The fine structure of the cyst wall of the metacercaria of *Fasciola hepatica*

By K. E. Dixon and E. H. Mercer

(From the Department of Zoology and the Electron Microscope Unit, Australian National University, Canberra, Australia)

With 3 plates (figs. 1 to 3)

Summary

Observations with the electron microscope have shown that 4 major layers can be distinguished in the cyst wall:

(a) an outer tanned-protein layer, consisting of a meshwork of irregular bodies made up of cigar-shaped particles;

(b) a predominantly mucopolysaccharide, finely-fibrous layer, closely adherent to the tanned layer;

(c) an inner, mainly mucopolysaccharide layer, which can be resolved into two layers differing in fine texture;

(d) a dense, compact layer, composed of numerous protein sheets stabilized by disulphide linkages. This layer is formed from tightly wound scrolls, developed in intracellular vacuoles, which are unrolled at the surface of the animal after secretion.

Introduction

The cyst wall which encloses the metacercaria of *Fasciola hepatica* L. has already been studied by histochemical techniques and conventional light microscopy (Dixon, 1964). An outer tanned protein layer and an inner mucopolysaccharide layer form the outer cyst wall, which may be removed mechanically. The inner cyst is composed of an outer, predominantly mucopolysaccharide layer, in which may be distinguished several sub-layers differing in their chemical characteristics, and an inner keratinized layer stabilized by disulphide linkages. On the inner and outer surfaces of the latter a thin layer of material that reacts positively with certain lipid colouring agents suggests that lipids may also occur in small quantities. A further investigation has been carried out with the electron microscope, with the object of elucidating the fine structure of the cyst wall. The formation of the cyst wall by the cercaria is also being studied, but only such details of this work as are relevant to this account of the structure will be described here.

Material

The methods by which the cysts were obtained, stored, and processed before fixation have been described by Dixon (1964). Both the entire cyst and the inner cyst, obtained by mechanical removal of the outer wall, and some cysts with partly formed walls, were used in this study.

Preparation for microscopy

Preliminary experiments with dyes and fixatives showed that the cyst was virtually impermeable to reagents, and the cysts were therefore punctured with a microscalpel or razor blade as quickly as possible after transfer to the fixative. This procedure facilitated the penetration of the fixative and the infiltration of the embedding material. Some of the cysts were treated with 0.5 M thioglycollic acid in acetate buffer at pH 5.5 (Rogers, 1959) to reduce the disulphide linkages of the keratinized layer.

Fixation. Standard buffered (7.2) 1% solutions of osmium tetroxide or Baker's formaldehyde-calcium (Baker, 1960) were used. Cysts were fixed in the osmium solution for 2 to 12 h and in the formaldehyde-calcium for 24 h. After fixation the cysts were rinsed in tap water and dehydrated in successive changes of 70% and absolute ethanol, each of 1 h. Infiltration was carried out in a 1:1 mixture of araldite and ethanol followed by pure araldite, both at 60°C for 1 h. The cysts were finally embedded in a further change of araldite and allowed to harden at 60°C for 48 h. Sections, passing roughly through the centre of a cyst, were cut on a LKB microtome with a diamond knife and were mounted on copper grids without a supporting film.

Staining. Sections were stained by floating grids on staining solutions for 30 min at 40 to 50°C. Good results were obtained with lead solutions (Kar novsky, 1961; Millonig, 1961) and use was also made of 1% solutions of uranyl acetate or potassium permanganate.

Electron microscopy. Sections were studied and photographed in an Elmiskop 1 electron microscope at instrumental magnifications of 2,500 to 40,000.

Results and discussion

No difficulty was experienced in recognizing from their locations the main layers of the cyst wall, and these are shown in fig. 1, A, a low power survey-micrograph. Their relative thickness depends on the part of the wall examined (dorsal or ventral) and need not concern us here.

The outer cyst wall

The tanned layer (fig. 1, A, t). The external layer of tanned protein is the thickest of the layers in the dorsal and lateral regions, and has the most open...
Dixon and Mercer—Cyst wall of metacercaria of Fasciola

texture. It consists of spongy aggregations of dense, irregularly shaped bodies whose character will be perhaps best appreciated by reference to the electron micrograph, fig. 2, A, which shows these bodies at a higher magnification than fig. 1, A. Closer examination of sections cut in different directions confirms that these aggregations consist of numbers of dense, cigar-shaped objects, apparently held together in a matrix of somewhat less dense material (fig. 2, A). Their appearance is essentially the same (though less apparent) in unstained material as in material stained with uranium or lead. The surfaces of these irregular masses fuse when they are in contact, giving the impression of being sticky. The entire layer, however, in no place appears compact, numerous interconnecting cavities remaining. The impression gained is that it would be rather porous, its value to the encysted animal being to add to the mechanical protection and perhaps (as suggested to us by Dr. W. L. Nicholas) to act as a barrier to bacterial and fungal penetration.

Rothschild (1936), discussing the formation of the outer layer of the cyst of an unknown cercaria, has described similar particles in a ‘primitive epithelium’, which later disintegrates leaving a layer of loosely adhering particles. Some support for her views has been obtained from sections of cercariae where these characteristic bodies can be seen within a bounding cell membrane.

The mucopolysaccharide layer (fig. 1, A, s1). This layer appears as a feltwork of very fine, poorly staining filaments. No structural distinction was observed between the parts of the layer known to give the reactions of acidic and neutral mucopolysaccharide. These reactions presumably are due to chemical end-groups distributed in varying amounts in a structural framework of macromolecular dimensions, visible by the electron microscope.

The inner cyst wall

The mucopolysaccharide layer (fig. 1, A, s2). On the ventral surface of the cyst this layer appears rather homogeneous, but on the lateral and dorsal surfaces it may be resolved into an outer, slightly denser layer, with a finely granular appearance, and an inner, structureless layer. The outer one is thought to be the mucoprotein layer IIIa described by Dixon (1964). The inner is probably

---

**FIG. 2 (plate).** A, the tanned-protein layer showing one of the aggregations of cigar-shaped bodies, cut roughly in longitudinal section at c. Osmium tetroxide; lead hydroxide.

B, the keratinized layer, k, showing the fine lamellae of which it is composed. pm, plasma membrane; m, edge of mitochondrion. Osmium tetroxide; lead hydroxide.

C, the keratinized layer after reduction of the disulphide bonds with buffered thioglycollate solution. Note the greater separation of the lamellae compared to those in fig. 2, B. Several free ends are visible in the lower portion. Osmium tetroxide; uranyl acetate.

D, imperfections at the outer edge of the keratinized layer. An incompletely opened whorl, w, may be compared with those shown in fig. 3, B. Some free ends may be seen at e. Osmium tetroxide; uranyl acetate.

E, high-magnification micrograph of the lamellae in the keratinized layer after treatment with buffered thioglycollate solution. The lamellae are rather widely separated and each lamella consists of a double strand. Osmium tetroxide; uranyl acetate.

F, longitudinal section of a scroll within the body of the cercaria. Osmium tetroxide; uranyl acetate.
made up of acidic and neutral mucopolysaccharide. The chemical differences may correspond to the structural differences evident in fig. 1, B at a.

The keratinized layer (fig. 1, A, k). Of the major layers this one is the most distinct, compact, and dense. At low power it appears homogeneous except for an occasional splitting into sheets. This effect is also visible with the light microscope, where the appearance suggests that this layer is coarsely laminated.

At higher powers (fig. 2, B) the formation is resolved into a succession of tightly compacted, fine, uniform lamellae each about 2.5 μ thick and separated by less dense layers of similar dimensions, composed of a finely particulate material. This structure is usually very regular, but some separation of the fine lamellae is occasionally apparent and other defective areas are not uncommon (figs. 1, A; 2, D; w). Some of these may have resulted from the process of formation (see below).

In wider gaps between the lamellae the less dense amorphous substance sometimes appears in larger amounts, particularly near the outer surface of the entire formation. This inter-lamellar component may conceivably function as an adhesive holding together the sheets of the lamellar component.

In sections of cysts pretreated with buffered thioglycollate solution the lamellae are more widely separated (fig. 2, c) and at higher magnifications (fig. 2, e) each lamella appears as a double strand. This suggests that the disulphide bonds occur predominantly between the lamellae.

This structure has some resemblance to the keratinized formations of the vertebrate epidermis. It is recognized that vertebrate keratin contains two components, a fine fibrillar material (α-keratin) and an amorphous matrix (γ-keratin), which are closely bonded together by the formation of disulphide bonds mainly located in the γ-component (Mercer, 1961). In the cyst wall we find fine sheets presumably bonded by a second component.

Superficially the keratinized layer of the cyst recalls the myelin sheath of myelinated nerves and the spherulites formed in water by phospholipids, although, of course, there is no chemical similarity. A structural distinction is the presence of 'free ends' in cross-sections of the keratinized lamellae (fig. 2, D, e). Such ends are almost never found in myelin formations.

Some further light on peculiarities of the structure of the keratinized layer comes from a consideration of its formation. Cells containing the secretion which is the precursor of the layer are found in the cercaria, where they may be recognized by their characteristic inclusions. In cross-section the inclusions appear circular and relatively uniform in diameter, and form closely packed hexagonal arrays in the cells. At higher powers a tightly wound spiral can be

---

Fig. 3 (plate). A, some details of the unwinding of a whorl, w, to form a further addition to the keratinized layer, k. pm, plasma membrane of an outer cell of the metacercaria. Osmium tetroxide; lead hydroxide.

B, details of the formation of the keratinized layer, k. Scrolls in various stages of secretion may be seen. w₁, the scroll deep within the cell is the most tightly wound; w₂, a later stage is closer to the surface and slightly larger in diameter; it is composed of fewer turns; w₃, the scroll is outside the cell and the final unwinding process has begun. Osmium tetroxide; uranyl acetate.
Fig. 3

K. E. Dixon and E. H. Mercer
resolved (fig. 3, B; w). In longitudinal and near longitudinal sections the secretion bodies appear as in fig. 2, F. The structure is evidently a tightly wound scroll, which appears as a small rod at low magnifications.

Nearer the cell surface the diameter of the scroll increases and it appears to be more open. A count of the number of turns of the spirals in cross-sections shows that these are less than in the compact forms, i.e. the scroll is unwinding. At the surface, the vacuole containing the secretion fuses with the cell membrane and its contents escape. The spiral scroll now appears to open more completely (fig. 3, A, B) and to produce a long, thin strip of material identical with the lamellae of the keratinized layer. The length of this strip is about 30 μ. The whorls which often occur as defects in the layer are recognizable as ‘scrolls’ which have not or have only in part unwound (fig. 2, D; w).

One of us (K. E. D.) held a Commonwealth Post-Graduate Scholarship while this work was being carried out.

References
Rothschild, M., 1936. Parasitology, 28, 56.