LIGHT AND TRANSMISSION ELECTRON MICROSCOPICAL
STUDIES AND AMINO ACID ANALYSIS OF THE
METACERCARIAL CYST OF ZYGOCOTYLE LUNATA
(TREMATODA)

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Abstract—ROBBINS S. H., HAMMETT M. and FREED B. 1979. Light and transmission electron microscopical studies and amino acid analysis of the metacercarial cyst of Zygocotyle lunata (Trematoda). International Journal for Parasitology 9: 257-260. Light microscopical studies indicated that the cyst of Zygocotyle lunata consists of outer, inner and ventral cyst walls. Transmission electron microscopical studies showed that the outer cyst and the ventral cyst each consist of two layers. The inner cyst is lamellated and contains a specialized ventral region designated the ventral lid. Amino acid analysis of cyst walls showed only trace amounts of cysteine, indicating that disulphide bonds are not used to stabilize the inner cyst of Z. lunata.

INDEX KEY WORD: Trematoda; Zygocotyle lunata cysts; light microscopy; transmission electron microscopy; automated amino acid analysis; proteins; cysteine; disulphide bonds.

INTRODUCTION

METACECRAE of Zygocotyle lunata encyst in the open on aquatic vegetation, snail shells, walls of laboratory containers and on Saran wrap (Willey, 1941; Fried, Robbins & Nelson, 1978). Fried et al. (1978) described this cyst as a dome-shaped hemisphere containing an outer and inner cyst. The ventral lid which is present following excystation was not observed in whole or sectioned cysts. Histochemical studies indicated that the outer cyst contains acid mucopolysaccharides in the dome, but not the ventral region, whereas the inner cyst is mainly proteinaceous.

The present study uses light and transmission electron microscopy (TEM) and automated amino acid analysis to further characterize the structure and composition of this cyst.

MATERIALS AND METHODS

Cysts were obtained from Saran wrap (Fried et al., 1978). For light microscopy studies, individual cysts were removed from the wrap, oriented under a dissecting scope, and embedded in an inert embedding compound ('O.C.T. Compound', Ames Co., Elkhart, IN). Cysts were frozen at -20°C, sectioned on a cryostat at 8-10 μm, postfixed in formalin fumes and stained with Toluidine blue-O, Gomori's trichrome (Humason, 1972, or safranin-O (Lillie, 1965). Whole and empty cysts (empty cysts were obtained following chemical excystation, Fried et al., 1978) were prepared for TEM as follows: individual cysts removed from wrap were fixed for 2 h at 4°C in 2.5% glutaraldehyde + 1% osmium tetroxide (1:2); cysts were embedded in Epon 812 following dehydration in ethanol and propylene oxide. Additional whole cysts were fixed in 3% glutaraldehyde, bisected with a razor blade, postfixed in 1% osmium tetroxide, dehydrated as above, and embedded in Spurr's (1969) medium. Thick (1 μm) sections were cut on a Sorvall Porter-Blum MTII ultramicrotome and stained with toluidine blue-O (Humason, 1972). Thin (60-90 nm) sections cut on an LKB III ultramicrotome with a diamond knife (MJO Co., Ft. Washington, PA) and collected on 75 mesh copper grids coated with 0.4% paralodion, were stained with 8% alcoholic uranyl acetate and 0.2% lead citrate. Sections were viewed on a JEOL 100 C electron microscope at 60 kV. For the amino acid analysis 10 whole cysts were bisected with a razor blade and the metacercariae were removed with a dissecting needle. The empty cysts were hydrolyzed with 6 N-HCl at 108°C for 24 h under nitrogen in a partially evacuated glass tube. The HCl was removed, leaving a dried hydrolysate which was dissolved in 0.2 M-sodium citrate buffer (pH 2.2). The analysis was made on a Beckman 121 M amino-acid analyzer using the standard program for collagen analysis (see application notes for Collagen Program on 121 M analyzer; published by Spinco-Division of Beckman Instruments Inc., Palo Alto, CA 94304, October 1973).

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Explanation of Figs. 1-6—Photomicrograph and electron micrographs of metacercarial cysts of *Lycocotyle lunata*. Abbreviations: I = inner cyst; DI = dorsal aspect of inner cyst; O = outer cyst; 01 = layer I of outer cyst; 02 = layer II of outer cyst; V1 = ventral aspect of inner cyst; V = ventral cyst; V3 = layer III of ventral cyst; V4 = layer IV of ventral cyst; VL = ventral lid; ZD = zone of dehiscence. Scale bars = 10 μm in Figs. 1 & 2, 1 μm in Figs. 3, 4 & 6; and 0.1 μm in Fig. 5.
RESULTS

Light microscopical observations indicated that the cyst of *Z. lunata* consists of outer, inner and ventral cyst walls (Fig. 1). TEM observations indicated that the outer and ventral walls can be further subdivided (Figs. 2, 3 & 6) as discussed below.

The outer wall, present in the dorsal and lateral regions of the cyst, consists of the external layers of the dome and flange regions (Figs. 1 & 2, and Fig. 2 in Fried *et al.*, 1978). This wall stained safranin-O positive and β-metachromatically with toluidine blue. Fried *et al.* (1978) reported that this wall contains acid mucopolysaccharides. Ultrastructurally, the outer wall consists of two layers, I and II (Fig. 3). Both layers are granular, and often contain cellular debris apparently entrapped in the matrix during encystment. Layer II is in close association with the inner cyst and is more finely granular than layer I.

The inner cyst wall is ellipsoidal, and is continuous dorsally and ventrally except for a small portion of the wall that splits laterally and remains attached to layer II of the outer cyst (Figs. 1–3). The ventral aspect of the inner cyst is thickened, stains intensely with Gomori’s trichrome and contains the ventral lid (Fig. 1). In a previous study (Fried *et al.*, 1978), the ventral lid was seen only during excystation. The inner wall stains with mercuric bromphenol blue and fast green indicating it is proteinaceous (Fried *et al.*, 1978). TEM observations indicated that the inner wall is lamellated (Figs. 4–6). Whereas the lamellae in the dorsal and lateral aspects are fine, those in the ventral region are thick and dense.

The ventral aspect of the inner cyst contains the ventral lid which opens following excystation (see Fig. 4 in Fried *et al.*, 1978). Following excystation broken fibers are apparent in the zone of dehiscence associated with the ventral aspect of the inner cyst (Fig. 4).

The ventral cyst wall is ventral and lateral to the inner cyst wall (Figs. 1 & 2). It is bordered dorsally by the ventral aspect of the inner cyst and laterally by the area of the inner cyst that splits as described previously. The ventral cyst wall was not described previously, and was thought to be a void between the inner and outer cysts (see Fig. 1 in Fried *et al.*, 1978). Ventrally this layer forms the base of the cyst and is in contact with the substratum on which encystment has occurred. This wall contains two layers which are seen ultrastructurally and with differential staining (Figs. 1 & 6). The outer layer (III) contacts the substratum; it is coarsely granular and electron dense (Figs. 1 & 6). The inner layer (IV) extends laterally about half-way up the cyst (Figs. 1 & 2) and is thicker in the periphery of the cyst than under the ventral lid. Layer IV did not react distinctly with any stains used in this study (Fig. 1). This layer is more finely granular and less electron dense than layer III (Fig. 6). TEM observations of empty cysts following *in vitro* excystation showed only fragments of the ventral cyst suggesting that this wall may be partially digested.

The amino acid analysis of 10 fresh cysts devoid of metacercariae is presented in Table 1. Hydroxyproline and hydroxylysine were not detected indicating the absence of collagen in the *Z. lunata* cyst.

DISCUSSION

The cyst of *Z. lunata* is more complex than

| Table 1—Amino Acid Composition of 10 Cysts of *Z. lunata* Following Removal of Metacercariae |
|-----------------------------------------------|-----|-----|-----|
| Amino acid | Residues (/1000) | Amino acid | Residues (/1000) |
| Aspartic acid | 82 | isoleucine | 73 |
| Threonine | 66 | leucine | 80 |
| Serine | 113 | tyrosine | 31 |
| Glutamic acid | 77 | phenylalanine | 29 |
| Proline | 68 | ornithine | 4 |
| Glycine | 113 | lysine | 43 |
| Alanine | 101 | histidine | 15 |
| Valine | 57 | arginine | 50 |
| Cysteine | trace |

**FIG. 1.** Photomicrograph of cryostat section stained with Gomori’s trichrome. Note outer, inner and ventral cyst layers; note splitting of the inner cyst in the dorso-lateral margin; note intense staining in the ventral lid area.

**FIG. 2.** Electron micrograph of lateral region of the cyst near the flange. Note outer cyst layers, splitting of the inner cyst, and thickness of ventral layer IV near the flange.

**FIG. 3.** Area demonstrated in Fig. 2 at higher magnification to show layers in the lateral region of the cyst.

**FIG. 4.** Electron micrograph of an empty cyst following chemical excystation. Note disrupted fibers in the zone of dehiscence in the ventral aspect of the inner cyst.

**FIG. 5.** High magnification of ventral aspect of inner cyst and ventral layer IV. Note thickness of lamellae in the inner cyst.

**FIG. 6.** Electron micrograph through ventral aspect of the cyst. Note layers of the ventral cyst and the ventral inner cyst.
previously reported by Fried et al. (1978). Ultrastructurally, it consists of 5 layers and therefore in terms of numbers of layers is similar in complexity to other metacercariae that encyst in the open, i.e., 3 layers for Parorchis acanthus (Cable & Schutte, 1973), 4 layers for Cloacitrema narrabeenensis (Dixon, 1975) and 5 layers for Fasciola hepatica (Dixon & Mercer, 1964).

The lamellated inner cyst of Z. lunata is very similar to that observed in numerous trematodes that encyst in the open or in intermediate hosts. Histochmical studies on numerous trematodes (reviewed in Dixon, 1975) indicate that inner cysts often contain protein layers which are stabilized by disulphide bonds. Our amino acid analysis detected only trace amounts of cysteine in the Z. lunata cyst. Therefore, it is unlikely that the lamellated layer is stabilized by disulphide bonds.

Trematodes that encyst in the open often have a specialized structure on the ventral surface of the cyst called a ventral plug (for a discussion of the ventral plug see Dixon, 1975). Z. lunata contains a specific area in the inner cyst previously designated a ventral lid (Fried et al., 1978). This lid opens during excystation, often remains attached to the cyst and is analagous to the operculum of the trematode egg. Factors responsible for the opening of the ventral lid in Z. lunata are currently under investigation.

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REFERENCES


