POSTRESECTION CHANGES IN LIVER MORPHOLOGY MONITORED BY THE SOVIET SURGICAL ULTRASONIC ASPIRATOR

T. V. Savvina, V. A. Vishnevskii, R. Z. Ikramov, and A. R. Abul'khanov

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The increasingly more extensive resections of the liver carried out in various pathological conditions demands the development of new types of apparatus which will allow such operations to be performed, and also will reduce the severity of complications during and soon after the operation. One new trend is the use of ultrasonic surgical aspirators for this purpose. Their clinical efficacy is reflected in previous publications [2, 3]. The character and intensity of the morphological chan along the edge of resection of the liver, revealed by different types of apparatus, vary. Little attempt has been made to study these changes [1].

The aim of the present investigation was to study morphological changes in the liver tissue at the edge of resection, during performance of operations with the Soviet ultrasonic URSK-7N-18 apparatus, which has been modified for use in resection of the liver and fitted with a special working part with vacuum pump and a liquid supply channel.

EXPERIMENTAL METHOD

Experiments were carried out on 13 mongrel dogs of both sexes, weighing 20-25 kg, under general anesthesia. The oscillation frequency of the working part of the apparatus was 26.5-39 kHz and its resonance frequency 26.5 kHz. The volume of parenchyma resected did not exceed 30-60%. The animals were killed immediately after ultrasonic treatment, and on the 7th and 15th days and after 1 and 3 months. After completion of the resection of the liver with the aid of the ultrasonic surgical aspirator, parenchymatous bleeding from the incision was slight, and not sufficient to require suture of the parenchyma to produce hemostasis. Large ducts were ligated. The material was fixed in 12% formalin and pieces of parenchyma, excised along the edge of the resection, were embedded in paraffin wax. Sections 7 μ thick were stained with hematoxylin and eosin, by Van Gieson's method, and by Perls' reaction, and were stained for fat with Scharlach red.

EXPERIMENTAL RESULTS

The edge of the liver resection immediately after ultrasonic destruction was rough, but there was no visible hemorrhagic seepage or necrosis. Vessels 1 mm or more in diameter on the border of the resection were ligated. Microscopic examination immediately after ultrasonic destruction revealed ligated vessels, which had undergone considerable changes in their shape, and also small deposits of necrotic tissue, with the appearance of homogeneous pinkish-gray masses on staining with hematoxylin and eosin, on the surface of the liver. Vessels, nerves, and ducts located actually in the zone of ultrasonic destruction, preserved their normal structure (Fig. 1a). The surface layers of the liver parenchyma on the boundary with the zone of necrosis formed a zone of destruction consisting of denuded stroma, saturated with blood, and no liver cells were present. The thickness of the zone of destruction did not exceed 0.5 mm. The staining properties of the hepatocyte cytoplasm were considerably altered both on the boundary with the zone of destruction and also, more especially, in the center of the lobules at depths down to 2-5-10 mm (the

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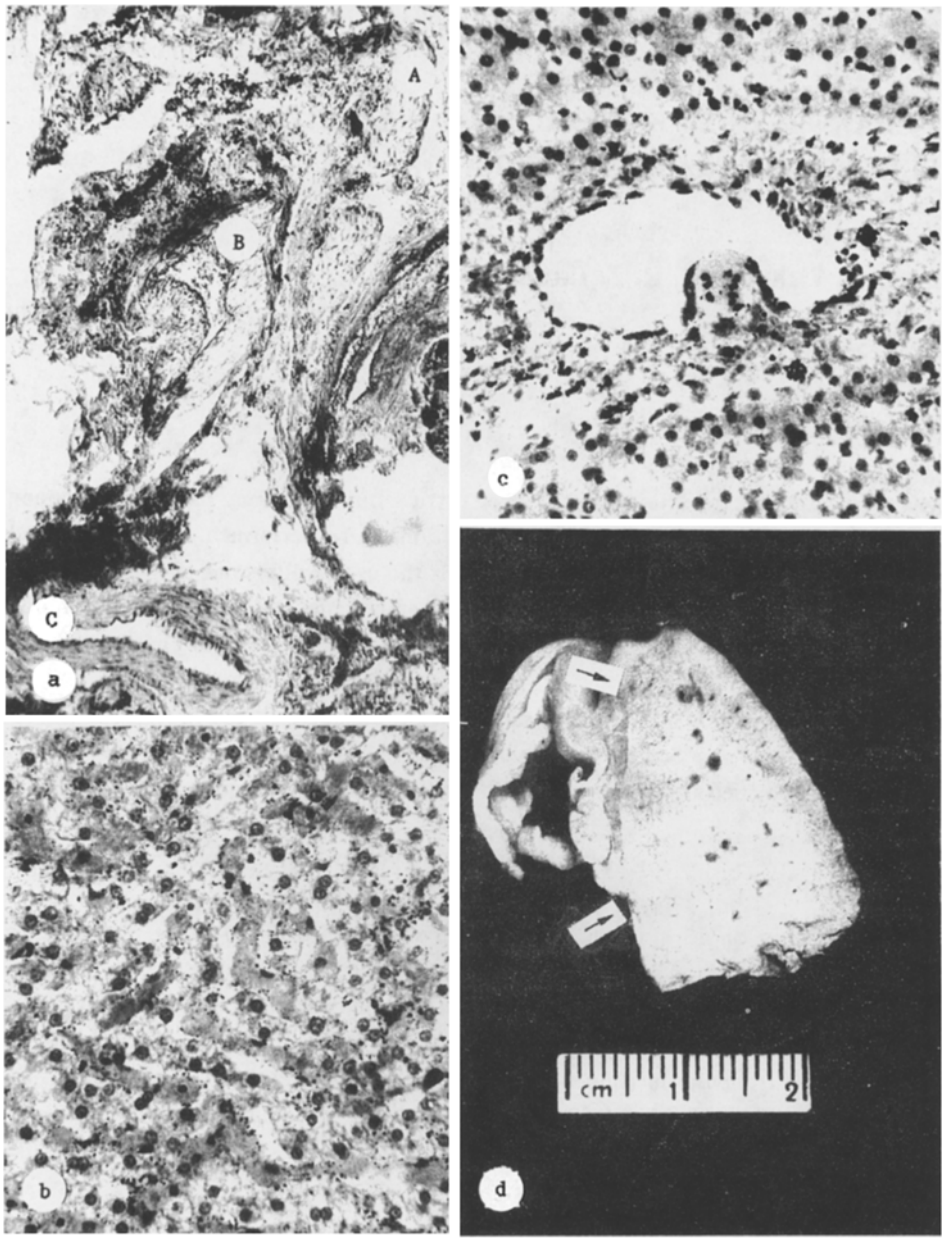

Fig. 1. Time course of morphological changes in liver after resection by the Soviet ultrasonic surgical aspirator: a) a neurovascular bundle, preserving its structure, lies among necrotic tissue: A) nerve trunk, B) vein, and C) artery immediately after. Ultrasonic treatment; hematoxylin-eosin, 90×; b) focal thrombosis of sinusoids immediately after ultrasonic treatment; hematoxylin-eosin, 160×; c) edema and hemorrhages around central vein. Focal subendothelial edema in wall of central vein with disturbance of integrity of endothelium and formation of cushionlike projections into lumen of vessel immediately after ultrasonic treatment; hematoxylin-eosin, 260×; d) specimen of liver 3 months after ultrasonic treatment. Line of resection is smooth, omentum adherent to it.

zone of action of the ultrasonic waves) Most of the cells had become palely stained, their cytoplasm was foamy, the nuclei palely stained, and the complex structure of the hepatocytes was disturbed; boundaries between the majority of cells were ill-defined. Focal disturbances of the circulation were distinctly visible in the form of congestion of the sinusoids, central veins, hepatic veins, and vessels of the portal tracts. Signs of stasis of blood in the above-mentioned vessels, and also signs of thrombosis, which predominated in the sinusoids (Fig. 1b) and were focal in character in the vessels of the portal tracts, were equally well marked. The development of edema with evidence of hemorrhagic seepage around the central veins was conspicuous (Fig. 1c). Immediately after ultrasonic treatment, changes were found in the walls of the central and hepatic veins of small caliber. Signs of focal

subendothelial edema with the formation of cushions, projecting into the lumen, and in some places with disturbance of the integrity of the endothelium (Fig. 1c), were found in the walls of the central veins. In the walls of the small hepatic veins, foci of basophilia and concentrations of smooth-muscle fibers were observed, with the formation of cushionlike swellings into the lumen of the vessels in these areas. The hepatic veins were surrounded by foci of perivascular hemorrhages, varying in severity.

Analysis of the results of the morphological study of the liver parenchyma along the edge of resection immediately after exposure to the ultrasonic aspirator shows spreading of the ultrasonic wave taking place along the course of the central and hepatic veins, thus explaining the damage to the hepatocytes and disturbances of the microcirculation of the liver at a depth of between 2 and 10 mm.

By the 7th day, circulatory disturbances in the form of congestion of the vessels and sinusoids, blood stasis, and signs of thrombosis were partially resolved. By this time focal degenerative and necrobiotic changes in the hepatocytes were more marked throughout the thickness of the zone subjected to the action of the ultrasonic wave. The hepatocytes differed in size and shape, the cell boundaries were distinct, the cytoplasm was strongly vacuolated, the nuclei sometimes palely stained, sometimes sharply reduced in size, compact, and hyperchromic, and the sinusoids were dilated. Hyperplasia of the stellate endotheliocytes was observed, and these cells were packed with hemosiderin granules. Solitary foci of necrosis were seen in the thickness of the lobules, infiltrated with macrophages, lymphocytes, and single polymorphonuclear leukocytes. In some places, in a zone of previous necrosis, the formation of a connective-tissue scar could be observed. Considering that the ultrasonic aspirstor removes all necrotic tissues from the site of its direct action, there was no leukocytic infiltration in the zone of destruction. Many macrophages loaded with hemosiderin and fat appeared in that zone, with marked proliferation of fibroblasts, oriented horizontally. Changes in the walls of the central and hepatic veins were still present.

By the 15th day of the experiment the histologic picture in the zone of exposure to ultrasonic waves corresponded basically to the picture on the 7th day, but the macrophagal reaction in the superficial layers of the liver was more intensive, and the connective-tissue covering the edges of resection was thicker. Its thickness increased to 1 mm, but its area was reduced by shrinkage. Sclerosis was observed around the central veins, in places where cushionlike projections appeared in the walls of the central and hepatic veins foci of sclerosis and hyalinosis could be identified.

One month after the experiment, the area of scar tissue covering the liver at the site of exposure to ultrasound could be seen macroscopically to have contracted by two-thirds compared with its initial size, and its thickness did not exceed 2.5 mm. Immured hyalin thrombi with a marked macrophagal reaction around them could be seen in the substance of the scar tissue, accompanied by giant cells and foreign bodies In some places encapsulated foci of necrotic tissue could be seen, homogeneous in appearance, and stained pinkish-gray with hematoxylin and eosin. Around the encapsulated foci of necrosis there were single small areas of round-cell infiltration. In the deep layers of the scar tissue on the boundary with the liver there was a thick layer of macrophages, loaded with hemosiderin and fat. In the superficial layers of the liver focal thrombosis of the sinusoids was still present In the deep parts of the zone of exposure to ultrasound sclerosis could be seen along the course of the portal tracts, around the central and hepatic veins, and single small fibrous scars were visible in the substance of the lobules. Structural changes were still present in the central and hepatic veins. It must be emphasized that all these changes were focal or mosaic in character.

When 3 months had elapsed after resection of the liver by means of the ultrasonic surgical aspirator, the resection line was smooth. Where the surface was covered with omentum, the latter was firmly adherent to the liver surface (Fig. 1d). Histologically, in the latter case a very thin layer of fibrous tissue with no signs of inflammation was present on the boundary between the liver parenchyma and the omentum, and no vessels or macrophagal reaction were present. The omentum was normal in structure. In the superficial layers of the liver adjacent to the zone of resection the portal tracts and central veins appeared close together because of shrinking and thinning of the scar tissue covering the liver surface. Small single scars also were present in the substance of the parenchyma. No circulatory disturbances were observed in the parenchyma. In some fields of vision in the zone of exposure to ultrasound waves areas of angiomitosis were observed to be appearing, mainly on account of preceding lysis of hepatocytes in the zones of thrombosis of the sinusoids. Cushionlike projections of the walls of the central and hepatic veins and into their lumen, consisting of hyalinized connective tissue and bundles of muscle fibers, were still present.

The results of the morphological investigation showed that the ultrasonic aspirator selectively destroys the parenchyma of the liver without damaging blood vessels, nerves, or ducts, and ensures hemostasis in the early stages because of changes in the microcirculatory bed, and it promotes rapid healing of the wound surface as a result of aspiration of necrotic tissues.

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MORPHOLOGICAL CHARACTERISTICS OF INTESTINAL ENDOCRINE CELLS IN MICE

T. G. Barkhina, Yu. G. Parkhomenko, I. M. Salakhov, and O. E. Bogatyreva

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Much evidence has now been obtained to show that endocrine cells are present in nonendocrine organs belonging to the extensive APUD-system, or in other words, in the composition of the diffuse endocrine system [2-6]. According to the modern classification the following types of these cells are distinguished: A, B, D, D_1, EC_1, EC_2, ECII, ECL, G, I, K, L, N, P, PP, S, X, IG, TG, MO, YY. Thanks to the widespread use of highly sensitive immunocytochemical methods combined with electron-microscopic analysis, more than 20 types of these cells, containing biogenic amines and peptides, and located in the mucous membrane of organs of the gastrointestinal tract, have been identified in different species of animals [3, 7]. However, there has been no attempt at morphological identification of these cells of the mouse intestine used in the different experiments, and that was accordingly the aim of the investigation described below.

EXPERIMENTAL METHOD

Pieces of small and large intestine from BALB/c mice were fixed in a 10% solution of neutral formalin and embedded in paraffin wax. Histologic sections 5-7 μ thick for quantitative analysis of endocrine cells were stained with silver nitrate by Grimelius' method and the cells in 1 mm^2 of intestinal section were counted. In this way it was possible to determine that the cells belonged to the APUD-system, although it was not possible to identify precisely their types. This defect was largely compensated by electron-microscopic analysis, which enables the types of endocrine cells to be determined not only by the shape and size of the granules, but also with respect to various other ultrastructural data. Student's test was used for statistical analysis. Material for ultrastructural study was fixed in a mixture of 1% glutaraldehyde and 4% formaldehyde in 0.05 M cacodylate buffer, and then in 1% osmium tetroxide solution, and then dehydrated and embedded in Vestopal. Ultrathin sections were stained with lead citrate and examined in the JEM-100C electron microscope.

EXPERIMENTAL RESULTS

Endocrine cells (EC) were found to be most numerous in the duodenal mucosa The number of EC in 1 mm^2 section of the small intestine was: in the duodenum 243 ± 15, jejunum 175 ± 16, ileum 87 ± 7; in the large intestine the distribution was: in the ascending colon 33 ± 4, transverse 39 ± 6, descending 24.4, and in the rectum 53 ± 1 in 1 mm^2. There was a tendency for the number of EC to diminish from the proximal to the distal parts of the intestine.

Laboratory of Infectious Pathology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 3, pp. 314-317, March, 1991. Original article submitted March 22, 1990.

