Surface modifications of hypodermal and trophosome cells from parasitic juveniles of mermithid nematodes

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SUMMARY

The present paper reports an ultrastructural investigation of the exposed surfaces of the hypodermal and trophosome cells from two parasitic juvenile mermithids, *Empidomermis riouxi* Doucet *et al.* and an undetermined species from *Porcellio scaber*.

Villi were found on the outer surfaces of the hypodermal cells of both mermithids. Villi and another type of specialized surface were also found lining the well-defined lumen of the trophosome.

The observation of hypodermal modifications supports earlier findings of transcuticular uptake of nutrients by mermithid nematodes. The purpose of specialized cell surfaces lining the trophosome lumen in both mermithid species is not known, but it is hypothesized that digested host material passes through the cuticle, hypodermis, pseudocoelom and trophosome cells into the lumen to be further digested and converted into storage material that is then reabsorbed by the trophosome cells.

RÉSUMÉ

Modifications superficielles des cellules de l'hypoderme et du trophosome des juvéniles parasites chez les nématodes mermithides

Cet article traite de l'ultrastructure de la surface active des cellules de l'hypoderme et du trophosome chez les juvéniles parasites de deux mermithides (*Empidomermis riouxi* Doucet *et al.* et une espèce indéterminée provenant de *Porcellio scaber*). Des villosités ont été observées à la surface externe des cellules hypodermiques des deux espèces. Par ailleurs des villosités et un autre type de surface spécialisée bordent le lumen bien défini de leur trophosome. Les modifications hypodermiques observées renforcent les remarques précédentes (Poinar & Hesse, 1977) sur l'absorption de substances nutritives à travers la cuticule chez les mermithides. Le rôle exact de la surface des cellules modifiée en relation avec l'absorption chez ces deux mermithides n'est pas connu ; on peut cependant supposer que certains matériaux digérés provenant de l'hôte passent, à travers la cuticule, l'hypoderme, le pseudocoelome et les cellules du trophosome, dans le lumen de ce dernier pour y subir une digestion complémentaire et y être convertis en matériaux de réserve qui seront alors réabsorbés par les cellules du trophosome.

Mermithids, a group of adenophorean nematodes that attack invertebrates, show some specialized morphological features as a result of their parasitic existence. As the nematodes initiates growth inside the host, the intestine separates from the pharynx and body wall (anus) and swells up into a food storage organ, that is known as a trophosome. Only the second and third stage juveniles take up nourishment ; all other stages are free-living and survive on stored up nutrients. There is no evidence that mermithid nematodes take up food through their mouth and the thin, narrow pharyngeal tube. As with many other internal parasites resting in a liquid environment, mermithids apparently absorp nutrients directly through their cuticle into the sublying hypodermal cords by what appears to be an active transport system.

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Further, it has generally been assumed that the lumen of the trophosome has either been lost or greatly reduced and very few references have been made to it (Chitwood & Chitwood, 1937 ; Rubtsov, 1967 ; Batson, 1979 *b*).

The present study demonstrates the presence of villi on the outer surfaces of the hypodermal cells of the parasitic stages of two mermithids as well as microvilli and other specialized surfaces lining the well-defined lumen of the trophosome, respectively.

Materials and Methods

The parasitic juvenile stages of two mermithid species were examined in the present study. One was *Empidomermis riouxi* Doucet *et al.*, 1979, a parasite of the mosquito, *Aedes detritus* in Southern France. The other was an undetermined parasite of the sowbug, *Porcellio scaber*, in California. The specimens were removed directly from their hosts and prefixed in 4% glutaraldehyde in 0.1 M phosphate buffer for one hour. They were then washed in buffer, fixed in 1% osmium tetroxide for 1 hr, dehydrated in an ethanol series and embedded in Araldite. Sections cut with a Porter-Blum MT2 ultramicrotome were stained with uranyl acetate and examined in a Philips EM-300 electron microscope.

Results

Cuticle and Hypodermis

In *E. riouxi* the cuticle was attenuated and composed of three basic layers. The surface of the underlying hypodermal cells was modified on the cuticular side into villi (Fig. 1). These villi were closely packed and interdigitated, containing ribosomes in some areas and frequently fine fibrils (Fig. 2, arrow). At the base of the villi some vacuoles were observed closely associated with endoplasmic reticulum (V. Fig. 2). The villi were quite extensive and often extended to the inner layer of the cuticle (C, Figs, 1, 2). The mermithid from *Porcellio* possessed a defined cuticle composed of three basic layers, which, in turn were subdivided (C, Figs. 3, 4). The outer surface of the hypodermal cells was modified into closely compacted villi, which also contained fine fibrils (Figs 4, arrows) but lacked ribosomes.

Intestine

The intestine of both parasitic juveniles had already become detached from the pharynx and body wall, and lacking any connection to the outside, it could be properly called a trophosome.

The trophosomes of both species were similar in being polycytous (composed of several hundred cells), homocytous (a similar cell character), isocytous (cells similar in size) and in possessing a well defined lumen for most of their length. A thin layer or basement membrane surrounded the outside of those cells of the trophosome that were adjacent to the body cavity. Nuclear divisions were evident and this probably accounts for the polynucleate condition of the cells.

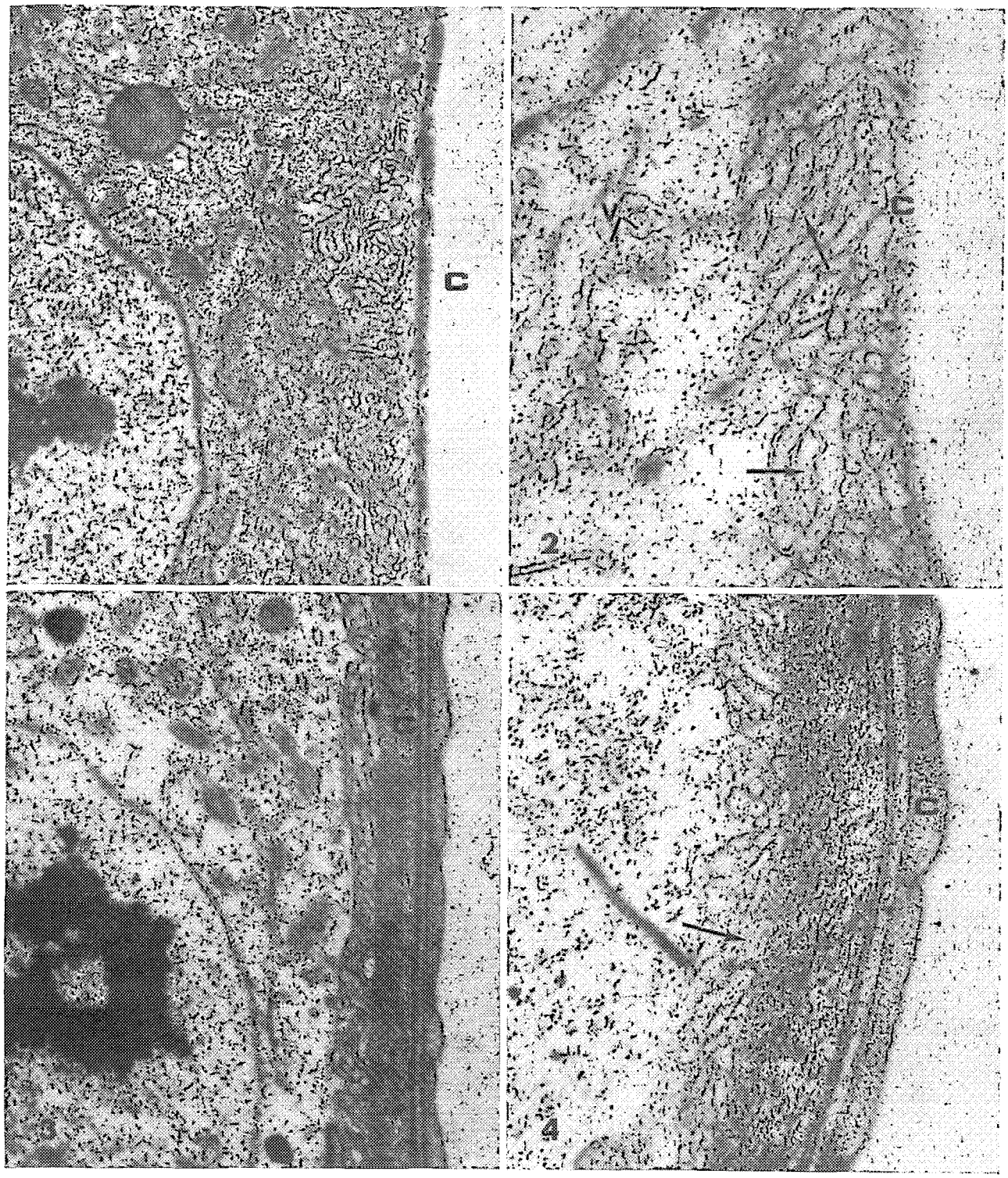
In *E. riouxi*, the surface of the cells bordering on the lumen was extended into a fine network of twisting interconnecting cytoplasmic projections (Figs. 5, 6).

These projections were present on all the cells surrounding the lumen. Frequently, the enclosed vesicles which were formed extended well out into the center of the lumen (P, Fig. 6), which was filled with granular material, presumably break down products from the cytoplasmic projections (G, Fig. 5). Just basal to the cytoplasmic projections were bundles of fine fibers which surrounded the lumen of the trophosome (F, Fig. 6). These fibers were oriented primarily parallel to the longitudinal axis of the lumen.

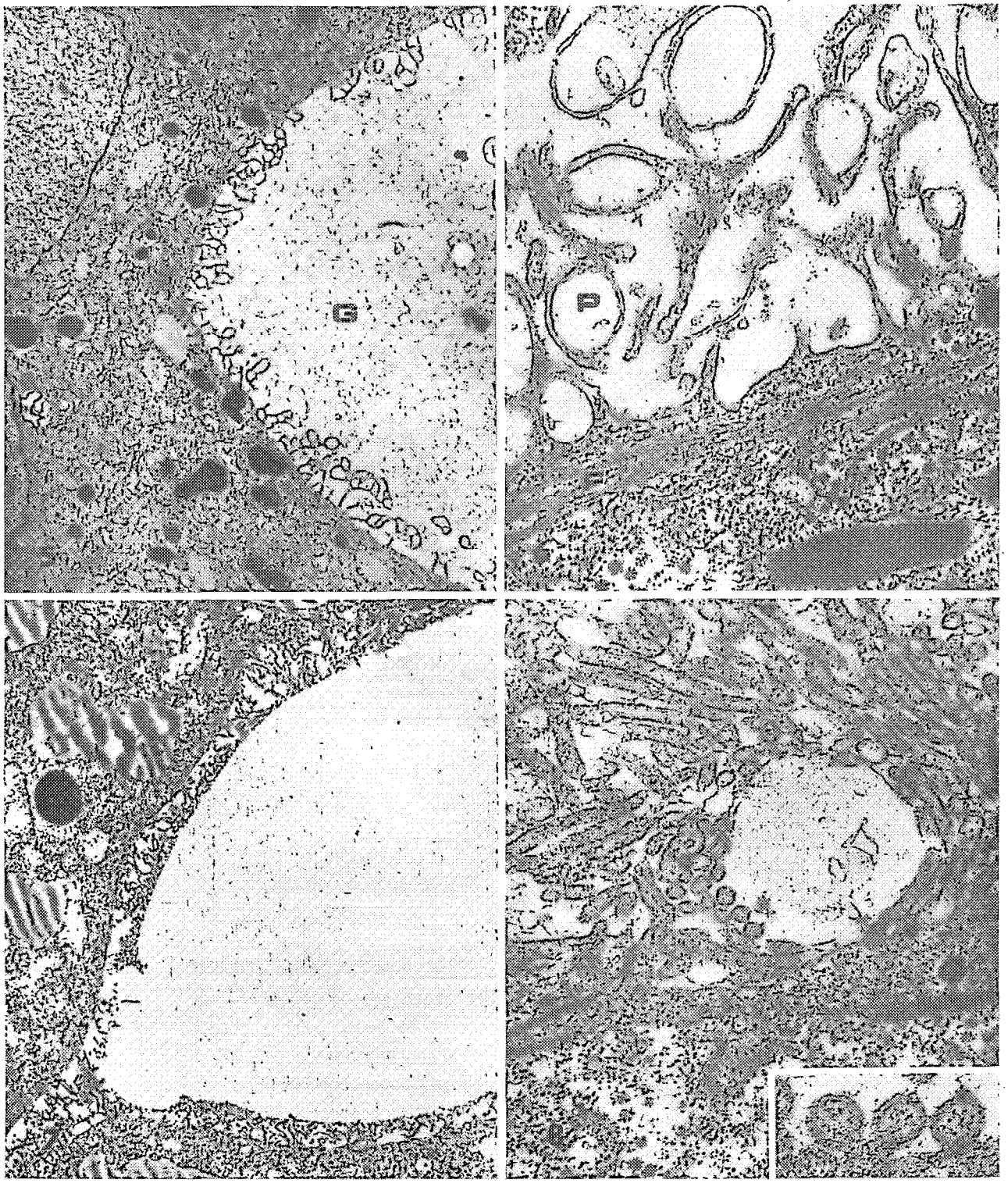
In the parasite from *P. scaber*, the surface of the cells facing the lumen was covered with elongate microvilli, similar to those in the intestine of microbotrophic free-living nematodes (Figs 7, 8). These villi were roughly the same length throughout the lumen, which appeared empty at this stage. The microvilli were approximately 100 nm in diameter and up to 1.5 μm in length, and did not appear to possess a glycocalyx covering externally.

Discussion

In the intestine of most nematodes, the surface of the cells of the mesenteron facing the lumen



Figs 1-4 ; 1 : Hypodermal cells and cuticle of *E. riouxi*, C, attenuated cuticle, 11,550 X ; 2 : Higher magnification of villi on hypodermal cells of *E. riouxi* showing the fine fibrils extending into the villar processes (arrows) and vacuoles at the base of the villi (V). C, attenuated cuticle. 41,000 X ; 3 : Hypodermal cells and cuticle of the parasite from *P. scabra*. C, cuticle. 20,000 X ; 4 : Higher magnification of the hypodermal cells with villi and the cuticle of the mermithid from *P. scabra*. Fine fibrils are observed within the villar processes (Arrow). 40,500 X.



Figs 5-8 ; 5 : Section of lumen bordered by cytoplasmic extensions of the trophosome cells of *E. riouxi*. G. granular material observed in lumen. 5,500 X ; 6 : Detail of cytoplasmic extensions adjacent to the lumen in *E. riouxi*. F, fine fibrils in area just below lumen. P, vesicles formed in cytoplasmic projections. 43,000 X ; 7 : Villi of the trophosome cells lining the lumen of the parasite from *P. scaber*. 4,950 X ; 8 : Villi of trophosome cells ad jacent to the lumen of the parasite from *P. scaber*. 37,800 X. Insert : Higher magnification of villi from Fig. 8 showing filaments in villar cytoplasm. 108,000 X.

is modified into villi of various lengths, depending on the species and stage, which absorb digested food material taken orally.

However, some of the entomogenous nematodes are known to have unusual methods of food uptake. Morphological evidence for nutrient uptake directly through the body wall has been shown for members of the Allantonematidae (Riding, 1970 ; Nicholas, 1972). At least one species of Sphaerulariidae absorbs nutrients through the inner surfaces of the everted uterine cells (Poinar & Hess, 1972). Mermithids are also known to take up substances through their cuticle (Rutherford & Webster, 1974). On the basis of experimental evidence, Rubtsov (1967) and Poinar and Hess (1977) concluded that nutrients pass through the thin cuticle of the parasitic stages of mermithids, thus avoiding any contact with the modified alimentary tract. The latter authors also showed that after passing through minute holes in the cuticle, ferritin particles were absorbed by the microvillar surface of the underlying hypodermal cells of the parasitic juveniles of *Romanomermis culicivorax*. Villi on the outer surface of the hypodermal cells in the two mermithids in the present investigation lend support to these earlier findings of transcuticular uptake of nutrients by mermithid nematodes. Other mermithids have also been shown to have their outer hypodermal cells modified into an absorptive surface (Batson, 1979 *a*). Although Poinar and Hess (1977) could not demonstrate an outward flow of secretions from the mermithid into the host, Rubtsov (1967) felt that cellular secretions acting as digestive enzymes passed out through cuticular canals into the host's body where extracorpeal digestion occurred. It was Rubtsov's contention that osmocytes (= stichocytes) drew in partially digested host hemolymph which then spread throughout the pseudocoelom along the trophosome.

Rubtsov (1967) characterized the mermithid trophosome as having no entry nor exit and lacking a lumen and Batson (1979 *b*) found no lumen in the synaptial trophosome of *Gastromermis boophthorae*. However it is now apparent that at least some species do possess a lumen. A small lumen in the anterior portion of the mermithid trophosome was first noted by Rauther (1909) in *Mermis* sp. and then briefly discussed by Steiner (1933). A lumen was observed in the anterior portion of the intestine of the infective-stage juveniles of *Filipjevimermis leipsandra* and *Gastromermis viridis* (Poinar & Hess, 1974) and was observed in the parasitic juveniles of the former species (Poinar, 1968).

In the present investigation the lumen of both mermithids investigated extends over half the length of the trophosome and all the cells lining it contain absorptive structures on their surfaces.

The purpose of the lumen and the absorptive surfaces of the adjacent cells are difficult to explain in the light of the "closed" condition of the modified intestine. Being a modified food storage organ, the trophosome grows tremendously in volume during parasitic development of the mermithid. Since there is no opening, it is assumed that the nutrients to be stored are absorbed directly from the body cavity of the nematode. However if the trophosome is obtaining and storing nutrients from the body cavity, it would appear that the absorptive surfaces should be on the outer layer of trophosome cells, and not on those facing the lumen. Clearly the villi and cytoplasmic projections lining the lumen are absorptive in function, yet it is a mystery as to just what they are absorbing.

It may be possible that partially digested host material passes through the cuticle, hypodermis, pseudocoelom and the trophosome cells into the lumen to be fully digested and converted into storage material that is then reabsorbed by the trophosome cells. This would explain the active appearance of the absorptive surfaces which tends to discredit the view that these villi are a vestige of earlier times, when, from the standpoint of evolution, the free-living ancestor of the mermithids was still taking in food material through the alimentary tract, and the absorptive surfaces were performing their natural function.

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