Z. Parasitenk. 54, 289–297 (1977)

Zeitschrift für
Parasitenkunde
Parasitologic Research


# Stereoscan Studies of Rediae, Cercariae, Cysts, Excysted Metacercariae and Migratory Stages of *Fasciola hepatica*

Marianne Køie,[1]* Peter Nansen,[2] and Niels Ørnbjerg Christensen[3]

[1] Marine Biological Laboratory, DK-3000 Helsingør, Denmark; [2] The Danish Agricultural and Veterinary Research Council, Parasitological Research Group, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Copenhagen, Denmark; [3] Danish Bilharziasis Laboratory, Jægersborg Alle 1D, DK-2920 Charlottenlund, Denmark

**Summary.** The external surface of the redial body of *Fasciola hepatica* is provided with microvillus-like projections or short lamellae, and short cilium-like structures are common anteriorly.

The anterior part of the cercarial body possesses a pattern of regularly arranged small depressions each containing a spine. Both long and short cilium-like structures occur anteriorly. The tail is spineless and provided with dorsolateral folds.

The outer cyst wall is formed by granules secreted from the tegument all over the body apart from the ventral sucker. Most granules transform into fibrillae which form the thick outer spongy layer.

The precursor of the inner cyst wall is at the beginning closely attached to the metacercarial surface, but later the membrane-like cyst wall extends, and when fully formed the metacercaria lies free in the flattened circular inner cyst. The ventral plug is formed by the ventral sucker.

The tegument of newly excysted metacercariae is provided with simple pointed spines, but later during migration in the mouse the spines become flattened and multipointed. Very young migratory stages may be attached with host cells.

## Introduction

Stereoscan electron microscopical studies have provided a considerable amount of information on newly excysted metacercariae and older developmental stages of *Fasciola hepatica* during growth and maturation in the mouse (Bennett, 1975a, b). The purpose of the present study was to provide additional information on surface morphology of *F. hepatica* rediae, cercariae, cysts, excysted metacercariae and migratory stages in mice. The main emphasis was put on the description of the different developmental stages of the cyst.

* To whom offprint requests should be sent

## Materials and Methods

*Fasciola hepatica* rediae, cercariae and older developmental stages were obtained from experimentally infected laboratory bred *Lymnaea tomentosa*. Shells of infected snails were crushed and removed, and the digestive gland gently teased apart. The liberated rediae and free-swimming cercariae were fixed immediately after the dissection of the snails, whereas the successive stages of the cyst formation were fixed at various intervals after the encystment process had initiated. The cercariae encysted on cellophane. Metacercariae were excysted in vitro by the method of Wikerhauser (1960). White female mice (NMRI strain) were infected using stomach tube, and the juvenile flukes were removed from the peritoneal cavity and the hepatic parenchyma at 24 h and 5 days after infection respectively according to the procedure described by Bennett and Threadgold (1975).

The material was fixed in glutaraldehyde in cacodylate buffer following the method of Nørrevang and Wingstrand (1970) and studied in a Cambridge 600 electron microscope.

## Results

### Rediae

The external surface of the redial body is provided with transverse circumferential folds (Figs. 1–3). High magnification revealed that the folds are provided with microvillus-like projections or short lamellae (Fig. 3). The collar is provided with small knobs, and short cilium-like structures are numerous anteriorly, especially on the sphincter muscle which surrounds the oral aperture (Fig. 1). When closed the pharynx appears as two jaw-like parts.

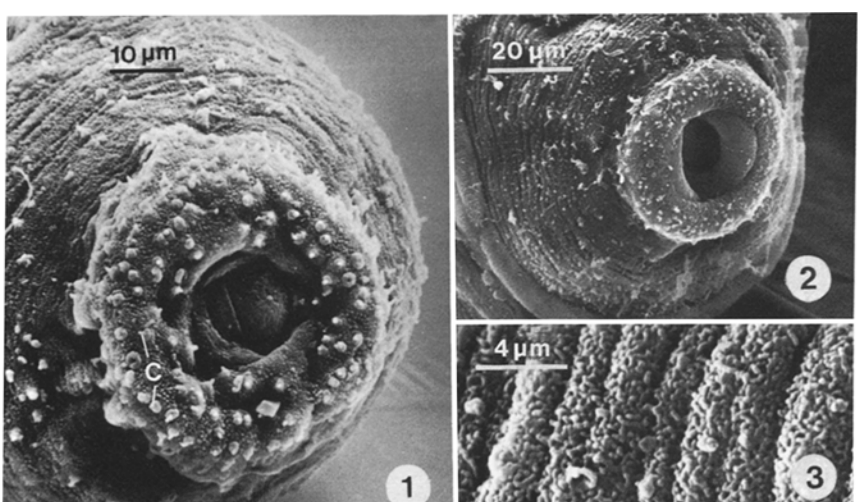


**Fig. 1.** Redia of *Fasciola hepatica* with closed pharynx. Short cilium-like structures (*C*) and microvillus-like projections are seen around the mouth

**Fig. 2.** Redia with open pharynx. Part of the collar is seen

**Fig. 3.** The surface of a redia with microvillus-like projections

## *Cercariae*

On the anterior surface of the cercarial body a pattern of regularly arranged small depressions is observed. Spines, which do never protrude above the general level of the tegument, are separately situated in the bottom of the small depressions. The spines could not be observed using the stereoscan microscope, but their existence was revealed using the light microscope. Cilium-like structures having a length of a few μm to about 10 μm occur anteriorly. The majority of these cilium-like structures is seen in the region of the oral sucker, and the longest of the cilium-like structures occur dorsally (Fig. 5). On the rim of the oral sucker small protuberances with about 0.3 μm long cilium-like structures are seen, and two large bulges without cilium-like structures are found dorsally in the oral aperture.

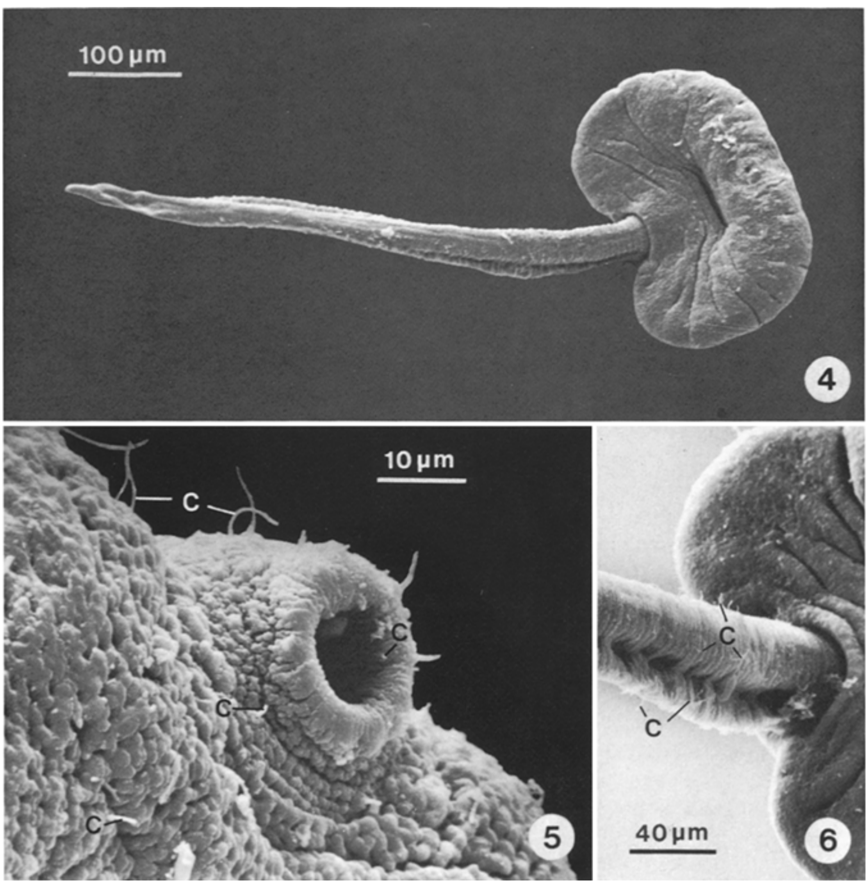


**Fig. 4.** Ventral view of a cercaria of *Fasciola hepatica*

**Fig. 5.** The anterior end of a cercaria showing the mouth and short and long cilium-like structures (*C*)

**Fig. 6.** Ventral view of the proximal part of the tail, showing one of the two dorso-lateral folds and short cilium-like structures

The cercarial tail is spineless. It is proximally provided with two dorso-lateral folds which fuse ventrally near the distal end (Fig. 4). Cilium-like structures about 10 μm long are observed on the proximal part of the tail (Fig. 6).

*The Cyst Wall of the Metacercaria*

Dixon and Mercer (1964, 1967), Mercer and Dixon (1967), and Dixon (1968) have given an intensive description of the ultrastructure of the cystogenic cells of the cercaria and the cyst wall during construction. In the following the classification of the different layers given by these authors is used.

*A. The Outer Cyst Wall.* Immediately after attachment to the substratum by the ventral sucker the dorsal surface of the cercaria is covered by cystogenous material (Fig. 7). The precursors of the external layer (layer I) are spherical granules 1–2 μm in diameter (referable to the tanned protein granules of Mercer and Dixon, 1967). These granules are released from the tegument all over the body apart from the ventral sucker (Figs. 10, 11). A few minutes later most of the granules transform into filaments (Fig. 11). Apparently this transformation makes the external layer inflate. Fully developed cysts have an irregular surface with transverse furrows (Fig. 8). Higher magnification of the rough surface reveals its porous nature (Fig. 9).

Fracture of the fully developed outer cyst wall is seen in Figures 12 and 13. Layer I is composed of a thick spongy meshwork of filaments attached to the thin and more homogenous layer II. Apart from a small aperture ventrally (Fig. 14) the outer cyst wall surrounds the inner cyst.

*B. The Inner Cyst Wall.* Following the construction of the outer cyst wall there is a pause in activity before the secretion of the inner cyst is initiated. A gap occurs between the outer and inner cyst walls which means that the inner cyst can always be easily removed from the outer cyst. The precursors of the external layer of the inner cyst (layer III) are secreted from the tegument all over the body (Fig. 15). The external surface of the newly formed layer III is provided with numerous pores 1–3 μm in diameter and narrow fissures in the slightly dented surface (Fig. 16). Layer III is at the beginning closely attached to the metacercarial surface (Figs. 15, 16), but after or during formation of layer IV layer III extends enormously and deep furrows appear all over the surface (Fig. 17). An opening is apparently formed in the area corresponding to the position of the ventral sucker. This area later develops into the ventral plug.

The precursors of layer IV consist of keratin rodlets (Fig. 16) which are released from the tegument. When the inner cyst is completed a cavity occurs between the inner cyst wall and the metacercaria. The inner cyst is circular in outline, slightly flattened, and the ventral plug appears as a circular thickening (Fig. 18).

*Excysted Metacercariae*

Compared with that of the cercaria, the external surface of the newly excysted metacercaria has greatly changed. Single-pointed spines are regularly arranged anteriorly and more irregularly scattered posteriorly (Fig. 19). The spines protrude about 2 μm

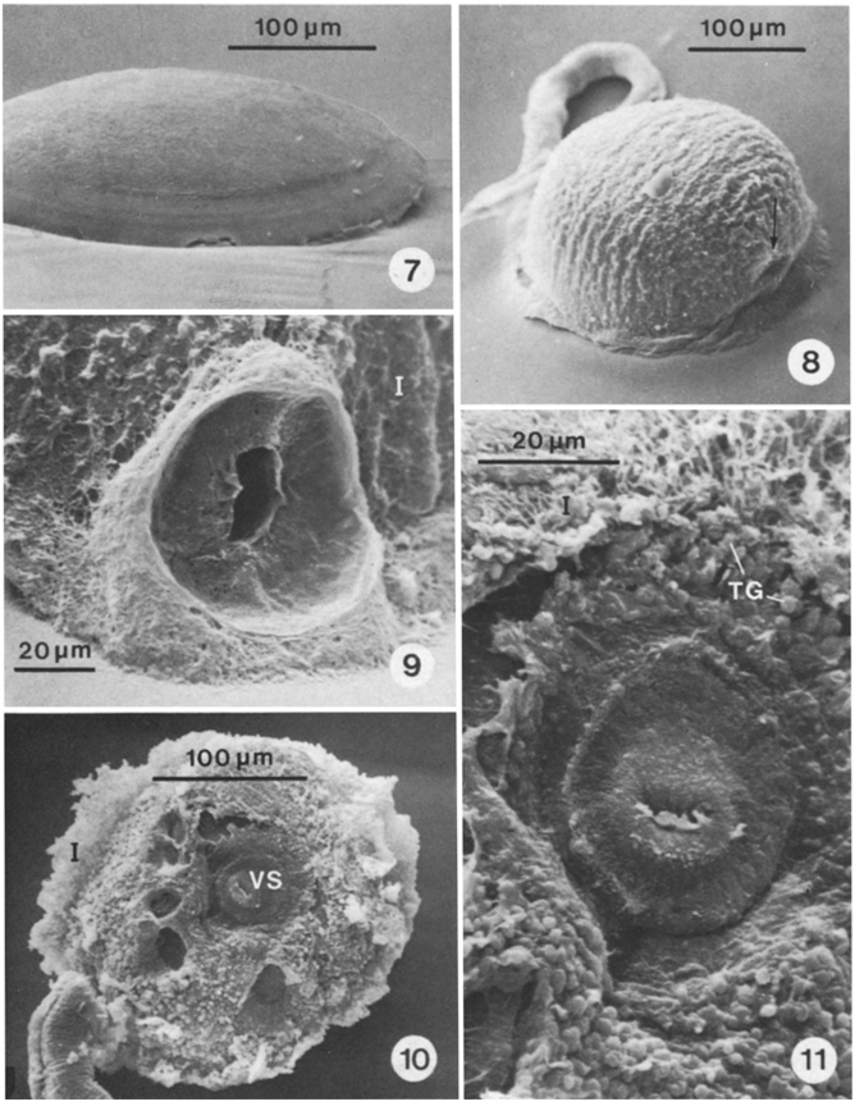


**Fig. 7.** The surface of the "cyst" just after attachment of the cercaria

**Fig. 8.** A few minutes later than Figure 7. The outer cyst is fully formed, but the tail is still attached. The arrow shows mark after the oral sucker

**Fig. 9.** "Scar" in the outer cyst after the shedding of the tail

**Fig. 10.** Ventral view of a cercaria shortly after attachment and secretion of the first cystogenous material. The "cyst" is removed from the substratum. The ventral sucker (*VS*) is not covered with cystogenous material

**Fig. 11.** Detail of Figure 10 showing the ventral sucker and the tanned protein granules (*TG*) during secretion from the tegument. At the top some granules are seen during transformation into filaments

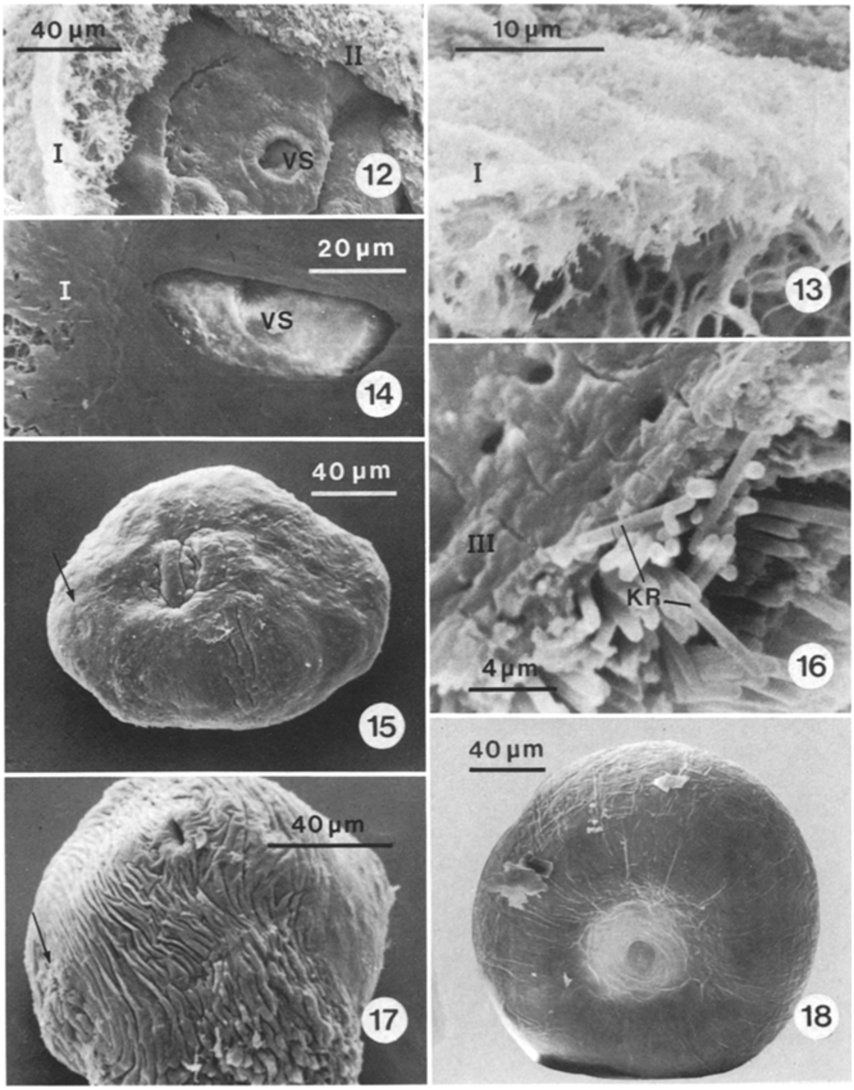


**Fig. 12.** Cyst damaged when removed from the substratum. The outer cyst wall (*I* + *II*) is completed, but the secretion of the inner cyst wall has not started

**Fig. 13.** Detail of Figure 12 showing the spongy external layer (*I*)

**Fig. 14.** Cyst removed from the substratum after the completion of the outer cyst wall. The ventral sucker (*VS*) of the metacercaria is seen through the opening in the outer cyst wall. The inner cyst is not formed

**Fig. 15.** The outer cyst is removed. Ventral view of the not yet completed inner cyst. Arrow shows the mouth covered with cyst material

**Fig. 16.** Metacercaria, similar to that shown in Figure 15, broken into pieces. The external layer of the inner cyst wall (*III*) is seen. The keratin rodlets (*KR*), the precursors of the internal layer of the inner cyst wall, are seen in the metacercarial tegument

anteriorly and about 1.5 μm posteriorly. The long dorsal cilium-like structures of the cercaria have disappeared, and hemispherical protrusions with short cilium-like structures are found in the same place as the short cilium-like structures of the cercaria.

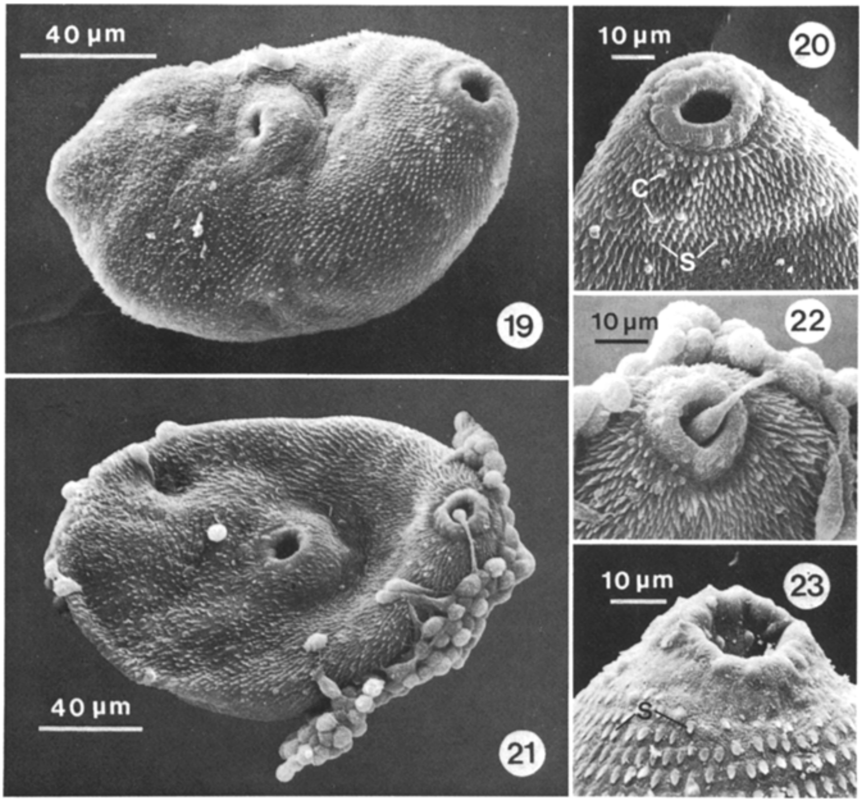


**Fig. 19.** Excysted metacercaria of *Fasciola hepatica*

**Fig. 20.** Ventral view of the anterior end of a 24-h-old specimen harvested from the peritoneal cavity of a mouse. Single-pointed spines (*S*) cover the body

**Fig. 21.** A 24-h-old specimen with adherent host cells

**Fig. 22.** Detail of Figure 21

**Fig. 23.** The anterior end of a five-days-old juvenile from the liver of a mouse. The spines are slightly flattened and the surface is covered by a thin glycocalyx

**Fig. 17.** Later developmental stages of the inner cyst wall, which has extended and forms deep furrows. Arrow shows the still recognizable mouth. The cyst material forms an opening at the place of the ventral sucker

**Fig. 18.** Ventral view of a several-days-old inner cyst, showing the ventral plug

### *Juveniles Recovered Intraperitoneally 24 h After Infection*

About half of the juveniles recovered were attached with host cells. The cells were especially found dorsally (Figs. 21, 22). The overall structures, arrangement of spines etc. (Fig. 20) did not differ essentially from those of the newly excysted metacercaria.

### *Juveniles Recovered From the Hepatic Parenchyma 5 Days After Infection*

During migration the spines gradually widen and become provided with increasing numbers of points (Fig. 23). Five-day-old specimens are covered by a distinct glycocalyx. The presence of this glycocalyx is revealed as a "woolly" cover (compare Fig. 20 and Fig. 23).

## Discussion

Earlier observations by Køie et al. (1976) showed that the sporocyst stage of *F. hepatica* is not provided with cilium-like structures. The existence of cilium-like structures of the redia is presumably explained by the fact that the redial stage represents a more active phase than the sporocyst. The increased surface, also observed by Erasmus (1972) using the transmission electron microscope, is presumably an adaptation to the transtegumental metabolic activity.

The tegument of developed cercariae is about 8 μm thick (Dixon and Mercer, 1967), whereas the tegument of newly encysted metacercariae is about 1 μm thick between the spines (Bennett and Threadgold, 1973). The great difference in thickness is presumably due to the release of cystogenous products from the tegument. This explains the appearance of spines and hemispherical structures with cilium-like structures. The observation by Dixon and Mercer (1967) that the tegument (= embryonic epithelium) is shed during the formation of the outer cyst wall was not confirmed in the present study.

Dixon and Mercer (1964) suggested that layer I, in addition to help to resist mechanical injury, may also act as a barrier against bacterial and fungal attacks. The stereoscan observations of this study make it likely that the porous structure of this layer may also protect the metacercaria against desiccation as this layer may absorb water or dew and store the water in the thick spongy mass.

The sensory structures of the metacercaria and the transformation from simple pointed spines of the early migratory stages into flattened multi-pointed of the more stationary stages of *F. hepatica* are described and discussed by Bennett (1975a, b). These observations have been confirmed in the present study. A similar morphological transformation of spines has been observed for other digenetic trematodes, e.g. *Cryptocotyle lingua* (see Køie, 1977).

## References

Bennett, C.E.: Surface features, sensory structures, and movement of the newly excysted juvenile *Fasciola hepatica* L. J. Parasit. **61**, 886–891 (1975a)

Bennett, C.E.: Scanning electron microscopy of *Fasciola hepatica* L. during growth and maturation in the mouse. J. Parasit. **61**, 892–898 (1975b)

Bennett, C.E., Threadgold, L.T.: Electron microscope studies of *Fasciola hepatica*. XIII. Fine structure of newly excysted juvenile. Exp. Parasit. **34**, 85–99 (1973)

Bennett, C.E., Threadgold, L.T.: *Fasciola hepatica*: Development of tegument during migration in mouse. Exp. Parasit. **38**, 38–55 (1975)

Dixon, K.E.: Encystment of the cercaria of *Fasciola hepatica*. Wiad. Parazyt. **14**, 689–701 (1968)

Dixon, K.E., Mercer, E.H.: The fine structure of the cyst wall of the metacercaria of *Fasciola hepatica*. Quart. J. micr. Sci. **105**, 385–389 (1964)

Dixon, K.E., Mercer, E.H.: The formation of the cyst wall of the metacercaria of *Fasciola hepatica* L. Z. Zellforsch. **77**, 345–360 (1967)

Erasmus, D.A.: The biology of trematodes. London: Edward Arnold 1972

Køie, M.: Stereoscan studies of cercariae, metacercariae and adults of *Cryptocotyle lingua* (Creplin, 1825) Fischoeder, 1903 (Trematoda, Heterophyidae). J. Parasit. **63** (1977)

Køie, M., Christensen, N.Ø., Nansen, P.: Stereoscan studies of eggs, free-swimming and penetrating miracidia and early sporocysts of *Fasciola hepatica*. Z. Parasitenk. **51**, 79–90 (1976)

Mercer, E.H., Dixon, K.E.: The fine structure of the cystogenic cells of the cercaria of *Fasciola hepatica* L. Z. Zellforsch. **77**, 331–344 (1967)

Nørrevang, A., Wingstrand, K.G.: On the occurrence and structure of choanocyte-like cells in some echinoderms. Acta zool. (Stockh.) **51**, 249–270 (1970)

Wikerhauser, T.: A rapid method for determining the viability of *Fasciola hepatica* metacercariae. Amer. J. vet. Res. **21**, 895–897 (1960)

*Received June 13, 1977*