The tegument and associated structures of 
Fasciola hepatica

By L. T. THREADGOLD

(From the Department of Zoology, The Queen's University, Belfast, 
Northern Ireland)

With 7 plates (figs. 2 to 8)

Summary

The cuticle of light microscopy is shown by electron microscopy to be a surface layer of protoplasm which is an extension of areas of nucleated protoplasm lying deep in the parenchyma. The cuticle therefore exists at two levels. The external level is syncytial, consisting of plateaux separated by branching valleys. This level contains apical pinocytotic vesicles, numerous mitochondria, endoplasmic membranes, large basal and other vacuoles, and dense spines. Tube-like evaginations from the base of the external level connect it to the individual areas of flask-shaped protoplasm which compose the internal level. Each of these areas of protoplasm contains a nucleus, great numbers of mitochondria, some vacuoles and diffuse inclusions, and the Golgi bodies.

The histochemistry and function of the cuticle is discussed in the light of this new knowledge of cuticular ultrastructure, and a comparison is made between the cuticle of Cestoda and Trematoda.

Introduction

The structure of the cuticle of parasitic Platyhelminthes has been a controversial subject during the last half-century. In Cestoda this controversy has been resolved by the electron-microscope studies of the cuticle of Hymenolepis diminuta, H. rana, and Raillientina cesticulus (Kent, 1957), H. diminuta (Rothman, 1959, 1961), H. diminuta and R. cesticulus (Read, 1955), and Dipylidium caninum (Threadgold, 1962). The cuticle was shown to be a protoplasmic zone, containing mitochondria, membranes, and vacuoles, and to be connected by protoplasmic tubes to a nucleated area of protoplasm lying deep in the parenchyma. The cuticle was, therefore, a surface extension of interparenchymal cuticular cells.

Despite the electron-microscope study of Schistosoma mansoni by Senft and others (1961), the real structure of the cuticle in Trematoda is not known. Senft and his colleagues stated that the schistosome cuticle is 'a vast spongy layer' and 'an acellular amorphous covering'. These statements are in sharp contrast to the findings for Cestoda and leave the 5 major theories of cuticular structure in Trematoda, as outlined by Hyman (1951), still extant. These 5 theories can be condensed into 4, as follows:

1. The cuticle is an altered and degenerate epidermis.
2. The cuticle is the basement membrane of a former epidermis.

Fig. 1. Diagrammatic drawing of the structure of the tegument of F. hepatica. bm, basement membrane; bv, basal vacuoles; cm, circular muscle; cv, cytoplasmic vacuoles; df, diffuse mass; er, endoplasmic reticulum; g, Golgi bodies; it, interstitial material; iv, invaginations of plasma membrane; lm, longitudinal muscle; m, mitochondria; n, nucleus; pa, parenchymal cell; pt, protoplasmic tubes; pv, pinocytotic vesicles; sp, spine; v, valley.
3. The cuticle is the outer layer of an epidermis, the cells and nuclei of which have sunk beneath the subcuticular musculature.

4. The cuticle is a secretion of specialized or ordinary mesenchymal cells (parenchyma).

A decision about these theories is impossible unless the structure of the cuticle is known in detail. This knowledge has importance beyond these theories, however, because it concerns the physiology of Trematoda, especially their ability to resist destruction by their hosts. A further problem is the homology of the cuticle. If this structure has a mesodermal origin, it would be an exception to the general germ-layer hypothesis.

This electron-microscope study of *Fasciola hepatica* was undertaken to elucidate some of these points.

**Materials and methods**

Living *Fasciola* were dissected from sheep livers, placed in a drop of Palade's or Dalton's fixative (pH 7.4), and sliced into thin sections with a new razor blade. The slices were then left in fresh fixative at 0° C for 1 h. The tissue was embedded in 15:85 butyl:methyl methacrylate and sectioned with glass knives on a Cambridge ultramicrotome.

Sections were mounted on carbon-coated grids and viewed in an Akashi electron microscope. Some sections were stained in lead hydroxide and sandwiched with a methacrylate membrane. Negatives were made at ×2,000 to ×12,000 and enlarged as required.

**Observations**

The chief results of this investigation are summarized in fig. 1.

The 'cuticle' is about 15 to 21 μ thick and consists of plateaux separated by valleys (fig. 2). Its external surface has a definite plasma membrane which is invaginated frequently along its length (fig. 3, A). Immediately below these narrow indentations are pinocytotic vesicles, apparently pinched off from the invaginations. Around the larger vesicles are great numbers of small membrane-limited vacuoles and vesicles, and packed granules. The concentration of vacuoles, vesicles, and granules gives the cuticle a superficial zone of increased electron density (figs. 2; 3, A; 4, A).

Occasionally the external cuticular surface is evaginated into balloon-shaped bodies containing fine granular material of low density. These evaginations are apparently nipped off into the environment (fig. 3, B).

Below the superficial dense zone the cuticle is composed of a background material containing the following:

1. Smooth-surfaced endoplasmic membranes, of variable length and perhaps derived from the external and internal plasma membranes. These membranes are usually aligned at right angles to the cuticular surface (figs. 2; 4, B).
2. Numerous oval or elongate mitochondria, of which the longer ones are sinuous. These mitochondria often form strings of 4 or more units and are usually aligned at right angles to the cuticular surface. The mitochondrial matrix is dense and crossed by only one crista, running longitudinally (figs. 2; 4, B).

3. A multitude of small vesicles, the number of which decreases from the exterior of the cuticle inwards (figs. 4, A, B).

4. Numerous ovoid vacuoles of variable size. The more basal vacuoles are large and may be incompletely surrounded by a membrane, which often arises from the basal plasma membrane. More distal vacuoles are smaller than the basal ones and are limited by a complete bounding membrane. Usually such bodies are in contact with endoplasmic reticular membranes, as though arising from them. Some of these ovoid bodies are filled with either a homogeneous material of low electron density or with a more granular material. They are rarely completely empty (figs. 2; 4, B).

The cuticle is bounded on its interior surface by a plasma membrane and lies on an amorphous narrow basement membrane (fig. 4, B).

The valleys, which separate the plateaux areas, are deep, narrow invaginations, penetrating about one-third the depth of the cuticle. From the central valley arise short side branches, this arboreal form being surrounded by a bulbous electron-dense area, which is continuous with the superficial dense zone (fig. 4, A).

Cuticular spines are spaced irregularly through the cuticle. These are extremely electron-dense. They are amorphous, with sharp points and flat bases, the latter being narrower than the widest part of the spine. The spines, although they project beyond the general level of the cuticle, are always contained within the outer and inner plasma membranes (fig. 5, B).

The base of the cuticle is everted at intervals into tubes which penetrate...
the basement membrane, the muscle-layers, the interstitial material, and the parenchymal cells. The tubes bifurcate and anastomose so that sections show a confused picture of numerous ducts cut in various planes (figs. 2; 5, A). The walls of the tubes are extremely thin, consisting only of the basal plasma membrane, except where accompanied by an inner layer of granular cytoplasm. Near the cuticle the lumina of the tubes are clear, except for occasional mitochondria and small areas of protoplasm (fig. 5, A). Deeper within the parenchyma and approaching the cuticular cells, the tubes are wider, and the amount of protoplasm and the number of mitochondria increases, whereas the number and size of the vacuoles is reduced (fig. 6).

The cuticular cells occur either singly or in groups, often mixed with muscle cells. Interstitial material surrounds and separates the cuticular cells from the parenchyma (figs. 6, 8). The individual cells are flask-shaped, the narrow necks tapering into the tubes leading to the cuticle (fig. 6). The nucleus lies centrally and has an undulating outline. The nuclear membrane is bulbous in places where the inner and outer membranes are separated by an expanded middle area (fig. 8). A single very dense nucleolus is usual, but other large and irregular electron-dense patches also occur.

The cytoplasm is filled with numerous mitochondria, which are mostly short or round (figs. 7, 8). The mitochondrial matrix is of medium electron density, with a fibrillar appearance. Cristae are rare, generally not more than one in each mitochondrion, running in a longitudinal direction. A number of Golgi bodies occur near the nucleus and consist of circular areas of membranes and vesicles (fig. 8).

In addition the cytoplasm contains first, endoplasmic membranes, both rough, near the nuclear membrane, and smooth elsewhere; second, amorphous electron-dense areas containing a few vacuoles and surrounded by a zone of vacuoles; and finally vacuolated areas, mainly in contact with the plasma membrane (figs. 9 and 10).

Discussion

The results presented in this paper show clearly that the accepted structure of the external covering of *F. hepatica* is incorrect. This external layer is neither a secreted inert structure, nor a much altered and degenerate epidermis. Theory 3 of the theories presented in the introduction is essentially correct, although dismissed by Hyman (1951). The cuticle is a true cellular tegument, which is specialized in the following ways:

1. It is organized at two levels, external and internal. These two layers, although well separated, are connected together by protoplasmic strands. Such strands were described as canaliculi by Prenant (1928), but he did not realize their significance.
2. The external layer, which functions as the tegument, is syncytial and without nuclei. Alvarado (1951) and Pantelouris and Gresson (1960)
claimed to have observed nuclei, but probably mistook massed mito-
chloridia (which are occasionally seen in electron micrographs), for
nuclei, for such masses are very large.

This layer contains mitochondria, smooth endoplasmic membranes,
numerous vacuoles, and electron-dense spines. The extreme surface
membrane has either pinocytotic invaginations or bulbous evaginations.
Despite the lack of nuclei, therefore, this external layer is protoplasmic.

3. The internal level is nucleated and composed of separate cells, which are
either well separated by intervening parenchymal cells or associated in
compact groups. In addition to nuclei, the usual cytoplasmic organoids
are present, including the Golgi bodies.

4. The internal and external levels are connected by protoplasmic tubes,
which are mainly hollow, but contain mitochondria and protoplasm in
increasing quantities as the interior level is approached.

Senft and his colleagues (1961) may be mistaken in concluding that the
cuticle of *Schistosoma* is a vast spongy layer and an acellular amorphous
covering. Indeed, a careful examination of their electron micrographs sug-
gests that the cuticle contains mitochondria, that evaginations arise basally,
and that their epithelializing cells are really cuticular cells which are joined
to the cuticle by protoplasmic tubes. Positive confirmation of these points
cannot be made because of the low resolution of the photographs, but it is
tentatively suggested that the cuticle of *Schistosoma* is essentially similar to
that of *Fasciola*.

These findings have a bearing on the function of the cuticle. Mansour
(1959) showed that a gut ligature did not prevent the uptake of glucose by
*Fasciola*. The cuticle must, therefore, be able to absorb glucose and probably
does so by pinocytosis, for which there is now morphological evidence from
electron micrographs. Pantelouris and Gresson (1960) demonstrated that
some of the radioactive iron injected through the mouth eventually appeared
in the cuticular cells (myoblasts) and the cuticle itself. They considered the
cuticle either excretory or secretory in function, serving to eliminate excess
metabolites or to secrete mucus. The presence of evaginations and pinched-
off extrusion bodies in electron micrographs may very possibly indicate the
process whereby metabolites are secreted. Equally, such bodies might be
evidence of the release of mucus over the cuticular surface, although no such
mucus layer was seen. Clearly the pinocytotic vesicles and extrusion bodies

---

**Fig. 6 (plate).** Protoplasmic tube projecting towards cuticle from cuticular cell. Stained with
lead.

*df,* diffuse mass; *it,* interstitial material; *m,* mitochondria; *n,* nucleus; *pa,* parenchymal cell;
*pt,* protoplasmic tube.

**Fig. 7 (plate).** Cuticular cell.

*cv,* cytoplasmic vacuoles; *df,* diffuse mass; *g,* Golgi bodies; *it,* interstitial material; *m,*
mitochondria; *n,* nucleus; *pa,* parenchymal cells.

**Fig. 8 (plate).** Cuticular cell with organelles.

*cv,* cytoplasmic vacuoles; *df,* diffuse mass; *g,* Golgi bodies; *it,* interstitial material; *m,* mito-
chondria; *n,* nucleus; *pa,* parenchymal cell.
Fig. 8

L.T. THREADGOLD
are indicative of considerable cellular activity by the external layer of the cuticle.

It is worth while attempting to equate cuticular fine structure with the results of the histochemical tests of Monné (1959) and of Björkman and others (1963). Using enzymatic tests Björkman and his colleagues concluded that the cuticle was proteinaceous. Monné described the cuticle as comprising alternating fibrillar and granular regions, perpendicular to the cuticular surface. The fibrils were collagenous and Monné considered the cuticle to be neither keratinized nor tanned. All these results are to be expected because of the protoplasmic nature of the cuticle, and Monné's fibrils are probably represented in electron micrographs by the columns of protoplasm with mitochondria which arise between the basal and other vacuoles.

Glucose, non-glucose polysaccharide, and protein associated with polyphenol-quinones also occur within the cuticle, but are difficult to homologize with ultrastructures. Björkman and his colleagues stated that the cuticle had a mucopolysaccharide or mucoprotein rim, and Monné that the external cuticular layer consisted of a protein associated with polyphenol quinones. This mucoprotein rim or external cuticular layer seems to correspond to the electron-dense region of pinocytotic invaginations and small vesicles. The many profiles of plasma membrane plus polysaccharides which may possibly be within the pinocytotic vesicles would give a mucoprotein reaction.

Monné's granules which lie between the fibrils are non-glycogen polysaccharide and Björkman and his colleagues found glycogen granules distributed throughout the cuticle. Perhaps the smaller vesicles scattered through the external level are the glycogen of Björkman, and the larger basal vacuoles and relatively large vacuoles elsewhere in the cuticle represent the non-glycogen polysaccharide. In addition Monné reported an acid mucopolysaccharide layer outside the cuticle. He considered that neutral polysaccharides were secreted through the cuticle and changed to acidic polysaccharides outside. The evaginated balloons seen in electron micrographs may represent the secretion of this altering polysaccharide.

A comparison of the cuticles of *Dipylidium* and *Fasciola* reveals both their essential similarity and their significant differences. Microtriches and porecanals are absent in *Fasciola* but its exterior level appears more vacuolated and contains relatively more mitochondria of a smaller size. The cuticular cells of *Fasciola* contain no fatty or crystalline inclusions, but do have considerably more mitochondria and Golgi bodies; the latter were absent in *Dipylidium*. The absence of inclusion-bodies in the cuticular cells of *Fasciola* and their presence in *Dipylidium* are consistent with the probable digestive function of the cuticle in the latter, an unnecessary function for the cuticle of a trematode like *Fasciola*.

The detailed morphological studies of the cuticle of this one trematode and a number of cestodes have shown this structure to be essentially cellular and similar to the epidermis of the related group, the Turbellaria. There seems no reason, therefore, to continue the use of the term 'cuticle' for this structure.
Threadgold—Tegument of Fasciola

The combined external and internal layers should be termed a tegument, the term epidermis being avoided until the embryological origin of the tegument is known for certain.

I wish to thank the Wellcome Trust and the Royal Society for the use of an Akashi electron microscope and ancillary equipment made available by a grant to Professor R. A. R. Gresson. Thanks are also due to Mr. W. Ferguson for enlarging the electron micrographs, and to Mr. A. Lyness for technical assistance.

References

Alvarado, F., 1951. Trabajos del Instituto José de Acosta, serie biológica, III. Madrid.


