METHODS

A New Method for Inducing Echinococcosis in the Liver

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Creation of human disease models is important for studies of pathogenesis and morphogenesis, for elucidation of the mechanisms of drug action, and for developing surgical methods.

Our purpose was to develop a new method for inducing echinococcosis (EC) of the liver. Current methods of inducing EC [1,2] fail to reproduce the hydatid form. We propose a method for inducing EC in rabbit liver.

MATERIALS AND METHODS

In contrast to previous methods, before implantation, invasive EC liquid containing 100-200 live protoscolices and scolices was injected in liver parenchyma. Liver EC cannot be induced with less than 100 live scolices, while injection of more than 200 live scolices leads to morphostructural changes in liver parenchyma eventuating in its dystrophic changes.

Upper median laparotomy was carried out in rabbits weighing 2200-3100 g under calypsol narcosis (0.2 ml/100 g) after preliminary injection of penicillin in a dose of 5000 U in 0.5% Novocain into the site of incision. For inducing EC, 1-2 live mature daughter EC cysts 1-1.2 cm in diameter were used (operative material). Invasive EC fluid (1.5-2 ml) dissolved in normal saline so that the inoculate contained 100-200 live protoscolices and scolices was injected into the liver parenchyma between the right and left lobes (site of implantation of daughter EC cysts) with a blunt needle with inner diameter 0.5 mm. The site of liver puncture was covered with MK-2 or MK-6 medical glue or a hemostatic sponge. Then 1-2 live daughter EC cysts were implanted into this zone, after which provisional catgut sutures were made between the right and left lobes of the liver and the wound was sutured layer by layer.

The development of EC was verified and time course of structural changes in the liver studied by histological (Van-Gieson hematoxylin-eosin staining) and histochemical (Brachet and Schick tests) methods. EC cysts with fibrous capsule, liver tissue adjacent to fibrous capsule, and remote sites of liver parenchyma were examined.

On day 3 after induction microscopic examination showed the formation of a white thin capsule at the site of inoculation and implantation. It contained a chitin vesicle with transparent amber-colored liquid, easily detached from the capsule. The color and consistency of the liver did not change. Histological examination showed marked edema of the interstitial tissue in the site adjacent to the implant, dilatation of Disset's space, and vacuole dystrophy of hepatocytes with decreased glycogen content. Leukocytes and monocytes formed a demarcation bank immediately round EC.

On day 7 after inducing EC of the liver, autopsy showed that the liver increased in size and was dark-brown with smooth surface and numerous daughter parasitic cysts from 0.2×0.2 to 0.5×0.5 cm in size on the diaphragmatic surface (Fig. 1). Fibrous capsule

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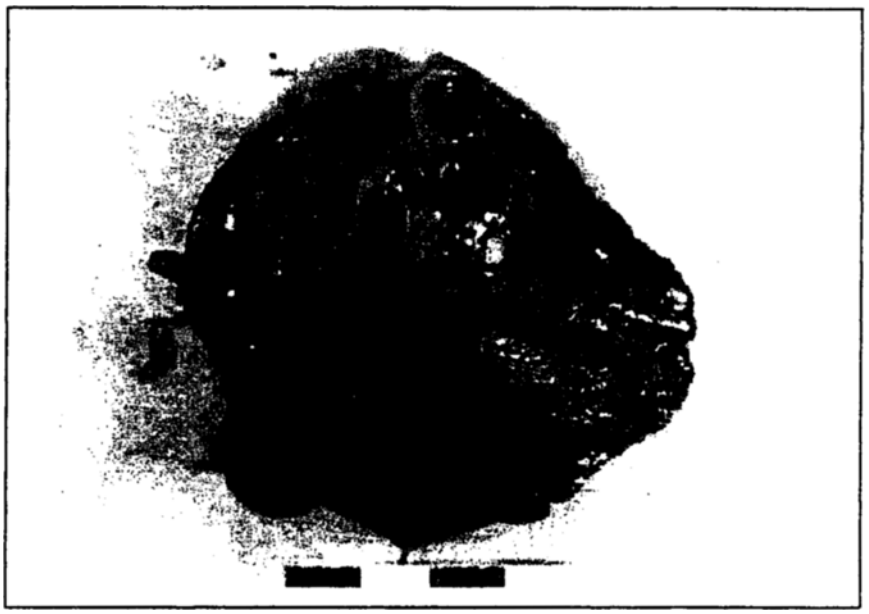

Fig. 1. Liver macropreparation on day 7 of experimental echinococcosis.

with two EC cysts containing transparent amber-colored fluid formed between the lobes at the site of implantation of live daughter cyst. Numerous EC cysts (inseminated) were found in the mesentery and on serous surface of large and small intestine and on the parietal leaf of the peritoneum. Histologic examination showed that the leukocytic bank was thickened, with necrotic mass inside and lymphohistiocytic infiltration on the surface (Fig. 2). Hepatocytes were in a state of deep vacuole and hyaline-droplet dystrophy with focal necrosis (Fig. 3). Lymphohistiocytic infiltration appeared round portal tract and, more so, round biliary tract.

On day 14 of experiment, a compact fibrous capsule was found at the site of cyst implantation, chitin coating of the cyst was strained, in some animals perforation of the cysts and contamination of the entire peritoneum were observed. Histological study of the liver and bile ducts near the focus of implantation showed oncospheres and small EC vesicles. Em-

Fig. 2. Microscopic changes in the liver on day 7 of experimental echinococcosis. Hematoxylin and eosin staining, ×140.

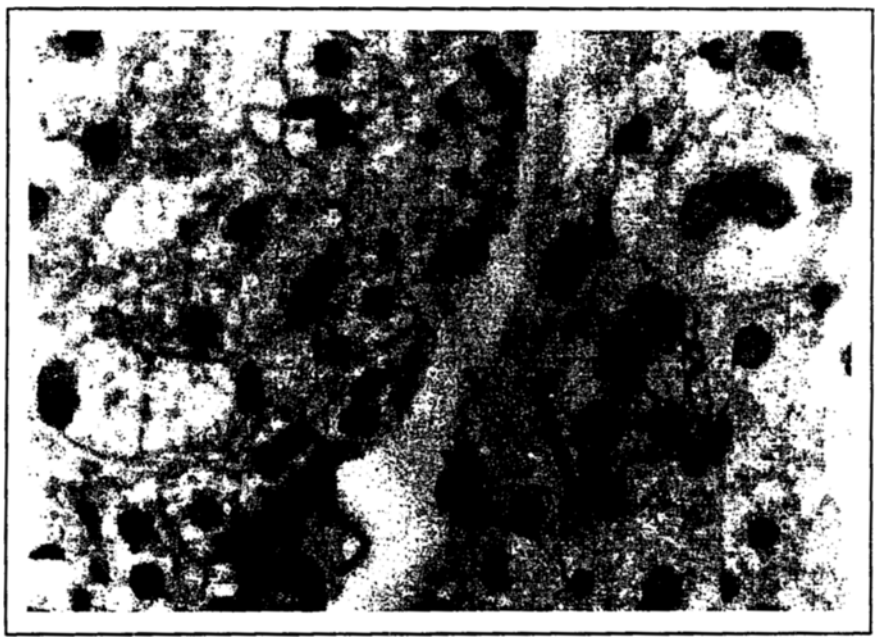

Fig. 3. Microscopic changes in hepatocytes on day 7 of experimental echinococcosis. Hematoxylin and eosin staining, ×280.

bolization of interlobular bile ducts with EC vesicles was seen (Fig. 4), with expressed lymphohistiocytic infiltration round these vesicles. Pyogenic layer of the capsule round implanted focus was clearly seen, with fibrinoid connective tissue necrosis.

On days 21-28 experimental animals lost weight, were adynamic; autopsy showed total involvement of the right and of two segments of the left lobe of the liver with EC cysts 1.5×1.5, 1×1, and 0.5×0.5 cm in size. The contents of fibrous capsule was caseous, the capsule was cut with a crunch, and live EC cysts were found at some places. The entire surface of the parietal leaf of the peritoneum was contaminated, as well as the surfaces of the large and small intestine in a "grape-like" pattern. Histologically, on day 21 of experimental EC, the primary focus of implantation was characterized by necrosis of EC vesicles and thickening of fibrous capsule, although the internal

Fig. 4. Microcyst invasion in small biliary ducts on day 14 of experiment. Hematoxylin and eosin staining, ×140.

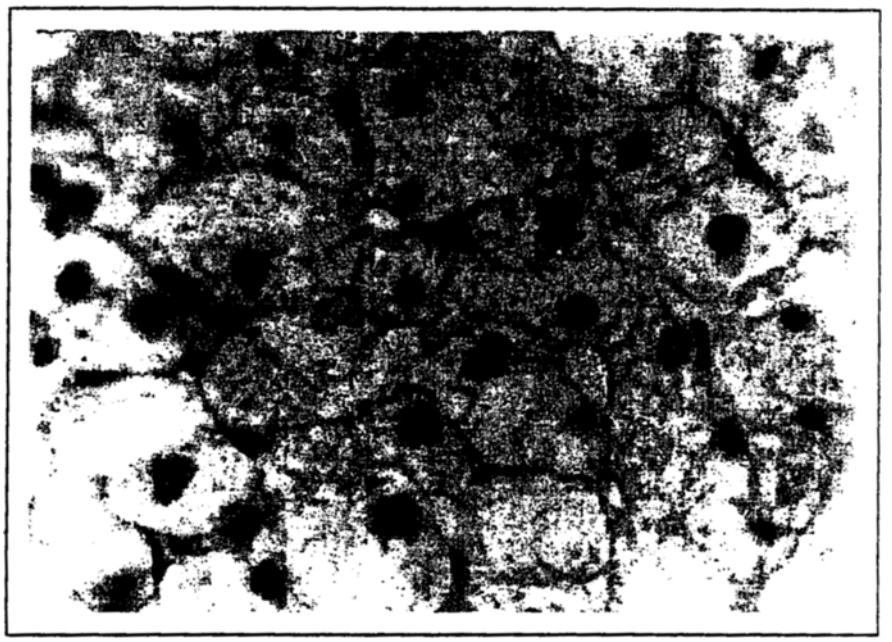

Fig. 5. Changes in hepatocytes on day 21 of experiment. Hematoxylin and eosin staining, ×280.

pyogenic layer was intact; there was perifocal inflammation manifesting itself as lymphohistiocytic infiltration and serous edema of the interstitium. EC eggs entered liver parenchyma through biliary capillaries, hepatocytes were in the state of vacuole dystrophy and necrosis (Fig. 5).

On day 28, invasion of EC eggs and vesicles was observed almost in the entire liver, which was confirmed by the presence of EC in many bile ducts and in the parenchyma with diffuse periportal and intrahepatic lymphohistiocytic infiltration. At this period numerous focal necroses of liver tissue were observed, the structure of the liver was impaired, and small false nodules appeared.

Thus, we propose a simple method for reproducing EC in the liver in experimental animals; the development of EC involves reactive and structural changes in liver parenchyma.

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