blood total destruction of the cytolemma of CPE and CPA was observed less frequently than in the control, and total destruction of endotheliocytes, type I alveolocytes, and the thin part of ABB was never observed. Thus if severe hypoventilation hypoxia is treated by the "Sever-OMR" MO, the ultrastructural changes in ABB of the lungs are less profound and widespread in character, evidence of the beneficial effect of ECMO on the state of the lung tissue. However, when the "Sever-OMR" MO was used, a tendency was observed for disturbances of the rheologic properties of the blood to be increased, and this was reflected morphologically in the formation of multiple intravascular groups of agglutinated erythrocytes, leukocytes, or a mixture of both kinds of cells. Further research must therefore be undertaken in order to obtain new and improved types of gas-exchange systems.

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# MORPHOMETRIC ANALYSIS OF CHANGES IN HEPATOCYTES OF RABBITS WITH EXPERIMENTAL HYPERCHOLESTEROLEMIA AND THEIR REVERSIBILITY


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Cholesterol is synthesized and converted into bile acids with high intensity in the liver [7, 9]. These processes are modified if intake of this steroid into the gastrointestinal tract is increased for a long period of time [9]. Considerable abnormalities are found under these circumstances both in the morphology and the metabolism of heaptocytes. This statement is confirmed by an increase in the lipid content, changes in karyometric parameters, and changes in the intensity of oxidation–reduction and of hydrolysis, evidence of deviations of many functions of the hepatocytes from normal [10]. Changes arising in experimental hypercholesterolemia in rabbits are similar in many of their parameters to those in patients with atherosclerosis [1]. It is now possible, in principle, largely to correct disturbances of lipid metabolism characteristic of atherosclerosis [6, 8]. The question accordingly arises whether normalization of lipid metabolism is followed by regression of the morphological changes developing under conditions of hypercholesterolemia.


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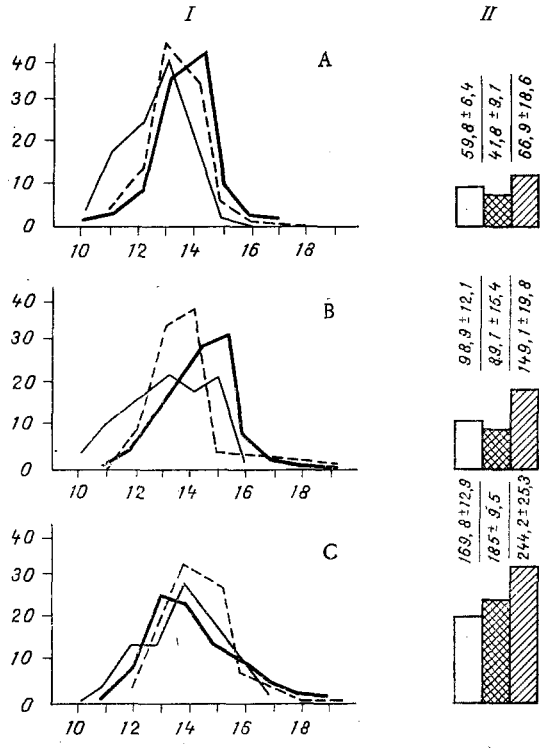


Fig. 1. Changes in size of nuclei (I) and number of binuclear hepatocytes (II) in central (A), middle (B), and peripheral (C) zones of lobules of rabbit liver under the influence of cholesterol. Abscissa, classes of nuclei; ordinate, number of nuclei (in %). Unshaded columns and thin line denote intact animals (group 1); cross-hatching and broken lines denote administration of cholesterol for 14 days (group 2); oblique shading and bold line denotes 1.5 months after end of cholesterol feeding (group 3).

This problem deserves attention also from theoretical (cytologic) standpoints, because comparison of the state of the hepatocytes under normal conditions, when cholesterol intake is excessive, and on cessation of its intake may provide a model of working of the cell when containing different quantities of cholesterol, one of the most important structural and metabolic components of the hepatocyte.

There is evidence in the literature that, on cessation of excessive uptake of cholesterol by animals, gradual normalization of the parameters of lipid-cholesterol metabolism in the blood and liver takes place [1, 4].

Information on the possibility of regression of morphological changes in the liver arising during hypercholesterolemia after termination of the cholesterol intake of the animals could not be found in the accessible literature. Accordingly, the aim of this investigation was to undertake a morphometric analysis of the state of the hepatocytes in the rabbit liver during excessive cholesterol intake and after its termination.

## EXPERIMENTAL METHODS

Experiments were carried out on adult rabbits divided into three groups: 1) intact (eight animals), 2) rabbits receiving cholesterol in a dose of 250 mg/kg daily for 14 days and killed 24 h after the last dose (eight), 3) rabbits receiving cholesterol for 14 days (as in group 2) and killed 1.5 months after termination of its uptake (eight). The experimental animals were killed simultaneously with intact, in the mornings, by air embolism. To judge the size of the hepatocytes in sections stained with hematoxylin and eosin, their number was counted in 100 standard fields of vision. The number of binuclear cells (per 1000) and the area of central cross-sections of 50 nuclei were counted in lobules of the liver, in the central, middle, and peripheral zones respectively in all cases. The area of cross section of the hepatocyte nuclei was measured by means of a karyometric measuring rule, made by the method described in [12] and based on the logarithmic principle of division

of areas of cross section of nuclei into classes. Stencils corresponding to classes 10-19 were found to be suitable for their determination in rabbit liver hepatocytes; their areas are as follows: class 10, 29.6 $\mu^2$; class 11, 32.2 $\mu^2$; class 12, 37.2 $\mu^2$; class 13, 40.8 $\mu^2$; class 14, 46.6 $\mu^2$; class 15, 51.4 $\mu^2$; class 16, 58.6 $\mu^2$; class 17, 65.3 $\mu^2$; class 18, 73.1 $\mu^2$; class 19, 82.1 $\mu^2$. Quantitative ratios between the classes of nuclei were expressed in %. The results were subjected to statistical analysis. During their analysis. data obtained previously in experiments on the same rabbits — according to which administration of cholesterol for 14 days led to a more than tenfold increase in its concentration in the blood (461 ± 67 compared with 42 ± 5.5 mg %), and that this parameter is normalized in animals killed 1.5 months after termination of the uptake of cholesterol [11] — were taken into account.

## RESULTS

Administration of cholesterol to the rabbits caused virtually no change in size of the hepatocytes, as shown by the results of counting them zone by zone in a standard field of vision of the sections (their number varied from 25.9 ± 0.69 to 26.6 ± 0.77), but it caused considerable deviations from karyometric values regarded as normal. In animals killed 24 h after the last dose of cholesterol, a significant decrease in the percentage of small and an increase in the percentage of large nuclei were observed in the central and middle zones of the hepatic lobules. The shift of curves reflecting the relative percentages of nuclei in the various classes in the liver of rabbits of the experimental group to the right compared with the control values can be clearly seen in Fig. 1. Nuclei with an area of cross-section of 5.14 $\mu^2$ (class 15) were seen most frequently, whereas under normal conditions this position is occupied by nuclei with an area of 40.8 $\mu^2$ (class 13). Nuclei of classes 17, 18, and 19 (areas 65.3, 73.1, and 82.1 $\mu^2$ respectively) were observed. In the peripheral zone of the hepatic lobules the shift of the curve to the right was less demonstrative. A considerable decrease in the percentage of nuclei in classes 10 and 11 was observed ($P < 0.01$) with the appearance of large nuclei belonging to class 18. Counting the number of binuclear cells showed that both in intact animals and in rabbits receiving cholesterol (groups 2 and 3) their largest number was observed in the peripheral part of the hepatic lobules, and their smallest in the central part (Fig. 1). A tendency for the number of binuclear hepatocytes to decrease in the central and middle zones and to increase in the peripheral zone of the lobules was found in the liver of the rabbits of group 2 under these circumstances. However, these deviations from normal were not statistically significant ($P > 0.05$).

From our point of view the most interesting results were those obtained in a study of the liver of the animals of group 3, killed 1.5 months after the last dose of cholesterol. In none of the zones of the liver lobules had the karyometric indices returned to their initial values. In the central and middle zones of the lobules of the liver the curves showed a shift to the left relative to those for the animals of group 2, but the percentages of small and large nuclei differed from those in intact rabbits (Fig. 1). For instance, in the central zones of the liver lobules of the last group of rabbits the number of nuclei in class 10 was 2.8 ± 0.24% and in class 11 it was 16 ± 4.74%, whereas in animals of group 3 there was no nuclei in class 10, and only 3.2 ± 0.44% in class 11 ($P < 0.02$). The number of nuclei in class 14 increased from 16.8 ± 4.04% in the control to 33.6 ± 0.62% in the experiment ($P < 0.001$). The number of nuclei in class 15 increased from 2 ± 0.4 to 6.8 ± 2.6% ($P > 0.05$). There were 1.6 ± 0.37% of nuclei in classes 17 and 18, whereas none were found in the liver of intact animals. Similar changes were found in the distribution of nuclei by classes in the middle zone of the hepatic lobules also.

No nuclei of classes 10 and 11 were found in the peripheral zone of the lobules 45 days after termination of cholesterol feeding, the fraction of nuclei in class 12 was reduced, whereas the number of nuclei in class 14 and, in particular, in class 15 was increased (19.6 ± 4.9% in the control and 27.6 ± 4.86% in the experiment, $P > 0.1$). In all zones of the hepatic lobules of the animals of group 3 an increase in the number of binuclear cells was observed. This took place particularly intensively in the middle (from 98.9 ± 12.08 to 149 ± 14.8%, $P < 0.02$) and peripheral (from 169.8 ± 12.9 to 244.2 ± 25.3%, $P < 0.02$) zones.

Administration of cholesterol to rabbits, causing an increase in its concentration in the blood and liver [1, 2, 4, 10], thus causes long-lasting changes in the karyometric parameters of the liver. Taking into account data in the literature on correlation between the volume and ploidy of nuclei in the liver parenchyma [2, 5], the increase in the number of cells with large nuclei in the glands of the experimental animals can be regarded as an in-

dication of a higher level of polyploidization of the hepatocyte nuclei. We know that the functional activity of polyploid hepatocytes is higher than that of diploid [2, 5]. Accordingly, the data showing an increase in the number of large nuclei in the liver cells of experimental rabbits can be interpreted as a reflection of changes in the intensity of metabolism in the hepatic parenchyma of animals with an excessive uptake of cholesterol, an important structural and metabolic component of hepatocytes. Meanwhile the increase in the percentage of hepatocytes with large nuclei can also be regarded from the standpoint of the role of polyploidy in the compensation of an unbalanced genome [3]. The results now obtained are evidence of incomplete return of the parameters studied to normal 1.5 months after the end of excessive uptake of cholesterol, although by this time the parameters of lipid metabolism in the blood and liver have largely returned to normal [4, 11]. Some of the changes discovered in a study of the liver of the rabbits of group 3 (shift of the curve reflecting the size of the hepatocyte nuclei to the right) can be regarded as "residual" from changes observed in the animals of group 2. Meanwhile, the presence of a significant increase in the number of binuclear hepatocytes, which was not found in a study of the organ in the animals of group 2, was characteristic of the liver of the rabbits of group 3. Data on the increase in number of binuclear cells can be regarded from the standpoint of the concept of the "change of generations" of mononuclear and binuclear cells [3], particularly in connection with the reduction in the fraction with the largest nuclei in the liver of these animals (when compared with group 2).

These data are evidence of the desirability of studying the state of the nuclear apparatus of the hepatocytes for even longer times after termination of cholesterol uptake.

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