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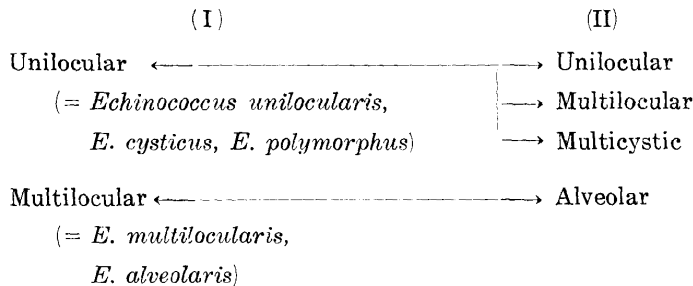
**STUDIES ON ECHINOCOCCOSIS V.
EXPERIMENTAL INFECTION OF THE SHEEP**

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It is a well-known fact that *Echinococcus* or hydatid has been classified into two types according to morphological characters: *Echinococcus unilocularis* and *E. multilocularis* or *E. alveolaris*. However, on the other hand, the term "multilocular cyst" is also used for a form of *Echinococcus unilocularis* by some authors. Such circumstances are listed below.



Having taken many particulars into consideration, the present authors are adopting the former (I) system. Consequently they comprehend the *Echinococcus alveolaris* as a synonymous term of *E. multilocularis*, and they avoid terming a form of unilocular hydatid cyst as "multilocular."

The authors in this report deal with the development of hydatid cyst within the body of sheep which were experimentally infected by *Echinococcus granulosus*. In the preceding report, it was stated that the hydatid cyst of mouse developed rapidly and established a spherical vesicle. In the sheep, however, the hydatid cyst manifested a different mode of development in contrast to the cyst in the mouse, namely—the hydatid cyst of the sheep showed a shape for which a suitable term would be "*Echinococcus polymorphus*."

MATERIALS AND METHODS

Hydatid sand of Australian strain of *Echinococcus granulosus* of case No. 2 described in the authors' first report was given orally to dogs; adult cestodes and eggs were obtained.

These eggs and gravid proglottids were swallowed by sheep.

The eggs were given to six 2-year-old sheep on January 26 and February 2, 1955 respectively and the sheep were also infected on March 11, 1955 with the proglottids of the cestode collected from the intestines of a dissected dog.

Cases No. 1 and No. 2 died on May 18 and 21 respectively, 112 and 115 days after the first ingestion of *Echinococcus* eggs. However, death was due to conditions other than any caused by the parasites. On February 29, 1956, case No. 3 was killed, 399 days after the first infection.

These 3 sheep were autopsied immediately after the death and macroscopical investigations were carried out as minutely as possible. Tissue materials from various organs were fixed by formalin and main organs were searched for the hydatid foci by making cut surfaces at intervals of 3~5mm.

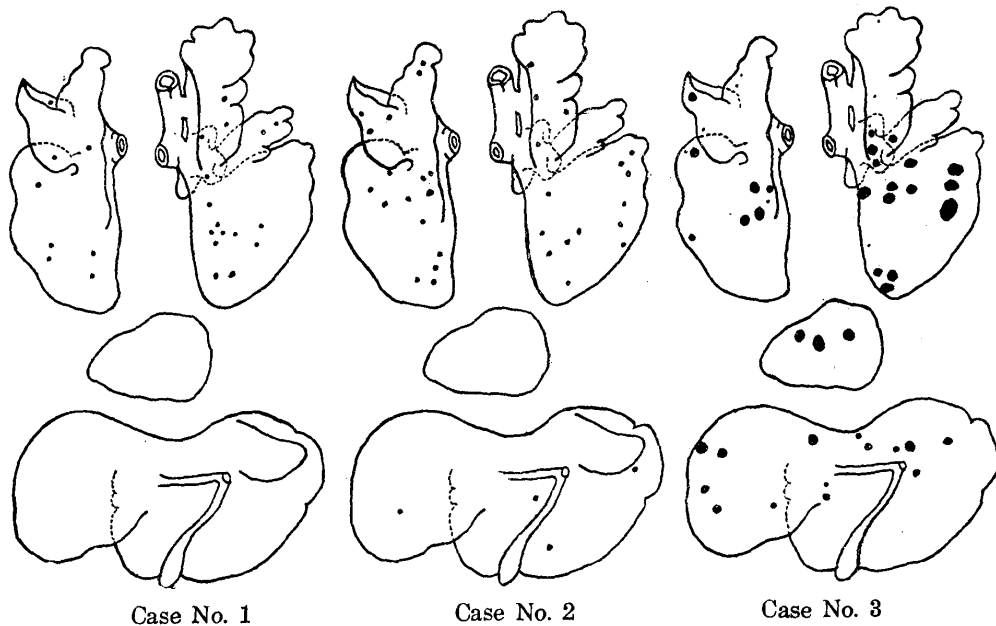
RESULTS

1. Macroscopical Findings

Cases No. 1 and 2 In these two cases, hydatid cysts manifested similar development. As to the distribution of hydatid cysts, the lungs were most densely occupied by them the right lobe being more often invaded than the left (Fig. 1). The cysts existed right beneath the serosa and deep in the parenchyma. They were so small, about 1~2mm in diameter, that they were likely to be overlooked unless they were searched for painstakingly. Particularly the pulmonary cysts bore resemblance to the cross section of bronchioli and blood vessels.

The hydatid cyst had a distinct cystic structure, enclosing liquid substance; the cyst

FIG. 1. *Distribution of Hydatid Cysts*



Studies on Echinococcosis V.

wall was very thin. The polymorphic structure was already found and some cysts had small pouching. No remarkable change in the pericystic host tissue was found. Connective tissue layer of cyst wall in the liver was thicker than that of the lungs.

Case No. 3 The hydatid cysts were more fully developed than in the above two cases. The foci were detected in the lungs, liver and spleen (Fig. 1).

The hydatid cysts showed various sizes; the small cyst was $2 \times 2 \times 2$ mm and the large $15 \times 10 \times 10$ mm, but generally they ranged between red-bean- and soybean-size. The cyst existed both superficially and deeply in the host tissue; the superficial cyst was convexed on the serosa. The cystic focus was considerably indurative. On cut surfaces, the cysts did not show a round shape but polymorphic; cystic structure with small or large pouching was remarkable and the complicated cyst was botryoidal. Even though the focus was seen as a collection of small cysts, the lumen of them was not divided.

The cyst was formed of adventitious connective tissue layer of grey color and the hydatid vesicle laid within this layer. The inner part of the former was a greyish-green opaque layer with somewhat caseous appearance. Calcification was frequently observed. The wall of the hydatid vesicle was clearly visible; there was a gelatinous thin membrane with transparent character and within the vesicle transparent fluid was filled.

The pulmonary hydatids manifested various sizes and their shapes were complicated. The hydatids in the liver, on the other hand, were small in size: the largest one was 7 mm in diameter, generally 3~4 mm. The proliferation of adventitious connective tissue or granulation tissue was more active than in other organs. In the spleen, three foci were discovered. Their sizes on cut surfaces were 6×4 , 7×5 and 9×6 mm respectively; they showed remarkable polymorphic shape.

The hydatid focus was clearly demarcated from the parenchyma of host organs and no remarkable changes were observed in the pericystic area of connective tissue layer. In one pulmonary focus, however, congested edema and atelectasis were found in the perifocal zone. In the lungs and liver, very minute nodules of less than 1 mm diameter could be found; they were grey or greyish-green and indurated; these are the regressive form of hydatid which died at an early stage of development.

2. Microscopical Findings

Cases No. 1 and 2 The hydatid vesicle was enclosed by immature connective tissue layer which was poor in collagenous fiber but rich in nuclei, that is granulation tissue. At the outer part of the connective tissue, lymphocytic cell accumulation existed, but no eosinophile cell was detected. The hydatid vesicle possessed one or some inflated projections or pouchings and the pouching invaded outwards into the connective tissue layer. The pouching became narrow at the portion opening into the vesicle proper; the pouching can be called "herniation." At the portion of herniation, lymphocytic reaction was remarkable close to it. Adventitious connective tissue and parenchymatous tissue were rather clearly demarcated and in the latter there existed no marked changes other than the changes caused by the pressure of the hydatid vesicle such as perifocal atelectasis in the lungs.

In some foci, remarkable lymphocytic cell accumulation appeared between the hydatid

vesicle and connective tissue, and the cells were involved in regressive changes. In this case, the inner zone of connective tissue layer was also influenced and a necrotic layer was established which was homogeneously stained by eosin and sometimes indicated presence of degenerated nuclei by scattered shady spots. These changes were generally slight in the lungs but severe in the liver. The hepatic focus had more thick adventitious connective tissue than the pulmonary focus, but herniation were rare in the hepatic focus.

The size of hydatid vesicles measured on the section preparations ranged from 0.5×0.7 to 0.9×1.7 mm; the thickness of adventitious connective tissue layer was $0.1 \sim 0.4$ mm. The cuticular membrane of hydatid vesicle was $10 \sim 20 \mu$ in thickness, scarcely stainable, however, the laminated structure was very distinct. The germinal membrane was so thin that it could only be distinguished by sporadic existence of small nuclei. The brood capsules and scolices were naturally absent.

Case No. 3 There were no endogenous daughter cysts, brood capsules or scolices. The structure of a hydatid vesicle was similar to that in the above cases but more highly developed. The thickness of cuticular membrane was increased, namely, the thin membrane was $20 \sim 25 \mu$ and the thick more than 70μ , in general about 40μ . Its laminated figure was more remarkable. The adventitious connective tissue layer became more thick, the number and thickness of collagenous fibers increased. Lymphocytic cell layer at the outer portion was not very thick. The necrotic layer increased its thickness as much as $0.25 \sim 0.7$ mm, stained homogeneously by eosin; calcified foci frequently appeared. In the necrotic layer thick collagenous fibers were sparsely detected. Between the vesicle wall and the adventitious layer, there existed no other element but sometimes a space as a technical product. The border line between the necrotic layer and the connective tissue sometimes indicated an irregular serration and degenerated connective tissue nuclei remained there. Some blood vessels in the connective tissue layer showed thickened wall.

The hydatid vesicle manifested polymorphy such as botryoidal shape. Such shape, the authors consider, had resulted from herniation and subsequent expansive development. At this stage, newly formed active herniation of various grades could be found. In some cysts, the homogeneous necrotic layer was immediately thinned in a limited portion where the vesicle wall showed inflation outwards, and where the adventitious tissue had marked cell accumulation. In more advanced cases, the herniation had occurred and projected outwards; a fresh granulation tissue with active cell reaction at the periphery of projected herniation and sometimes even giant cells appeared. The necrotic layer which enclosed the original vesicle did not prolong to the peripheral tissue of herniation; it can be said that herniation occurred at a hole in the necrotic layer. In the adventitious portion of herniation, however, the necrotic layer was immediately formed and the slender projection inflated. In hepatic hydatid cysts, adventitious connective tissue formation was remarkable and the necrotic layer was very thick similar to that of the previous cases.

The authors wish to describe the regressive changes of a hydatid cyst. The minute nodules which could be observed macroscopically were originated from dead parasites. In the center of the nodule, there existed eosinophile homogeneous substance with intricate collagenous fibers; its outer layer was connective tissue. When the hydatid died at the stage of herniation, cellular and connective tissue reaction became extremely remarkable,

regressive cell element appeared within the vesicle and calcification was found. This process was very rare, that is to say—the death of a hydatid cyst usually occurs in the early stage of development and is very rare in the median stage.

DISCUSSION

The present authors could not obtain any knowledge concerning the development of a hydatid cyst at its very early stage, because 3 months elapsed before the examination in both cases Nos. 1 and 2. It was an unexpected result that the diameter of a hydatid cyst even at 3 months after infection was only about 1 mm. Murine hydatid cyst at 3 months after infection was 1 cm in diameter in the authors' experiment; DEW (1925) reported porcine cases in which hydatid cysts required 3 months to grow to the size in 4~5 cm. In the cases of the present sheep, hydatid cysts required more than 1 year to reach approximately 1 cm in size. It is very interesting that the growth of a hydatid cyst is slow in ovine body.

The shape of the hydatid cyst in the sheep is fairly characteristic, namely—the vesicle scarcely manifests a simple spherical shape, but it becomes a polymorphic vesicle worthy of the term, "*Echinococcus polymorphus*." Such a polymorphy of ovine hydatid cysts is a well-known fact. FAIRLEY & WRIGHT-SMITH (1929) also reported the result of detailed investigations in which they clarified this fact. They named the cysts "multilocular cyst."

The present authors ascertained that perifocal host tissue reaction occurred severely in the hydatid case of sheep. That is to say, a thick layer of granulation tissue surrounds the hydatid vesicle and a necrotic layer at the inner portion of the granulation tissue frequently accompanied by calcified focus. The cell element in the focus is composed of lymphocytes. Consequently it can be said that the tissue reaction is not very acute. In the focus, therefore, a severe host tissue resistance against the hydatid vesicle exists with the result that the smooth and rapid enlargement of the vesicle is naturally impeded. The above-stated relation is easily understood when the sheep hydatid is compared with the authors' mouse hydatid which grows rapidly without any tissue reaction. Because resistance occurs, the mechanism of development of hydatid cyst in ovine body comes characteristic, that is, herniation and following enlargement are repeated and a polymorphic hydatid cyst appears as described above. With regard to the polymorphism of hydatid cysts, some investigators are giving attention to it and the fact is also described in text books. As to herniation, many authors explain that the hydatid vesicle projects through a weak point because of the resistance offered by hard tissue found in the blood vessels or biliary ducts. The present authors, however, consider that there naturally appears a weak point due to the

contrariety of balance between the pressure by outward development of hydatid vesicle and the resistant power of surrounding connective tissue, especially the necrotic layer; herniation thus occurs through such a point. It can be considered that usual connective tissue and parenchymatous tissue do not hinder a uniform development of a vesicle, but the necrotic tissue as found in the present cases is liable to be invaded irregularly. Such interpretation of some investigators that so-called exogenous daughter cyst formation is nothing but herniation is agreeable to the present authors, who, however, wish to leave the problem concerning daughter cyst formation for future consideration.

Characteristic changes in the ovine hydatid cyst are the appearance of lymphocytes and necrotic layer as noted above. So far as the authors have experienced, the eosinophile cells do not play a role in ovine case. The fact is quite peculiar in comparison with DEW's experimental porcine cases in which he assigns a leading role to the eosinophile cells. With regard such a difference, the species disposition can also be considered.

Pressure necrosis according to the development of hydatid vesicle can be attributed to the origin of appearance of necrotic layer, but such a remarkable necrosis cannot be explained only by pressure necrosis. It is thought that the irritation which provokes proliferation of granulation tissue participates in necrosis. In spite of slowness of development and severe tissue reaction, it seems quite certain that the sheep is an ideal intermediate host for *Echinococcus granulosus*. It can be imagined that the energy which must be used for the enlargement of a vesicle is diverted to scolex or brood capsule formation.

As to the distribution of the hydatid cysts in the present cases, the cysts are more plentiful in the lungs than in the liver; the right lung lobe is preferred to the left. This fact has been already pointed out by FAIRLEY and WRIGHT-SMITH in their detailed statistic investigations of numerous cases.

In short, the authors learned that the speed of development of hydatid vesicles and the character of tissue reaction show differences according to the kind of host animal.

CONCLUSION

In the sheep, the hydatid vesicle grows slowly, connective tissue reaction around the vesicle is remarkable and the vesicle shows polymorphic shape through herniation. Some characteristic changes can be found in the ovine hydatid cyst and the authors would emphasize that there are differences of development and reaction according to the difference of host species.

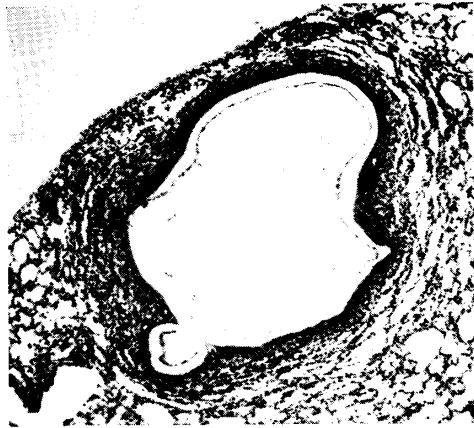
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REFERENCES

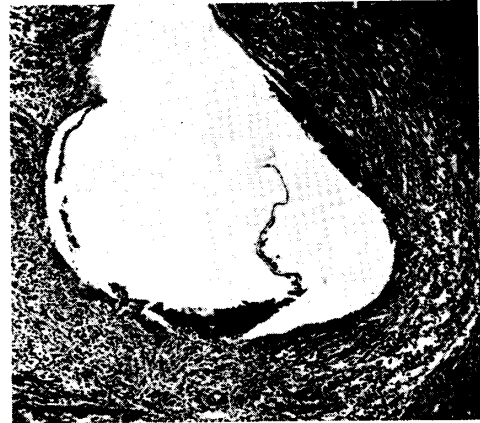
- 1) CAMERON, T. W. M. (1951): *The Parasites of Domestic Animals*. A. & C. BLACK, London.
- 2) DEW, H. R. (1925): *Med. J. Aust.*, **1**, 101.
- 3) FAIRLEY, N. H. & R. J. WRIGHT-SMITH (1929): *J. Path. Bact.*, **32**, 309.
- 4) HOSEMANN, G., E. SCHWARZ, J. C. LEHMANN & A. POSSELT (1928): *Die Echinokokkenkrankheiten*. F. ENKE, Stuttgart.
- 5) YAMASHITA, J., M. OHAYASHI & S. KONNO (1956): *Jap. J. vet. Res.*, **4**, 1 & 123.

EXPLANATION OF PLATE

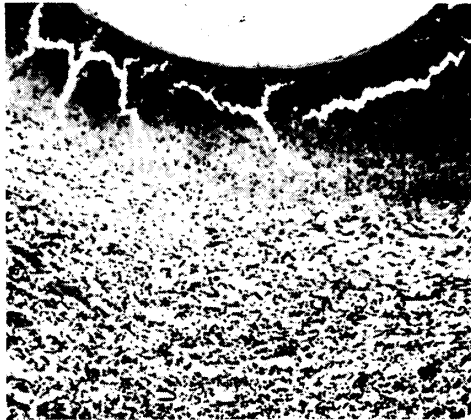
- Fig. 1. Case No. 1. Lung; hydatid cyst with small herniations. Hematoxylin-eosin (H.-E.). $\times 30$.
- Fig. 2. Case No. 2. Liver; hydatid cyst with thick adventitious connective tissue layer. H.-E. $\times 30$.
- Fig. 3. Case No. 2. Lung; initial stage of appearance of necrotic layer. H.-E. $\times 80$.
- Fig. 4. Case No. 3. Lung; polymorphic hydatid cyst underneath pleura. $\times 4$.
- Fig. 5. Case No. 3. Liver; beginning of herniation—necrotic layer interrupted at the portion of herniation. H.-E. $\times 80$.
- Fig. 6. Case No. 3. Liver; herniation with marked reaction. H.-E. $\times 80$.
- Fig. 7. Case No. 3. Spleen; herniation with necrotic layer formation. H.-E. $\times 80$.
- Fig. 8. Case No. 3. Lung; herniation with regressive change. H.-E. $\times 80$.



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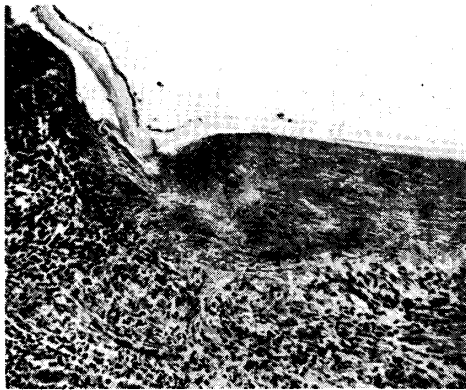
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