An electron microscope study of the tegument and associated structures of *Dipylidium Caninum*

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With 4 plates (figs. 1–4)

Summary

The ultrastructure of the 'cuticle' of *Dipylidium caninum* is described. The presence of mitochondria within the 'cuticle' and the connexion between it and subcuticular cells indicates that the outer covering of this cestode is of living cells forming an epidermis. On its external surface the epidermis is folded into finger-like microthriches and its thickness is pierced in places by pore-canals ending at the basement membrane. The sunken epidermal cells contain mitochondria, endoplasmic reticulum, fatty or glycogen inclusions, and protein crystalloids. It is suggested that such cells act as both storage and synthesizing units.

The possible bearing of the present morphological findings on cestode physiology is discussed, especially in reference to cestode resistance to digestion and the ability of the 'cuticle' to absorb.

Introduction

Because of their peculiar environment, tapeworms are highly specialized both anatomically and histologically, a fact well known. It is reasonable to assume, therefore, that the high resolution of the electron microscope might reveal finer morphological adaptation, having some bearing on the specialized physiology of these parasites and enabling them to exist in their unusual environment.

Such a possibility has already been recognized by Kent (1957), Rothman (1959, 1960), and Read (1955). Kent carried out electron microscopy on *Hymenolepis diminuta*, *H. rana*, and *Raillientina cesticillus*, Rothman on *H. diminuta*, and Read on both *H. diminuta* and *Raillientina cesticillus*. With respect to the 'cuticle' these authors have shown that there are surface projections resembling microvilli (microthriches—Rothman); mitochondria present in the 'cuticle', suggesting that it is living material rather than a secretion (hence the preference for the term tegument—Rothman, 1959, 1960); and that it is permeated by pore-canals, the entrance and termination of which are not surely known.

The studies of Rothman and of Read were preliminary in some respects, and I therefore decided to study the 'cuticle' of *Dipylidium caninum* (L.).

Material and methods

Proglottides, from an adult tapeworm obtained from a cat, were fixed in Palade’s fixative at pH 7.4 and embedded in methacrylate. Sections were cut on a Porter-Blum ultramicrotome with a diamond knife and micrographed at magnifications varying from 2,000 to 8,000 on a Philips E.M. 75.

Observations

Fig. 1 shows a view of the structures lying immediately above the general parenchyma. These layers will be described from the exterior inwards. The tegument is electron-dense and of variable thickness, has numerous vacuoles of varying sizes, and scattered mitochondria, which tend to be concentrated basally (figs. 2, B, C; 3, B). The external surface is raised into finger-like projections, microthriches, whose outer layer is continuous with the membrane covering the tegument surface elsewhere. Immediately inside this limiting membrane is a thin, electron-dense region (fig. 2, A). The interior of a microthrix is continuous with the general mass of the tegument and its distal region is pointed and more electron dense than its proximal portion (fig. 2, A). In cross-section the distal region of a microthrix is round or oval and the proximal region diamond-shaped, changing to approximately square as the tegument is approached (fig. 2, D).

The tegument is pierced in places by more or less continuous pores (fig. 3, A, B). At higher magnification these pores are seen to lie between sections of integument which are bound by membranes (fig. 3, A). Tenuous strands cross such pores and the basement membrane is exposed to the exterior. Discontinuous pores show at their outer or inner closed end two opposed membranes resembling the typical interface between two cells (fig. 2, B, C), although such membranes are indistinct and become lost in the middle region of the tegument. In regions without pores such double membranes can be seen running through the tegument (fig. 2, A to C), but they tend to be discontinuous or to disappear among the numerous small vacuoles. In addition there are short infoldings of the basal plasma membrane (figs. 2, B; 3, C).

The tegument lies on a thick basement membrane of amorphous material containing granular inclusions. The basement membrane appears to be continuous with the general filling material which lies between parenchymal...
FIG. 1

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FIG. 2

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cells of the interior of the proglottis (fig. 3, c). Below the basement membrane are two layers of smooth muscle, the outer occurring as circular bundles and the inner as longitudinal bundles of variable thickness (fig. 1). Immediately below the muscle-layers are cells of three types. First, muscle-cells, some of which run from the outer muscle-layers into the interior of the parenchyma (fig. 1). Second, 'light' cells, somewhat more electron-dense than the general background filling and having long pseudopodial-like extensions (fig. 1).

The third cell-type is the 'dark' cell, which will be considered in detail, because this cell is intimately connected with the tegument. Dark cells are generally elongated; both the cytoplasm and the nucleus are very electron-dense and hence the latter is difficult to distinguish from the former (fig. 4, A). The nucleus usually contains a relatively large nucleolus, as well as darker masses of chromatin. The cytoplasm and plasma membrane are granular. Mitochondria are rare and have few cristae. Short lengths of endoplasmic reticulum are common and sometimes this cell organelle is coiled, its individual membranes being separated by vacuoles of variable size (fig. 4, A, B). Conspicuous in the cytoplasm are crystalline bodies of relatively large size (fig. 4, A, B), usually in contact with strands of endoplasmic reticulum; and osmiophil, presumably fatty, inclusions, which are in many cases shrunken or partly dissolved away (fig. 1).

The dark cells have protoplasmic processes, containing mitochondria, extending from the cell-pole facing the exterior of the proglottis. These processes traverse the muscle layers and basement membrane to connect with the tegument itself (fig. 3, c). Such processes on approaching the tegument contain vacuolated, intermittent protoplasm, and frequently, just prior to their junction with the tegument, are hollow tubes, the sides of which are the plasma membranes (fig. 3, c). There is no doubt that the tegument is merely an extension of the dark cells.

Discussion

In general the present findings agree with those of Kent (1957), Rothman (1959, 1960), and Read (1955) for Hymenolepis and Raillientina, namely in that the surface of Dipylidium is covered with microthriches and in that the 'cuticle' is of living matter and, therefore, is better called a tegument.

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**Fig. 3 (plate).** A, a pore-canal. Note opposed membranes at arrow. *m*, mitochondria; *cm*, circular muscle; *bm*, basement membrane; *ct*, part of a connexion 'tube' between dark cell and tegument.

B, transverse section of cuticle to show incomplete pore-canals (*ipc*). *bm*, basement membrane; *mi*, microthriches.

C, connecting 'tubes' between dark cells and tegument. *dc*, dark cells; *ct*, 'tube'; *cm*, circular muscle; *x*, opposed membranes between tegument derived from different dark cells.

**Fig. 4 (plate).** A, dark cell. *n*, nucleus; *nl*, nucleolus; *m*, mitochondrion; *pr*, protein crystalloids; *er*, endoplasmic reticulum; *f*, fatty or glycogen inclusions.

B, *pr* protein crystalloid; *er*, endoplasmic reticulum.
**Threadgold—The tegument of Dipylidium caninum**

*Dipylidium*, however, differs in some structures from the species cited above. The microthriches do not have a central cavity in their proximal half but have, instead, an electron density similar to the tegument itself. These microthriches are undoubtedly extensions of the tegument.

It is evident that the tegument is not a continuous structure over the whole surface of the proglottid, but is interrupted by membranes very similar to those occurring at interfaces between cells in other animals. Similar opposed cell membranes are shown in Read’s paper (in his fig. 10). It must be pointed out, however, that such membranes in *Dipylidium* are usually indistinct and discontinuous and it has not been possible in the present study to follow them throughout the entire thickness of the tegument.

The connexions between tegument and dark cells, and the presence of mitochondria throughout the tegument substance, strongly suggest that this structure is made up of portions of cells forming a surface, squamous-like epithelium, joined by protoplasmic extensions to the main cell-bodies which are sunk deep in the parenchyma. This interpretation is shown in fig. 5. Studies of the embryological development of cysticerci of *Taenia* by Schiller (1960) is further support for such an interpretation, though it must be noted that Schiller suggests that the cuticle is a cement secreted by subcuticular cells.

The arrangement of the tegument and dark cells is suggestive of a peculiarly everted gut. It is reasonable to suppose that the epithelial cells have not only the protective function, common to all teguments, but have assumed also the absorptive function of digestive cells, since cestodes are without an alimentary canal.

Rothman and also Read mentioned pore-canals or tube-like structures which pierced the tegument and passed from its inner border into the sub-tegument, then branched and disappeared into the parenchyma. Such canals occur in *Dipylidium* and are of two types. The first are due to the more or less complete separation of portions of tegument arising from two adjacent dark cells (fig. 3, A). Such canals stop at the basement membrane. The second type of canal occurs in the protoplasm connecting the main body of the dark cells to the base of the tegument. Such canals are not continued into either the tegument, the main part of the cell, or the microthriches and do not connect with the usually larger canal of the first type (see fig. 3, C). It is tempting to suggest physiological functions for both types of canal but this is perhaps somewhat premature.

What bearing do the present morphological findings have on the problems of cestode physiology? First, the microthriches, assuming the tegument has the ability to absorb, would greatly increase the surface area. Their dense distal ends, however, suggest a mechanical function, perhaps to prevent expulsion of the worm, by interdigitation with the microvilli of the host’s cells. Microthriches could also act as an abrasive, destroying host cells and thereby adding nutritives in high concentration within a localized narrow zone close to the parasite.
FIG. 5. Diagrammatic representation of a section through the tegument and neighbouring structures of *Dipylidium*. *mi*, microthriches; *m*, mitochondria; *v*, vacuoles; *pc*, pore-canal; *bm*, basement membrane; *ct*, connecting tube between dark cell (*dc*) and tegument; *cm*, circular muscle; *lm*, longitudinal muscle; *n*, nucleus; *er*, endoplasmic reticulum; *pr*, protein crystalloid; *f*, inclusions of fat or glycogen; *op*, opposed membranes; *ipc*, incomplete pore-canal.
Second, the finding that the 'cuticle' is of living protoplasm offers a solution to the problem of cestode resistance to digestion. Anti-enzyme systems or a highly resistant 'horny cuticle', which both raise difficulties with regards to absorption by the cestode itself, are unnecessary. A cellular 'cuticle' would merely be replaced as a whole, or renewed, probably the latter on present evidence, especially as the major portion of the cell would be unaffected, lying as it does deep in the parenchyma. A similar idea was previously suggested by Watson (1960), though he believed the cuticle was of inert material re-formed by the subcutaneous glands or the epidermis.

Third, the existence of numerous vacuoles, especially in the distal part of the tegument and the presence of pores ending at the basement membrane, would facilitate the penetration of substances in solution. The vacuoles could be the result of 'cuticular pinocytosis', as suggested by Rothman (1960), although they may be only structural features.

Finally, the electron-dense cytoplasm, the presence of mitochondria and endoplasmic reticulum, and the existence of crystalline and storage bodies (fat or perhaps glycogen) in the cytoplasm of dark cells suggests that such cells may subject absorbed substances to synthesis and either store or pass on these synthesized substances to the parenchyma.

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References
—— 1960. Ibid., 46, 5 (suppl.), 10.