Journal of Nematology 17(3):261–269. 1985.

Permeability of the Body Wall of *Romanomermis culicivorax* to Lanthanum[1]

ANN PLATZER[2] AND EDWARD G. PLATZER[3]

Abstract: Ultrastructural study of the body wall of preparasitic, parasitic, and postparasitic stages of *Romanomermis culicivorax* showed that the cuticle of all three stages was permeable to lanthanum. The cuticle of the parasitic stage was the thinnest and showed the greatest permeability. Lanthanum accumulated on the apical surfaces of the hypodermal cells but was not found intracellularly. The negative staining characteristics of lanthanum enhanced the detection of numerous smooth septate junctions in the hypodermis of the parasitic stage.

Key words: Culex pipiens, hypodermis, mermithid ultrastructure, nematode ultrastructure, septate junctions.

In contrast to earlier studies on cuticular permeability in nematodes (37), Rutherford and Webster (31) demonstrated unequivocally that *Mermis nigrescens,* a mermithid nematode, assimilated small molecular weight nutrients by means of transcuticular uptake from the hemolymph of its insect host. Because mermithid nematodes lack a complete digestive tract, this mode of nutrient assimilation is considered normal for mermithids. Although Gordon and Webster (9) and Schmidt and Platzer (34) could find no biochemical evidence of protein uptake by the parasitic stage of *M. nigrescens* and *Romanomermis culicivorax,* Poinar and Hess (26) reported ultrastructural evidence of cuticular pores permeable to high molecular weight proteins.

The purpose of this study was to search for potential pores involved in nutrient uptake in the body of three life stages of the mermithid nematode, *R. culicivorax,* a parasite of mosquito larvae, using lanthanum, an electron dense element.

MATERIALS AND METHODS

R. culicivorax was maintained in *Culex pipiens* as described earlier (25). The following nematode stages were examined: free swimming preparasites within 1 day after hatching, parasitic stages after 3, 4, and 5 days inside the host, and postparasites 7–30 days after emergence from mosquito hosts.

Control nematodes were fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 day at 25 C, postfixed in 1% osmium tetroxide in the same buffer for 1 hour at 4 C, dehydrated in an ascending ethyl alcohol series and propylene oxide, and embedded in Spurr's epoxy (35). Nematodes were exposed to lanthanum by the following two methods: One series of experimental nematodes were fixed and dehydrated in the same manner as the controls except that 2% w/v lanthanum nitrate was added to all fixatives and dehydrating solutions but not the final 100% alcohol (19) and propylene oxide steps. A second series of experimental nematodes were placed in 2% w/v lanthanum nitrate for 1–2 hours, rinsed, and fixed as above except the cacodylate buffer was replaced with 0.1 M sodium phosphate buffer, pH 7.2, which precipitated the lanthanum. The same results were obtained by both methods. Dehydration and embedding were performed as for the control nematodes. Sixty to ninety nanometer sections were prepared with a Sorval MT-2B ultramicrotome. Control sections were stained with lead citrate with or without prior staining with aqueous uranyl acetate. Sections from lanthanum treated animals were not stained. Sections were examined with either a Philips 300 or 400 electron microscope calibrated with a grating replica.

RESULTS

The basic nomenclature for the cuticle proposed by Bird (4) will be used here.

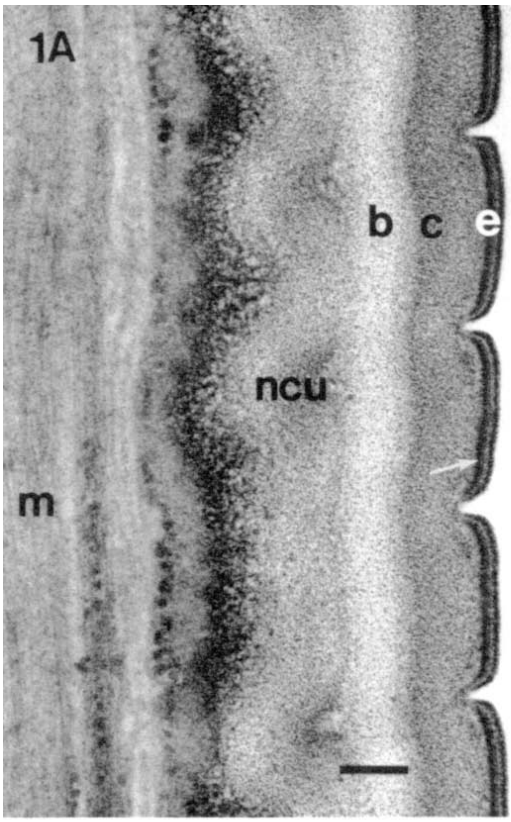
Preparasite control (Fig. 1A–D): The cuticle of the preparasite is about 300 nm

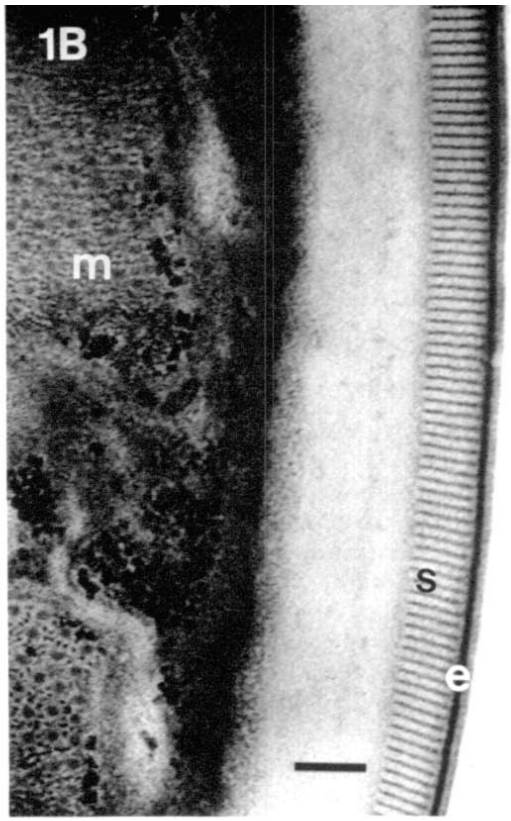
Received for publication 20 August 1984.

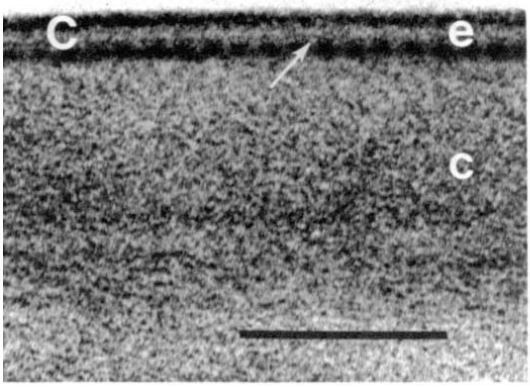
[1] This study was supported in part by Research Grant AI-15717 from NIAID, National Institutes of Health.

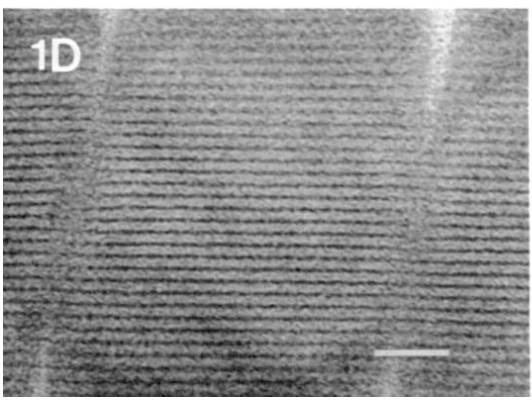
[2] Department of Biology, University of California, Riverside, CA 92521.

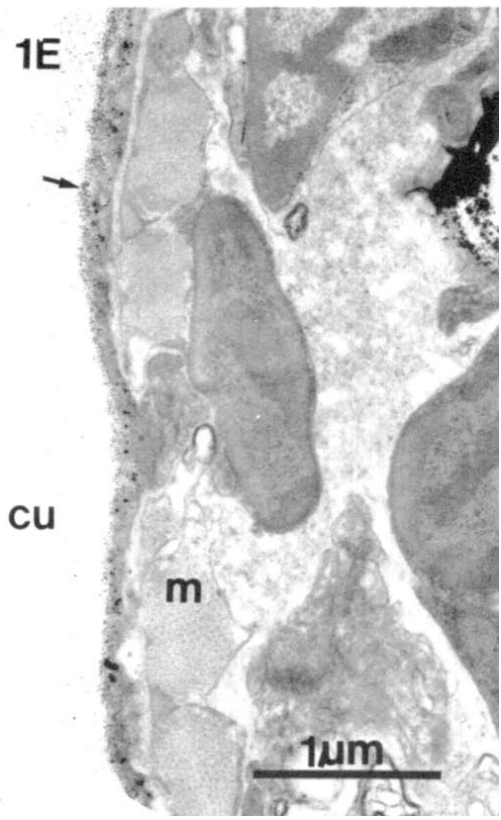
[3] Departments of Nematology and Biology, University of California, Riverside, CA 92521.


1A
b c e
ncu
m


1B
m
s
e


C
e
c


1D


1E
cu
m
1µm

thick and consists of an epicuticle, cortical zone, and basal zone.

The epicuticle, about 25 nm thick, is trilaminar under low magnification. With higher magnification, the inner dark osmiophilic layer appears to be composed of a thin, dark outer layer (Fig. 1A, C—arrow) and an inner thicker chain of closely packed globular units about 14 nm thick.

The cortical zone is an amorphous, moderately osmiophilic dense layer about 100 nm thick. This region contains a single fibrillar layer consisting of regularly arranged radial thick (4 nm) and thin striations which circumscribe the worm (Fig. 1B, D). The striations are in very close association with the epicuticle. Thick striations are spaced about 16 nm apart. A longitudinal surface view shows that these striations run uninterrupted except superficially in the furrows of annulae which indicates they are laminae (Fig. 1D) as proposed by Popham and Webster (28). A finely granular basal zone of uneven thickness often appears to blend into the hypodermal cells.

The hypodermal cells are packed with rough endoplasmic reticulum (as in parasitic stage, Fig. 2A). Thin interchordal cytoplasm (5) runs underneath the basal zone, and close junctions occur between plasma membranes of adjacent cells. Microvilli are numerous on the apical surfaces. In some nematodes examined an anlage of a new cuticle is evident (Fig. 1A).

Lanthanum treated preparasites (Fig. 1E): The cuticle in this stage is somewhat permeable to lanthanum. Small dots (approximately 1.5 nm as shown in Fig. 3C) are scattered in the cuticle, and a few deposits occur in furrows. Moderate amounts of lanthanum occur in the apical hypodermal zones. Although large accumulations of lanthanum coat cellular membranes in the esophageal and amphid areas where lanthanum entered directly, the element did not occur intracellularly.

Parasitic controls 3–4 days after infection: The cuticle is thin and indistinctly zoned; striations are absent (Fig. 2A, B). A faint double layer of fibers runs parallel to the long axis. An amorphous area occurs external to the epicuticle. In contrast to the cuticle, the interchordal cytoplasm of the hypodermis is frequently thick and contains abundant rough endoplasmic reticulum. Microvilli are numerous on the apical surface.

Parasitic control 5 days after infection: The cuticle is about 800–1,000 nm thick and resembles the postparasite (Fig. 2C, D). The cortical zone is amorphous and not striated. Next there are two layers each composed of alternately pale and dark bundles of filaments which run parallel to the cuticle. The next region consists of a mixture of granules and more clumps of longitudinally arranged filaments. In oblique view, the bundles form a loose basket weave. Cross bandings, with a major periodicity of about 64 nm (18), indicate that they are composed of collagen (Fig. 2D). Interchordal cytoplasm is usually thick and contains numerous clusters of pale vesicles sometimes bound by a single membrane (Fig. 2C) and often in intimate association with rough endoplasmic reticulum. Microvilli are more compact than at 3–4 days.

Lanthanum treated parasites 3–5 days after infection (Fig. 3A–E): Lanthanum penetrates freely along the length of the cuticle of the 3–5-day parasitic nematodes (Fig. 3A, B). Some accumulations occur in the channels of the cuticular annulations. Numerous dots 1.5 nm in diameter occur in the cuticle (Fig. 3C). These minute accumulations of lanthanum, which also occur in preparasites, may represent porous regions through which ions pass, but more work is necessary to determine the presence or absence of pores. Large deposits of lanthanum accumulate mainly in the intercellular spaces of the hypodermal cells (Fig. 3A, B). On the apical surface of the hypodermal cells are extensive microvilli, indicative of active absorptive surfaces (Fig.

←

FIG. 1. Electron micrographs of the body wall of *Romanomermis culicivorax*, preparasites. A–D) Controls. E) Lanthanum treated. A, D) Longitudinal sections. B, C, E) Transverse sections. Distinct thick and thin striations (s) are present in the cortical zone (c). Underneath is a basal zone (b) but no medial zone. An anlage of a new cuticle (ncu) is present. E) Lanthanum deposits (arrows) occur in the apical zone of the hypodermis beneath the indistinct cuticle (cu). Muscle (m), epicuticle (e). All bars equal 0.1 μm, except 1E.

3A, B). When microvilli make contact with one another and with the basal zone of the cuticle, small localized deposits of lanthanum are seen on the surface of microvilli and cuticle. Lanthanum treatment revealed numerous smooth septate junctions at the apical surface of the hypodermal cells (Fig. 3A, D, E). In transverse section, the intercellular space (20 nm) is filled with a dense material. A faint dense axial line is sometimes apparent (Fig. 3D, E). Gap junctions are also numerous, and tricellular junctions may be seen where three cells abut (Fig. 3E). Septate junctions were seen only in the ampulla regions of the preparasites and were absent in the postparasites.

Postparasite control: The cuticle of the 7–30-day-old postparasites (Fig. 4A) is about 2,200 nm thick and is multilayered like that of the 5-day parasite. In addition, there is an inner diffusely vacuolated and granular region, the vacuoles of which are reminiscent of the vesicles seen in the interchordal cytoplasm of the hypodermis of the 5-day parasite. A new cuticle is developing external to the hypodermis. Occasionally a triple membrane separating old and new cuticles is apparent (Fig. 4B—arrow). Beneath is a granular zone followed by a narrow layer of "struts" (4) consisting of a thick vertical fiber sandwiched between two thin fibers (Fig. 4A, B). The hypodermal cell is filled with stacks of rough endoplasmic reticulum, also characteristic of earlier stages, and the moderately thick extensions have microvilli and contain mitochondria and rough endoplasmic reticulum.

Lanthanum treated postparasite: Lanthanum permeates the cuticle of intact worms and large deposits occur intermittently in the hypodermal region (Fig. 4C). Additional structures were seen when some nematodes were nicked during preparation to facilitate fixation. When examined in transverse view, vertically arranged striations were apparent in the lower cortical zone, a few of which extended to the epicuticle (Fig. 4D).

Discussion

Mermithid nematodes have poorly developed alimentary tracts; therefore, a transcuticular means of nutrient uptake has been proposed (30,31). The multilaminar epicuticle possesses a number of features in common with cell membranes, such as selective permeability (20), transport of amino acids (32), net negative surface charge (13), and a high concentration of mucopolysaccharides on its surface (12). Some differences indicate that it is not a classical membrane. For example, freeze fracture studies of the epicuticle of microfilaria from *Onchocerca volvulus* indicate that membrane particles are absent, although particles do occur on the hypodermal membrane (21). The *Onchocerca* study also suggests that a layering of fibrillar material on the outer surface of the hypodermis forms the epicuticle (21).

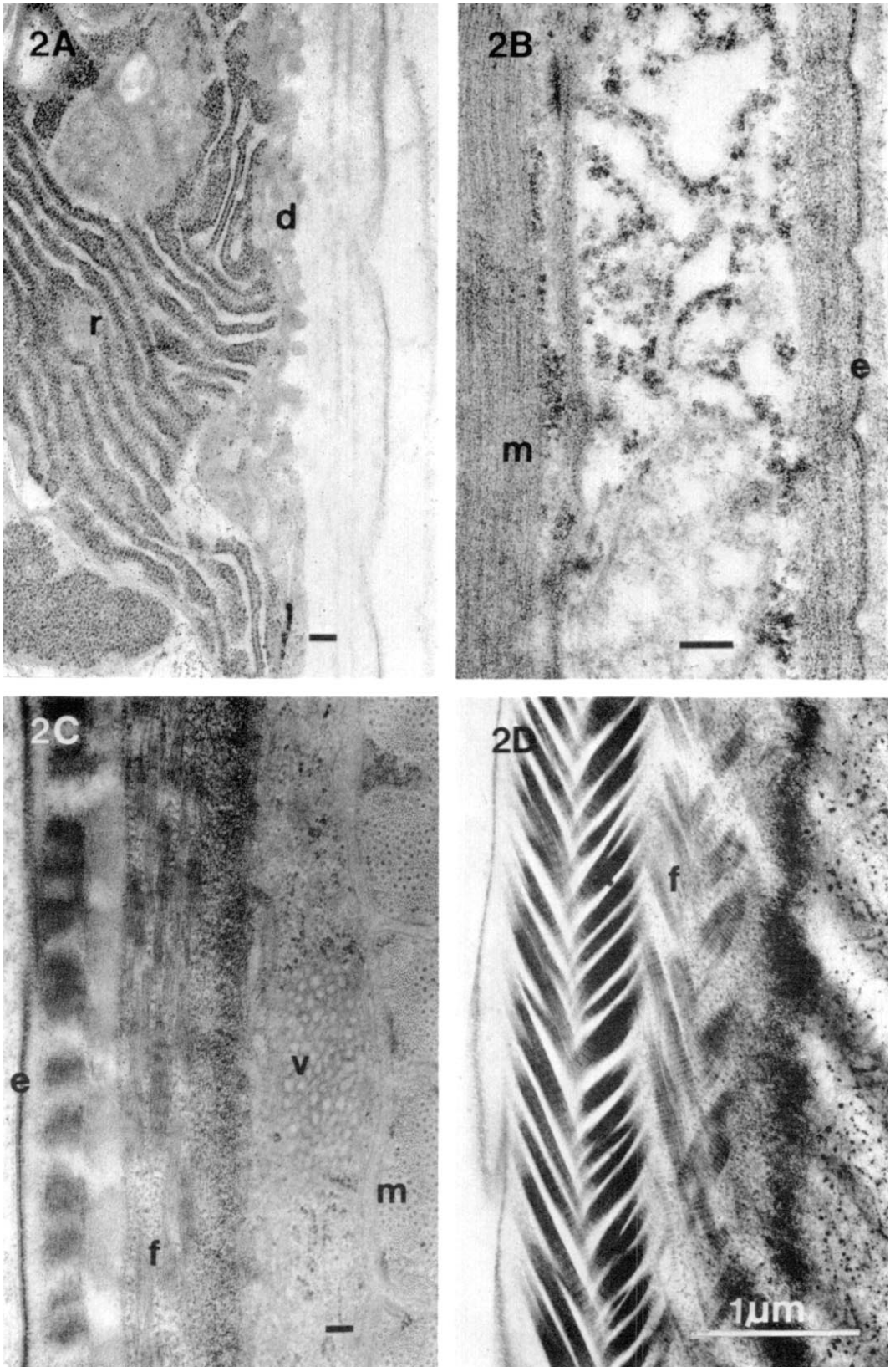
Our study of *R. culicivorax* also indicates

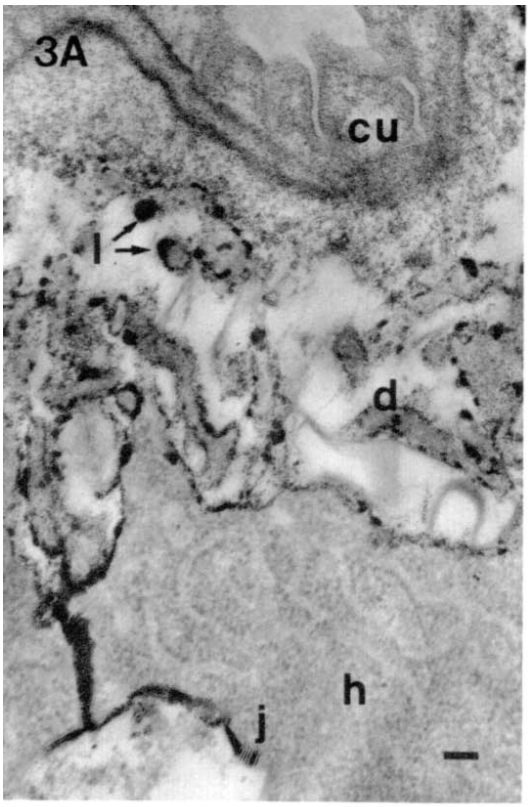
→

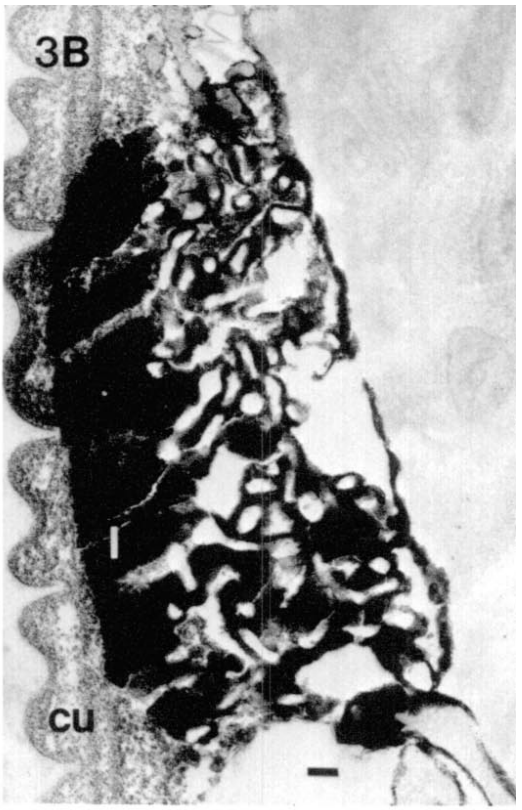
Fig. 2. Electron micrographs of the body wall of *Romanomermis culicivorax*, control parasites 3–5 days old. A, B) 3–4 days in longitudinal section. C, D) 5 days in transverse (C) and oblique (D) views. Cuticle is thin and zones are indistinct at 3–4 days (A, B) but are thick and distinct at 5 days (C). Thick hypodermal processes contain rough endoplasmic reticulum (r), microvilli (d) and vesicles (v). D) Fibrils (f) exhibit cross banding with a 64-nm periodicity characteristic of collagen. Muscle (m), epicuticle (e). All bars equal 0.1 μm except 2D.

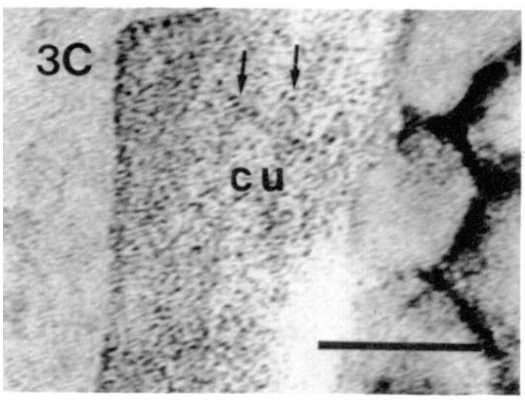
Fig. 3. Electron micrographs of the body wall of *Romanomermis culicivorax*, lanthanum treated parasites 3–5 days old. Lanthanum (l) penetrates the cuticle and accumulates in the intercellular spaces of hypodermal cells. Microvilli (d) contain localized deposits of lanthanum (3A—arrows). C) Small deposits of lanthanum (arrows) about 1.5 nm in diameter occur in the cuticle (cu). A, D, E) Smooth septate junctions (j) are numerous in the apical region of the hypodermis (h) at this stage. E) Tricellular (t) and gap (g) junctions are also present. All bars equal 0.1 μm.

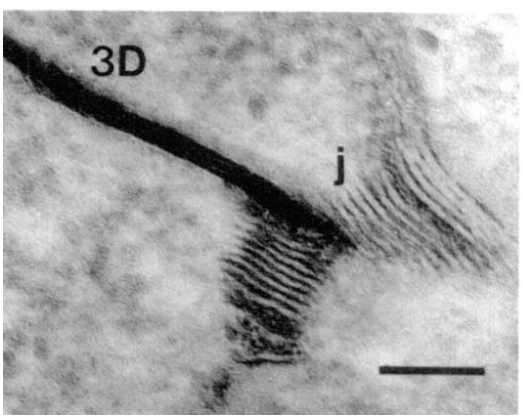
Fig. 4. Electron micrographs of the body wall of *Romanomermis culicivorax*, postparasites 7–30 days old. A, B) Controls. C, D) Lanthanum treated. Compare vacuoles (va) in lower zone of thick cuticle with vesicles in Fig. 2C. New cuticle (ncu) is forming and rests on hypodermal processes (p). Well-developed muscle layer (m) is present. D) Faint striations (s) are evident in cortical zone of a nicked lanthanum treated cuticle but lack the thick and thin repeating pattern present in preparasite (1B, D). Lanthanum deposit (l). All bars equal 1.0 μm except 4B which equals 0.1 μm.

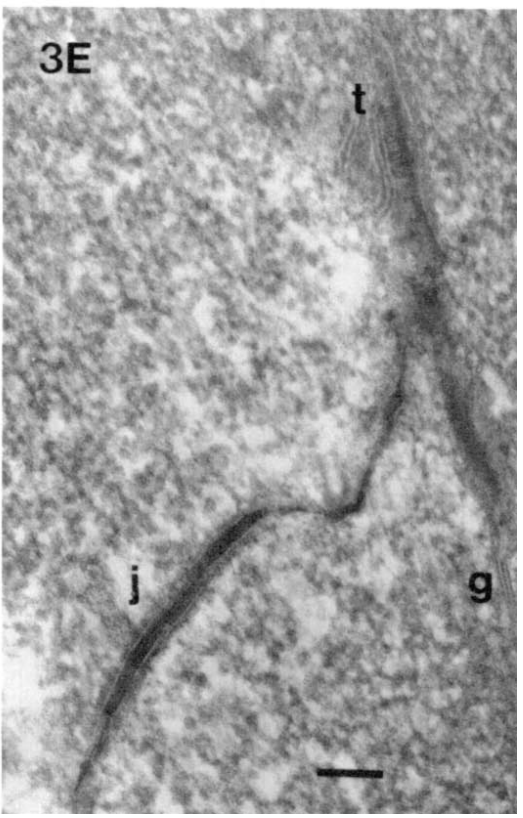

2A
d
r
2B
e
m
2C
e
v
m
f
2D
f
1µm

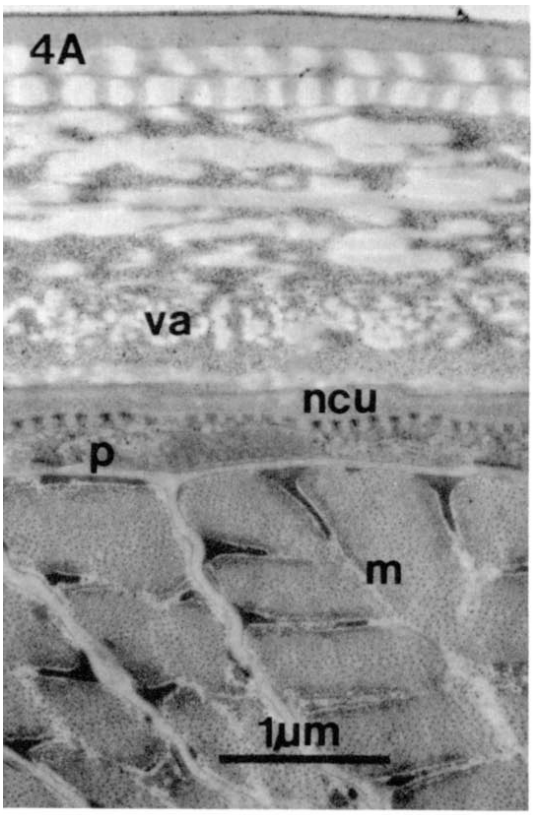

3A
cu
l
d
h
j

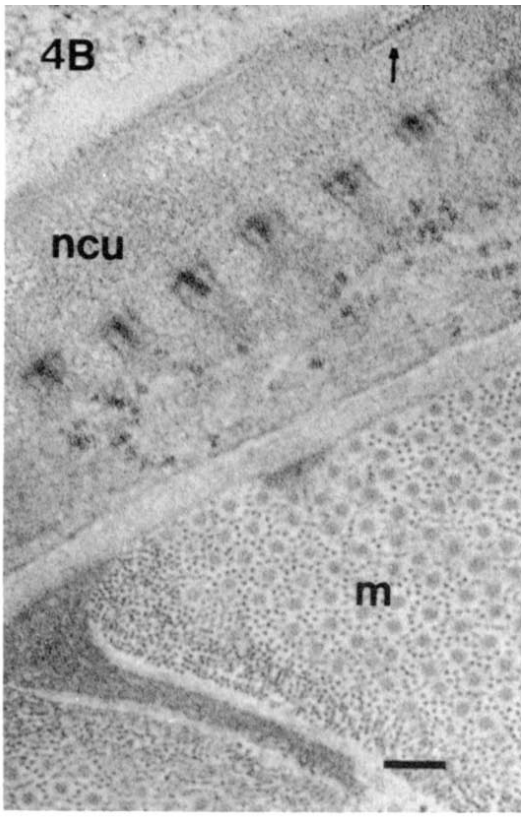

3B
l
cu

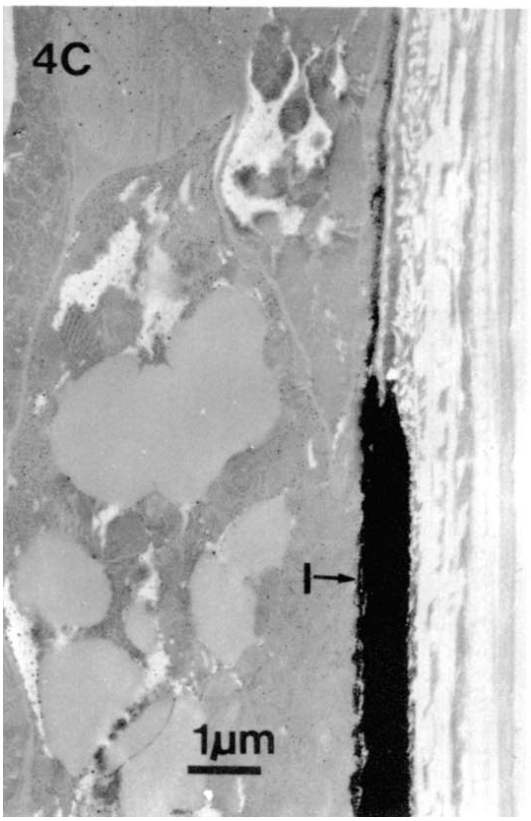

3C
cu

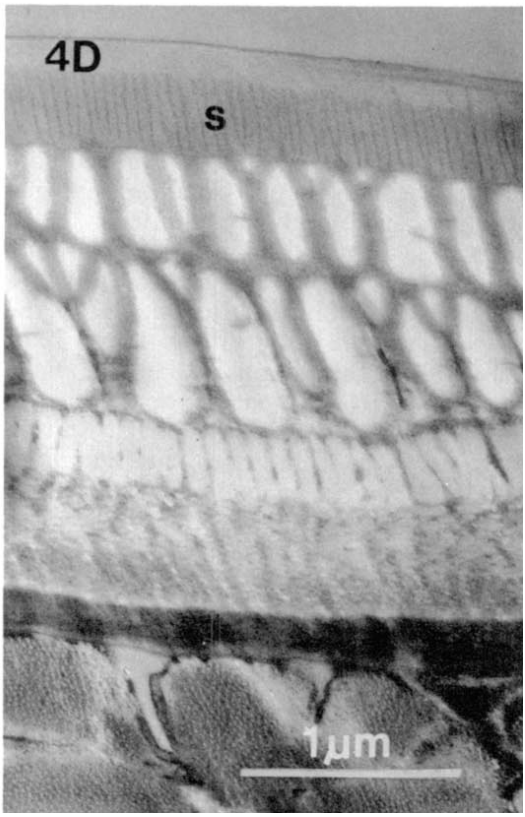

3D
j


3E
t
j
g


4A
va
ncu
p
m
1µm


4B
ncu
m


4C
l
1µm


4D
s
1µm

that the epicuticle and the plasma membrane of the hypodermis differ because only the cuticle was permeable to lanthanum ions. Maximum permeation of lanthanum occurred during the early parasitic stages when the cuticle was thin and the interchordal cytoplasm of the hypodermis was thick. At this time, lanthanum was especially prevalent on the surfaces of the microvilli, but the ion was absent intracellularly.

Poinar and Hess (26) reported uptake of ferritin through 7–11-nm pores in the cuticle of 3–5-day-old *R. culicivorax*. Our lanthanum studies revealed no such pores. Negative contrast staining indicated only the possible presence of 2.5-nm "pores," but these were considered artifactual since Spurr's epoxy resin external to the cuticle exhibited a similar configuration (Fig. 3C). If pores exist, other methodology will be necessary for their demonstration.

Lanthanum has been used extensively as a negative contrast stain, especially to demonstrate extracellular spaces and junctional complexes. The hydrated lanthanum ion is 0.9 nm in diameter (22), and, when in solution below pH 7.8, it is predominately in the ionic form (33). Although lanthanum ions can permeate spaces 2 nm wide or narrower, it does not enter undamaged cells (29). The use of lanthanum in this study has shown that the apical surfaces of the epithelial cells of the hypodermis contain smooth septate junctions in addition to gap junctions and tricellular junctions. These junctions, which stand out in bold relief, are numerous in the parasitic stage but have been observed only in the ampulla of the preparasite and not at all in the postparasite. More observations are needed, however, to verify the sparsity or absence of these junctions in nonparasitic stages. Smooth or continuous septate junctions (36) have been described in arthropod tissue (10,23), Onychophora (17), Tardigrada (11), and also in the intestinal cells of *Ascaris* (7).

The concept of cuticular nutrient uptake in nematodes has been further supported by radiography studies on the epithelial cells of the hypodermis. For example, studies performed on infective *Brugia pahangi* larvae showed that adenosine was taken up by the cuticle, with the greatest incorporation occurring in the hypodermis (6). Alkaline phosphatase activity occurs beneath the cuticle of *Gastromermis boophthorae*, suggesting an active absorptive surface (1). Ultrastructural investigations by others (1,5,26,27), as well as this study, show that the interchordal cytoplasm of the hypodermis contains numerous microvilli on the apical surfaces and is thus modified into an absorptive surface (1,26).

Most investigators consider lanthanum a nonspecific stain, but some studies provide evidence that lanthanum forms associations with polysaccharides of the extracellular matrix (24) or replaces calcium in external cell membranes (8). In our study, the microvillar surfaces of the hypodermal cells of *R. culicivorax* frequently exhibited small dense deposits of lanthanum. That these might represent selective binding sites cannot be confirmed at this time.

The cuticles of preparasitic juveniles of various nematodes contain regularly arranged vertical rows of striations in the basal layer (2,14,38,39) which are thought to give rise to a resistant protective layer (3). Unlike the aforementioned secernentean species, these striations are found in the cortical layer of *R. culicivorax*, *G. boophthorae* (1), and *Trichinella spiralis* (16). A less regularly arranged layer of striations is present in the postparasite. These striations were observed only in the preparasitic stage of *G. boophthorae* (1), and in *R. culicivorax* they may represent a stage-specific adaptation to increase cuticular strength during exposure to the vicissitudes of the nonparasitic habitat; i.e., movement through the particles of the substratum in a pond. Kondo and Ishibashi (15) observed that the striations in the basal layer of *Bursaphelenchus lignicolus* varied directly with the mobility and survival characteristics of the dispersal stage.

Literature Cited

1. Batson, B. S. 1979. Body wall of juveniles and adult *Gastromermis boophthorae* (Nematoda: Mermithidae): Ultrastructure and nutritional role. International Journal of Parasitology 9:495–503.

2. Bird, A. F. 1968. Changes associated with parasitism in nematodes. III. Ultrastructure of the egg shell, larval cuticle, and contents of the subventral esophageal glands in *Meloidogyne javanica* with some observations on hatching. Journal of Parasitology 54: 475–489.

3. Bird, A. F. 1971. The structure of nematodes. London: Academic Press.

4. Bird, A. F. 1980. The nematode cuticle and its

surface. Pp. 213–235 *in* B. M. Zuckerman, ed. Nematodes as biological models. Aging and other model systems, vol. 2. New York: Academic Press.

5. Bonner, T. P., and P. P. Weinstein. 1972. Ultrastructure of cuticle formation in the nematodes *Nippostrongylus brasiliensis* and *Nematospiroides dubius*. Journal of Ultrastructure Research 40:261–271.

6. Chen, S. N., and R. E. Howell. 1979. *Brugia pahangi*: Uptake and incorporation of adenosine and thymine. Experimental Parasitology 47:209–221.

7. Davidson, L. A. 1983. A freeze fracture and thin section study of intestinal cell membranes and intercellular junctions of a nematode, *Ascaris*. Tissue and Cell 15:27–37.

8. Doggenweiler, C. F., and S. Frenk. 1965. Staining properties of lanthanum on cell membranes. Proceedings National Academy of Sciences U.S. 53:425–430.

9. Gordon, R., and J. M. Webster. 1972. Nutritional requirements for protein synthesis during parasitic development of the entomophilic nematode *Mermis nigrescens*. Parasitology 64:161–172.

10. Green, L. F. B., P. R. Bergquist, and S. Bullivant. 1980. The structure and function of the smooth septate junction in a transporting epithelium: The malpighian tubules of the New Zealand glow-worm *Arachnocampa luminosa*. Tissue and Cell 12:365–381.

11. Greven, H. 1976. Some ultrastructural observations on the midgut epithelium of *Isohypsibius augusti* (Murray, 1907) (Eutardigrada). Cell and Tissue Research 166:339–351.

12. Himmelhoch, S., and B. M. Zuckerman. 1978. *Caenorhabditis briggsae*: Aging and the structural turnover of the outer cuticle surface and the intestine. Experimental Parasitology 45:208–214.

13. Hudson, R. J., and W. D. Kitts. 1971. Electrostatic forces affecting in vitro adherence of polymorphonuclear leukocytes to the cuticle of *Protostrongylus* larvae (Nematoda). Journal of Parasitology 57:808–814.

14. Ibrahim, I. K. A., and J. P. Hollis. 1967. Cuticle ultrastructure of the *Meloidogyne hapla* larvae. Proceedings Helminthological Society of Washington 34:137–139.

15. Kondo, E., and N. Ishibashi. 1978. Ultrastructural difference between the propagative and dispersal forms in pine wood nematode, *Bursaphelenchus lignicolus*, with reference to the survival. Applied Entomology and Zoology 13:1–11.

16. Kozek, W. J. 1971. The molting pattern in *Trichinella spiralis*. II. An electron microscope study. Journal of Parasitology 57:1029–1038.

17. Lavallard, R. 1967. Ultrastructure des cellules prismatiques de l'épithélium intestinal chez *Peripatus acocioi* Marcus et Marcus. Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, Serie D: Sciences Naturelles 264:929–932.

18. Leeson, T. S., and C. R. Leeson. 1981. Histology, 4th ed. Toronto: W. B. Saunders Company. P. 110.

19. Lewis, P. R., and D. P. Knight. 1977. Staining methods for sectioned material. P. 311 *in* A. M. Glauert, ed. Practical methods in electron microscopy, vol. 5. Amsterdam: North-Holland Publishing Co.

20. Marks, C. F., I. J. Thomason, and C. E. Castro. 1968. Dynamics of the permeation of nematodes by water, nematocides and other substances. Experimental Parasitology 22:321–337.

21. Martinez-Palomo, A. 1978. Ultrastructural characterization of the cuticle of *Onchocerca volvulus* microfilaria. Journal of Parasitology 64:127–136.

22. Nightingale, E. R., Jr. 1957. Phenomenological theory of ion solvation, effective radii of hydrated ions. Journal of Physical Chemistry 63:1381–1387.

23. Noirot-Timothee, C., and C. Noirot. 1980. Septate and scalariform junctions in arthropods. International Review of Cytology 63:97–140.

24. Overton, J. 1968. Localized lanthanum staining of the intestinal brush border. Journal of Cell Biology 38:447–452.

25. Platzer, E. G., and A. M. Stirling. 1978. Improved rearing procedures for *Romanomermis culicivorax*. Proceedings of the California Mosquito and Vector Control Association 46:87–88.

26. Poinar, G. O., Jr., and R. Hess. 1977. *Romanomermis culicivorax*: Morphological evidence of transcuticular uptake. Experimental Parasitology 42:27–33.

27. Poinar, G. O., Jr., R. Hess, and M. Doucet. 1981. Surface modification of hypodermal and trophosome cells from parasitic juveniles of mermithid nematodes. Revue de Nematologie 4:35–40.

28. Popham, J. D., and J. M. Webster. 1978. An alternative interpretation of the fine structure of the basal zone of the cuticle of the dauerlarva of the nematode *Caenorhabditis elegans* (Nematoda). Canadian Journal of Zoology 56:1556–1563.

29. Revel, J. P., and M. K. Karnovsky. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. Journal of Cell Biology 33: C7–C12.

30. Rubtsov, I. A. 1967. Organs and process of extraintestinal digestion in mermithids. Izvestiya Akademii Nauk Azerbaidzhanskoi SSR, Seriya Biologicheskikh Nauk 6:883–891.

31. Rutherford, T. A., and J. M. Webster. 1974. Transcuticular uptake of glucose by the entomophilic nematode, *Mermis nigrescens*. Journal of Parasitology 60:804–808.

32. Rutherford, T. A., J. M. Webster, and J. S. Barlow. 1977. Physiology of nutrient uptake by the entomophilic nematode *Mermis nigrescens* (Mermithidae). Canadian Journal of Zoology 55:1773–1781.

33. Schatzki, P. F., and A. Newsome. 1975. Neutralized lanthanum solution: A largely noncolloidal ultrastructural tracer. Stain Technology 50:171–178.

34. Schmidt, S. P., and E. G. Platzer. 1980. An investigation of possible *Romanomermis culicivorax* proteins in the hemolymph of *Culex pipiens*. Journal of Invertebrate Pathology 36:149–158.

35. Spurr, A. R. 1969. A low-viscosity resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26:31–43.

36. Staehelin, L. A. 1974. Structure and function of intercellular junctions. International Review of Cytology 39:191–283.

37. Von Brand, T. 1973. Biochemistry of parasites, 2d ed. New York: Academic Press.

38. Wisse, E., and W. T. Daems. 1968. Electron microscopic observations on second-stage larvae of the potato root eelworm *Heterodera rostochiensis*. Journal of Ultrastructure Research 24:210–231.

39. Yuen, P. H. 1967. Electron microscopical studies on *Ditylenchus dipsaci* (Kühn). Canadian Journal of Zoology 45:1019–1033.