STUDIES ON ECHINOCOCCOSIS IV. EXPERIMENTAL INFECTION OF THE WHITE MOUSE

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The authors, in the preceding papers,[6] reported experimental results that dogs were infected with the hydatid sands obtained from a hydatid cyst in the liver of a sheep imported from Australia and that the adult tapeworms of *Echinococcus granulosus* were confirmed. As the authors could obtain numerous eggs and tapeworms, they have continually carried out experiments to induce the hydatid infection in other animal species, the intermediate hosts. The experiments were undertaken using sheep, white mouse, squirrel (Asiatic chipmunk; *Eutamias asiaticus lineatus* TEMMINCK), white rat and voles (*Clethrionomys rufocanus bedfordiae* THOMAS and *Apodemus speciosus ainu* (THOMAS)). Satisfactory results were obtained from the experiments made on the sheep and mouse.

There were two objects in carrying out the experiments. The first was to preserve the *Echinococcus* strain for detailed experiments in the future; for this object, sheep were used. This was done because the sheep is an ideal intermediate host for *Echinococcus*, in particular, the sheep is susceptible to *Echinococcus* without exception and the scolices in the hydatid cyst develop in high ratio. The other object was to obtain a small animal suitable for the experiment of *Echinococcus*. Among various animals, the mouse was the only one which presented a positive result in low infective ratio. Very interesting findings were obtained in connection with the experiment on mice.

The authors wish to describe the results obtained from the experiments made on mice.

MATERIALS AND METHODS

Twenty mice of *dd*-strain were used in 2 groups of 10 mice respectively.

As for the inocula, the eggs of *Echinococcus granulosus* obtained from the feces of experimental dogs and adult tapeworms collected from the intestines at the autopsy of experimental dog were selected. The gravid proglottids were divided into fine pieces by needles in the physiological saline solution. About ten eggs were swallowed by each mouse.

The mice were dissected after a certain period, the hydatid cysts were searched for

minutely and histological investigations were made.

RESULTS

Group I. The eggs collected from the feces were administered to 10 mice orally with the saline solution by the pipette on January 26, 1955.

Two mice were dissected on March 11 and 22 respectively and the remaining 6 mice were investigated on June 27. None of these cases showed the hydatid cyst formation.

Group II. The materials taken from the proglottids obtained from experimental dog No. 4 were swallowed by 10 mice on March 11, 1955.

All were killed and dissected on June 30, the 111th day from the infection. Of the 10, two mice gave a positive result for the hydatid cyst.

Case No. 1. Three hydatid cysts were confirmed. A spherical unilocular cyst was localized in the right hepatic lobe facing the diaphragm right underneath the hepatic capsule. The cystic focus was convex on the hepatic surface with 10 mm diameter and thin tense wall containing transparent liquid. A similar unilocular cyst with 10 mm diameter was found superficially in the portal area of liver; it was protruded on the surface and its half part was buried in the hepatic parenchyma.

Another unilocular hydatid cyst was found in the peritoneal cavity, free from the viscera; its diameter was 10 mm. No abnormality existed on the peritoneal fluid.

Histologically the hepatic hydatid cyst was situated under the hepatic capsule, the hepatic parenchyma was mechanically pressed by the cyst and thin subcapsular parenchyma was atrophic. The wall of the hydatid cyst showed a typical structure; the cuticular membrane had a thickness of 20~30μ, it was laminated and enucleate, and the innermost germinal membrane was very thin with nuclei scattered in one line, but no scolices were found. No histological reactions such as proliferation of connective tissue or cell infiltration were observable.

The histological structure of the free hydatid cyst in the peritoneal cavity was identical to that of the hepatic one, the cuticular membrane was 50~60 μ in thickness, surrounded by a thin layer of lymphocytes and histiocytes, and the outermost layer was identified as the serous membrane.

Case No. 2. A spherical unilocular hydatid cyst with a diameter of 10 mm was found in the right pleural cavity. The cavity contained some bloody substance and the right lung and the diaphragm were pressed.

The histological findings were similar to those in the free cyst of case No. 1, the cuticular membrane had a thickness of about 40μ and the cyst was covered with serous membrane in company with some cellular elements. Atelectasis and cell accumulations were sporadically demonstrated in the right lung; the focus of fibrous pleural thickening with cell infiltration was found in the posterior lobe.

DISCUSSION

It can be said with certainty that the above described cystic structures are hydatid cysts, because the laminated and enucleate cuticular membrane and the germinal membrane are completely characteristic. It is because of the absence

of any scolices that the authors, in order to make sure, compared the above cysts to the cysticerci of other cestode species. Comparative investigations were made between the structure of cyst wall of hydatid cyst and that of other types of cysts using section preparations stained by hematoxylin-eosin.

TABLE 1. *Cysticerci Investigated*

PARASITE (ADULT : CYSTICERCUS)	INTERMEDIATE HOST
Multiceps serialis (GERVAIS) : *Coenurus serialis* GERVAIS	*Lepus timidus ainu* BARRETT-HAMILTON
Taenia solium LINNAEUS : *Cysticercus cellulosae* (GMELIN)	*Sus scrofa domesticus* BRISSON
Taenia hydatigena PALLAS : *Cysticercus tenuicollis* RUDOLPHI	*Sus scrofa domesticus* BRISSON
Taenia taeniaeformis (BATSCH) : *Cysticercus fasciolaris* RUDOLPHI	*Rattus norvegicus* (LINNAEUS)
Taenia pisiformis (BLOCH) : *Cysticercus pisiformis* BLOCH	*Oryctolagus cuniculus* (LINNAEUS)
Taeniarhynchus saginatus (GOETZE) : *Cysticercus bovis* COBBOLD	*Bos taurus* LINNAEUS

The histological structure of the cyst wall of these cysticerci is quite different from that of the hydatid cyst. The wall of cysticerci is composed of the homogeneous thin cuticular layer and thick parenchymatous tissue. The latter is no more than the elongation of the parenchyma of immature proglottid, that is to say, the canalicular system (osmoregulatory system), calcareous granules and even the musculature are distributed in the loose reticular parenchymatous tissue. Among these cysticerci, *Cysticercus tenuicollis* is apt to be confused macroscopically with a large unilocular hydatid cyst, but differs in the histological structure; the cysticercus cyst shows even expansive and contractive movement in fresh live state. *Coenurus serialis* sometimes forms the endogenous daughter cysts, but their structures differ from that of hydatid cyst. In conclusion, it can be said that the structure of the wall of hydatid cyst is specific.

Echinococcus granulosus is parasitic on many mammalian species. According to the investigation made by YAMASHITA, one of the present authors, the terminal hosts are 11 species of carnivorous mammals and there are 46 species of mammals belonging to 6 orders as intermediate host. The mouse, *Mus musculus* LINNAEUS, is also an intermediate host. With regard to the literature so far as the authors know up to date, it can be said, however, that there are no experimental cases to which the *Echinococcus* eggs have been administered orally and only a few reports can be found[1,2,5] on the intraperitoneal inoculation test with the scolices.

Moreover, it is a well-known fact that some differences are noticeable about the internal distribution of hydatid cyst according to the specific difference. As for the mouse, DÉVÉ states that the hydatid cyst develops only in the lung or pleura in this animal. At any rate, the mouse is said to be affected with hydatid cyst very rarely.

The authors' experiments also resulted in finding a low infective ratio as 10%. They cannot make a definite assertion by reason of the scantiness of swallowed eggs but it can be said without doubt that the mouse is an unsuitable intermediate host. However, it is a very interesting fact that the hydatid cysts were found in the present study in the liver and independently in the peritoneal cavity.

In the past reports, whether of human or animal cases, the peritoneal hydatid cyst is said to be of secondary origin, that is, when the scolex or brood capsule in the primary cyst got out into the peritoneal cavity for some cause, the secondary hydatid cyst developed. Concluding from the fact that the cyst is covered by cellular layer with the serous membrane, the authors consider that the hydatid cysts found in the peritoneal and pleural cavities of the mice as described in the present paper had originally developed in the subserosa of some viscera and that the cyst, which resulted from their rapid growth, dropped into the body cavities. As to case No. 2 above, the authors cannot assert that the focal lesions such as pleuritis and lesions in the right posterior lobe of the lung or the bloody contents in the right pleural cavity have no relation with the genesis of free cyst.

One of the peculiar findings observed on the mice is the histological reaction of the host against the hydatid cyst. That no histological reaction is demonstrable in the liver would be considered to be unaccountable comparing to that which has been described regarding echinococcosis of other animal species. It is supposed that the hydatid cyst developed smoothly and rapidly without any resistance of the host tissue.

CONCLUSION

The authors investigated the experimental hydatid cyst infection of the mouse using the Australian strain of *Echinococcus granulosus*. Unilocular cysts were confirmed in the liver, peritoneal and pleural cavities of the mice in the ratio of 10 %.

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EXPLANATION OF PLATE

Figs. 1~3. Mouse No. 1.

Fig. 1. Macroscopical view of the hepatic and free hydatid cysts.

Fig. 2. The hepatic cyst showing the typical structure of the wall and lacking in reaction. Hematoxylin-eosin.

Fig. 3. The free cyst in the peritoneal cavity showing the wall structure. H.-E.

Fig. 4. Mouse No. 2.

Focal pleuritis in the right posterior lobe of the lung. H.-E.

Figs. 5~9. The histological structure of the wall of the cysticerci other than hydatid cyst. H.-E.

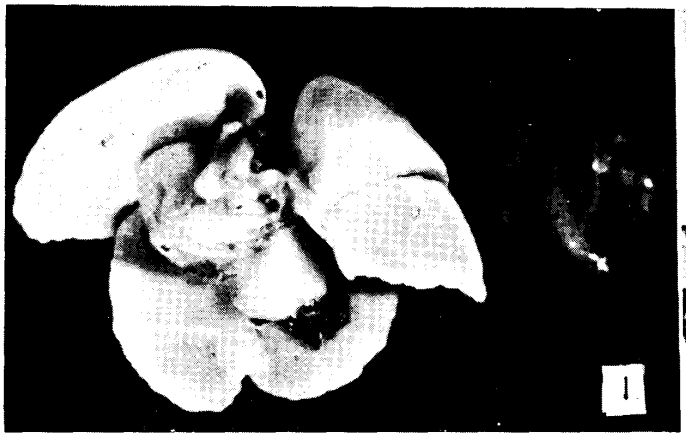
Fig. 5. *Cysticercus cellulosae.*

Fig. 6. *Cysticercus fasciolaris.*

Fig. 7. *Cysticercus tenuicollis.*

Fig. 8. Mother cyst of *Coenurus serialis.*

Fig. 9. Daughter cyst of *Coenurus serialis.*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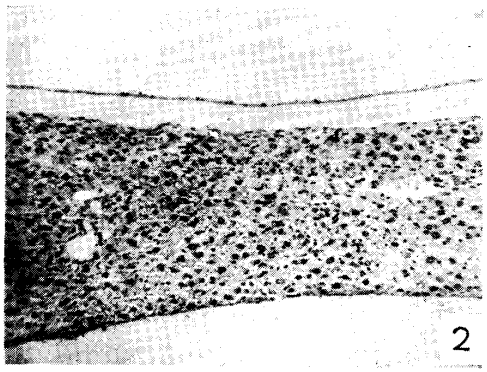
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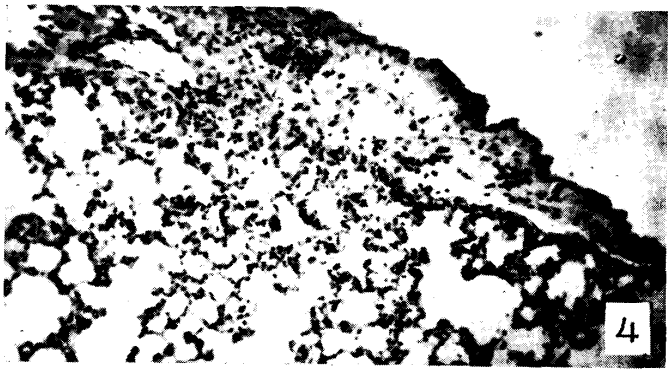
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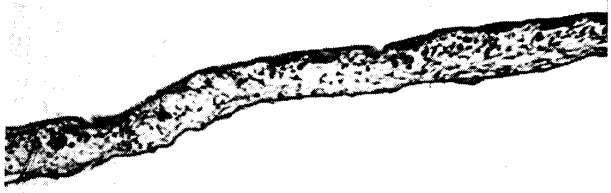


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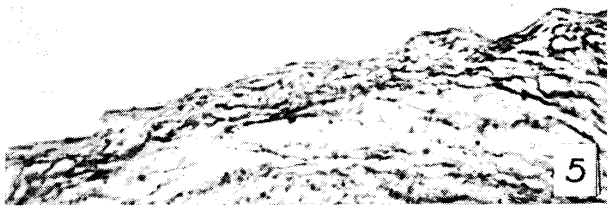
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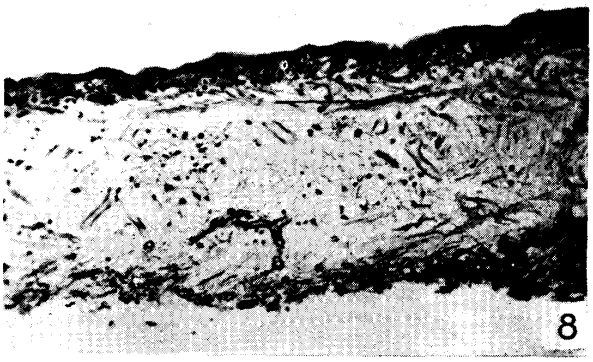
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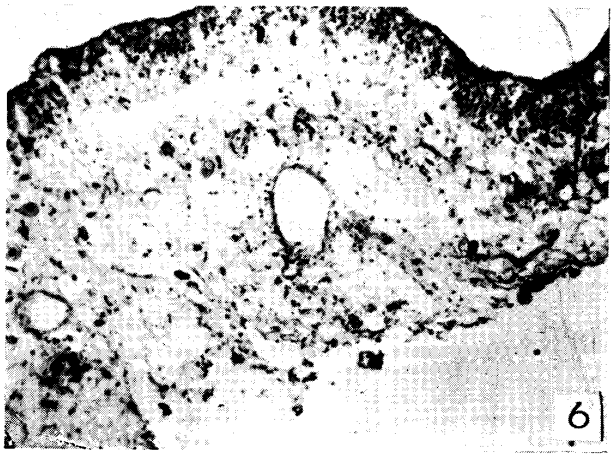
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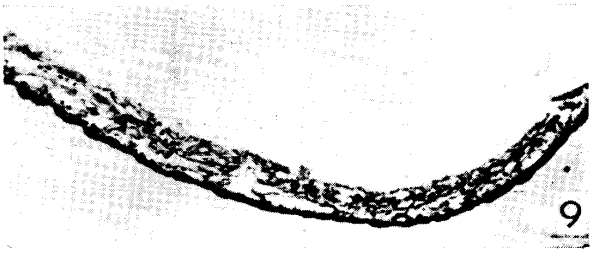
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