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Research Note

Murine Model for Hepatic Alveolar Hydatid Disease without Biohazard

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Many species of *Cricetidae* and several strains of mouse are susceptible to larval *Echinococcus multilocularis,* and used as the laboratory models for studies of alveolar hydatid disease (Ohbayashi *et al.*, 1971; Kamiya, 1973; WHO, 1984). These animals are infected experimentally by oral administration of eggs or intraperitoneal injection of hydatid homogenate. For the substitutional model for human disease, it is desirable that the hydatid cysts develop in the liver. By using eggs, the animals harboring hepatic hydatid cysts are easily prepared, however, the biohazard control is very difficult in ordinary laboratories. For making the liver infection without using eggs, the intrahepatic injection of hydatid homogenate has been carried out (Yamashita *et al.*, 1963; Liance *et al.*, 1984), however, the accidental metastases frequently occurred in this technique. In order to overcome this defect, the method of trans portal injection was devised to make the liver infection of rats in our laboratory (Ohnishi, 1984). Furthermore, we improved this surgical technique to apply for the liver infection of mice.

More than 10 mice of each inbred strain, DBA/2, BALB/c and C57BL/6 (Clea, Japan) were used for experiments. An isolate of larval *E. multilocularis,* obtained from naturally infected *Clethrionomys rufocanus bedfordiae* captured in Akkeshi town, Hokkaido has been maintained by intraperitoneal transfer using Chinese hamsters (*Cricetulus griseus,* CHA colony). The hydatid mass obtained from Chinese hamsters was minced in sterile phosphate-buffered saline (PBS, Nissui, Japan) containing kanamycin sulfate at 60 μg/ml. Minced pieces were well mixed by repeated pipetting, and passed through 210 μm mesh. The sediment containing protoscolices, minute vesicles and calcareous corpsucles was washed 3 times with PBS, and 5–10% suspension (volume/volume) was made. Mice were anesthetized by intraperitoneal administration of sodium pentobarbital (Nembutal, Abbott, USA). Following ventral celiotomy, 0.1ml of the sediment suspension was injected with a 27 gauged insulin syringe (Terumo, Japan) into the mesenteric vein (Fig. 1). After injection, bleeding was protected by covering the vein with a sterile gelatin sponge (Gelfoam, Japan-Upjohn). The bleeding stopped after pressing the sponge (Fig. 2), and the abdomen closed with silk sutures.

One to 5 months later, the hydatid cysts developed in the liver of all observed mice, and the metastasis did not occur. Macroscopic and histological features in 3 inbred strains were given in Figs. 3–8. DBA/2 showed the rapid proliferation of vesicles, and protoscolex formation was observed 2 months later. In BALB/c, most vesicles were sterile, and few were fertile. C57BL/6 harbored the sterile hydatids even 5 months later. The developments of sterile hydatids in BALB/c and C57BL/6 were morphologically similar to human lesions. Our technique without biohazard will be available for biological,

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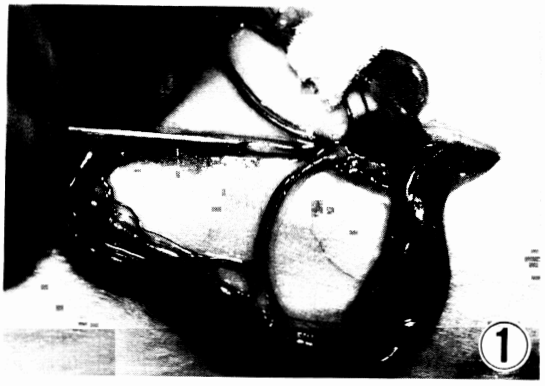
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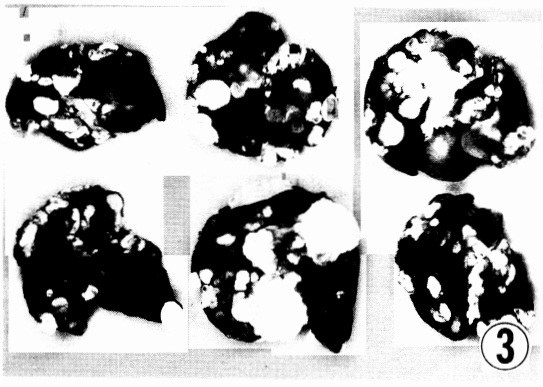
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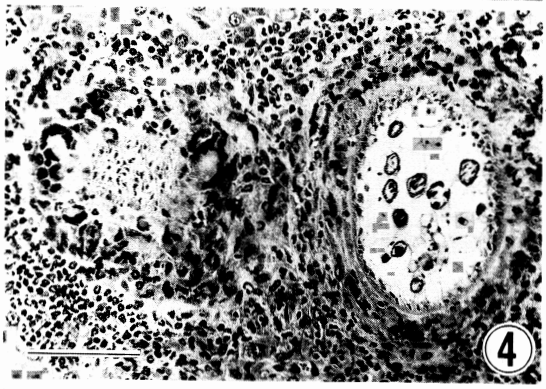
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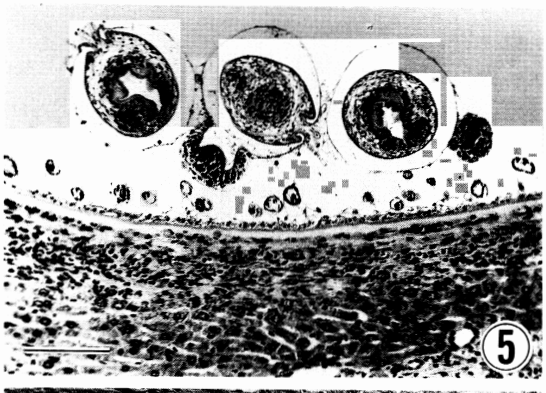
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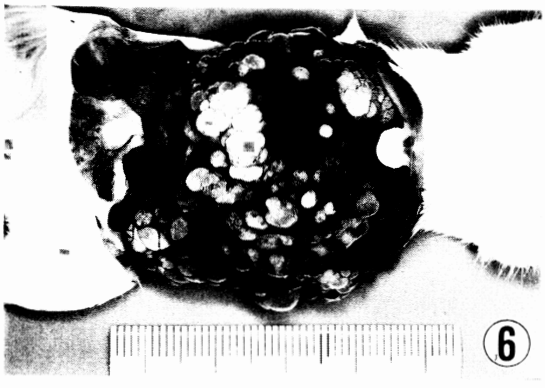
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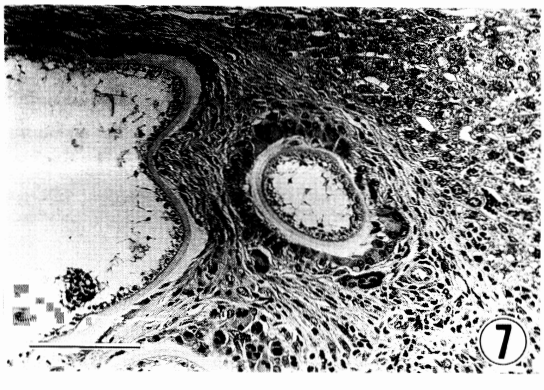
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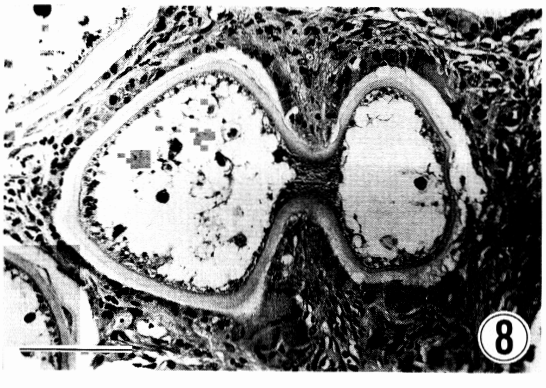
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immunological and chemotherapeutical studies of hepatic alveolar hydatid disease.

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Explanation of Figures (Histological sections were stained by Hematoxylin and eosin. Scale bars represent 100 μm.)

Fig. 1 Injection into mesenteric vein of mice.
Fig. 2 Hemostasis by using a gelatin sponge.
Fig. 3 Liver of female DBA/2 4 months after infection.
Fig. 4 Hepatic lesion of female DBA/2 2 months after infection. Giant cells surrounded the protrusion of germinal cells (left). Calcareous corpsucles existed in the cavity (right).
Fig. 5 Protoscolex formation occurred in female DBA/2 2 months after infection.
Fig. 6 Enlarged liver of male BALB/c 5 months after infection.
Fig. 7 Hepatic lesion of male BALB/c 4 months after infection. Epitheloid cells surrounded the vesicles.
Fig. 8 Hepatic lesion of female C57BL/6 3 months after infection. The vesicle seemed to be dividing.