

To sum up it can be concluded that induction of SEBP is an androgen-dependent process, and accordingly this protein may be interesting not only as a possible mediator for one of the pathways of action of estrogens on the liver, but also as a marker of the action of androgens on this organ.

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#### REGENERATION OF THE LIVER WITH EXPERIMENTAL CIRRHOSIS AFTER QUADRUPLE RESECTION

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KEY WORDS: regeneration; cirrhosis of the liver; resection of the liver.

Many investigations demonstrating the ability of the cirrhotic liver to undergo regeneration have now been published. Several workers [2-7, 9, 11] have noted that resection of the cirrhotic liver favors reversibility of the pathological changes. The study of the compensatory and regenerative powers of the cirrhotic liver after multiple repeated resections is of great interest.

The object of this investigation was to study the course of regeneration of the cirrhotic liver after four resections.

#### EXPERIMENTAL METHOD

Experiments were carried out on 250 albino rats weighing 160-180 g. Cirrhosis of the liver was produced by subcutaneous injection of carbon tetrachloride (0.2 ml in an oily solution) into each animal four times a week for 5 months. Resections of the liver were carried out at intervals of 45 days. The animals were killed on the 2nd, 3rd, 7th, and 45th days after each operation. Rats with experimental cirrhosis not undergoing resection and intact animals of the same age were sacrificed at the same time. At the first operation the left lateral lobe, weighing 5.11 g (43.3% of the weight of the liver in rats with experimental cirrhosis) was removed. At the second operation the left and right lobules of the central lobe, weighing 3.27 g (42% of the liver after a single resection) were removed. At the third operation the right lateral lobe of the liver, weighing 2.76 g (34.2% of the twice resected liver) was removed. At the fourth operation the caudate lobe of the liver, weighing 1.94 g (24.9% of the thrice resected liver) was removed. The total weight of liver tissue removed in the course of the four operations was equivalent to 144.4% of the weight of the liver of the control rats with cirrhosis.

Pieces of liver taken during the operations and at sacrifice were fixed in neutral 10% formalin and Carnoy's fluid and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, with Water blue, by Van Gieson's method, with Sudan III, and by Mc-

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Manus' method with amylase control. Films of liver tissue were fixed in alcohol and stained with hematoxylin-eosin. The number of mitoses and of binuclear cells was counted in 7000 hepatocytes. The mitotic index (MI) and number of binuclear cells (NBC) were expressed in promille. To estimate the quantity of connective tissue the hydroxyproline content in the liver was determined by the method in [10], in the modification in [1]. The content of connective tissue also was determined by a quantitative method of comparison of systems using an ocular attachment for full stereologic measurements of microscopic objects [8].

#### EXPERIMENTAL RESULTS

The body weight of the rats 45 days after each operation did not differ significantly from the body weight of intact animals of the same age. The values of the absolute, relative, and dry weights of the liver 45 days after each operation also differed only a little from the corresponding values in intact animals. As a result of injection of carbon tetrachloride marked cirrhosis developed in the liver, and was manifested as a combination of degenerative and regenerative changes, diffuse proliferation of connective tissue, circulatory disturbances, and the presence of foci of necrosis. Connective tissue, in coarse thick bands, divided the parenchyma into pseudolobules of different sizes and shapes, sometimes consisting of several hepatocytes. The hydroxyproline content in the liver was 0.037%. The most marked morphological changes were found in the early periods after the operations. Vasodilatation was observed in the portal and venous tracts, afferent vessels, and sinusoids. Stasis of blood and microhemorrhagic foci, accompanied by death of hepatocytes, were noted in some places. The architectonics of the liver trabeculae was disturbed. Blood vessels were surrounded by areas of connective tissue. The quantity of connective tissue 2-3 days after the first operation still remained high. After all subsequent operations it showed a tendency to decline. Cloudy-swelling degeneration (granular, balloon degeneration), fatty infiltration (small or large drops), and a reduction in size of the glycogen granules, which remained only in individual hepatocytes at the periphery of the pseudolobules, were revealed by histochemical methods in the cytoplasm of the hepatocytes. Cells in a state of hydropic degeneration, with pycnotic nuclei and oxyphilic cytoplasm, were observed. Hepatocytes with unchanged cytoplasm were preserved mainly in the zone of the portal tracts. After one week, fewer cells were observable in the connective tissue. The quantity of connective tissue decreased a little at this period after all operations by comparison with the preceding period, as reflected in the results of stereometric determination — 1) 4.05, 2) 1.66, 3) 1.64, and 4) 1.54 — and the hydroxyproline content — 1) 0.039, 2) 0.025, 3) 0.025, and 4) 0.022%. A trabecular arrangement of the hepatocytes was observed over wide areas of the liver parenchyma. Polymorphism of the hepatocytes and their nuclei as regards both size and shape remained well marked. Fatty infiltration by small droplets was observed, although in some hepatocytes the droplets were large. Staining for protein revealed cloudy-swelling degeneration. The content of glycogen granules in the hepatocytes was greater than at the preceding period. Cells in the periportal zone were particularly rich in glycogen granules. Hepatocytes with unchanged cytoplasm were located mainly around the central zones of the portal acini. Individual hepatocytes showing degenerative changes and foci of necrosis containing anuclear cell bodies, pycnotic nuclei, and fragments of nuclei, scattered haphazardly among the amorphous mass, were seen in the liver parenchyma around the periphery of the pseudolobules. A decrease in the quantity of connective tissue compared with the previous time, the contrast being particularly great after the first operation, was noted 45 days after the operations. Thin connective-tissue bands bounded much larger pseudolobules and sometimes changed into "highways" of connective-tissue infiltration, consisting mainly of lymphocytes, histiocytes, and single macrophages. The hydroxyproline content — 1) 0.022, 2) 0.025, 3) 0.023, and 4) 0.027% — and the connective tissue content, determined stereometrically — 1) 2.06, 2) 2.39, 3) 2.47, and 4) 2.74) — were lower than in the control animals with cirrhosis (0.037%; 5.47). The architectonics of the hepatic trabeculae was restored around the central veins. In individual rats the structure of the trabecular complexes was not restored. In regions of the triads, proliferation of cells of the bile ducts was observed. In the liver parenchyma areas of organizing necrosis could be seen. No fatty infiltration of the hepatocytes was detected. In individual hepatocytes along the periphery of the lobules, granular degeneration was observed. The content of glycogen granules in the hepatocytes was a little below normal.

The increase in MI of the hepatocytes observed on the 2nd day was much greater after the first and third operations than after the second and fourth operations (Fig. 1). On the 3rd and 7th days MI was sharply reduced. MI of the hepatocytes was very low 45 days after all the operations. While MI of the hepatocytes on the 2nd day after all operations was raised, NBC

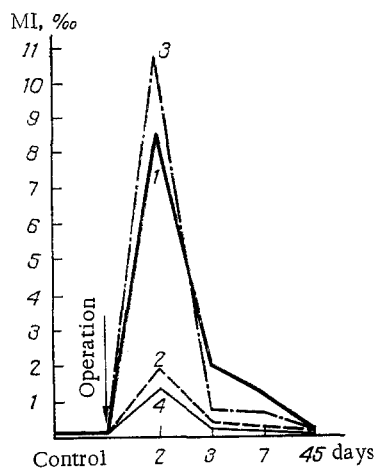


Fig. 1

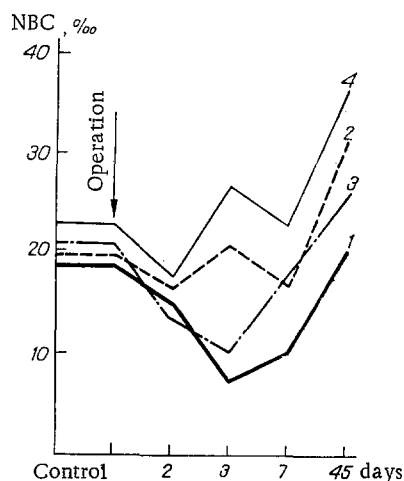


Fig. 2

Fig. 1. Mitotic activity of hepatocytes after single (1), double (2), triple (3), and quadruple (4) resection of the liver. Here and in Fig. 2: ordinate, days after operation.

Fig. 2. Number of binuclear hepatocytes after single (1), double (2), triple (3), and quadruple (4) resection of the liver.

was lower than in intact animals (Fig. 2). NBC was increased on the 3rd and 7th days after the second and fourth operations. On the 3rd day after the first and third operations a further decline was observed in NBC, and not until the 7th day was a tendency observed for it to rise. NBC 45 days after all the operations was higher than its value in intact animals of the same age.

The results are evidence that after multiple resections of the cirrhotic liver a tendency toward normalization of the structure of the parenchyma and a decrease in the quantity of excessively proliferating connective tissue are observed. The decrease in the quantity of connective tissue was particularly marked after the first operation. After further repeated operations the subsequent decrease in the quantity of connective tissue was very small.

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