



Title	STUDIES ON ECHINOCOCCOSIS X. : HISTOLOGICAL OBSERVATIONS ON EXPERIMENTAL CASES OF MULTILOCLAR ECHINOCOCCOSIS
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Citation	Japanese Journal of Veterinary Research, 8(1-4), 134-160
Issue Date	1960
DOI	10.14943/jjvr.8.1-4.134
Doc URL	<a href="http://hdl.handle.net/2115/4676">http://hdl.handle.net/2115/4676</a>
Type	bulletin (article)
File Information	KJ00002373268.pdf



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**STUDIES ON ECHINOCOCCOSIS X.**  
**HISTOLOGICAL OBSERVATIONS ON EXPERIMENTAL CASES**  
**OF MULTILOCLAR ECHINOCOCCOSIS**

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(Received for publication, March 29, 1960)

INTRODUCTION

Few reports have been hitherto published pertaining to histopathological or histogenetical studies on experimental cases of multilocular echinococcosis. RAUSCH (1954) reported the results obtained from experiments on *Microtus pennsylvanicus* and MANKAU (1955, 1956, 1957) on laboratory mice. As well as the description of lesions in the central nervous system of *Microtus agrestis* investigated by VOGEL and SCHUMACHER (1957), some patho-anatomical and -histological data can be found in papers presented recently by RAUSCH, VOGEL, YAMASHITA and others.

The present writer, in the 8th report on echinococcosis co-authored with YAMASHITA et al. (1958)<sup>35)</sup>, described investigations on the susceptibility of 10 uniform mouse strains and 8 other rodent animals. Two types were classified according to combination of morphology of the parasite and host tissue reaction. In type 1, the parasite developed rapidly, individual cysts were large in size, scolices could be detected at 1.5~2.5 months and host tissue reaction was slight in degree. In type 2, however, the parasite developed slowly, individual cysts were minute, more than 5 months was needed for scolex formation and severe tissue reaction of the host side was demonstrable.

The author carried out histological investigations on experimental cases of multilocular echinococcosis. Materials were obtained from cases of vole *Microtus montebelli montebelli*, mice (strains dba, CF #1 and C 57 BL/6) and cotton rat *Sigmodon hispidus*. *Microtus* and mouse cases were the ones used in the above noted paper by YAMASHITA et al. (1958)<sup>35)</sup> and those newly induced for the present work. The susceptibility of cotton rat was reported by SADUN et al. (1957), but they described only anatomical findings while histological ones were omitted.

MATERIALS AND METHODS

Investigations were conducted on 21 voles, 18 cotton rats, 18 dba mice, 17 CF # 1 mice and 29 C 57 BL/6 mice. The *microtus*, cotton rat and dba are animals which manifest type

1 lesions and CF#1 and C 57 BL/6 do those of type 2. The two types were classified and described in detail in the 8th report above noted. The animals were obtained from the breeding stocks of the Institute for Infectious Diseases, University of Tokyo. The oral infections were conducted in the period between September, 1957 and January, 1958; the Alaskan strain of *Echinococcus multilocularis* was used the same as in the preceding investigations. The inocula which contained echinococcus eggs were obtained from the feces of experimentally infected dogs by the centrifuge using 0.6% saline solution. As the inoculations were conducted several times, the number of eggs in inocula differed from time to time; the range was from 120 to 1,000 per animal.

After the inoculation, 1~625 days, the animals were killed at desired intervals except a small number of dead cases. The tissue materials were fixed with 10% formalin solution, serial paraffin sections were made and stained with hematoxylin-eosin and other routine procedures were followed.

#### RESULTS AND DISCUSSION

Numerous papers dealing with multilocular or alveolar echinococcosis have been published, however, the majority of them were limited to natural cases of human beings with chronic course. As the experimental animals for the suitable intermediate host in connection with multilocular echinococcosis were unknown until 1950, there can be found few reports about experimental echinococcosis.

It is a well-known fact that human multilocular echinococcosis usually manifests a chronic lethal course as 5~10 years. It can be said that the greater part of the knowledge obtained from human cases by many investigators was derived from chronic cases and that the characteristics of multilocular echinococcus have been known only to a limited extent. As one characteristic of multilocular echinococcus, multilocular vesiculation by exogenous budding occurs continuously. Therefore, observation of a portion where the exogenous budding occurs is somewhat useful to analyse the initial stage of development of the parasite, but it is merely an indirect method. It must be remembered, as a remarkable characteristic of human cases, that the scolex formation is very rare and individual cysts are minute. As the studies of multilocular echinococcus have been limited to human cases alone, the findings might be considered as typical. Judging from the biological point of view, however, it must be considered as typical when the echinococcus manifests sufficiently brood capsules with scolices. In the body of a suitable intermediate host, the brood capsule formation has a close relation to development of the germinal layer and starts in an enlarged cyst at the beginning. Consequently, the author considers, when multilocular echinococcosis in various animals has been fully investigated in the future, it will become known that human multilocular echinococcosis is a particular type and must be discussed with an introductory remark of "in human case".

The author, in the present paper, obtained many interesting results and would like to describe and discuss them under the following headings A to I.

A. *Initial Stage of Development* It was clarified, in the present experiment, that echinococcal foci become visible to the naked eye on the 5th day after the inoculation. Microscopically, however, the spherical or ovoid parasites can be found even in cases only 24 hours old after the inoculation except for C57BL/6 strain of mouse. The parasite is composed of a thin "larval membrane" and a mass of germinal cells; a narrow space is seen between them. The nucleus of a germinal cell is spherical, pale, and possesses a distinct nucleolus. The cytoplasm is stained basophilic. The size of the parasite does not exceed 20  $\mu$ . Unilocular vesiculation occurs in 3~4 day cases when the parasite is 30~60  $\mu$  (normally 40  $\mu$ ) in diameter, that is to say—a vacuolar structure appears at the central portion of the germinal cell mass and germinal cells manifest ring-shaped arrangement in one layer. The days required for unilocular and multilocular vesiculation respectively are: in microtus 3 and 10 days; cotton rat 3 and 5; dba 4 and 7; CF #1 4 and 10; and C57BL/6 3 and 7 (Table 1).

TABLE 1. *Days Required for Unilocular Vesiculation, Multilocular Vesiculation, Cuticular Layer Formation and Brood Capsule Formation*

SPECIES OR STRAINS OF ANIMALS	UNILOCULAR VESICULATION		MULTILOCULAR VESICULATION		CUTICULAR LAYER FORMATION		BROOD CAPSULE FORMATION	
	—	+	—	+	—	+	—	+
	Microtus	1	3	5	10	20	26	33
Cotton rat	1	3	3	5	14	18	18	20
dba	3	4	5	7	20	30	30	49
CF #1	3	4	5	10	10	30	60	90
C57BL/6	2	3	5	7	20	26	120	150

At the time of multilocular vesiculation, the above-mentioned larval membrane shows granular or indistinct appearance and disappears. In between the stages of the disappearance of the larval membrane and formation of the cuticular layer, therefore, the vesicle wall is made up of a thin germinal cell layer alone.

It was made clear that the parasite invades the liver by way of the portal circulation system. For instance, the parasitic foci are found at the portions nearby the portal branches such as in the interlobular veins or GLISSON'S capsule; in some cases, these structures are included in the foci. The blood vessel wall often indicates circumscribed cell accumulation and sometimes even thrombus or intima granuloma formations. A cell accumulation which connects the blood

vessel and focus can also be found in a few cases. On the contrary, no close relations can be observed between the liver capsule and parasitic foci in the initial stage.

The initial focus begins with a focal cell accumulation. The cell element which plays an important role is that of activated liver sinusoid endothelia of which the nucleus indicates polymorphism. At the same time, some polymorphonuclear cells (neutrophil and eosinophil) and degenerated liver cells participate. Accumulated cells tend to fall into degeneration in contact with the parasite; degeneration is slight in cotton rat but remarkable in CF #1 and C 57 BL/6 mice. In the latter two mouse strains, therefore, the multilocular vesiculation occurs embedded in a detritus mass. The liver cells usually manifest perifocal regressive changes, however, in the 5 day cases of CF #1 and C 57 BL/6, the cellular reaction is severe and a stratiform degeneration of liver cells can be seen at the portion of the intermediate layer of the focus; the focus becomes as large as 400~700  $\mu$  in diameter in contrast with the size of 200~300  $\mu$  in the focus of the other three animals.

Before long the host tissue reaction changes into granulation tissue formation; this stage coincides with that of initial multilocular vesiculation. Cotton rat cases indicate these changes 5 days after the inoculation, the other experimental animals within 7~10 days. In this stage, a remarkable number of so-called epithelioid cells are observed with intermingled giant cells including many LANGHANS type cells. Lymphoid cells and eosinophil cells accumulate at the peripheral portion of the granulation tissue.

Degenerative findings of the parasite itself are rarely observable. In a 4-day case of CF #1, a unilocular parasite manifested regressive change, namely—karyopycnosis of germinal cells and eosinophil cell invasion into vesicle lumen were observed. No parasites can be found in some foci irrespective of the similar character in comparison with other foci; these findings are thought to be the result of death of parasites.

As for systematic histological investigations on multilocular echinococcosis using experimental animals, only papers on the results with *Microtus pennsylvanicus* by RAUSCH (1954) and with mouse (without description of its sort of strain) by MANKAU (1955, 1956, 1957) are to be found. In RAUSCH's microtine cases, the parasite can be found in 26-hour cases and a 20-hour case shows cellular foci alone. The characteristics of accumulated cells are similar to those of the present cases, but RAUSCH describes the cell element as "irregularly-shaped leukocytes which may have their origin in the von Kupffer cells of the sinusoids" which correspond to "activated liver sinusoid endothelia" in the present paper. He offers no description on the larval membrane and states that unilocular and multilocular vesiculations

are observable in 3 and 9-day cases respectively. Results obtained by MANKAU are almost analogous to the above, however, multilocular vesicles are found in 10-day cases and appearance of fibrocytes in 6-day cases. He gives no descriptions on minute structures of the parasite in the initial stage.

The parasite, in the present experimental cases, is covered by very thin larval membrane and its distinct outline can be seen in about 4-day cases. Between the larval membrane and germinal cell mass, there exists a narrow space, however the latter occasionally shows delicate cytoplasmic projections against the former. The author could not find any descriptions about the larval membrane in the earlier literature, although it was observed clearly in the author's serial section preparations.

B. *Multiplication of Cystic Structure* The multilocular vesiculation progresses by means of exogenous budding or herniation. This phenomenon is identical with so-called exogenous daughter cyst formation as YAMASHITA et al. (1957)<sup>32)</sup> and many other investigators have stated. The term "daughter cyst" must be used only for an endogenous daughter cyst and a so-called exogenous daughter cyst is to be corrected to "an exogenous budding". In the multilocular echinococcus cyst, circumscribed exogenous protrusion takes place very frequently and finally the echinococcus manifests a complicated botryoid or coralloid aggregation of numerous minute cysts of which the lumina communicate with each other. The sizes of individual cysts are normally less than one millimeter, the majority of them about 200~300 $\mu$  in diameter and some smaller ones only 50 $\mu$ . Communicating portions among the cysts are sometimes extremely narrow and others show only a constriction. The former finding is frequently observed in microtus cases; two cysts are connected by a narrow tube-like part. The latter, on the contrary, can often be found in dba cases; it indicates a figure like the *Echinococcus polymorphus* in the unilocular echinococcus.

The exogenous budding occurs repeatedly in the multilocular echinococcus. A newly formed exogenous bud itself produces a bud while the first bud does not show enlargement and, on the other hand, the original vesicle also produces other buds—thus the multilocular vesiculation progresses complicatedly. The speed of multilocular vesiculation, however, is different according to the difference of species or strains of experimental animals. At about the 20th day after inoculation, on a section preparation, a focus of microtus cases is made up of 40~70 small cysts, cotton rat about 100 cysts, however, mouse cases usually less than 10 cysts.

With the progress of multilocular vesiculation, some cysts indicate enlargement in size, but in some portions active small-cyst multiplication takes place; the structure of a multilocular echinococcus pursues a course of remarkable

complication. These enlarged cysts have a close relation to brood capsule and scolex formation. YAMASHITA et al. (1958)<sup>35)</sup>, with present author as a co-worker, classified multilocular echinococcosis into two types. In their type 1 animals, enlarged cysts can be found in a considerably early stage, but in type 2 animals in a late. In 30, 20 and 50-day cases of microtus, cotton rat and dba respectively, enlarged cysts of more than about 3 mm are found. In CF #1 and C 57BL/6, however, a small number of cysts of 2 mm size are observed in 60 and 120-day cases respectively and appearance of cysts more than 3 mm in size is delayed further. As to initial brood capsule formation, it is first detected in a 44-day case of microtus, 20-day of cotton rat, 49-day of dba, 90-day of CF #1 and 150-day of C 57 BL/6.

As to the fact that the multilocular vesiculation is due to exogenous budding, RAUSCH and JENTOFF (1957) carried out an interesting experiment. Using three kinds of voles, they observed the propagation of the larval *Echinococcus multilocularis* in vitro and made it clear that the multilocular vesiculation occurred only as a result of exogenous budding. On the other hand in microtine cases reported by RAUSCH (1954) and mouse cases of MANKAU (1957), "endogenous budding" is described in a very early stage of development such as about 10 days after the inoculation. No detailed morphological descriptions are given, but the present author has some doubt on the existence of endogenous budding in such an early stage. He also would like to discuss the matter in the article below on daughter cyst formation, but so far as the present cases are concerned, daughter cyst formation has no relation to multilocular vesiculation and the phenomenon of daughter cyst formation might be found at a time after the scolex formation. Although the echinococcus manifests an extraordinarily complicated multilocular figure, it can be demonstrated, through studies on serial section preparations, that the multiplication is resultant from exogenous budding. It must be added that RAUSCH and JENTOFF (1957) use the term "endogenous budding" in the same meaning as "endogenous daughter cyst formation." As one of the reasons that indistinctness of a figure of exogenous budding is brought about, the present author wishes to describe a change named by him "multilocular vesiculation in closed area." In the area surrounding an echinococcus, there exists connective tissue resulting from a reaction of host tissue (adventitious tissue). Large cysts have a tendency to be encapsulated by an adventitious layer of regular thickness with dense collagenous fibers. Multilocular vesiculation by minute cysts, on the contrary, is normally noted within rather loose granulation tissue and the exogenous budding invades into this adventitious tissue. Sometimes, however, active multilocular vesiculation takes place in an area surrounded by dense adventitious layer similar to that of a large cyst. This fact means that a large cyst produces

many exogenous buddings simultaneously and, as a result, the cysts are packed closely side by side in a spherical area surrounded by an adventitious layer. The walls of the cysts are close together and sometimes a small quantity of detritus material is interposed. As the wall is very thin with scarce nuclei of germinal cells, the focus seems as if it were a large cyst with septa dividing it into many loculi and, in some sections, a structure can be seen as if a minute cyst were afloat in the lumen of a larger cyst. In these occasions, however, septum-like structure or wall-like one of the minute cyst above-stated are made up of walls of two cystic structures, namely—they show doubleness.

By reason of lacking of the cuticular layer for a definite period from the beginning of multilocular vesiculation, the wall of an echinococcus cyst is composed of the thin germinal layer alone. The cuticular layer can be demonstrated as a distinct layer of  $1\sim 2\mu$  thickness for the first time in a 26-day case of microtus, and 18-day cotton rat, 30-day dba, 30-day CF #1 and 26-day C57BL/6 cases respectively. It can be said, therefore, that the actual time of cuticular layer formation is earlier than these days. The present author's results pertaining to the time of cuticular layer formation are similar to those of other investigators; RAUSCH (1954) and MANKAU (1957) find the cuticular layer formation at about the 20th day after the experimental infection. Characteristic laminated figure is naturally invisible in an early stage.

*C. Wall of Echinococcus Cyst* The wall of a fully developed multilocular echinococcus cyst is established by the outer laminated cuticular layer without nuclei and inner germinal layer. The latter produces brood capsules and scolices.

As far as experimental cases have shown, the cuticular layer appears about 20 days after the inoculation as above stated; the layer is usually very thin. Its maximum thickness is  $12\mu$  according to RAUSCH (1954). The cuticular layer of the present cases is  $1\sim 2\mu$  in thickness at the beginning, normally about  $5\mu$  in advanced cases and rarely  $10\sim 12\mu$ . The characteristic laminated figure becomes distinct when the layer is thickened to a certain extent. The layer is stained slightly reddish and yellow by eosin and picric acid respectively. It is a clearly ascertained fact that the cuticular layer is produced by the germinal layer and, also in the present cases, there often exist findings further to support that fact. The nuclei of the germinal layer are distributed more densely in the outer portion than the inner and fine reddish granules are sometimes deposited in contact with the cuticular layer. It, therefore, can be easily supposed that the germinal cells are capable of producing the substances which compose the cuticular layer. The thickness of the latter is considerably varied having no connection with the elapsed time after infection. It has, however, a tendency to be thin when small-cystic multilocular vesiculation is active and to be thick in such

a large cyst as one where scolex formation is demonstrable.

The most interesting findings in respect to the experimental multilocular echinococcosis are observed in the germinal layer. This layer appears in an initial stage of development as a thin mono-layered wall. The germinal cells indicate as characteristics to be flattened and to have indefinite cell limits. The nucleus of a germinal cell is a chromatin-poor round structure of  $5\mu$  diameter with a distinct nucleolus, but it becomes a pycnotic nucleus of  $3\sim 4\mu$  size at the time of multilocular vesiculation. Although RAUSCH (1954) states that the cytoplasm is acidophilic, that of the present author's cases is preferably basophilic, stained pale purplish showing a fine granular character; the stainable substances are gradually decreased according to the progress of development. Distribution of the nuclei of germinal cells becomes extremely sparse as multilocular vesiculation advances, however the nuclei increase again simultaneously with thickening of the germinal layer. That layer is mono-layered and very thin at the beginning, but in advanced cases such when as brood capsule formation takes place, it gradually shows thickening to as much as  $20\sim 40\mu$ . Thickening of the germinal layer is limited to some large cysts at the beginning; it has a close relation to brood capsule formation; so-called calcareous corpuscles appear simultaneously with the thickening. The thickened germinal layer manifests a fine reticular structure with small dark-stained nuclei and before long numerous brood capsules appear which contain scolices. As the thickness of the germinal layer increases remarkably in this stage, this layer, in the end, lines the wall of large cyst as a layer of  $300\sim 800\mu$  thickness or even fully fills the lumen of a small cyst. The thickness, indeed, is influenced by density of distribution and degree of development of brood capsules; the germinal tissue is normally seen as the one which fills spaces among numerous brood capsules.

The calcareous corpuscle (laminated body, Chitinkugel) is a structure which can be invariably found in cases of experimental multilocular echinococcosis. The calcareous corpuscle shows round contour, usually laminated and contains a nuclear structure in the center. Its size is about  $20\mu$  in diameter, rarely as large as  $30\mu$ ; immature ones are small. Although it is named "calcareous corpuscle", it can hardly be stained by hematoxylin and the results of staining of calcareous corpuscles show similarity with those of staining of cuticular layer. The corpuscle, however, has a tendency to contain a calcareous substance preferably as a senile or regressive change. In the germinal tissue, there appear numerous calcareous corpuscles. The majority of them show a character of just filling up the mesh of the reticular structure of germinal tissue and, when the layer is thin, the corpuscle protrudes toward the lumen of the cyst. The calcareous corpuscles both in the germinal tissue of a cyst and the parenchymatous tissue of a scolex

are different morphologically, size and shape, but the cells of germinal and parenchymatous tissues are derived from the same origin. The author, therefore, would like to give the same physiological meaning for the present to the calcareous corpuscles of germinal tissue and scolex.

Pertaining to the animal experiments, MANKAU (1957) described the increase of thickness of germinal layer and found numerous calcareous corpuscles in cases where more than about 4 months had elapsed since inoculation. RAUSCH (1954) also reported the thickening of the germinal layer and existence of calcareous corpuscles. No sufficient discussions have been presented on the relations among appearance of calcareous corpuscles, brood capsule formation and thickening of germinal tissue, and detailed morphological descriptions were also absent in these reports. However, the existence of calcareous corpuscles has been described in reports concerning human cases of multilocular echinococcosis. The corpuscles, however, are likely to appear very rarely in human natural cases and their existence is apt to be unmentioned in text books. POSSELT (1928) who discussed in detail the echinococcus from the viewpoint of dualism also scarcely referred to this structure. As far as the present author investigated section preparations obtained from a human case\*, the germinal layer is poorly formed and merely a few calcareous corpuscles are found in a cyst which possesses some brood capsules with scolices. As the calcareous corpuscles appear rarely and the regressive changes of the echinococcus itself and host tissue reactions are complicated in chronic human cases, it can be said that many investigators have a tendency to strain the meaning of the calcareous corpuscle. MELNIKOW-RASWEDENKOW (1901) dealt with many human materials and described them in detail, but as for the calcareous corpuscle found in human cases of alveolar echinococcosis, he discussed it under the same category as his "ovoider Embryo" notwithstanding an understanding that it had better be named as "Chitinkugel" and might be homologous to the cuticular layer. MITA (1918) also believed that it might correspond to an anlage of the cyst. With regard to the calcareous corpuscle found in a fox case, ISHINO (1941) considered it should be named "laminated body", however, he regarded it histogenetically as the remains of an under-developed hydatid.

The present author would like to consider the calcareous corpuscle as a normal histological component of the multilocular echinococcus and this consideration can be easily understood in such experimental cases as those discussed in the present report. Although the germinal layer manifests certain particular characteristics in a sense, it can be naturally placed in the same category as the parenchymatous

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\* A patient from Rebus Island: Reported by RAUSCH & YAMASHITA (1957)

tissue of the wall of cysticerci and the scolex. It, therefore, is not strange that the calcareous corpuscles are found as a normal structure in the germinal tissue of the multilocular echinococcus.

D. *Brood Capsule and Scolex Formation* The scolex formation takes place in the brood capsule without fail and the latter is formed in the germinal tissue layer. The time at which brood capsule formation begins varies according to the taxonomical differences of host animals, namely—brood capsule formation is found for the first time in 44-day case of microtus, 20-day case of cotton rat, 49-day case of dba, 90-day case of CF #1 and 150-day case of C 57 BL/6. Development thereafter is also different by animal species or strain. Some animals require a long time before fully developed brood capsules and scolices can be found and others need a short period for simultaneous appearance. More specifically, microtus cases of 53~60 days have numerous completed brood capsules with scolices, cotton rat needs the same number of days as microtus for completion after a considerably long period when the mature and immature types of brood capsules are mingled. Among mouse hosts, 60~90 day cases of dba strain exhibit brood capsules and scolices with some mature ones and 150-day cases have numerous mature ones. In CF #1 cases less than 5 months after the inoculation, on the other hand, the brood capsule formation has scarcely takes place and, if any, the majority are still immature. Five-month cases of C 57 BL/6 mice show brood capsule and scolex formation for the first time and mature scolices can be seen in the cases of more than 7 months.

YAMASHITA et al. (1958)<sup>35)</sup> investigated the susceptibilities of various rodent animals to multilocular echinococcosis as already stated. Classifying the present cases according to their method, microtus and dba manifest type 1 lesions and CF #1 and C 57 BL/6 type 2. Even if the analysis is restricted to the brood capsule and scolex formation, the differences can be recognized, but it is an undeniable fact that dba cases manifest an under-developed state. RAUSCH (1954) found the brood capsule formation in *Microtus pennsylvanicus* cases of more than 34 days and their number was great in a 5-month case. MANKAU (1957), on the other hand, could observe the formation of brood capsules and scolices in mouse cases of more than 4 months. As to the observations by VOGEL (1955)<sup>24)</sup>, 5-month cases of *Microtus oeconomus* showed many brood capsules with scolices, although 3-month cases of mice had no brood capsule according to his figures and description.

The brood capsule formation begins from an accumulation of germinal cells in the germinal layer. Dark-stained nuclei accumulate densely and form a clearly demarcated focus of about 40  $\mu$  diameter. The focus is usually embedded and rounded in the germinal layer tissue where the layer is thick, but in thin layer, it is recognized as a hill-like thickening or a remarkable projection into the cyst

cavity. This accumulation of nuclei is followed by the formation of a minute lumen in its central portion and the focus grows into a vesicular structure of  $60\sim 80\mu$  size enclosed by a thick wall with enlargement of the lumen. A thin layer of cuticle is formed on the inner surface of the wall. Sometimes the nuclei of germinal cells accumulate irregularly in contact with the calcareous corpuscle, but the author considers this finding has no relation to the brood capsule formation. The anlage of a scolex appears with changes of brood capsule while the lumen enlarges, the wall is thickened partially and the distribution of nuclei of other portions becomes loose. The initial stage of a scolex manifests a dense accumulation of nuclei, it projects into the lumen of the capsule as a hill-like, spherical and then ellipsoid structures successively. The nuclei are distributed more densely at the outer portion of the scolex than the inner. The immature scolex develops to a mature form in company with the brood capsule. The completely developed scolex normally shows an ellipsoid invaginated form; its major axis is  $150\sim 170\mu$ ; it attaches to the capsule wall by a short peduncle of one end and is covered by a thin cuticle layer. A tubular structure is produced owing to the invagination of the scolex and the tubule invades into the central portion of the scolex along the major axis from the end opposite to the peduncle. The bottom of the tubule, the interior part, is widened and provided with hooklets. The suckers are found around the tubule. The parenchymatous tissue is delicately reticulated in which dark-stained minute nuclei and ellipsoid-shaped calcareous corpuscles are scattered; the latter are about  $10\mu$  in size. The size and shape of fully developed brood capsules varied according to the number of scolices contained; usually they were  $200\sim 300\mu$  in diameter and sometimes more than  $600\mu$ . The wall of a brood capsule is very thin with sporadically distributed nuclei and the inner surface is lined with thin cuticle extended from that of the scolex. The number of scolices contained in one brood capsule is  $1\sim 2$  in early stage, 5 or so in fully developed and on rare occasion more than 10. The brood capsule is normally embedded in the germinal tissue where it offers an appearance as if a hole existed in the germinal tissue.

The brood capsules and also scolices are formed in an enlarged cyst for the first stage, however, with multiple formation of completed structures, they become found also in minute cysts. The lumen of small cysts is frequently filled by one brood capsule and in some cases a budding with the brood capsule and scolices can be noted.

Investigators in the past have tended to pay no attention to the close relations between the scolex and brood capsule and the latter and germinal tissue respectively. The present author considers that MANKAU (1957), however, was considerably interested in the relations of brood capsule and germinal layer. ISHINO

(1941) reported a natural fox case of multilocular echinococcosis, in which he seemed to take up too much space with discussions on analogy with findings of human cases. He, therefore, made no valuable contribution in regard to normal brood capsule and scolex formation and on the relation between these phenomena and germinal layer. MELNIKOW-RASWEDENKOW (1901), on the other hand, with respect to his human cases supposed some structural analogy between the larval multilocular echinococcus and adult echinococcus tapeworm. He attached importance to "ovoid Embryo" as a structure which played an important role in multilocular vesiculation. His ovoid embryo, however, contained various structures such as calcareous corpuscles, endogenous daughter cysts etc. He pointed out the ovoid embryo as an important structure which could be explained as having invaded into the surrounding host tissue and developed into the hydatid at the time of multilocular vesiculation. The present author considers that MITA also followed the opinions of MELNIKOW-RASWEDENKOW.

With respect to human cases of multilocular echinococcosis, it can be said, in short, that discussions has seemed always to be limited to secondary changes of chronically progressed cases. The author considers that there has been available only poor knowledge about the morphological characteristics which are observable at the stages previous to the secondary changes. There has been, on the other hand, plentiful knowledge obtained from experimental and natural cases of unilocular echinococcosis, but it may be reasonable to conclude that to explain the findings of multilocular echinococcosis by means of results from the unilocular has been unreasonable.

E. *Regressive Changes* The term "regressive change" in this chapter is that of the echinococcus itself. Generally speaking, the regressive changes of an echinococcus in experimental cases are found very rarely except in those of cotton rat. The cases dealt with in this report, indeed, are those less than 5~7 months after the inoculation except for one C 57 BL/6 case of 21 months. It needs to be taken into consideration that there are no other cases with so long duration after the inoculation. RAUSCH (1954) and MANKAU (1955, 1956, 1957) described nothing of regressive changes of echinococcus tissue in their experimental animal cases, however in their cases a not very long time had elapsed after the inoculation—it is a reasonable result. It must also be a not negligible fact that the majority of experimental cases, especially those of animals which manifest type 1 lesions of multilocular echinococcosis, do not show any remarkably long clinical course on account of the intense development of the echinococcus with lethal termination of the life of the host animal.

Existence of fluid substance in the lumen of an echinococcus cyst is easily recognizable by the naked eye and, therefore, it can be supposed that the lumen

of the brood capsule also contains fluid. Any stainable substance is unrecognizable within the above-noted lumina in normal cases, but a stainable substance appears with the appearance of regressive changes of an echinococcus; these findings are remarkable in cotton rat cases. Among the structures which make up the echinococcus, the scolex manifests regressive findings considerably frequently. The changes are noted after the completion of scolices. Necrobiosis, necrosis and dissolution of the scolex and falling of hooklets can be seen. These changes, however, are not very conspicuous and the brood capsule tends to contain stainable fluid substance in its lumen. A 21-month case of C 57 BL/6 showed wide-spread necrotic area where severe calcification had taken place. In short, it can be said easily that in experimental cases the regressive changes of an echinococcus are rarely found while the multilocular vesiculation is progressing.

As for cotton rat cases, the regressive changes of an echinococcus are particularly remarkable findings; these changes have a close relation to the daughter cyst formation described below. To be brief, the regressive change of cotton rat case is "pseudo-retention cyst formation" or "collapse" of echinococcus cyst. That is to say, the wall of cyst desquamates from fibrous adventitious layer and the echinococcus tissue manifests necrobiotic or necrotic change. These findings bear some resemblance to those of a retention cyst, so that the author describes it under the term pseudo-retention cyst formation. This phenomenon is found in cotton rat cases alone except for a part of the microtus cases; in cotton rat, it is frequently demonstrable in cases more than about 30 days after the inoculation. In cotton rat, large cysts of more than 5 mm appear from about 20 days after the inoculation and thereafter large cystic structures of as much as 20 mm diameter are also found. In a portion of these structures, desquamation between the adventitious layer composed of connective tissue and the wall of the echinococcus cyst is observed. The desquamation can occur both widely and partially; the space between the two tissue caused by desquamation retains homogeneous eosinophil fluid with mingled histiocytes derived from the inner area of the adventitious layer. In some cases, desquamation between the cuticular layer and germinal layer takes place. Regressive cyst wall manifests undulation or, sometimes, destruction and floats in the fluid contents like trabeculae or flaps. These findings can be interpreted as collapse of the cyst. The cyst wall itself manifests necrobiotic or necrotic changes; the cuticular layer becomes homogeneous and the germinal layer is led into edematous soakage. In some cases, the whole area of the germinal layer falls into necrosis, tissue structures are seen shadily and hooklets of scolices remain, but in a severe case the tissue changes into a necrotic detritus mass. The lesion of germinal layer is conspicuous, namely —with the edematous change, there can be seen numerous hyalinous

globules of about  $20\mu$  size. The globules are considered to be derived from calcareous corpuscles. The brood capsule shows irregular form, frequently enlarges to as much as  $500\sim 800\mu$  in diameter, contains stainable fluid substance in the lumen; the scolex has a strong tendency to dissolve and disappear. The brood capsule which shows above-stated changes has an inner cuticular layer and outer germinal layer. As the composition is opposite to that of the daughter cysts, the author named this regressive brood capsule a "pseudo-daughter cyst." The pseudo-daughter cyst is apt to be mistaken for a daughter cyst.

As to the genesis of pseudo-retention cyst formation or collapse of echinococcus cyst in cotton rat, the author cannot make a clear statement. However, the phenomenon is supposed to have close relations to the following facts:—the multilocular echinococcus of cotton rat shows extremely rapid development as compared with that of other experimental animals, it provokes remarkable abdominal expansion caused by filling of the abdominal cavity and many large cysts are produced. For example, one animal died of torsion of a hepatic lobe in which an enormously enlarged cyst was located. In an area where an enlarged cyst exists as above-noted, the adventitious layer is fibrous but as very thin as  $10\sim 30\mu$ . The adventitious layer is undoubtedly in a state liable to manifest circulatory disturbances on account of pressure of cysts upon each other and of extreme thinness of the layer. It, therefore, is considered that the echinococcus falls into anoxia easily in cotton rat and thus collapse takes place. In small cysts, the adventitious layer is considerably thick, the communication among lumina of cysts is hardly disturbed by pressure and, consequently, regressive changes are scarcely found.

F. *Daughter Cyst Formation* There are many reports in which daughter cyst formation is discussed, however almost all of them deal with the problem as regards the unilocular echinococcus. Knowledge on daughter cyst formation of the multilocular echinococcus seems to be insufficient.

That multilocular vesiculation of the multilocular echinococcus results from exogenous budding has already been stated in this paper. The daughter cysts were classified into endogenous and exogenous ones in the past; however, it is well-known that the latter is not a daughter cyst in a narrow sense but is only an exogenous budding. Consequently, the term "daughter cyst" must be used for so-called "endogenous daughter cyst" alone.

DEW (1926), in his paper concerned with the unilocular echinococcus, described the daughter cyst formation and concluded that such a cyst is formed only endogenously from the germinal layer and brood capsule usually and can be derived from the scolex rarely. He presented his discussions from the viewpoint of monism; his materials are obtained from specimens of unilocular echinococcus.

Accordingly, in order to make clear whether or no his conclusion is equally applicable to the multilocular echinococcus, it seems that further knowledge is needed. As far as the results obtained from cotton rats are concerned, indeed, the author would like to attach importance to the endogenous daughter cyst derived from the scolex. No daughter cyst formation was found except in cotton rat cases.

It goes without saying that multilocular vesiculation is a result of exogenous budding. POSSELT (1928) also lay emphasis on this fact from a dualistic point of view, although he states at the same time that no daughter cyst formation can be seen in multilocular echinococcosis. Through surveying of reports pertaining to human cases of multilocular echinococcosis, however, some descriptions can be found in which daughter cyst formation originating from scolices is supposed. For instance, among the structures which were described as "embryo" by MELNIKOW-RASWEDENKOW (1901), a structure which can be thought to be transformed from a scolex was found together with calcareous corpuscles and immature or mature scolex. His embryo seems to have a close relation to metastasis.

Speaking of the report by DEW (1926), he concluded that the germinal layer and brood capsule play important roles in daughter cyst formation. His conclusion, however, is inapplicable to the present work pertaining to cotton rats.

Although a number of pseudo-daughter cysts are recognized in the present author's cotton rat cases, it can be said that these structures do not develop into daughter cysts. Judging from the composition of a pseudo-daughter cyst, this conclusion is acceptable; the cuticular layer lines the inner surface contrary to a true daughter cyst. The pseudo-daughter cysts are embedded usually in the germinal layer tissue. They occasionally contain degenerative or dissolutive scolices during the time of formation and later there are found no structures except for fluid substance in the lumen. Sometimes they remain free with loosening or dissolution of germinal tissue and degenerative changes appear. Finally, the pseudo-daughter cysts fall into degeneration and disappearance. As to the causal genesis, the author can conclude that the pseudo-daughter cyst is formed as a result of collapse of the echinococcus cyst as a degenerative change.

The process of development of daughter cysts from scolices can be traced morphologically. Daughter cysts are found after the time when scolices have fully developed and in the area where collapse of the echinococcus cyst or formation of pseudo-retention cyst is observed. Such a cyst is found existing freely or embedded in degenerated germinal tissue; sometimes it is attached to necrosed tissue which originated from germinal layer. At whatever time, the wall of a daughter cyst is composed of thin outer cuticular and inner germinal layers. The latter layer, with progress of development, might produce calcareous

corpuscles and brood capsules which contain scolices just like the mother cyst. The scolex manifests swelling and loosening of its parenchymatous tissue for early metamorphic change and thereafter lumen formation in the central portion, falling of hooklets, etc. are seen until the daughter cyst is fully formed. Some scolices in early developmental stage of a daughter cyst are yet recognizable in brood capsule, however others exist free. At any rate, daughter cyst formation can occur in cotton rat cases more than about 2 months old after the inoculation.

It can be concluded that daughter cyst formation in multilocular echinococcus is a rare finding except for cotton rat cases and that daughter cysts are formed starting from a part of existing scolices in company with regressive changes of the echinococcus cyst.

G. *Metastasis* It is well-known fact that metastatic multilocular echinococcosis is a common occurrence in human cases. As to metastases of animal cases, THOMAS et al. (1954) described a natural case of *Microtus oeconomus*, RAUSCH (1954) experimental cases of *Microtus pennsylvanicus* as above stated and YAMASHITA et al. (1958)<sup>35)</sup> experimental cases of *Clethrionomys rufocanus bedfordiae*. Generally speaking, it can be said that primary foci of echinococcosis are limited to the liver in almost all experimental cases and metastatic development is very rare.

MANKAU (1956) found primary foci developed not only in the liver but in other organs and stated that brood capsules, scolices or other germinative parts of the primary hydatid cyst are carried to another site by the lymph or blood system. He also concluded that brood capsules, scolices and germinal membrane derived from the intraperitoneal rupture of a primary or a secondary cyst are implanted at new infection sites. The present author, as far as his materials are concerned, came to the conclusion that primary echinococcus foci can be found only in the liver and metastasis must be classified as a very rare phenomenon except for cotton rat cases.

About a half of cotton rat cases which are more than about 50 days old after the inoculation manifest metastatic foci in the present cases. The metastatic foci are localized mainly in the greater omentum and there are recognizable accumulated or scattered minute cysts showing granular appearance. The cysts, histologically, are surrounded by a poorly proliferated adventitious layer and the cuticular and germinal layers are thin. It is an interesting fact in evidence of metastasis that development of the parasite in the greater omentum shows an earlier stage as compared with that in the liver; some cysts still remain in a unilocular stage and others have immature brood capsules contrary to a primary focus with fully developed brood capsules and scolices. At any rate, it seems that no metastases can be observed until mature hepatic foci of primary nature

accompanied by fully developed scolices are clearly established and a characteristic collapse phenomenon occurs. It cannot be asserted that daughter cysts derived from scolex or intact scolices play a role in the production of metastatic foci. Metastases of both origins, however, can be supposed, because the matter has close relation to the time of daughter cyst formation in the liver. Moreover, the scolex, as observable in experiment of secondary echinococcosis, can develop to echinococcus focus when introduced into the abdominal cavity. As to the route of the metastasis, it is better to consider it a contact metastasis judging from extensive development of the echinococcus in the liver and the anatomical relation between the liver and greater omentum.

H. *Changes of Host Tissue* Changes of host tissue can be classified into two groups: productive changes of connective tissue as a reaction against the development of echinococcus and changes of hepatic parenchymatous tissue around a focus. The former changes are simultaneously accompanied by reactive accumulation of cell elements and degenerative changes of both connective tissue and accumulated cells. There exist essentially no differences of host tissue changes among experimental animals, namely—they can be explained in the same category; however, considerably remarkable variations are found according to animals in combinations of histological components and mode of progression.

Changes of host tissue between the initial focus formation and the beginning of multilocular vesiculation have been described above. And it seems that no particular differences are found between the present author's findings of host tissue changes and those of other investigators' such as RAUSCH (1954) and MANKAU (1956, 1957) when descriptions of experimental cases are compared.

Although some variations are found according to host animals, to state it briefly, foci of cell accumulation are established and cell elements derived from the reticulo-endothelial system of the liver play the leading role accompanied by some emigrated cells. Thereafter the cell reaction alternates with chronic granulation tissue reaction. Granulation tissue is found in abundance without formation of a layer of definite thickness and the echinococcus is embedded in granulation tissue while small-cystic multilocular vesiculation is taking place. With large cyst formation in some portions, however, the large cysts come to be surrounded by a circular layer of connective tissue which is not very thick. During these changes, epithelioid cells are found conspicuously in the granulation tissue frequently accompanied by giant cells of which the majority are of LANGHANS type. Especially in the outer portion of the granulation tissue, dense cell accumulation composed of lymphoid cells, plasma cells and eosinophils can be observed nearby the parasite and sometimes lymphocytes are conspicuous. Reaction tissue manifests remarkable regressive changes in contact with the

parasite and a necrotic layer is produced. The necrotic layer is a detritus mass derived from accumulated cells or granulation tissue having fallen into necrosis and the detritus mass belongs to a production in earlier stage.

It has already been stated above that multilocular vesiculation takes place being embedded in a detritus mass of necrosed cells in *microtus* cases. Surrounding the detritus mass, immature granulation tissue with giant cells proliferates and lymphoid cell accumulation is seen. The necrosed cell mass exists in cases of less than 20 days and afterwards decreases gradually until about the 30th day after inoculation when the granulation tissue itself falls into tissue necrosis. A layer of epithelioid cells with giant cells can be demonstrated at the inner portion of adventitious layer which encloses a large cyst, but this epithelioid cell layer disappears in cases of about 5 months. The granulation tissue in which the group of small cysts is contained shows comparatively invariable progression accompanied by central tissue necrosis, cell accumulation and occasional calcification. In cotton rat cases, granulation tissue reaction is by far less than that in *microtus* cases. The granulation tissue around small cysts is poor and the adventitious layer enclosing a large cyst is very thin as 10~30  $\mu$  in most cases. Tissue reaction in contact with an echinococcus is also found frequently, but appearance of detrited cell mass is almost insignificant. Consequently, at the time of initial multilocular vesiculation, cystic structures or buddings pass through immature granulation tissue in which epithelioid cells are conspicuous and some buddings come in contact with hepatic parenchyma directly. Findings of *dba* cases are approximately identical to those of *microtus* and the initial multilocular vesiculation or exogenous budding occurs in a detrited cell mass, however reactions of adventitious tissue are more predominant than those of *microtus* in quality and quantity.

As compared to above-stated three animals which show type 1 of YAMASHITA et al. (1958)<sup>35)</sup>, CF #1 and C57BL/6 mice, type 2, manifest granulation tissue reaction of higher degree; abundant adventitious tissue with conspicuous cell accumulation is found and tissue necrosis at the central area is expanded, although the two types are of the same nature. The thickness of adventitious tissue layer of the large cyst is as much as 100~200  $\mu$  in 5-month cases of CF #1 but is generally not so thick as in C57BL/6. Detritus masses can be detected in contact with cyst wall even in animals of chronic course such as 4 or 5 months after the inoculation.

Contrary to granulation tissue reaction, changes of hepatic parenchyma are comparatively uncomplicated. Degeneration of liver cells is found in the initial stage of focus formation, but pathological changes are hardly demonstrated in the liver cell cords except for pressure atrophy at the time when adventitious

layer of granulation tissue is produced. In some cases, however, groups of liver cells or branches of hepatic duct are found showing an island-like appearance in adventitious tissue. Slight pericellular cirrhosis can be found rarely in the area adjacent to echinococcus focus. Circulatory disturbances are remarkable in lethal cases. It can be said that degenerative changes as above-stated are not very severe and one of the cause of mild parenchymatous changes is slow development of the echinococcus. Even in cases in which the liver seems to be replaced totally by echinococcus tissue, the total volume of remaining island-like liver parenchyma is not so decreased; it can be explained that compensatory hypertrophy of liver parenchyma takes place on the one hand.

I. *Comparison of Unilocular and Multilocular Echinococcoses* Discussions on the differences between the unilocular and multilocular echinococcoses were presented in detail by many investigators from the monistic and dualistic points of view in the past. With regard to monographic papers, one can find the report of DEW (1953) as a monistic work and POSSELT (1928) as a dualistic. However, it is regrettable that materials of multilocular echinococcosis were restricted to human cases and no results of experimental echinococcosis were reported. This is why clarification of the life cycle of the multilocular echinococcus was expected.

Dualism has been settled recently. The morphological differences of adult tapeworms have been adduced as proof of dualism by RAUSCH (1952, 1953, 1956), RAUSCH and SCHILLER (1954), VOGEL (1955, 1957), YAMASHITA et al. (1956<sup>30</sup>), 1958<sup>34,36</sup>) and YAMASHITA (1959); these investigators discussed the matter in detail. As the present paper is concerned with clarification of larval stage, the writer would like to discuss mainly the morphological findings of the echinococcus in larval stage.

The relation between human and animal cases of multilocular echinococci have to be taken into consideration at the time of comparison of the unilocular and multilocular echinococci. *Echinococcus multilocularis*, of which the suitable intermediate hosts are voles such as *Microtus*, is undoubtedly the causal worm of typical human multilocular echinococcosis on the other hand. This fact is obvious through the results obtained by VOGEL (1957). At the time of comparing the findings of experimental animal case of multilocular echinococcosis with those of human case, however, one would feel as if both cases were provoked by different causal worms, namely—the morphological findings of the two echinococcoses are different. The multilocular echinococcus of voles, contrary to human cases, shows a poor cuticular layer, remarkably well-developed germinal layer and numerous brood capsules with scolices. Consequently, characteristics of human multilocular echinococcus, which were considered as important particulars for differentiation from unilocular echinococcus in the past, need to be reconsidered. For instance, POSSELT (1928) states that multilocular echinococcus cyst contains

no fluid substance, scolex formation is very rare, daughter cyst formation does not occur and so on. The present author's experiment on voles, however, yielded results opposite to POSSELT'S.

Among experimental animals, some animal strains or species are found which manifest findings of echinococcus of the same category as human case; through the precise investigation, it becomes clear that the multilocular echinococci of human and animal cases should have no essential difference.

The multilocular and unilocular echinococci of human cases are quite different in their morphological characteristics of larval stage. The similar fact was found by YAMASHITA et al. (1956<sup>31</sup>), 1958<sup>35</sup>) in cases of dd mice, namely—dd mouse has susceptibility to the two echinococci and the infection rates are both about 10%, but the unilocular shows a simple spherical cyst and the multilocular manifests type 2 similar to the human type.

It can be said that animals show opposite susceptibilities to the two echinococcus species in general. And animals, which are susceptible to both simultaneously but rarely, manifest the larval echinococci of quite different morphological characteristics. The author would like to consider the differences of the two below.

Susceptibilities of host animals to the multilocular were investigated by RAUSCH, YAMASHITA, VOGEL and others. Especially reports by RAUSCH and SCHILLER (1956), VOGEL (1955)<sup>24,25</sup>) and YAMASHITA et al. (1958)<sup>35</sup>) dealt with this problem. Shortly speaking, animals susceptible to the unilocular echinococcus are those belonging to ungulate represented by ruminants and, on the other hand, those susceptible to the multilocular are rodent represented by murines, although infection rates are various. Hosts which are simultaneously susceptible to both species such as mouse and man are found. It can be concluded that the above-stated differences of susceptibilities to the two echinococci are one of grounds to support dualism.

As to morphological differences of larval stage echinococci, the author wishes to restrict discussion to two or three points, although they can be discussed from various viewpoints. Further he would like to add that the presence or absence of scolices, daughter cysts, metastatic foci, etc. is considerably varied according to the host species or strains. The differences of these findings, therefore, give no absolute ground for differentiation between the unilocular and multilocular.

In experimental cases of multilocular echinococcosis, unilocular vesiculation can be demonstrated 3~4 days after inoculation for the first time and multilocular vesiculation 5~10 days. DEW (1925) states that vesiculation takes place 14 days after the inoculation in experimental unilocular echinococcosis of swine. The unilocular echinococcus of sheep manifests in general a shape of so-called

*Echinococcus polymorphus* and this shape is also produced by exogenous budding morphogenetically the same as an initial multilocular vesiculation of multilocular echinococcus. DENAPOLITANO et al. (1953) found no exogenous budding in ovine cases until about one month had elapsed and in experiment of sheep by YAMASHITA et al. (1957)<sup>32)</sup> a few of exogenous buddings were detected in 4 month cases. Consequently, it can be concluded that the vesiculation of multilocular echinococcus begins from very early stage as compared with the unilocular and that exogenous budding of the unilocular, even if it might occur, takes place after some delay.

Active multilocular vesiculation by exogenous budding can be found in the multilocular echinococcus and a number of buds are produced from which new formation of exogenous buds, while an original bud does not so enlarge. Through the repetition of this process of multiplication, an echinococcus composed of minute cysts of less than 1 mm is formed. The majority of unilocular echinococci, however, increase in size with a spherical shape and, even in cases of polymorphous echinococcus, a bud enlarges parallel with enlargement of the original cyst without repetition of active successive buddings. Therefore, it is obvious macroscopically that lumina of cysts of polymorphous echinococcus in sheep communicate with each other and the communicating portions are normally not very narrow.

The above-mentioned relation is easily comprehensible on the grounds of the experiment on dd mice by YAMASHITA et al. (1956<sup>31)</sup>, 1958<sup>35)</sup>). Their cases of unilocular echinococcus developed to a spherical cyst of 10 mm in about 3 months when the cuticular layer was 20~40  $\mu$  in thickness and the nuclei of germinal layer were scattered sporadically in one layer. In a 4-month cases of the multilocular echinococcosis, contrary to the unilocular, the echinococcus focus occupied an area of about 3 mm in diameter and was made up by a number of very minute cysts of generally 200~300  $\mu$  size, the cuticular layer was only 5~10  $\mu$  in thickness and the focus was embedded in abundant connective tissue of which the central portion fell into tissue necrosis. In short, dd mouse manifests echinococcus foci the same as human cases.

Finding which attract attention of the author in the present experimental cases of the multilocular echinococcosis are those concerned with the germinal layer. Those findings are observed frequently especially in cases of active brood capsule and scolex formation. At the time when some of minute cysts begin to enlarge, the germinal layer of enlarged cyst starts to be thickened accompanied by initial brood capsule formation. Brood capsules with scolices are embedded in thickened germinal layer the maximum thickness of which often reaches to 600~800  $\mu$ . By reason of these findings, it can be said easily that so-called hydatid sand formation found in the unilocular echinococcus cannot be produced in the multilocular. In practice, the author experienced the fact that to make inoculum

with scolices for secondary echinococcosis experiment was not so easy contrary to the case of unilocular. Calcareous corpuscles are the very characteristic structure and they are produced numerously with thickening of the germinal layer. Although calcareous corpuscles are inconspicuous in an area where small-cystic multilocular vesiculation is taking place, they are scattered numerously in the germinal layer accompanied by brood capsule formation. The appearance of calcareous corpuscles is inconspicuous in human multilocular echinococcosis and, therefore, the corpuscle has been likely to be neglected by many investigators, although the present author can detect it in his material. It, however, seems to be difficult to differentiate the multilocular and unilocular echinococci of human materials simply by means of presence or absence of calcareous corpuscles. The presence of calcareous corpuscles in the germinal layer of unilocular echinococcus is doubtful. Although the germinal layer of the unilocular seems to show no thickening, the findings thought to be calcareous corpuscles can be seen in photographs attached in some text books, but no descriptions are found. FISCHER (1930) states that at the outer portion of the germinal layer the calcareous corpuscles are found and very frequently in the multilocular echinococcus in human cases. As far as the present author investigated, there could be found no calcareous corpuscles and the germinal layer was very thin in unilocular echinococcosis of sheep and cattle. Regarding the unilocular echinococcus, it, therefore, can be said without doubt that the germinal layer is not so thick and discovery of calcareous corpuscles is very difficult.

POSSELT (1906, 1928) attached importance to the morphology of the scolex, especially that of hooklets as to differentiation and VOGEL (1957) and YAMASHITA et al. (1958)<sup>36)</sup> also investigated in detail about the hooklets.

The author would like to conclude that both the unilocular and multilocular echinococci have characteristics by which differentiation can be done in their larval or hydatid stage as it can be in adult stage. He considers, however, that synthetic investigations are needed in respect of the larval stage.

#### SUMMARY AND CONCLUSION

Experimental multilocular echinococcosis caused by *Echinococcus multilocularis* LEUCKART, 1863 was investigated histologically as respects microtus, cotton rat and mice (dba, CF #1 and C 57 BL/6 strains). Results obtained are summarized as follows.

Unilocular vesiculation, multilocular vesiculation, cuticular layer formation, brood capsule and scolex formation, etc. take place successively after the inoculation. The periods after the inoculation needed for manifestation of these phenomena are systematized together with host tissue reactions according to

species or strains of experimental animals. It was redemonstrated that the combination of larval echinococcus and host tissue reaction can be classified into two types.

Initial echinococcal foci are localized in the liver and noticeable to the naked eye from the 5th day after the inoculation. The focus, however, can be demonstrated microscopically in one day cases as an accumulation of histiocytes derived from the reticulo-endothelial system of the liver with some intermingled degenerated liver cells and leukocytes. At the central portion of the focus, there exists a parasite which possesses a larval membrane; the parasite manifests unilocular vesiculation in 3~4 day cases.

In cases of 5~10 days after inoculation, multilocular vesiculation takes place when the larval membrane disappears. Cuticular layer formation starts in the 18~30 day case. The animal which manifests these findings in the earliest stage is the cotton rat. Multilocular vesiculation is attributed to exogenous budding and the parasite begins to manifest a complicated form, however the speed of vesiculation varies by animal species or strains.

Brood capsule formation of microtus, cotton rat and dba mouse, type 1, is recognized within 50 days cotton rat being the earliest at 20 days. Contrary to type 1 animals, CF #1 and C 57BL/6 mice, type 2, show brood capsule as much later as 3~5 months. The brood capsule is noticed for the first time in an enlarged cyst with thickened germinal layer and scattered calcareous corpuscles. The brood capsule starts from a dense accumulation of germinal cells in the germinal layer tissue and, after sack formation, terminates in scolex formation. The brood capsule is usually embedded in the germinal tissue. The calcareous corpuscle is spherical, about  $20\mu$  in size and manifests laminated figure. It is derived from the germinal cell and contains no calcareous substance at the early stage. The calcareous corpuscle is considered to be a normal element.

In cotton rat cases, some enlarged cysts frequently manifest desquamation between the cyst wall and adventitious layer, retention of fluid substance, regressive changes of the tissue of cyst wall, pseudo-daughter cyst formation of the brood capsule, etc. These changes are summarized as pseudo-retention cyst formation or collapse. In respect to the cause, the author attaches importance to circulatory disturbances due to extremely rapid development and, consequently, anoxia.

Daughter cyst formation in the true sense can be found in cotton rat alone. As it is noticeable only in the area of pseudo-retention cyst, the close relation to regressive changes of echinococcus cyst must be taken into consideration. The process of daughter cyst formation starting from the scolex is traceable and it can be denied that other structures such as brood capsule and germinal layer

participate to the formation.

Metastasis is observed almost limited to the cotton rat cases. Cotton rats manifest metastases in high ratio later than the stage of scolex completion and it is considered to be a metastasis which originated from scolices.

Host tissue changes after the multilocular vesiculation are those of chronic granulation tissue reaction and, at the same time, necrotic changes are conspicuous. The appearance and course of reaction show differences according to host animals. Regressive changes of parenchymatous cells of the liver are not very remarkable. In some cases, multilocular vesiculation is found in a closed area surrounded by adventitious connective tissue layer.

It has been recently clarified that the multilocular and unilocular echinococci are caused by *Echinococcus multilocularis* LEUCKART, 1863 and *E. granulosus* (BATSCH, 1786) respectively on the basis of investigations on adult tapeworms and susceptibilities of intermediate hosts. In the present paper, the materials are studied from the histological viewpoint and it is concluded that the above two species can be differentiated also in their larval stage through synthetic consideration of characteristics such as mechanism of multilocular vesiculation, findings of the cyst wall, especially the germinal layer, and others.

The author could recognize again the fact of the existence of two types stated in the preceding paper, namely—parasitic lesions manifest differences according to differences of host animals. Among many items of knowledge obtained, existence of larval membrane in the initial stage of development, characteristics of the germinal layer, pseudo-retention cyst formation, pseudo-daughter cyst formation, daughter cyst formation derived from the scolex, etc. are thought to answer the problems which had remained at the termination of the preceding study.

The author wishes to express his cordial thanks to Prof. S. YAMAGIWA, Department of Veterinary Pathology, Prof. K. TAKAHATA, Department of Veterinary Anatomy and Prof. J. YAMASHITA, Department of Parasitology, for their direction of this study and review.

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EXPLANATION OF PLATES

All photographs except for figs. 7, 34 and 35 are those of hematoxylin-eosin stained materials.

PLATE I.

- Fig. 1. Host C57 BL/6, 1 day after the inoculation. Magnification  $\times 650$ . Cell accumulation in initial focus.
- Fig. 2. Cotton rat, 1 day,  $\times 210$ . A parasite in loose nodule composed of reticulo-endothelial cells.
- Fig. 3. dba, 1 day,  $\times 650$ . Loose cell focus with a parasite: liver parenchyma intact.
- Fig. 4. *Microtus*, 3 days,  $\times 650$ . Initial unilocular vesiculation.
- Fig. 5. C57 BL/6, 5 days,  $\times 650$ . A parasite with larval membrane embedded in dense cell accumulation; initial unilocular vesiculation.
- Fig. 6. C57 BL/6, 4 days,  $\times 110$ . Thrombosis and cell accumulation of a branch of portal vein.
- Fig. 7. Cotton rat, 10 days, v. GIESON stain,  $\times 320$ . Intima granuloma formation of a branch of portal vein.
- Fig. 8. *Microtus*, 10 days,  $\times 160$ . Active multilocular vesiculation in detrited cell mass; communication of cysts by very narrow tube-like portions.

PLATE II.

- Fig. 9. Cotton rat, 10 days,  $\times 160$ . Multilocular vesiculation by exogenous budding; some cysts pass through histiocytic cell layer and invade surrounding tissue.
- Fig. 10. Cotton rat, 18 days,  $\times 50$ . Multilocular structure with some enlarged cysts; slight host tissue reaction.
- Fig. 11. dba, 30 days,  $\times 70$ . Conspicuous granulation tissue formation with central regressive changes; inconspicuous multilocular vesiculation.
- Fig. 12. CF #1, 10 days,  $\times 160$ . Initial multilocular vesiculation; necrosed liver parenchyma in the center.
- Fig. 13. C57 BL/6, 90 days,  $\times 70$ . Progressed multilocular vesiculation with remarkable granulation tissue reaction; exogenous budding of large cyst.
- Fig. 14. *Microtus*, 26 days,  $\times 320$ . Initial cuticular layer formation.
- Fig. 15. *Microtus*, 44 days,  $\times 160$ . Early stage of brood capsule formation.
- Fig. 16. *Microtus*, 53 days,  $\times 160$ . Well-developed germinal layer tissue with brood capsule formation.

PLATE III.

- Fig. 17. *Microtus*, 103 days,  $\times 70$ . Numerous brood capsules with scolices embedded in germinal tissue; small cysts are quite filled with germinal tissue.
- Fig. 18. Cotton rat, 30 days,  $\times 70$ . Many immature brood capsules with scolices; poor adventitious tissue.

- Fig. 19. CF #1, 150 days,  $\times 320$ . Brood capsule is embedded in germinal tissue and contains a scolex.
- Fig. 20. C57 BL/6, 150 days,  $\times 70$ . Multilocular echinococcus accompanied by brood capsule and scolex formation; degeneration of granulation tissue in the central portion.
- Fig. 21. CF #1, 150 days,  $\times 70$ . Multilocular vesiculation in a closed area surrounded by fibrous adventitious layer.
- Fig. 22. C57 BL/6, 5 days,  $\times 160$ . Conspicuous stratiform degeneration of liver parenchyma.
- Fig. 23. C57 BL/6, 10 days,  $\times 140$ . Fresh granulation tissue and tissue necrosis in contact with parasite.
- Fig. 24. dba, 7 days,  $\times 320$ . Degenerative change of accumulated cells.

## PLATE IV.

- Fig. 25. CF #1, 60 days,  $\times 320$ . Adventitious tissue with giant cells.
- Fig. 26. C57 BL/6, 30 days,  $\times 320$ . Detrited cell mass in contact with cyst wall.
- Fig. 27. dba, 60 days,  $\times 160$ . Partial tissue necrosis of granulation tissue.
- Fig. 28. Cotton rat, 20 days,  $\times 160$ . Epithelioid cell layer around a cyst.
- Fig. 29. C57 BL/6, 120 days,  $\times 320$ . Hematoidin crystals in intercystic necrosed adventitious tissue.
- Fig. 30. Cotton rat, 70 days,  $\times 320$ . Desquamation between adventitious layer and cyst wall and appearance of cell elements.
- Fig. 31. Cotton rat, 76 days,  $\times 70$ . Desquamative changes, regressive change of germinal tissue and initial pseudo-daughter cyst formation.
- Fig. 32. Cotton rat, 70 days,  $\times 160$ . Pseudo-daughter cysts.

## PLATE V.

- Fig. 33. Cotton rat, 70 days,  $\times 320$ . Necrosed germinal tissue with remaining hooklets in pseudo-retention cyst.
- Fig. 34. Cotton rat, 70 days,  $\times 3$ . Naked eye appearance of pseudo-retention cyst with daughter cysts.
- Fig. 35. Cotton rat, 70 days. Daughter cysts collected from pseudo-retention cysts; scale in mm.
- Fig. 36. Cotton rat, 62 days,  $\times 320$ . Scolices in brood capsules in desquamated degenerative germinal tissue. One scolex is degenerated and the other shows initial cystic metamorphosis (initial daughter cyst formation).
- Fig. 37. Cotton rat, 36 days,  $\times 320$ . Early stage of daughter cyst formation from scolex.
- Fig. 38. Cotton rat, 70 days,  $\times 42$ . Daughter cysts without thickening of germinal layer.
- Fig. 39. Cotton rat, 64 days,  $\times 60$ . Daughter cyst with initial brood capsule formation.
- Fig. 40. Cotton rat, 83 days,  $\times 42$ . Daughter cysts with brood capsule and scolex formation.









