytosine content in DNA (the addition of hydrophobic methyl groups) should lead to a weakening of the electrostatic interaction between DNA and the surrounding proteins [2, 3, 9]. If this is indeed the case, a rearrangement of the structural organization of the chromatin should take place. This rearrangement was in fact disclosed by means of electron microscopy.

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