# PATHOMORPHOLOGICAL HISTOCHEMICAL CHANGES IN ACUTE EXPERIMENTAL BILIARY STASIS AND CALCULOUS CHOLECYSTITIS

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The development of pathomorphological and histochemical changes in the liver, kidneys, heart, and spleen in acute experimental biliary stasis and cholecystitis in rabbits is described. Acute biliary stasis was found to cause the development of biliary cirrhosis of the liver as early as at the beginning of the 3rd week of the experiment. Degenerative changes developed in the kidneys and heart, and hyperplasia in the spleen. In the animals with cholecystitis, changes predominantly of cloudy swelling developed in the liver, kidneys, and heart, while proliferation of lymphoid tissue took place in the spleen.

Investigations by other workers [1-4, 7, 8], together with histological and histochemical studies of pieces of liver taken after operations on 76 patients with surgical diseases of the extrahepatic biliary tract, have shown [5, 6] that obstruction and inflammation of the bile ducts are frequently accompanied by severe degenerative changes in the liver. Subsequent proliferation of the connective tissue may lead to the development of cirrhosis [9]. Despite the importance of these pathological changes, the morphological and histochemical disturbances in the liver and other organs under these circumstances have so far been inadequately studied.

It was therefore decided to conduct an experimental investigation to ascertain the dynamics of the morphological and some histochemical changes in the liver, kidneys, heart, and spleen in obstructive jaundice and calculous cholecystitis.

## EXPERIMENTAL METHOD

Experiments were carried out on 42 chinchilla rabbits divided into three groups. Obstructive jaundice was produced in the animals (17) of group 1 by isolated ligation of the common bile duct with a silk ligature. In the animals (15) of group 2 the development of acute calculous cholecystitis was induced. For this purpose, pulverized concretions removed from patients with cholelithiasis were introduced into the gall bladder. The defect in the gall-bladder wall was sutured with thin Kapron ligatures and peritonized by sticking the peritoneum to it with BF-2 glue. All operations were performed under ether–oxygen anesthesia. During the operations and in the postoperative period no drugs (including antibiotics) were given. The animals (ten rabbits) of group 3 acted as the control. Observations were made on the rabbits to assess the clinical manifestations of the disease and to determine the blood bilirubin. The animals were sacrificed at various times after the beginning of the experiment. Pieces of liver, kidneys, heart, and spleen were fixed in 10% neutral formalin and Carnoy's fluid. Histological sections were stained with hematoxylin–eosin and by Van Gieson's method, for fat with Sudan III, for glycogen by Best's method, for DNA by the Feulgen reaction, and for RNA by Brachet's method. Succinate dehydrogenase (SDH) was also detected in frozen sections by Nachlas's method with nitro-BT.

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## EXPERIMENTAL RESULTS

Of the 17 animals of group 1 three died during the 1st day. The rabbits which survived the 1st day were lethargic, their appetite was poor, and they developed diarrhea. The blood bilirubin concentration rose, and the urine became reddish in color.

Congestion of the central veins and intralobular capillaries, small foci of necrosis, and degenerative changes were observed in the liver of the animals sacrificed after biliary stasis lasting 3-5 days. Frequently larger foci of necrosis were found, occupying several lobules and located chiefly along the course of the portal tracts. The necrotic foci were surrounded by pseudoeosinophils, macrophages, and Kupffer cells with numerous large and small formazin granules in their cytoplasm. The macrophages contained large quantities of glycogen, DNA, and RNA. The periportal spaces were abundantly infiltrated by round cells. These cells penetrated inside the lobules along the course of the sinusoidal capillaries. The bile capillaries were dilated and congested with bile. Bile pigment accumulated as tiny granules at the apical poles of the hepatocytes, and it was also found in the necrotic foci. The fibers of the myocardium were irregularly stained and some areas were basophilic with increased SDH activity. Congestion of the glomerular vessels was marked in the kidneys. In the spleen hyperplasia of the lymphoid tissue was observed.

After the 5th day regenerative processes began to predominate in the liver of these animals and connective tissue proliferated. Meanwhile fresh foci of necrosis surrounded by a leukocyte barrier appeared. In the hepatocytes which remained intact cloudy swelling and fatty degeneration were frequently seen, the SDH activity fell sharply, and the nucleic acid and glycogen content was reduced.

The most marked succinate dehydrogenase activity was found in the hepatocytes of the peripheral zones of the lobules. Together with numerous tiny formazan granules, large granules, sometimes extremely swollen, were found. Many parenchymatous cells contained two nuclei, and frequently the nuclei were large but poor in chromatin. The stroma was infiltrated with lymphocyte-like cells. In the kidneys the epithelial cells of the convoluted and collecting tubules were swollen and the cytoplasm was filled with tiny granules. The nuclei of many epithelial cells had broken up and disappeared, and the stroma was infiltrated with small cells. Hyperplasia of the lymphoid tissue and reticulo-endothelial cells was observed in the spleen.

Toward the end of the 2nd-3rd week of biliary stasis the periportal connective tissue in the liver showed marked proliferation and surrounded the hepatic tracts by wide, dense rings. The connective tissue proliferating at the site of the foci of necrosis and along the course of the sinusoidal capillaries penetrated into the depth of the hepatic lobules to form pseudolobules. Often localized areas of the hepatic trabeculi showed loss of their normal complex structure and many of the cells were necrotic. The cytoplasm of many hepatocytes contained large oxyphilic granules. The total content of glycogen, nucleic acid, and SDH was reduced. The number of Kupffer cells was increased. Meanwhile many binuclear cells with basophilic cytoplasm and characterized by a high content of glycogen, RNA, and DNA, and also by high SDH activity, were observed. In the kidneys and myocardium at this time a varied degree of granular degeneration was present, while in the spleen there was hyperplasia of the lymphoid tissue and reticulo-endothelial cells. In all these organs bile pigments were detected.

Acute biliary stasis in rabbits thus led to focal necrosis in the liver, in the origin of which the cytolytic action of the bile played a major role, and to the development of biliary cirrhosis, which became particularly well-marked in the 3rd week of the experiment. The disturbance of the normal structure and function of the liver also affected other organs: degenerative changes appeared in the kidneys and myocardium and hyperplasia in the spleen.

For the first 2-3 days after the operation the animals of group 2 were lethargic, their appetite was poor, and their blood bilirubin concentration was slightly raised. On the 5th day of the experiment the changes observed in the liver included congestion and focal granular degeneration of the hepatocytes, widening of the Desse's spaces, hyperplasia of the Kupffer cells, and infiltration of the periportal spaces by round cells.

On the 10th day of the experiment the congestion of the liver had disappeared, localized areas of the trabeculi showed loss of the normal structure, and degenerative changes and disintegration of the parenchymatous cells appeared. At the same time large and binuclear hepatocytes could be seen. The SDH activity in the hepatocytes was reduced, and glycogen was found mainly in the cells of the peripheral part of the lobules.

On the 15th day of the experiment the signs of loss of the normal structure of the liver trabeculae, the cloudy swelling and fatty degeneration of the hepatocytes, were more severe and small foci of necrosis appeared.

After 1 month the interior of the gall bladder was occupied by a large, dense calculus. In some places in the liver cloudy swelling of the parenchymatous cells, round-cell infiltration of the periportal spaces, and cirrhosis of the walls of the bile ducts were observed. The content of glycogen, DNA, and RNA and the SDH activity were reduced in the degenerating cells.

Throughout the experiment mainly evidence of granular degeneration was observed in the myocardium and kidneys and proliferation of lymphoid tissue in the spleen of the rabbits with calculous cholecystitis.

In experimental calculous cholecystitis in rabbits changes thus took place in the liver, heart, and kidneys which are characterized chiefly by evidence of granular degeneration.

The model of acute experimental biliary stasis and calculous cholecystitis can be used to study the pathological changes in these diseases in the liver and other organs over a period of time, and the results of the investigations described above show that patients with calculous cholecystitis and biliary stasis should receive operative treatment as early as possible.

## LITERATURE CITED


1. V. I. Antonovich, in: Current Problems in Liver Pathology [in Russian], No. 5, Dushanbe (1969), p. 164.
2. A. F. Blyuger and M. P. Sinel'nikova, in: Intravital Morphological Study of the Liver [in Russian], Riga (1962), p. 66.
3. G. A. Vadachkoriya and L. P. Chirgadze, in: Current Problems in Experimental Clinical Treatment [in Russian], Tbilisi (1965), p. 395.
4. V. I. Vitlin, Klin. Khir., No. 4, 26 (1964).
5. V. F. Gaevskii, Klin. Khir., No. 6, 67 (1972).
6. V. F. Gaevskii, V. G. Zorya, and P. F. Shamrai, in: Surgery of the Organs of Digestion [in Russian], Kiev (1972), p. 115.
7. L. L. Gromashevskaya, A. F. Kiseleva, and É. P. Gette, in: Current Problems in Liver Pathology [in Russian], No. 5, Dushanbe (1969), p. 188.
8. A. I. Klembovskii, E. E. Gupalo, and A. S. Loginov, in: Proceedings of the 2nd Conference on Clinical Biochemistry of Infectious Diseases [in Russian], Riga (1963), p. 239.
9. L. N. Popova, Vestn. Khir., No. 5, 26 (1958).