



Title	STUDIES ON ECHINOCOCCOSIS VIII : EXPERIMENTAL ECHINOCOCCOSIS MULTILOCULARIS IN VARIOUS RODENTS ; ESPECIALLY ON THE DIFFERENCE OF SUSCEPTIBILITY AMONG UNIFORM STRAINS OF THE MOUSE
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STUDIES ON ECHINOCOCCOSIS VIII.
EXPERIMENTAL *ECHINOCOCCOSIS MULTILOCULARIS*
IN VARIOUS RODENTS;
ESPECIALLY ON THE DIFFERENCE OF SUSCEPTIBILITY
AMONG UNIFORM STRAINS OF THE MOUSE

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The susceptibility of various animal species to *Echinococcus multilocularis* LEUCKART, 1863 has recently been investigated by RAUSCH and SCHILLER, VOGEL and other students. In those investigations, it was clarified that rodent animals are suitable intermediate hosts to *Echinococcus multilocularis*. Especially the voles belonging to genera *Microtus* and *Clethrionomys* show severe infection of the worm without exception and multilocular hydatid lesion can be found accompanied by numerous scolices.

In Japan, human multilocular echinococcosis can be found endemically on the island of Rebun, Hokkaido. It was made clear that this endemic disease, however, has been introduced to the island from the Commander Islands by way of the Middle Kuriles; a case of naturally infected multilocular echinococcosis in *Microtus oeconomus* PALLAS was also obtained from an island of the Middle Kuriles^{5,9)}. Although the adult tapeworms were confirmed in dogs in Rebun Island, the intermediate hosts other than human beings have not yet been discovered; the present authors suspect *Clethrionomys rufocanus bedfordiae* and *Eutamias asiaticus lineatus* which inhabit the island as possible intermediate hosts. In this regard, it is of importance that the susceptibility of various species of wild rodent animals in Japan be investigated experimentally and that species which can play the role of intermediate host to *Echinococcus multilocularis* be identified. One of the objects of the present investigation is the carrying out of such experiments.

The authors of the present paper make a comparison of the susceptibilities of various uniform strains of mice. In the literature up to this time, there are

to be found both kinds of reports in which positive results (VOGEL, MANKAU) and negative results (RAUSCH and SCHILLER, SADUN et al.) are respectively described pertaining to the experiments with mice. The present authors took interest in such inconsistency of experimental results in mice and suspected the difference of strains of mice as a reason for the inconsistency. It is emphasized, in the field of bacteriology and virology, that the difference in strains of experimental animals must be taken into consideration at the time of experimental study. Indeed, there can be found many examples where remarkably different results eventuated according to the strains. In the field of helminthology, on the contrary, no consideration of strains of experimental animals is taken into account and investigators, in general, do not suppose any different results among the strains. The present authors obtained much interesting experimental data concerned with comparison of various strains of mice. They, therefore, realized the fact that the strains of experimental animals must not be disregarded even in the field of helminthological study.

MATERIALS AND METHODS

The species of animals used in the experimental infection are as follows:

Clethrionomys rufocanus bedfordiae (THOMAS); *Clethrionomys rutilus mikado* (THOMAS); *Apodemus speciosus ainu* (THOMAS); *Apodemus geisha* (THOMAS); *Eutamias asiaticus lineatus* TEMMINCK; *Microtus montebelli montebelli* (MILNE-EDWARDS); *Meriones unguiculatus* (MILNE-EDWARDS); albino hamster, *Mesocricetus auratus* (WATERHOUSE); laboratory mouse, *Mus musculus* LINNAEUS. As for mice, use was made of 10 strains: AKR, dba, dd, C57BL/6, CF#1, b, CFW, A, BALB/C and C3H/He. *Meriones unguiculatus* and *Microtus montebelli montebelli* used in this experiment were obtained from the breeding stocks* of the Institute for Infectious Diseases, University of Tokyo.

These various animals were given oral inoculation of *Echinococcus multilocularis* eggs. The eggs were obtained from the feces of an experimentally infected dog by the centrifuge using physiological saline solution. The *Echinococcus multilocularis* in the present paper is that of Alaskan strain sent by Dr. RAUSCH as already described in the preceding papers. The eggs were introduced directly by a syringe with cannula and the number of eggs inoculated ranged from 15 to 750 per animal.

After the inoculation the animals were killed at regular intervals and were investigated closely with naked eyes or a magnifier, however, some animals were also dissected after death. Tissue materials were fixed with 10% formalin solution, paraffin sections were stained with hematoxylin-eosin for microscopy.

RESULTS

1. *Clethrionomys rufocanus bedfordiae*

* The stocks had been developed from the animals domesticated by Mrs. Masuko NOMURA and Miss Michi NOMURA, Central Laboratories for Experimental Animals.

The animals had been captured in the field. Eleven voles were orally inoculated with 15~750 eggs; all the animals revealed the development of typical lesions. The case in the earliest stage was one in which 44 days had elapsed after the inoculation.

Macroscopicals: In the 44-day case, the liver was greatly swollen by remarkable development of hydatid tissue; liver parenchyma remained sporadically. The hydatid tissue was composed of an aggregation of numerous cysts. The size of cysts was not more than 2 mm in diameter and the majority of them were so minute that one could barely distinguish the cystic structure. Each cyst contained transparent fluid and its wall was very thin. Remarkable metastatic development of hydatid tissue was observed in the mesenterium, dorsal portion of the abdominal cavity, gastro-lienal ligament, etc. Another 10 cases in which 58~293 days had elapsed after the inoculation showed strong tendency to manifest metastatic development of hydatid tissue with a few exceptional cases. In the liver of cases inoculated with a small number of eggs, there remained considerably much liver parenchyma, but the characteristics of hydatid tissue were the same as those of cases inoculated with a large number of eggs. In these cases, the cystic structure was already barely distinguishable by naked-eye observations, the hydatid tissue was composed of soft grayish-white medullary tissue which protruded on the surface of liver with coarse granular appearance and the subcapsular blood vessels were congested showing ramiform figure. On close observation, the hydatid tissue was seen to be in reality an aggregation of tissue masses of various sizes. Such tissue mass was about 2~3 mm in diameter, sometimes 5 mm; a large one often possessed a narrow lumen in the center. The masses were separated by thin partitions. Swelling of the liver was so severe that it was recognizable while the animals were alive. In two cases, metastatic development of hydatid tissue in the lung was found.

Microscopicals: In the case of 44-day development, an early stage of brood capsule and scolex formation was observed. The hydatid tissue showed spongy multilocular structure and was composed of an aggregation of numerous cysts of various sizes ranging from 500 μ to 2 mm; minute cysts showed a marked tendency to aggregate (daughter cyst formation). The cuticular layer was thin, 5~20 μ . The germinal layer was varied in development by cyst, filled the lumen in a minute cyst and had large lumen in a larger cyst. In the germinal layer, there existed aggregations of nuclei, lumen formation of the aggregation (early stage of brood capsule), protrusion formation in the lumen (scolex in early stage), etc. Scolex was immature. As for the host tissue reaction against the hydatid tissue, there was histiocytic reaction accompanied by formation of giant cells. There was found a wide-expanded focal necrosis which contained both liver and hydatid tissue. In a case in which 71 days had elapsed from the inoculation, the development of hydatid tissue was advanced, many scolices were mature; however on the other hand, active new formation of scolices was found. The thickness of the cuticular layer was the same as before and the germinal layer was thickened with fine reticular tissue including many calcareous corpuscles. The brood capsule was 200~300 μ in diameter and was buried in the germinal layer. In cysts of about 1.5 mm, the thickness of germinal layer reached even 500 μ , brood capsules were numerous and cystic lumina were very narrow. Minute cysts produced by active exogenous daughter cyst formation were found, but an endogenous

one was very rare. The tissue reaction on host side against the hydatid was revealed at loci of new cyst formation and the majority of large cysts were surrounded only by thin connective tissue layer. In cases where more than 88 days had elapsed, the hydatid cyst possessed numerous scolices which were matured and contained within brood capsules. The size of a brood capsule was the same as in the preceding stage. The size of cyst became considerably uniform; the majority of the cysts were 2~3 mm in diameter and sometimes reached 5 mm. In intercystic portions, there was found little host tissue or only a small amount of connective tissue. The majority of cysts were filled with numerous brood capsules and germinal tissue; the latter occupied spaces among brood capsules. The brood capsules contained scolices. The germinal tissue decreased in quantity according to increase of number of brood capsules. Some large cysts still had a lumen in the center and the germinal layer was even more than 1 mm in thickness. On the one hand regressive changes were found, on the other, daughter cyst formation could be observed. There was no evidence of remarkable host tissue reaction.

2. *Clethrionomys rutilus mikado*

A vole which had been captured in the field was inoculated with 750 eggs; it died 23 days after the inoculation.

Macroscopicals: The liver was considerably swollen, hydatid foci densely distributed in all lobi, the liver parenchyma could be seen partially and many hemorrhages were scattered. The liver showed a spongy appearance resultant from dense aggregation of numerous small cysts of less than 0.5 mm diameter.

Microscopicals: The liver tissue revealed severe congestive edema and hemorrhages, sinusoids dilated and atrophic liver cell cords showed dissociation. The hydatid tissue was spongy and was composed of various-sized cysts, active formation of exogenous daughter cysts was found in every portion and some portions were made of an aggregation of minute cysts of about 200 μ in diameter. The wall of a cyst was extremely thin, the germinal layer was mono-layered. The thickness of cuticular layer was only 1~2 μ . The tissue reaction against the hydatid tissue was poor; lymphocytic, histiocytic and eosinophile cell accumulation was observed in some places and accumulated cells often showed regressive changes.

3. *Apodemus speciosus ainu*

A vole captured in the field was given oral administration of about 20 eggs. The animal was killed 76 days after the inoculation and close investigation was carried out. No hydatid focus, however, could be discovered either macro- or microscopically.

4. *Apodemus geisha*

Seven voles captured in the field were used. Each animal was orally inoculated with about 20 eggs. Of these, 3 cases revealed hydatid foci in the livers.

Macroscopicals: One out of 3 cases in which 76 days had elapsed after the inoculation and 2 out of 4 of 100 days manifested positive results. Each of positive two cases showed an aggregation of large and small cysts in the left lateral lobus, one large cyst attained

about 5 mm in diameter and communication with each other among lumina of some cysts could be seen even macroscopically. The remaining one case revealed large cyst multilocular hydatid foci in the left lateral and right medial lobi. Each cyst was large-sized in this case, and contained transparent fluid in its lumen; the largest cyst was nearly 10 mm in diameter. These cysts had thin walls which presented semitransparent appearance and on the inner surface of which, in 100-day cases, scattered very minute white spots showed.

Microscopicals: The wall of cyst in the case of 76 days was thin, the cuticular layer was about 5μ in thickness and the germinal layer was comparatively well-developed showing a thickness of about 10μ . As for the matter of early stage of scolex formation, the germinal layer showed aggregations of nuclei and hill-like thickenings. Surrounding tissue was a thin-layered lympho- and histiocytic cell accumulation of about 50μ thickness and was accompanied by some eosinophiles. The liver parenchyma manifested lipoidosis. In cases of elapsed 100 days, the thickness of cuticular layer was the same as in the previous stage. The germinal layer was about $300\sim 500\mu$ in thickness, fine reticular and accompanied by numerous calcareous corpuscles. Numerous brood capsules with scolices were embedded in the germinal layer. A brood capsule was $300\sim 500\mu$ in diameter. The scolex of invaginated type was about $100\times 150\mu$ in size, therefore, each brood capsule did not contain very many scolices. Scolex was completely matured. In tissues surrounding the hydatid tissue, there was very slight host tissue reaction or almost insignificant.

5. *Eutamias asiaticus lineatus*

Two Asiatic chipmunks which were captured in the field were examined. One case was orally inoculated with about 80 eggs and the other case with 100 eggs. The former was killed 76 days after the inoculation and showed positive result. The latter, however, died 7 months after the inoculation and revealed no lesion at autopsy.

Macroscopicals: The hydatid foci were limited in the liver. The foci were distributed in all lobi and showed cystic structure which slightly protruded on the liver surface. A focus was composed of cysts and sometimes presented an appearance of complicated spongy structure which was divided by septa into various-sized lumina. A cyst contained transparent fluid and the inner surface of a cyst manifested fine granular appearance of white color. A large cyst reached as large a diameter as several millimeters.

Microscopicals: The hydatid tissue was composed of an aggregation of various-sized cysts and showed spongy appearance. A newly-formed minute cyst was only about 50μ in size. Adventitial tissue reaction was generally slight, but in certain portions, there existed foreign body giant cells in a line surrounding a comparatively minute cyst. The cuticular layer was $5\sim 10\mu$ in thickness and the germinal layer was rich in calcareous corpuscles. The early stage of brood capsule and scolex formation could be observed in every palce of the germinal layer. In section preparation, an aggregation of nuclei was seen to occur in the germinal layer and it became a ring which was an early stage of brood capsule with thick wall. Protrusions with abundant nuclei, immature scolices, were formed one by one at the wall of brood capsule toward its lumen, the brood capsule wall thinned and the lumen expanded.

6. *Microtus montebelli montebelli*

Fifteen voles, 2 months old, inbred in the Institute for Infectious Diseases, University of Tokyo were used. Each animal was given oral administration of about 120 eggs. All the animals presented typical hydatid infection; they were investigated 20~202 days after the inoculation.

Macroscopicals: In one 20-day case, the liver showed scattered grayish-white minute foci. Twenty-six-day cases presented considerably enlarged liver of which parenchyma was congested. Cyst with very thin wall of which size was about 1 mm and grayish-white foci of same size were found. These lesions were intermixed and, by close examination, it was clarified that the grayish-white focus was indeed no more than an aggregation of extremely minute cysts. The liver of a 53-day case was remarkably swollen and almost all the tissue of the liver was replaced by hydatid tissue except such portions as small portal area, a part of the right lateral lobe, etc. The liver had uneven surface, ramiform blood vessel dilatation was remarkable and the hydatid tissue showed a typical multilocular structure which was composed of numerous various-sized cysts. Such a cyst contained transparent fluid in its lumen. A large cyst was several millimeters in size but the minute one could not be identified as to its cystic structure by the naked eye. The large cyst had minute granular spots on its inner surface. In cases in which more than 2 months had elapsed, swelling of the liver progressed; it often reached even a hen's-egg-size, nearly all the parenchyma was replaced by grayish-white multilocular hydatid tissue; the liver presented irregular surface on account of various-sized granulations and blood vessels were congested ramiformly. The hydatid tissue was somewhat elastic and soft in consistency, and was composed of an aggregation of grayish-white medullary tissue masses which were 2~3 mm in diameter. In some certain masses, the cystic structure was yet recognizable, a central narrow lumen remained and the wall was lined with a thick medullary layer of grayish-white color.

Microscopicales: Island-like or wide-spread multilocular hydatid foci were found in the 26-day case. The focus was a reticular structure composed of numerous minute cysts of 20~100 μ diameter with which mingled a few cysts of about 1 mm size. The daughter cyst formation was remarkably active. Considerable cellular accumulation mainly composed of histiocytes was observed around hydatid cyst. The wall of the hydatid cyst was very thin and the germinal layer was rich in nuclei. Both 56- and 60-day cases manifested similar findings. The liver parenchyma was almost entirely replaced by spongy multilocular hydatid tissue, only a few islands of parenchyma remained sporadically and liver cells were often atrophic. The germinal layer of cyst was fine-reticular tissue with calcareous corpuscles and its thickness was 100~300 μ . In small cysts, the germinal tissue filled the lumina. Findings of active brood capsule formation with scolices could be identified in the germinal layer; however, those structures were not yet matured. The cuticular layer was about 10 μ in thickness. Considerable histiocytic reaction in tissue surrounding the hydatid tissue was revealed. Cases in which more than 77 days had elapsed after the inoculation showed similar findings. The majority of cysts were large, 1~3 mm in size, and there was little liver tissue in intercystic areas. Thin connective tissue layer with

occasional existence of histiocytes was found as a tissue reaction. The cuticular layer measured $10\sim 20\mu$ in thickness, however sometimes $50\sim 70\mu$ in advanced cases. The germinal layer was thick, $300\sim 700\mu$, and possessed numerous brood capsules with fully developed scolices. In large cysts, there remained a narrow lumen, but a small cyst was completely filled up by the germinal tissue. Regressive changes were often observed in the hydatid tissue.

7. *Meriones unguiculatus*

Two-month-old Mongolian gerbils were orally inoculated with the eggs. They received about 130 eggs each. Two animals were killed 60 days, 2 were killed 90 days and one was killed 200 days respectively after the inoculation. All the animals showed remarkable development of multilocular hydatid foci.

Macroscopicals: Foci were found in the hepatic tissue alone, the number of them being 7 to 10 per animal. A small focus was $4\sim 5$ mm and a large one was 20 mm in diameter. The focus protruded on the surface of liver and expanded deep in liver parenchyma; it was grayish-white in color and was rather soft in consistency. The focus consisted of large and small cysts, the large cysts, $5\sim 6$ mm in diameter, being conspicuous. The wall of cyst was thin, and the lumen contained transparent fluid. The inner surface of a cyst wall was covered with numerous minute grayish-white spots which were identical with scolices. In a prolonged case, the layer which contained scolices was thickened, the lumen became rather narrow and in the small cyst the lumen was filled up with grayish-white medullary tissue rich in scolices.

Microscopicals: The reaction of host tissue against the hydatid tissue could hardly be recognized except slight lympho- and histiocytic cell accumulation. In cases of 60 days duration, the cuticular layer was thin, $5\sim 10\mu$, and the germinal layer was $200\sim 300\mu$ in thickness. The latter was composed of fine reticular tissue with abundant calcareous corpuscles, in which brood capsules with scolices were embedded. The majority of scolices were immature. The thickness of germinal layer reached $500\sim 700\mu$ in 90-day cases and numerous brood capsules with matured scolices were found. The case in which 200 days had elapsed since inoculation presented still more advanced scolex formation and the thickened germinal layer which naturally filled up the lumina in small cysts. The thickness of cuticular layer was less than 20μ . Regressive changes were observable. The tissue reaction was insignificant similar to that of 90-day cases.

8. *Mesocricetus auratus*

Seventeen albino hamsters were given oral inoculation each with about 40 eggs. They were killed at intervals of 1, 2, 3 and 5 months after the inoculation. No animal, however, was found which presented the development of hydatid tissue.

Macroscopically, a small number of minute grayish-white spots were found in one each 1, 3 and 5 month case respectively. Histologically, however, these foci were merely small nodules which were composed of central homogeneous necrotic substance and surrounding fibrous tissue layer with histiocytes. The focus, therefore, was considered as a nodule formation which had resulted from the organization of a dead parasite.

9. *Mus musculus*

Following 10 strains of mice were examined.

1) AKR

Four-month-old mice were orally inoculated with about 130 eggs respectively. All of the 6 cases showed multilocular hydatid tissue which consisted mainly of large-sized cysts.

Macroscopicals: Three cases, in which 2 months had elapsed from the inoculation, showed swollen liver with uneven irregular surface owing to the development of various-sized cysts. Out of these 3 cases, two had 9 and 14 foci respectively and the remaining one manifested numerous foci all over the liver. The focus was established by an aggregation of large cysts; the largest cyst was $7 \times 9 \times 5$ mm in size while the majority of them were 3~4 mm and contained transparent fluid. Minute cysts were also demonstrable among the large cysts, however, the former were inconspicuous. The blood content of liver was increased. Findings of two 3-month cases were similar to those of 2-month for the most part. In one certain place where the aggregation of large cysts was extremely conspicuous, the focus protruded on liver surface making an appearance like a bunch of grapes. The liver of a five-month case was extremely swollen through the existence of large cyst multilocular hydatid tissue. The inner surface of cyst wall was covered with a layer with minute white spots, that is to say, scolex formation could be seen even macroscopically.

Microscopicals: The cyst of 2-month case was very large. The cyst was surrounded by a connective tissue layer with histiocytes and a few eosinophiles as the tissue reaction and necrotic portion was occasionally found in contact with the cyst wall. The cuticular layer was $5 \sim 10 \mu$ in thickness and the germinal layer was less than 30μ . The stage of development was already that of early brood capsule formation which was indicated by accumulation of nuclei, lumen formation of the accumulation and occasional formation of immature scolices. The brood capsule, however, was protruded showing a hill-like figure toward the lumen of cyst owing to the thinness of germinal layer. In some places, there existed an exogenous daughter cyst formation, but not frequently. Liver cells manifested pressure atrophy. General state of hydatid tissue in cases of 3-months' development was almost the same as that of the 2-month cases, however exogenous daughter cyst formation became more active. The germinal layer revealed not much change, but its thickness reached rarely 100μ . Brood capsule and scolex formation remained in early stage and was shown to occur in large cysts. As for the 5-month case, the majority of hydatid cysts presented numerous brood capsules and scolices which were fully developed. The germinal layer was $300 \sim 500 \mu$ in thickness, fine reticular in construction and accompanied with calcareous corpuscles. The cuticular layer, however, was $10 \sim 15 \mu$ in thickness. Daughter cyst formation was demonstrable and, at the same time, minute cysts had abundant germinal tissue and such a minute one as 300μ in diameter often had scolices.

2) dba

About 4-month-old mice were used and about 130 eggs were given each. All cases, 6 mice, were demonstrated to have hydatid foci which were found in the liver alone.

Macroscopicals: Five cases which had 2 months history after the inoculation had multilocular hydatid foci. Out of these 5 cases, 4 showed diffuse distribution of numerous various-sized foci and the remaining 1 case showed only one multilocular focus of which the size was $7 \times 5 \times 4$ mm. The sizes of foci varied, a small cyst was less than 1 mm, but the majority of foci were 4~5 mm in size and presented an irregular-shaped area. The multilocular focus consisted of minute cysts with intermingled large number of large cysts which had thin wall, were about 1 mm in diameter and contained transparent fluid. A 5-month case presented remarkably swollen liver which showed irregular uneven surface due to development of large cyst multilocular hydatid tissue. Large cysts of 1~5 mm size were found predominantly in foci which were scattered in all lobi and the spaces among large cysts were filled with minute cyst multilocular hydatid tissue. Liver parenchyma at the surrounding portion of the hydatid tissue showed increase of blood content. The hydatid cyst contained transparent fluid and some cysts had translucent wall, however the majority of them showed grayish-white appearance due to the germinal layer with scolices which covered the inner surface of cyst wall.

Microscopicals: In cases of 2-months development, the cyst was large-sized. Although the tissue reaction was not very remarkable, connective tissue reaction with histiocytes could be seen and, occasionally, a laminiform necrosis was found in contact with cyst wall. The cuticular layer was about 5μ in thickness and the germinal layer was generally thin except for an occasional thick layer which was measured 20~50 μ . An early stage of brood capsule formation was found but the majority of such formations manifested sack-form thick-walled structure. They were about 50 μ in size and rarely had thickening or protrusion of the walls the initial stage of scolex formation. Daughter cyst formation was considerably active. Surrounding the hydatid cyst of 5-month cases, there could be found a thin connective tissue layer often accompanied by cell accumulation. The majority of cysts showed brood capsules which contained mature scolices. The germinal layer, commonly, was about 300 μ in thickness; in it brood capsules were sporadically demonstrated in a line. There were some cysts which had immature brood capsules and scolices. The thickness of the cuticular layer was only about 5 μ .

3) dd

Thirteen mice were examined and about 100 eggs were given each. Only one case 131 days after the inoculation revealed one focus in the liver.

Macroscopicals: The focus was about 3 mm in diameter, located in the left lateral lobe nearby the portal area and was grayish-white hard in character. The focus was composed of multilocular hydatid tissue which was established by an aggregation of extremely minute cysts.

Microscopicals: There was no scolex formation. The focus was composed of small cysts, of which the size was less than 1 mm and generally 200~300 μ in diameter. The tissue reaction was so conspicuous that the group of cysts was embedded in well-developed connective tissue with numerous histiocytes. The intercystic connective tissue in the center portion of focus manifested necrotic change. The shape of cysts was irregular in general, the cuticula layer was 5~10 μ in thickness and the germinal layer was as thin as 10~20 μ .

4) C57BL/6

Two-month-old mice were orally inoculated with about 120 eggs respectively. Fifteen cases out of 19 examined showed positive result. The development of hydatid tissue was commonly slow.

Macroscopicals: The hydatid foci were found in the liver alone and the number was generally small ranging from 1 to 12. Foci of the cases in which 1 month had elapsed after the inoculation showed minute cystic structure, less than 1 mm in diameter and many of the cysts were unilocular macroscopically. In 2-month cases, multilocular structure which was composed of minute cysts already appeared, however, the focus was only 1~2 mm in diameter. The majority of foci in 4-month cases were about 5 mm in size and, occasionally, foci of 8~10 mm size could be found. The focus was multilocular hydatid tissue composed of minute cysts, but any large cyst was very rarely detected. The focus had granular surface, hard, grayish-white and on cut surface, the focus was seen to contain many minute cysts. In the cases more than 5 months after the inoculation, the lesion became conspicuous and expanded, but the characteristics were similar to those of the former stage. In a certain case, the lobus of liver was totally replaced by the hydatid tissue. There was no metastatic development of hydatid tissue.

Microscopicals: In one-month cases, the majority of foci were spherical unilocular cysts and some of them showed early multilocular structure. The wall of cyst was so thin as to be almost indifferntiable. In surrounding portion of cyst, there existed marked connective tissue reaction; the tissue was accompanied by numerous histiocytes intermingled with lymphocytes and eosinophiles. The thickness of fibrous tissue layer was about 300 μ and regressive changes of accumulated cells appeared there. In 2-month cases, findings of multilocular hydatid became distinct. Remarkable tissue reaction was recognized; abundant cell elements such as lympho- and histiocytes were differentiable. Also these cell elements sometimes showed regressive changes, but there frequently existed tissue necroses in contact with hydatid cysts. Typical multilocular hydatid tissue was confirmed in cases in which 4 months had elapsed after the inoculation. The group of hydatid cysts was embedded in an area of connective tissue which was rich in histiocytes accompanied by lymphocytes, a small number of eosinophiles and some giant cells. Cysts were irregular-shaped and various-sized; they aggregated contacting or, in some places, communicating with each other respectively. The majority of cysts were minute in size. The cuticular layer was 5~10 μ in thickness and the germinal layer was about 10 μ . Focal necrosis in surrounding tissue was detected in contact with hydatid cyst. The cyst was lacking in scolex. Five-month cases indicated conspicuous multilocular figures. In wide-spread focus, intercystic connective tissue fell into necrosis. Early stadium of brood capsule and scolex formation could rarely be found for the first time in this stage and, in this case, the thickness of germinal layer was 20~50 μ . Active daughter cyst formation was observed. In cases which had lasted as long as 7 months, multilocular structure became extremely complicated and very active daughter cyst formation, mainly exogenous one, was seen. There was severe tissue reaction against the hydatid; necrotic changes of host tissue were conspicuous in some places. In some parts, there were found brood capsules with fully

developed scolices and, at the same time, the thickness of germinal layer was 200~300 μ and that of cuticular layer was 10~20 μ .

5) CF #1

Thirteen mice, 7 months old, were examined. About 130 eggs were orally administered to each. All animals presented positive results of hydatid infection.

Macroscopicals: Foci were limited in the liver. Two-month cases, 5 animals, manifested 2, 3, 5, 6 and 24 foci respectively. The majority of foci were minute grayish-white of about 0.5 mm size, however, case No. 5 presented a multilocular focus composed of minute cysts accompanied by large cysts. Four cases at 3 months after the inoculation showed 3, 4, 6 and 7 foci respectively. Foci were composed of various-sized irregular-shaped multilocular tissues; a small focus was about 1 mm in diameter while the largest one occupied an area of 15 \times 10 mm. The focus was commonly made up of multilocular tissue composed of minute cysts, however, a large focus was mixed with cysts as large as 1~5 mm in diameter. Five-month cases were characterized by well-developed hydatid tissue and the liver was enlarged. Foci protruded on liver surface with irregular granulation appearance and in one case the left lateral lobus had been almost entirely replaced by hydatid tissue. The focus was hard, it was multilocular tissue composed of numerous minute cysts and a few large cysts with transparent fluid were mingled.

Microscopicals: The wall of hydatid cyst in 2-month cases was thin, the cuticular layer was only 1~2 μ in thickness and the germinal layer was almost mono-layered. Strong tissue reaction indicated by proliferation of connective tissue accompanied by accumulation of large number of histiocytes and lymphocytes, and also giant cells, was demonstrated. Daughter cyst formation was conspicuous and necrotic change of surrounding tissue was found in contact with daughter cyst. In cases 3 months after the inoculation, tissue reaction against hydatid cyst was remarkable. The cyst was enveloped by thick connective tissue, of which the outer portion presented severe histio- and lymphocytic cell accumulation with occasional eosinophile infiltration. The cuticular layer was also thin. Large cysts rarely had thick germinal layer as much as 50 μ thick. Active daughter cyst formation was recognized. In 5-month cases, exogenous and endogenous daughter cyst formation was active and multilocular structure became conspicuously complicated. Some parts of connective tissue in the focus showed necrosis and stained well, homogeneously, with eosin. Scolex formation could rarely be observed but some scolices were matured.

6) b

Each of 8 mice, 4-month-old, was orally inoculated with about 130 eggs. Five cases out of 8 showed positive result and foci localized in the liver alone.

Macroscopicals: The number of hydatid foci ranged from 1 to 5. In 2 cases 2 months after the inoculation, one animal had 4 cysts of about 1 mm diameter; the other showed a focus composed of an aggregation of various-sized cysts in the left lateral lobus, the size was 9 \times 5 \times 3 mm. Of 2 cases which had continued 3 months, one case showed grayish-white multilocular focus of 2 mm diameter which was composed of minute cysts in the right lateral lobus; other case had 5 irregular-shaped foci of 1~10 mm size which were

composed of many cysts of less than 1 mm. The liver of a 5-month case showed swelling accompanied by severe hydatid lesion. Multilocular tissues composed of minute cysts were found in some parts of the left lateral and medial lobes, and in almost the whole of right half of the liver. The hydatid tissue protruded on liver surface showing granular appearance and was hard to the touch. The focus was mixed with some large cysts of 1~5 mm size which contained transparent fluid. The inner surface of the wall of a large cyst was covered by grayish-white layer.

Microscopicals: In 2-month cases, multilocular hydatid tissue was embedded in well-developed connective tissue which was accompanied by lympho- and histiocytes. Necroses were recognized in contact with hydatid cyst. The thickness of surrounding tissue of cyst was 200~300 μ . The wall of hydatid was very thin. Multilocular figure provoked by daughter cyst formation was conspicuous. Three-month cases presented remarkable multilocular figure due to daughter cyst formation. Reactive proliferation of connective tissue was found in a high degree, accumulation of numerous histio- and lymphocytes was revealed and the connective tissue had a tendency to be fallen into necrosis in contact with hydatid cyst showing thick layer. The wall of hydatid cyst was thin, the cuticular layer was 2~3 μ in thickness and the germinal layer was mere mono-layer with sporadic nuclei. As for 5-month cases, wide areas were replaced by multilocular hydatid tissues; various-sized cysts aggregated, their shape was irregular and some cysts showed communication of lumen with each other. Both exogenous and endogenous daughter cyst formations could be recognized. Connective tissue reaction was conspicuously observed and frequent appearance of giant cells was observed nearby the cyst wall. In the central portion of the focus, there existed necrosis of connective tissue. Brood capsule and scolex formation was observed occasionally. The cuticular layer was 5 μ in thickness and the thickness of germinal layer was various. The latter reached about 300 μ in thickness when brood capsules with matured scolices could be detected, however, in the germinal layer of 100~200 μ in thickness, the brood capsule and scolex were immature.

7) CFW

Twelve 2-month-old mice were used. About 130 eggs were orally given each. Of these animals, only 6 cases showed hydatid foci in the liver alone.

Macroscopicals: Two cases in which 2 months had elapsed after the inoculation presented 1 and 2 foci respectively. Sizes of these 3 foci were 3×2, 3.5×3 and 3×1.5 mm respectively. They had hard consistency and were multilocular hydatid foci which were made up of numerous minute cysts. In 3-month cases, only one animal out of 3 showed positive result; in it a soybean-size focus of irregular shape was detected in the left lateral lobe. The focus was multilocular tissue which was mixed with some large cysts. Three cases in which 5 months had elapsed manifested severe lesions. The focus was multilocular hydatid tissue which was composed of minute cysts accompanied by some large cysts, 1~5 mm in diameter. The focus occasionally occupied the greater part of a lobe and protruded on the liver surface showing coarse granulation. A large cyst contained transparent fluid and its wall was thin.

Microscopicals: The hydatid tissue of 2-month case showed multilocular structure

and daughter cyst formation. Individual cysts were minute. Thick connective tissue layer with abundant histio- and lymphocytes was observed surrounding the hydatid; its thickness reached even 500 μ . Considerable eosinophile accumulation was seen and conspicuous tissue necrosis could be found in contact with the parasite. Hydatid cyst wall was thin. In 3-month cases, the focus was multilocular hydatid composed of various-sized cysts, proliferation of connective tissue was remarkable, lympho- and histiocytes were abundant and, in contact with the parasite, eosinophile leucocytes accumulated. Neighboring hepatic parenchyma fell into pressure atrophy. Laminiform necrotic lesion was frequently recognized in contact with cyst wall. On section preparations, hydatid cyst wall was thin and often showed irregular undulations of which the surrounding tissue sometimes manifested conspicuous cell accumulation. In cases in which 5 months had elapsed after the inoculation, multilocular hydatid tissue composed of an aggregation of various-sized irregular-shaped cysts due to very active daughter cyst formation was proven to exist. Markedly increased connective tissue was found, in which hydatid tissue was embedded and accumulation of lymphocytes, numerous histiocytes and considerable number of eosinophiles was observed. In contact with the hydatid cyst, there existed cell and tissue necroses. The wall of hydatid cyst was thin and differentiation between the cuticular and germinal layer could hardly be made. No brood capsules nor scolices were detectable.

8) A

Fourteen male mice, 4 and half months old, were examined. Each mouse was orally inoculated with about 130 eggs. From among these 14 mice, 10 cases revealed hydatid infection in the liver. The characteristics of foci were comparatively uniform according to the lapse of time after the inoculation.

Macroscopicals: Five cases 2 months after the inoculation showed 1 to 5 grayish-white foci of 1~2 mm diameter some of which already manifested multilocular structure composed of minute cysts. In 2 positive cases among those 3 months after the inoculation, respectively 1 and 4 hydatid foci were detected, of which the characteristics were almost the same as those of 2-month cases. Among 5-month cases, 3 cases had 2, 5 and 7 foci respectively. The focus was a clearly-demarcated grayish-white one, of which the size was 2~4 mm in diameter. The focus slightly protruded on the liver surface showing fine granular appearance and was typical multilocular hydatid tissue made up of minute cysts; the cyst was so minute that one could barely differentiate its cystic structure by naked eye.

Microscopicals: In cases in which 2 months had elapsed after the inoculation, severe reactive proliferation of connective tissue was found, of which the thickness was 500~800 μ . With the connective tissue was associated an accumulation of histio- and lymphocytes mingled with a considerable number of eosinophile leucocytes. The hydatid tissue manifested multilocular structure produced by daughter cyst formation mainly of exogenous nature. The cyst wall was thin; the surrounding portion presented necrotic change in proliferated tissue. In 3-month cases, the multilocular character became conspicuous and the cuticular layer of hydatid cyst was about 5 μ in thickness. The cyst wall often manifested marked undulation and on such occasion, cellular reaction was remarkable accompanied by giant

cell appearance. In cases of 5-months development, multilocular figure became much more remarkable than that of 3-month cases, exogenous daughter cyst formation was extremely active and shape of cysts was conspicuously irregular. Connective tissue reaction was recognizable in high degree and necrotic change was seen in the central part of focus. The wall of cyst was thin and no brood capsules nor scolices could be detected.

9) BALB/C

Ten mice, 4 months old, were given oral administration of about 130 eggs each. In 6 cases, the livers showed small hydatid foci in small number.

Macroscopicals: One case, one month after the inoculation, presented 3 cysts, of which the size was about 0.5 mm. In 2 cases 2 months after the inoculation, one had a single focus in the left medial lobe and the other case showed 1 and 2 foci in the right and left lateral lobes respectively. These foci were multilocular tissue made of minute cysts and ranged from 1 to 4 mm in size. Among 3-month cases, one showed a multilocular focus, 2 mm in size, composed of minute cysts in the papillary process. In 5-month cases, one had 2 small-cystic multilocular foci of 2 mm size in the marginal portion of the left lateral lobe. Another case showed a focus, of which the size was 7×8 mm, located in the left lateral lobe showing small-cystic multilocular structure and it had some mingled large cysts of 1 mm size. Nearby this focus, another small focus, 1 mm in size, was found.

Microscopicals: Two-month cases revealed proliferation of connective tissue associated with accumulation of histiocytes and a few eosinophiles. The wall of hydatid cyst was thin and had multilocular figure owing to exogenous daughter cyst formation. In a 3-month case, the hydatid was surrounded by connective tissue layer of about 100 μ thickness, the cuticular layer was about 5 μ in thickness and the germinal layer, rarely thickened, showed increase of nuclei. Cases of 5-months development exhibited severe connective tissue proliferation surrounding the parasite accompanied by a large number of histio- and lymphocytes and considerable number of eosinophiles. Necrosis was recognized in contact with the hydatid cyst wall. There was active exogenous daughter cyst formation. The cuticular and germinal layers were thin.

10) C3H/He

Seventeen 4-month-old mice orally received about 130 eggs respectively. Thirteen cases showed hydatid foci in their livers.

Macroscopicals: In 4 cases which had progressed for 2 months, one to three small foci were sporadically found, of which the size was about a half millimeter. Out of 6 cases of 3-months development which showed positive, 4 cases manifested a few small foci of which the size was less than 1 mm in diameter, one case sporadically showed more than 10 small foci and the remaining one case showed a small-cystic multilocular focus, 10×5 mm in size, in the right medial lobe in addition to 5 small foci. Each of 3 cases which lived 5 months after the inoculation manifested several foci, 5~10 mm in size. Distribution of foci inclined to the right half of the liver and the focus was multilocular hydatid tissue composed of minute cysts, however, large cysts of 1~4 mm size which looked grayish-white were also intermixed.

Microscopicals: In 2-month cases, the hydatid tissue was enveloped by connective tissue layer with slight cell accumulation and regressive change showed in contact with hydatid tissue. The wall of cyst was extremely thin. Connective tissue reaction of 3-month cases was also remarkable, histio- and lymphocytic cell accumulation was found and necrosis was conspicuous at points in contact with the parasite. The hydatid tissue presented comparatively large cysts, daughter cyst formation was inactive and complication of hydatid structure was slight. The wall of a large cyst showed undulation. Although the hydatid cyst wall was thin, the cuticular layer occasionally reached 5 μ thickness and the germinal layer 10~20 μ . Cases which lived 5 months showed comparatively large irregular-shaped cysts, connective tissue proliferation was striking and marked necrosis was found in contact with the parasite. Early stage figure of brood capsule and scolex formation was detected very rarely.

DISCUSSION

As already stated, there are many species of animals which are susceptible to *Echinococcus multilocularis* infection. According to the literature more than 40 species have recently been investigated in regard to this problem. RAUSCH and SCHILLER (1956), especially, carried out experimental study on many species, and VOGEL (1955) and SADUN et al. (1957) also made experimental infection tests on various species. Synthesizing the results of these reports, it can be said that rodents, especially voles belonging to genera *Microtus* and *Clethrionomys*, manifest high susceptibility to the infection. The present authors, in their own experiment, also confirmed the fact, namely—all cases of *Clethrionomys rufocanus bedfordiae* and *Microtus montebelli montebelli* examined were surely infected by oral inoculation irrespective of the number of eggs. The hydatid tissue developed rapidly in these animals and it was recognized that the hydatid focus with numerous scolices was established. It is a well-established fact that many cases of human multilocular echinococcosis can be found as an endemic on Rebun Island, Hokkaido^{5,9)}. Although no natural case of intermediate host other than human has been discovered up to the present, it can be easily presumed that *Clethrionomys rufocanus bedfordiae* is the most possible animal species which should play a role as the intermediate host to make the disease endemic in Rebun. *Eutamias asiaticus lineatus* can be found also in Rebun and one can likewise suppose, judging from the present experiment, that this animal is able to play a role as an intermediate host.

The susceptibilities of various rodent animals investigated by the authors are tabulated below (Table I).

Although *Apodemus speciosus ainu* terminated in negative result, experiment was carried out on only one case. The authors, therefore, do not intend to conclude simply that this species is insusceptible to *Echinococcus multilocularis*, because

TABLE 1. *Experimental Results*

ANIMALS INVESTIGATED	NO. OF CASES OF WHICH RESULTS WERE		SUSCEPTIBILITIES (%)
	Positive	Negative	
<i>Clethrionomys rufocanus bedfordiae</i>	11	0	100
<i>Clethrionomys rutilus mikado</i> *	1	0	(100)
<i>Apodemus speciosus ainu</i> *	0	1	(0)
<i>Apodemus geisha</i>	3	4	43
<i>Eutamias asiaticus lineatus</i> *	1	1	(50)
<i>Microtus montebelli montebelli</i>	15	0	100
<i>Meriones unguiculatus</i>	5	0	100
<i>Mesocricetus auratus</i>	0	17	0
<i>Mus musculus</i>			
AKR	6	0	100
dba	6	0	100
dd	1	12	8
C57BL/6	15	4	79
CF #1	13	0	100
b	5	3	63
CFW	6	6	50
A	10	4	71
BALB/C	6	4	60
C3H/He	13	4	76

* : Species on which future study is desirable since only one or two animals were examined.

Apodemus geisha, a species of the same genus, showed considerable susceptibility. Among many species, albino hamster alone was the only one which showed quite negative result. In spite of the large number of cases which were investigated, no proof of development of hydatid tissue, either macro- and microscopically, could be obtained other than some organized parasitic nodules in a few cases. In conclusion, it may be said that the albino hamster possesses strong resistant power to the infection of multilocular hydatid. The result obtained by the authors concerning albino hamsters coincides with that by other investigators. Rodent species and mice strains other than the above two species were susceptible and showed various infection rates according to species and strain. It, however, can be said without doubt, by reason of facts discussed below, that the weight of role as the intermediate host should not be judged merely from the infection rate.

In comparison with human multilocular echinococcosis, the morphological

characteristics of multilocular hydatid tissue in voles of *Clethrionomys* and *Microtus*, the optimum intermediate hosts, give an impression as if the lesion was provoked by some other cause than that in human case. In short, there is remarkable difference of macro- and microscopical findings between human and vole cases. In a human multilocular case, as described in numerous cases by many investigators in the past, it is said that the majority of cysts which constitute hydatid tissue are extremely minute, that there is little or no fluid within cyst lumina, scolex formation is very rare or entirely lacking, evidence of host tissue reaction against the parasite is conspicuous and that severe connective tissue proliferation can be observed. These characteristics were also found in human cases in Rebus Island⁵⁾. In vole cases, on the contrary, the most characteristic finding is the formation of numerous brood capsules with scolices and these structures, together with the germinal layer, cover the inner surface of cyst wall or fill up the lumen entirely. The majority of cysts of vole case, indeed, are large in size and contain transparent fluid; the host tissue reaction against the parasite is insignificant in comparison with human case and the hydatid tissue is often nothing but aggregation of hydatid cysts. As a reason for appearance of characteristic hydatid structure and tissue reaction, RAUSCH and SCHILLER (1956) consider that man is an unsatisfactory intermediate host to *Echinococcus multilocularis*. Additional future investigation will be needed to clarify this interpretation; however, the results in the present experiment on mice provide ground to agree with their opinion to a certain extent.

On the susceptibility of the mouse, two sorts of results have been opposed to each other in the past; the one indicates that the mouse is susceptible (VOGEL, 1955; MANKAU, 1955, 1957) and the other that it is insusceptible (RAUSCH and SCHILLER, 1956; SADUN et al., 1957). Even in the former opinion, characteristics of hydatid tissue are different from those of vole case in spite of considerably high infection rate, namely—brood capsules and scolices were not described, or were found only in cases more than 4 months after the infection. Accordingly, there have been no mice which showed brood capsule and scolex formation in early stages of the infection, or there have been no mice which showed positive result. As the most possible reason for such inconsistency of result, the present authors took the difference of "strain" of mice into consideration. In this connection, there is to be found no description or consideration concerning the strain in literature hitherto published. The present experiment, as the authors had previously conjectured, produced the result that there were remarkable differences of susceptibility, 8~100%, among the strains investigated. Moreover, it was made clear that the hydatid could be classified into 2 types according to combination of host tissue reaction and morphology of hydatid cyst as follows.

Type 1: This type occurs in 2 mouse strains, AKR and dba. The hydatid tissue develops rapidly and multilocular tissue composed of numerous large cysts as large as several millimeters is established in 2 months when brood capsule and scolex formation is already taking place. The germinal layer develops well and becomes thick. The reaction of host tissue against the hydatid is not remarkable and slight proliferation of connective tissue with some cell element is merely recognizable. Infection rate of the 2 strains was both 100%, therefore, the strains can be satisfactorily used as experimental animals. The authors could also confirm that the hydatids of *Clethrionomys rufocanus bedfordiae*, *Microtus montebelli montebelli*, *Meriones unguiculatus*, *Eutamias asiaticus lineatus*, *Apodemus geisha* and undoubtedly *Clethrionomys rutilus mikado* should belong to type 1. These rodent animals show multilocular hydatid tissue which is commonly composed of large cysts and the development is very rapid. Numerous scolices are recognizable in two and a half months at the latest and some cases even in one month and a half. Host tissue reaction is slight. Accordingly, although some species show low infection rate, the above-named species are suitable intermediate hosts, judging from the characteristics of hydatid.

Type 2: This type is demonstrated by 8 mouse strains viz., dd, C57BL/6, CF#1, b, CFW, A, BALB/C and C3H/He. In these strains, the hydatid develops slowly, although the lesion often becomes severe in prolonged cases. For instance, C57BL/6 in which 2 months elapsed after the inoculation shows multilocular foci of only 1~2 mm size. The hydatid of this type has a strong tendency to become an extremely complicated structure due to active daughter cyst formation. Individual cysts are generally minute and large cysts are rare. Host tissue reaction is severe and a thick layer of connective tissue is observable accompanying accumulation of such cells as histiocytes, lymphocytes and sometimes eosinophile leucocytes. The central portion of connective tissue often falls into necrosis in contact with the hydatid cyst. Scolex formation is delayed and inactive, and the scolex usually requires more than about 5 months to start its development. In short, it can be said in a sense that these strains are unsatisfactory or insuitable intermediate hosts and that the host tissue suppresses the development of hydatid tissue, although some strains such as C57BL/6 and CF#1 show high infection rate. The present authors, however, consider that the strains of this type could also play some role as an intermediate host when scolices matured, although the development is slow and inactive. In other words, it seems that this type of hydatid should be discussed in the same category as human multilocular hydatids.

It was, as above described, experimentally clarified that *Echinococcus multilocularis* shows 2 types in the tissues of intermediate host. Type 1 is that one which has been hitherto recognized in vole cases by many investigators and

the present authors newly discovered 2 mouse strains in which hydatid belonged to this type. Type 2 is the one which has been known in infection experiment of mice up to the present and, as is also easily understood in the present study, the majority of mouse strains manifest this hydatid type. The authors know that there are differences of susceptibility to the hydatid, development of hydatid, host tissue reaction, etc. according to mouse strain. These facts, however, never have been taken into consideration in the past literature. It is a well-accepted fact in the fields of bacteriology and virology that the "strain" of experimental animal should be taken into consideration when experimental studies are carried out. In the field of helminthology these ideas have not attracted the attention of investigators. Differences of susceptibility by mouse strain were proven by the present authors in the experiment in regard to *Echinococcus multilocularis* which belongs to the helminthes. Consequently it can be said with certainty that interesting problems for the future are brought forward. In practice, the animal which shows the hydatid of type 1 will be valuable in passage of *Echinococcus multilocularis* and the mouse strain which presents the hydatid of type 2 should be used by reason of similarity to human case in therapeutic experiments together with type 1. The authors reported the experiment concerning secondary multilocular echinococcosis in dd strain in their 6th paper. However, the same experiment should be carried out using other mouse strains, especially the strain which shows the type 1, otherwise generalization cannot be made.

SUMMARY AND CONCLUSION

Susceptibility to *Echinococcus multilocularis* was investigated in various species of rodent animals and various strains of mice. Among these, the albino hamster showed entirely negative result in respect to development of hydatid tissue and the case of *Apodemus speciosus ainu* examined likewise produced negative result. Other species and strains, however, were susceptible to the parasite, although infection rates were varied by species or strain.

It was made clear that the hydatid can be classified into two types according to combination of the morphology of the hydatid and host tissue reaction. Type 1 was observed in *Clethrionomys rufocanus bedfordiae*, *Clethrionomys rutilus mikado*, *Apodemus geisha*, *Eutamias asiaticus lineatus*, *Microtus montebelli montebelli*, *Meriones unguiculatus* and two strains of mice, AKR and dba. In this type, the hydatid develops rapidly, individual cysts are large in size, scolices can be detected at 1.5~2.5 months and host tissue reaction is slight in degree. Type 2 was manifested by 8 mouse strains, dd, C57BL/6, CF #1, b, CFW, A, BALB/C and C3H/He. In type 2, the hydatid develops slowly, individual cysts are minute, more than 5 months is required for scolex formation and severe tissue reaction

on the host side is demonstrable. An animal which presents the hydatid of type 1 is a suitable intermediate host but an animal developing type 2 is not a very suitable one. It can be said without doubt that human cases manifest the latter type. It is a new fact that there are some mouse strains such as AKR and dba which show the hydatid of type 1.

As a reason for inconsistency in reported results of mouse experiments in the past, the present authors wish to emphasize the difference of strains of mice. In the field of helminthology up to the present, investigators have had no regard for the strain of experimental animals in experiments on susceptibility. Concluding from the results of experiment with *Echinococcus multilocularis*, consideration must be paid to the strain in helminthology hereafter.

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EXPLANATION OF PLATES

Microphotographs of preparations stained with hematoxylin-eosin, Figs. 8~15 and 20~25, are at the same magnification, $\times 50$. Figs. 6, 7 and 16 are also shown on the same scale, $\times 1.4$.

PLATE I.

- Fig. 1 *Clethrionomys rufocanus bedfordiae*, 78 days after inoculation; the hydatid of authors' type 1. $\times 7/10$.
- Fig. 2 *Microtus montebelli montebelli*, 60 days after inoculation; multilocular hydatid tissue composed of large cysts with thin walls; type 1. $\times 7/8$.
- Fig. 3 *Microtus montebelli montebelli*, 202 days after inoculation; grayish-white hydatid tissue with remarkable scolex formation. $\times 10/11$.
- Fig. 4 *Eutamias asiaticus lineatus*, 76 days after inoculation; large cyst hydatid foci; type 1. $\times 3/2$.
- Fig. 5 *Meriones unguiculatus*, 90 days after inoculation; large cyst hydatid foci; type 1. $\times 2/3$.

- Fig. 6 *Microtus montebelli montebelli*; comparison of livers (cut surfaces) showing development of hydatid foci of type 1; top, 26 days after inoculation; middle, 60 days; bottom, 120 days.
- Fig. 7 *Meriones unguiculatus*; comparison of livers; top row, 60 days after inoculation; middle, 90 days; bottom, 200 days; germinal layer gradually thickened.

PLATE II.

- Fig. 8 *Clethrionomys rufocanus bedfordiae*, 118 days after inoculation; fully developed hydatid tissue.
- Fig. 9 *Microtus montebelli montebelli*, 26 days after inoculation; early stage of development.
- Fig. 10 *Microtus montebelli montebelli*, 77 days after inoculation; scolex formation; in upper left is a part of large cyst.
- Fig. 11 *Microtus montebelli montebelli*, 202 days after inoculation; fully developed hydatid tissue.
- Fig. 12 *Apodemus geisha*, 100 days after inoculation; brood capsules and scolices; without tissue reaction; type 1.
- Fig. 13 *Eutamias asiaticus lineatus*, 76 days after inoculation; early stage of brood capsule and scolex formation.
- Fig. 14 *Meriones unguiculatus*, 60 days after inoculation; scolices are still immatured.
- Fig. 15 *Meriones unguiculatus*, 90 days after inoculation; matured scolices.
- Fig. 16 C57BL/6, comparison of livers; top row, 60 days; middle, 150 days; bottom, 210 days; small cyst hydatid foci; type 2 lesion.

PLATE III.

- Fig. 17 AKR, 90 days after inoculation; large cyst hydatid tissue; type 1. Natural size.
- Fig. 18 C57BL/6, 120 days after inoculation; small cyst multilocular hydatid tissue with severe connective tissue reaction; type 2 lesion. $\times 5/7$.
- Fig. 19 CF#1, 150 days after inoculation; severe type 2 lesion in the left lateral lobus. $\times 3/2$.
- Fig. 20 dba, 60 days after inoculation; initial stage of brood capsule and scolex formation; type 1.
- Fig. 21 dba, 150 days after inoculation; brood capsules with scolices in germinal layer.
- Fig. 22 dd, 131 days after inoculation; type 2 lesion remarkable.
- Fig. 23 C57BL/6, 120 days after inoculation; type 2 lesion.
- Fig. 24 C57BL/6, 150 days after inoculation; initial stage of brood capsule and scolex formation.
- Fig. 25 b, 150 days after inoculation; type 2 lesion with initial stage of brood capsule and scolex formation.





