RESTORATION OF CELL FUNCTION AND STRUCTURE OF THE LIVER AFTER OBSTRUCTION OF THE BILE DUCT IN DOGS

I. D. Mansurova, V. I Antonovich, and L. G. Kaletkina

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Despite the restoration of the structure and function of the liver during spontaneous recanalization of the common bile duct in dogs after its ligation, considerable metabolic disturbances were found in the hepatocytes in the late periods after restoration of the biliary flow.

As a result of recent work, ideas regarding the irreversibility of fibrous and cirrhotic changes have undergone a change, and this has stimulated the investigation of intracellular regeneration [7, 8]. The suggestion has also been made that the damaged and partially dedifferentiated cells of an injured organ do not lose their power of subsequent differentiation and functional modification [10].

TABLE 1. Enzyme Activity in Subcellular Structures of Dog Liver during Obstructive Jaundice and Recanalization of the Common Bile Duct

	Control	Obstructive jaundice	Recanalization
	AST		
Nuclei	13,1±0,9	18,4±3,0	16,4±3,1
Mitochondria	17,0±1,1	13,5±1,2	14,2±1,1
Microsomes	8,1±0,85	6,4±0,9	5,2±1,7
Cytoplasm	61,8±2,05	61,7±4,1	62,2±4,2
	ALT		
Nuclei	10,0±0,82	13,0±1,1	10,5±1,7
Mitochondria	14,2±1,0	12,5±2,6	15,6±1,6
Microsomes	6,2±0,55	3,6±0,6	3,8±1,0
Cytoplasm	69,6±1,82	70,9±3,5	70,1±2,3
	AP		
Nuclei	24,3±3,1	16,9±5,8	29,8±1,1
Mitochondria	25,5±3,0	30,1±2,8	25,4±6,0
Microsomes	14,8±1,52	21,5±5,1	15,9±5,4
Cytoplasm	35,4±3,25	31,5±6,4	28,8±3,2
	ACE		
Nuclei	26,0±2,2	19,4±5,3	25,9±1,8
Mitochondria	15,8±1,9	16,1±3,0	22,7±2,3
Microsomes	39,2±2,2	33,3±6,8	25,6±3,1
Cytoplasm	19,0±1,6	31,2±8,2	25,8±4,2
	HAL		
Nuclei	17,2±1,6	16,0±3,7	16,6±1,6
Mitochondria	38,3±1,6	15,4±3,9	23,9±2,8
Microsomes	14,2±1,3	22,8±4,2	15,5±1,9
Cytoplasm	30,3±1,2	45,8±4,8	44,0±3,2

It is therefore interesting to study the state of metabolism at the ultrastructural level during spontaneous recanalization of the common bile duct, a problem which has received insufficient attention in the literature.

In the combined investigation described below an attempt was made to examine the pathological state at the cellular level and to determine the morphological changes in the liver in the late stages after restoration of the biliary flow and ligation of the duct for a second time.

EXPERIMENTAL METHOD

The function and structure of the liver were studied in 14 dogs with ligation (6 dogs) and division (8 dogs) of the common bile duct. Recanalization of the duct took place in all dogs in which it was ligated. In 2 of the 6 dogs with spontaneous recanalization, the duct was divided 4-5 months after ligation, and in this case the biochemical and clinical manifestations corresponded to obstructive jaundice. The animals remained under observation for between 8 and 18 months after ligation. The results of the experiments on the dogs with recanalization of the common bile duct were compared with those in the period of mechanical jaundice after division of the duct.

Biochemical Department, Institute of Gastroenterology, Academy of Sciences of the Tadjik SSR, Dushanbe. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 5, pp. 43-47, May, 1971. Original article submitted August 5, 1970.

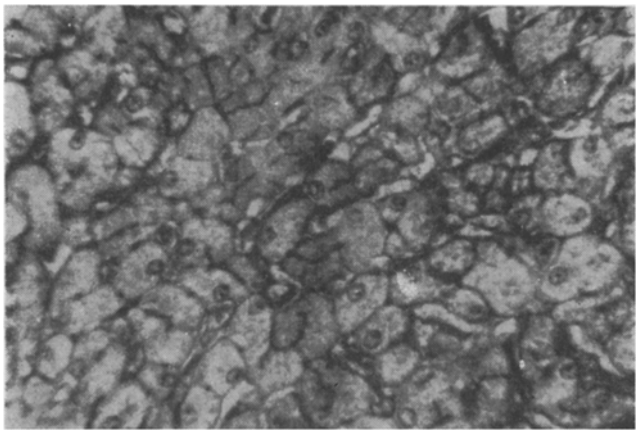

Fig. 1. Cellular polymorphism. High alkaline phosphatase activity in sinuses along the course of biliary capillaries and in cytoplasm of hepatocytes. Reaction for alkaline phosphatase, 140×.

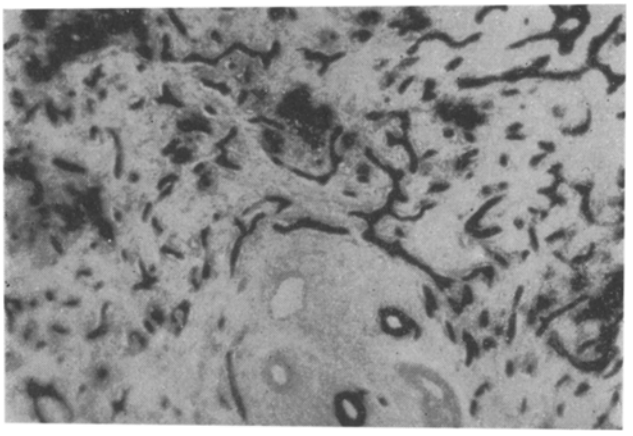

Fig. 2. Fibrous changes in liver tissue of dog 18 months after restoration of biliary flow. High alkaline phosphatase activity in proliferating bile ducts. Reaction for alkaline phosphatase, 140×.

Liver homogenates were fractionated in a TsLR-1 refrigeration centrifuge by Khesin's [12] modification of Hogeboom and Sneider's method, and nuclear, mitochondrial, microsomal, and cytoplasmic fractions isolated. Activity of the enzymes aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), histidine-ammonia liase (HAL), acetylcholinesterase (ACE), and alkaline phosphatase (AP) was investigated in the total homogenate and in its fractions. The activity of the enzymes was investigated by micromethods developed by Mansurova [1]. AST and ALT activity was expressed in micrograms pyruvic acid, HAL in micromoles × 100 of urocannic acid, ACE in milligrams acetylcholine, and AP in milligrams phosphorus per gram liver tissue. For the histological investigations paraffin sections were stained with hematoxylin-eosin, and for the histochemical demonstration of alkaline phosphatase Gomori's method was used.

EXPERIMENTAL RESULTS

The results showed that during spontaneous recanalization, which occurred in all the dogs with ligation of the common bile duct, the tendency toward restoration of the liver structure was slight and was not the same in all cases, despite the fact that the biliary stasis in the liver was reduced, necrotic foci were absorbed, and the inflammation subsided.

In one dog, 18 months after recanalization of the bile duct, focal changes characteristic of a severe pathological process were observed in one lobe of the liver. These local changes evidently corresponded to the severest type of destructive changes during the period of biliary stasis. Even in places where the course of repair was most favorable, the trabecular structure characteristically was irregular, the liver cells were arranged haphazardly, and they varied considerably in size. In the hypertrophied cells the nuceli were eccentric in position, and karyolysis and vacuolation of the karyoplasm were observed, indicating that the condition was not altogether favorable. Only a few binuclear cells were seen.

Alkaline phosphatase activity in the liver of this dog was high along the course of the biliary capillaries. It was found diffusely but irregularly in the cytoplasm of the hepatocytes in the form of tiny granules, the result of earlier severe damage to the hepatocytes in the period of biliary stasis (Fig. 1). Fibrous changes consisted of marked proliferation of connective tissue in the portal tracts. In other parts, where the changes in the past had evidently been less intensive in character, a large area of the parenchyma was replaced by zones of fibrosis, containing inumerable proliferating bile ducts showing high alkaline phosphatase activity (Fig. 2).

These observations show that recovery of the liver structure after degeneration induced by biliary stasis is very protracted in its course. An aggravating factor in this case is the disturbance of the circulation of blood in the lobules accompanying severe biliary stasis after ligation of the common bile duct.

The other animals were investigated in the earlier stages after recanalization of the duct, and no such marked fibrotic changes were found in their liver.

The degree of reversibility of pathological changes depends in each concrete case on the character of the pathological state. It has often been mentioned that in lesions of the liver accompanied by biliary stasis, despite the high regenerative ability of the hepatocytes, repair takes place slowly [3, 5, 9, 11].

At the same time, when poisoned with bile the liver cells become resistant to subsequent interference. Evidence of this resistance was found when division of the previously ligated bile duct did not induce cirrhotic changes in the liver, although such changes could have taken place. These results are interesting because the reactive state of the cells developing during biliary stasis was responsible for the resistance of the hepatocytes to the repeated toxic action of bile.

Hypertrophy and hyperplasia of the cell organelles have been demonstrated during cell hypertrophy by the electron microscope [6, 7]. The possibility of changes in the relationships between the intracellular membranes cannot be ruled out under these circumstances, and this could probably be connected with the redistribution of enzyme activity observed in these experiments in the organelles of the hepatocytes.

With respect to the character of the enzymic disturbances in the subcellular structures, the writers have previously reported considerable changes in activity of the enzyme systems during obstructive jaundice [4]. Investigation of enzyme activity in the total homogenate revealed a significant increase in alkaline phosphatase activity in the period of recanalization, when it was 1.5 times higher than in the control ($P < 0.02$).

HAL activity in the recanalization period was slightly higher than during obstructive jaundice, but the difference between these activities was not significant ($P > 0.2$), thus suggesting marked inhibition of the enzyme with restoration of the flow of bile.

The increase in ALT activity in the recanalization period likewise was not significant compared with its level in the period of obstructive jaundice ($P > 0.5$), although AST activity in this period was almost back to normal, namely 99.6% of its initial value.

Investigation of enzyme activity in the subcellular structures showed that the mitochondria of the hepatocytes had undergone the greatest damage, as shown by a decrease in HAL activity by more than half in the period of obstructive jaundice. During recanalization of the duct, its activity rose although it still remained much below the normal level. HAL activity in the cytoplasm remained essentially as high as in the period of obstruction to the flow of bile (Table 1).

Besides the changes in HAL activity, which is more specific for liver tissue, in the period of restoration of the biliary flow in dogs, the activity of the other enzymes in the organelles of the hepatocytes remained disturbed. For instance, marked depression of ALT activity and a less marked depression of AST activity was observed in the microsomes, while alkaline phosphatase activity in this fraction remained

slightly raised; finally, the ACE activity was increased in the mitochondria and depressed still more in the microsomal and cytoplasmic fractions compared with the period of obstructive jaundice.

Hence, the redistribution of enzyme activity observed in the hepatocytes, indicating profound intracellular disturbances under the influence of biliary stasis and the reduced oxygen saturation of the liver tissue [2], had not returned completely to normal at the stipulated periods of recovery of the biliary flow. At the same time, the character of distribution of enzyme activity in the cell organelles was altered.

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