COURSE OF WOUND HEALING IN THE RAT LIVER DEPENDING ON METHOD OF ARRESTING BLEEDING AND ESCAPE OF BILE

A. I. Volozhin, V. I. Pronin, and Yu. I Lomachenko

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To treat wounds of the liver physical methods, biological tissues, and synthetic materials are used [2, 4, 10]. This variety of methods of hemostasis and biliary stasis leads to the development of a combination of structural and functional changes in the liver and in the zone of the wound defect, in response to surgical intervention, and these determine the nature of the course of reparative processes and the time taken for their completion [1, 3, 6, 7, 9]. Comparison of the course of healing of liver wounds after their treatment by different methods can thus help to choose the optimal method of hemostasis and biliary stasis, and the investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 314 noninbred male albino rats weighing 170-250 g, kept on an ordinary diet. The animals were anesthetized with ether and a guillotine resection of the liver carried out (without strict observance of the architectonics of the blood vessels and bile ducts) amounting to 17-21% of the mass of the organ (40-50% when the proliferative response of the hepatocytes was studied). Hemostasis and biliary stasis were achieved by means of the Soviet preparation "Hemostopan," with leaving the polymer matrix of the preparation in situ (group 1) or removing it (group 2), and also by the use of methods of wound omentoplasty, using glue (group 3) and suturing (group 4). The course of regeneration in the liver was assessed by the time course of mitotic activity of cells of the hepatic parenchyma, and also restoration of the relative mass of the liver of the hepatectomized animals. The number of dividing hepatocytes was counted in histological sections stained with hematoxylin and eosin; the mitotic index was determined and expressed in promille. The tissue partial pressure of oxygen (pO_2) was determined in the resected and unresected lobes of the liver by a polarographic method [5], using an open needle electrode with diameter of the platinum thread of 80 μ, under hexobarbital anesthesia before and immediately after resection of the liver, and also 2, 5, 10, 20, and 30 days after the operation. The comparison electrode consisted of a standard Ag—AgCl electrode of the EVL MZ type. The pO_2 level was recorded during inhalation of atmospheric air and administration of an oxygen—air mixture through an oronasal mask. Pathomorphological investigations of the liver tissue were carried out at the same times after the operation. Preparations were stained with hematoxylin-eosin, and selectively with toluidine blue and picrofuchsine by Van Gieson's method. Material for electron microscopy was fixed with glutaraldehyde and OsO_4, and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead hydroxide and examined in the Tesla BS-540 electron microscope.

EXPERIMENTAL RESULTS

The study of cell proliferation in the liver after the operation revealed the optimal distribution of mitotic activity on the hepatocytes with time only in groups 1 and 4. The mitotic index 24-48 h after the operation showed only very slight

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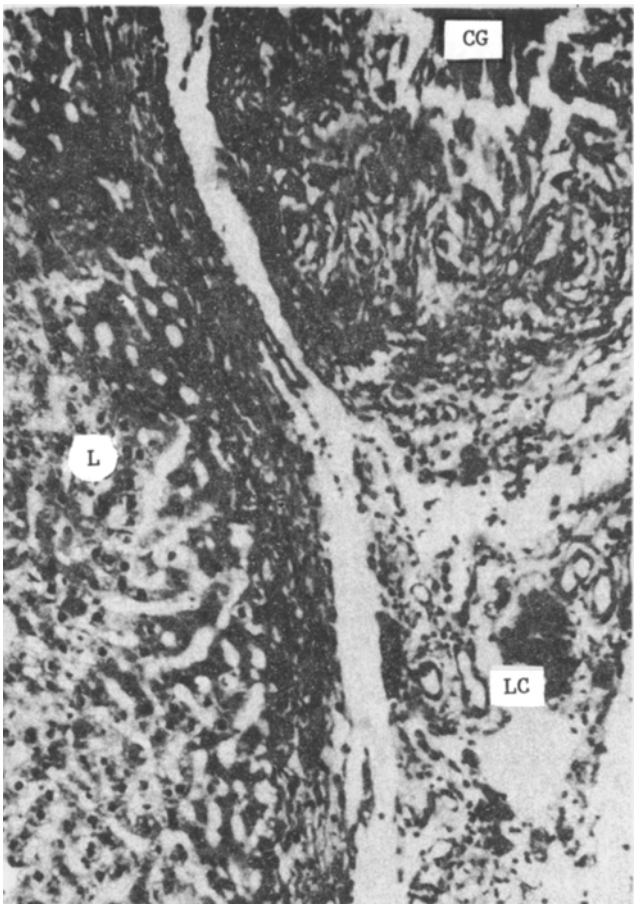

Fig. 1. Accumulation of degeneratively changed liver cells (LC), fragments of cat gut (CG) in zone of operation wound of the liver (L) on 30th day after resection of the organ (group 4). Hematoxylin-eosin. Object 10.0, ocular 6.3.

changes — from 0.3 to 37.2‰, reaching a maximum after 23 h. We know that in the intact rat liver the value is 0.1-0.2‰ [8]. In group 2 the dynamics of hepatocyte proliferation was characterized by low rates of increase of mitotic activity, reaching a maximum after 48 h (22.9‰). In group 3 mitotic division of the hepatocytes took place asynchronously and the mitotic index reached high values (the highest 95.1 and 72.2‰, 24 and 28 h respectively after the operation). Mitotic activity of the hepatocytes decreased irregularly, slowly, and was higher at all times of the investigation than in the other groups. Allowing for data on the distribution of dividing cells by phases of mitosis, the results can be explained by the presence of a constant stimulus to cell proliferation, the high level of which was due to the considerable tissue damage.

Wound healing was complete after the shortest time in groups 1 and 2, due to the fact that measures to secure hemostasis and biliary stasis were less traumatic than in the other groups. Fibers of the polymer matrix of the "Hemostopan" if left in the liver wound became encapsulated, and a flat connective-tissue scar formed in the region of the wound defect. In group 3, healing of the liver wound was a more prolonged process. Ingredients of the glue and their metabolic products evidently had an unfavorable influence on reparative processes.

A marked damaging action on the tissue also was found after omentoplasty of the wound surface of the liver using sutures (group 4). Insertion of a hemostatic hepatic suture was accompanied by the formation of zones of tissue necrosis and necrobiosis, which were gradually replaced by loose, structureless connective tissue. "Islets" of degeneratively changed liver cells remained in the zone of the wound defect until the 30th day after the operation (Fig 1).

After resection of the liver the tissue pO_2 fell in both resected and unresected lobes (at most, down to 64.9% of the initial level), reflecting worsening of the hepatic blood flow, and perhaps indicating postoperative hypovolemia. Meanwhile in group 4, in liver tissue adjacent to the line of the hepatic suture, an increase in tissue pO_2 was observed (at most by 87% of the initial level), evidently as a result of a disturbance of aerobic metabolism with a marked reduction of

TABLE 1. Changes in Tissue pO_2 (in mm Hg) of Liver before and 30 Days after Resection, Detected by Oxygen-Air Tests

Test object	Parameter	Before resection of liver	30 days after operation			
			group 1	group 2	group 3	group 4
Unresected lobe of liver	Initial pO_2 level	8,6±2,8	4,2	5,9	26,6	10,1
	Latent period after giving oxygen-air mixture, sec	10—30	5	20	25	10
	Rate of rise of pO_2, mm Hg/sec	0,3±0,1	0,3	0,1	0,1	0,1
	Maximal pO_2 level	20,1±7,5	25,1	9,9	29,7	14,1
	Increase in pO_2, % of initial level	364±130	595	168	111	138
	Rate of fall of pO_2, mm Hg/sec	0,4±0,2	0,8	0,1	0,1	0,1
Tissue adjacent to zone of scar formation	Initial pO_2 level	8,6±2,8	5,4	—	11,4	4,9
	Latent period after giving oxygen-air mixture, sec	Not more than 15	5	—	5	5
	Rate of rise of pO_2, mm Hg/sec	3,0±1,5	0,3	—	1,0	1,9
	Maximal pO_2 level	151,1±50,3	38,6	—	89,0	79,5
	Increase in pO_2, % of initial level	2065±836	717	—	780	1616
	Rate of fall of pO_2, mm Hg/sec	3,8±1,4	2,0	—	0,6	2,0

Legend. Data on resection of the liver correspond to formula MAV ± m, $p < 0.05$. In group 2, when liver tissue adjacent to zone of scar formation was tested, no change was noted in pO_2 in response to administration of oxygen—air mixture.

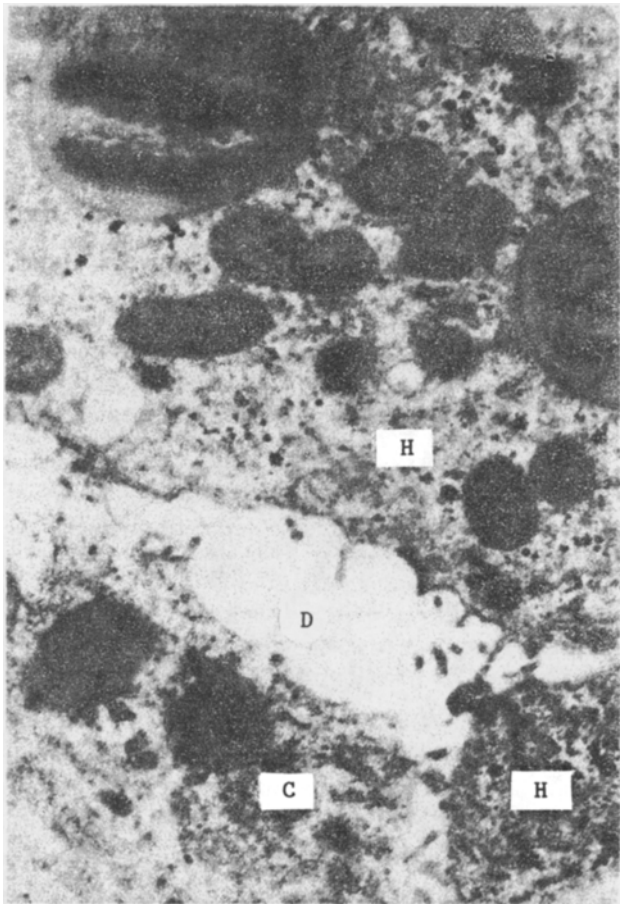

Fig. 2. Fragments of cytoplasm of adjacent hepatocytes (H) and cells (C) of sinusoid wall, bounding Disse's space (D). Reduction of microvilli of sinusoidal pole of hepatocytes can be seen Day 2 after resection of liver (group 4). 7000×.

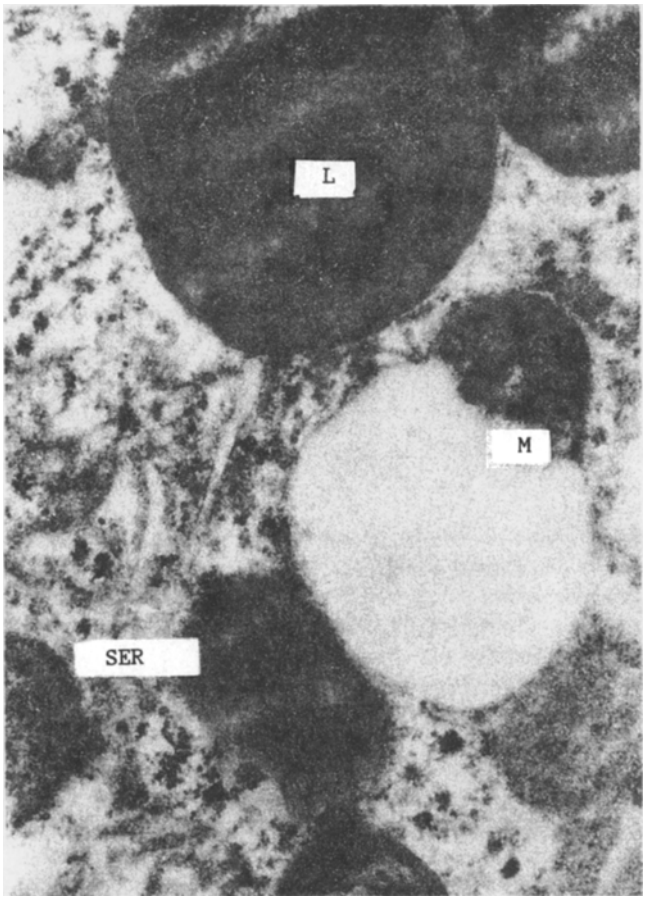

Fig. 3. Fragment of cytoplasm of a hepatocyte. Structures of smooth endoplasmic reticulum (SER), destroyed mitochondria (M), and concentration of acid lipids (L) can be identified. Day 10 after resection of liver (group 4). 9000×.

the tissue oxygen consumption. In the remaining cases, in which no suture was inserted, a fall of pO_2 was observed in the corresponding zone, probably due to a smaller defect of the oxygen utilizing system.

By the 30th day after resection the oxygen metabolism of the liver had not yet been restored to its initial level, evidence of incomplete healing. Meanwhile the ratio between the polarographic parameters, calculated during investigation in the unresected lobe of the liver of the group 1 rats (Table 1), indicates functional normality of the systems supplying oxygen to the tissues, and a high level of oxidation—reduction processes in it In the other groups of animals changes in pO_2 indicating functional disturbances in the oxygen transport and utilization systems were found. These changes were particularly marked in liver tissue adjacent to the zone of scar formation.

Electron-microscopic investigation of the liver tissue in the region next to the wound revealed changes of similar type in the Disse's spaces: a reduction in the number of microvilli at the sinusoidal pole of the hepatocytes, which normally fill the lumen of the Disse's spaces, and the onset of their partial reduction (Fig. 2). As a result of this the functionally active area of the cytoplasmic membrane of the liver cells was reduced, leading to restructuring of the metabolism of the hepatocytes and their change to the anaerobic metabolic pathway. At the ultrastructural level this was expressed as an increase in the number of lysosomes, the appearance of autophagolysosomes, some of which had a wide zone of lysis around their organelles, reduction of the number of glycogen granules or increased clarity of their outlines, changes in the number of lipid inclusions, and a decrease in the degree of condensation of peripheral chromatin in the nucleus. On the 2nd day of the postoperative period, in the 3rd and 4th groups, and on the 5th day in the 1st and 2nd groups, hyperplasia of structures of the smooth endoplasmic reticulum was observed in the cells, reflecting activation of the detoxication function of the hepatocytes. Meanwhile, in 211 cases the number of microvilli at the biliary pole of the hepatocytes,

responsible for their excretory function, was reduced. Only the ultrastructure of the biliary capillaries was restored to normal in most cases, by the 30th day of the postoperative period. The greatest changes in ultrastructure of the hepatocytes with signs of destruction of the cellular organelles were discovered in group 4 (Fig 3).

The study of recovery of the relative mass of the liver in the postoperative period showed that only in group 1, by the 30th day, had it reached the values observed in animals whose liver was not resected.

Thus comparison and analysis of the results of this investigation showed that methods of hemostasis and biliary stasis differ in the degree of additional damage done to the liver tissue after its resection. On the basis of all the changes discovered, the use of the preparation Hemostopan must be regarded as the optimal method for achieving hemostasis and biliary stasis. Methods of omentoplasty of the wound surface of the liver, using either sutures or MK-8 glue, lead to more severe damage to the structures and disturbance of the functional state of the liver, increasing the risk of postoperative complications.

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