[Jap. J. Parasit, Vol. 27, No. 3, 245-260, 1978]

The Ultrastructure of Early Larval Morphogenesis in *Brugia pahangi* (Nematoda : Filarioidea)

YASUMASA TONGU†, ALBERT L. VINCENT§
AND LAWRENCE R. ASH§

(Received for publication; April 14, 1978)

Introduction

The ultrastructural characteristics of microfilariae have been described by numerous workers including Johnson and Bemrick (1969), McLaren (1969, 1972), Kozek (1971), Johnston and Stehbens (1973), Kanagasuntheram *et al.* (1974a, b), Laurence and Simpson (1974), Singh *et al.* (1974, 1975a, b) Tongu (1974), Gibson *et al.* (1976), and Suguri (1977). Lee and Miller (1967, 1969), Harada *et al.* (1970), Maeda *et al.* (1970), and Vincent *et al.* (1975a, b) have studied the ultrastructure of adult filariae, while Beckett and Boothroyd (1970), Collin (1971), Ho and Kan (1972), and Singh *et al.* (1975a, b) have presented the ultrastructure of the infective larva.

The morphogenesis of filariae has been described by a number of light microscopists including Kobayashi (1940), Schacher (1962), Schacher and Khalil (1968), and Ash and Schacher (1971). In the mosquito the first molt occurs between four and five days and the second molt at about eight days, after which the larvae are immediately infective (Schacher, 1962). To date, however, there has been no account of the ultrastructure of developmental-stage larvae in the mosquito except the infective larva.

The present study describes the ultrastructural features of the developmental-stage larvae of *Brugia pahangi* from two days to seven days after infection of the mosquito, *Armigeres subalbatus*. The results are compared with those of Suguri (1977) on the ultrastructure of *B. pahangi* microfilariae, and development of the filarial alimentary tract is emphasized.

Materials and Methods

Armigeres subalbatus mosquitos were fed on the blood of jirds, *Meriones unguiculatus*, experimentally infected with *Brugia pahangi*. During larval development, mosquitos were maintained at 24 C. Larvae were collected at two, three, five, and seven days after the blood meal. They were dissected from the mosquito in Earle's balanced salt solution (Ash and Schacher, 1971) and fixed for one or two hours in cold 3.5% phosphate-buffered glutaraldehyde (pH 7.4) with 0.25M sucrose. After three or four rinses in buffer, the larvae were postfixed in buffered 1% osmium tetroxide solution for two hours or overnight, then dehydrated in ethanol by routine methods.

This investigation was supported by the Japan-United States Cooperative Medical Science Program.

† *Department of Parasitology, Okayama University Medical School, 2-5-1 Shikata-Cho, Okayama, Japan*

§ *Division of Epidemiology, School of Public Health, University of California, Los Angeles, CA 90024*

After passing through N-butyl glycidyl ether, the larvae were embedded in Araldite-Epon mixtures according to Mollenhauer (1964). The specimens were thin-sectioned with a LKB or a Poter-Blum ultramicrotome. Following the double-staining with uranyl acetate and lead citrate, the sections were examined with a Hitachi HS-8 electron microscope at an accelerating voltage of 50 KV.

Results

Cuticle, hypodermis, and somatic musculature:

At the anterior tip of the two-day larvae (112×15 μm) and three-day larvae (135×15 μm), before the first molt, a hook (Fig. 1, H) and the three spines (Fig. 1, SP) were seen. The hook was dense (Fig. 1, H), situated on the anterior edge of the head, and projected backward along the long axis of the body. Three shorter spines appeared opposite the hook (Fig. 1, SP). These were lost with the cuticle of the first stage. Five-day larvae (135×22 μm) showed none of these structures. Sausage-stage larvae from two days to five days were partially surrounded by an irregular, thin cuticle (Fig. 17), clearly visualized at the anterior extremity (Fig. 1). The cuticle consisted of only a fibrous layer and for the most part, did not show transverse striations on its outer surface. Deep, transverse striations (Fig. 22) were clearly evident, however, in the small tail of two- to five-day larvae; the tail appeared to be lost by the seventh day. Most of the body of seven-day larvae (600×30 μm) had no cuticle, only a sheath-like double membrane (Figs. 24, 25). In cross-sections of five- and seven-day larvae, seven or eight hypodermal cells in a single layer formed the circumference of the larvae. These cells showed many vacuole-like structures of varying sizes. A dense matrix was seen in these cells overlying the lateral chords. Here the hypodermal cells showed swollen mitochondria and there were many vacuole-like structures in the matrix. The hypodermal cells of the tail were thinner than those of the body. In the seven-day larvae the hypodermal cells demonstrated an external double membrane (Fig. 25). In some five-day specimens, several rickettsia-like bodies (Fig. 23) were seen in a hypodermal cell. These bodies measured approximately 1 μm in diameter; they appeared to be limited to the hypodermal cell and to be surrounded by a plasma membrane of the host cell.

The muscle cell (Figs. 3, 12; MU) of developmental stages, especially of the five-day larvae, had rudimentary myofilaments. Four muscle groups, usually of two cells each (Figs. 3, 12) were identified in cross-sections of the main body, but not in the extremities. The contractile portion with myofilaments occupied only a small proportion of the muscle cell area.

Central canal and inner body:

The central canal consisted of a long cylindrical tube 0.4 μm in diameter (Figs. 2, 3, 4, 5, 8; CC) which occupied the center of the worm. It ran from head to inner body and was present in two-, three- and five-day larvae. In the cross-section, the canal was surrounded by seven or nine cells (Fig. 5, CC), whose cell membranes were clearly joined by desmosomes. In the developing larvae, these cells were smaller between head and excretory cell than posterior to the excretory cell. In seven-day larvae a recognizable triradiate esophagus (Fig. 26) had differentiated from the canal cell, and the intestinal epitherium (Fig. 24) from the inner body. The inner body cell with much dense materials was not found in the sausage-stage larvae after three and five days. The inner body was replaced by two cells in cross-section (Figs. 10, 12; I). A small lumen (Figs. 10, 11, 12; arrow) differentiated within the enclosure of these cells. By seven days the lumen had enlarged considerably (Fig. 24, LU) and, in some parts, contained many mitochondria (Fig. 25, M), apparently ingested from the mosquito flight muscle. The intestinal epitherium (Fig. 25) was a single layer of flat cells containing much rough endoplasmic reticulum and vacuoles. Micro-

villi were, however, not observed on the luminal surface of the gut. Some development of pseudocoel was evident by two days, and further development was seen at five days. There was no such space around the buccal cavity (Fig. 1) and nerve ring (Fig. 5), however.

Excretory apparatus, R-cells, and anal apparatus:

The excretory cell (Figs. 7. 8 ; EC) showed a posterior nucleus (Fig. 7, N) and opened through the body wall in larvae from two to five days old (Figs. 7, 8). As the larvae approached five days, the cell became rounded and lost the backward-extending cytoplasmic bridge. The cell (Figs. 7, 8 ; EC) contained many membrane-bounded vesicle-like structures and dense bodies of varying sizes (Figs. 7, 8, 9). The pore stopped with a cap (Fig. 8, arrow) of dense materials. In five-day larvae the membrane-bounded vesicles increased in number, but microvillus-like projections were not seen.

In the cross-section of the five-day larva, two large cells (Fig. 14, R) were seen in the center of the area formerly occupied by the R-cells, but a lumen was not identified. A lumen was first seen among the R-cells at seven days of development. These cells had many vacuole-like structures and swollen mitochondria. Much pseudocoelomic space appeared between the R-cells and the body wall in the five-day larva. In the two-day, three-day, and five-day larvae, the anal pore (Figs. 18, 19; AP) bore a dense plug (Fig. 18, arrow) at its orifice. Vesicle-like structures bounded by the membranes, and microvilli (Fig. 19, MV), containing filaments (Fig. 21) were abundant in the lumen. The cell occupied nearly the whole width of the worm. A large Pseudocoelomic space (Fig. 16, arrow) was seen behind the anal pore in the five-day larva.

Nerve ring, amphid, and phasmid:

The nerve ring of the five-day larva (Figs. 4, 5, 6 ; NR) was composed of tightly packed nerve fibers containing neurofilaments, vesicles, and mitochodria. The central canal (Fig. 5, CC) was surrounded by a number of nerve fibers in this region. The nerve ring (Figs. 4, 6 ; NR), about 7 μm wide, occupied nearly the whole width of the body. The amphids (Figs. 1, 3 ; A) and phasmids (Fig. 13, P) originated as a cephalic and a caudal channel in the microfilaria and were observed throughout all larvae from the two-day to the five-day specimens. In the seven-day larva they were not observed in the present study. A pair of amphids (Fig. 3, A) containing several dense rod-like cilia were situated at the anterior extremity on both sides of the central canal. A pair of phasmids were located behind the anal pore ; they differed from the amphids in the presence of only one cilial rod.

Discussion

The external structures of microfilariae, such as the hook and spines, have been studied by electron microscopists including Kozek (1971), McLaren (1972), Tongu (1974), and Aoki and Katamine (1975). Using a light microsope, Laurence and Simpson (1968) demonstrated a hook and three spines at the head of microfilarial *B. pahangi*. Likewise, in the present study, the two-day and three-day larvae (first-stage) possessed a similar armature at the anterior tip but these structures were not visible in any five-day larvae (second stage) or further-developed larvae. It seems reasonable to assume, therefore, that hook and spines were lost during the first molt.

The cuticle of the microfilaria of *B. malayi* (Tongu, 1974) and *B. pahangi* (Laurence and Simpson, 1974 ; Suguri, 1977) consists of three layers and has transverse striations on the outer surface. Our observations show that the microfilarial cuticle is reduced during larval morphogenesis. Between two and five days the cuticle consists of only a thin, irregular, fibrous layer and striations are confined to the tail. The seven-day larva is partially nude, covered largely by a membrane-like structure. The lack of cuticular differentiation during this period may be

related to the rapid cycle of molting, to the protected intracellular environment of the larva, or perhaps to uptake of materials directly through the body wall. In any case, a well-defined, fibrous cuticle reappears with the infective stage (Collin, 1971). The hypodermis of the developing larva follows the basic pattern reported for the microfilaria, infective larva (Collin, 1971) and the adult worm (Lee and Miller, 1967, 1969; Vincent *et al.*, 1975a, b). In the five- and seven-day larvae, seven or eight nucleated hypodermal cells underlaid the thin cuticle or plasma membrane.

The muscle cells and myofilaments of sausage stages were fewer than those of the microfilariae (Laurence and Simpson, 1974; Suguri, 1977) or of the infective larva (Collin, 1971). Unlike these stages, the sausage forms are more quiescent and have little requirement for motility.

Tongu (1974) and Suguri (1977) showed that the central canal (pharyngeal thread) of the microfilaria of *B. malayi* and *B. pahangi* opened into the inner body cell, but that the larval alimentary tract had not yet differentiated. In *B. pahangi* the canal cell was well developed in the five-day larva as compared with microfilaria. The cuticle-lined canal was completely lost in the seven-day larva and was replaced by a triradiate esophagus, apparently between five days and seven days. Seven-day larvae had an esophagus and intestine which were completely patent and enclosed many mosquito mitochondria in the lumen. Ingested mitochondria have been reported in the last third of development in *B. pahangi* by Beckett and Boothroyd (1970) and in the third-stage larva of *Breinlia sergenti* by Ho and Kan (1972). As in the present study, they suggested that the host-muscle mitochondria are a source of nutrients for the larvae during the last part of their developmental phase. Collin (1971), however, believed that the intestine of infective-stage *B. pahangi* was not fully developed and that the alimentary tract was blocked at the level of the esophageal-intestinal valve.

Although the ultrastructure of the posterior alimentary canal has not been completely studied, it was noted that in five-day larva, no lumen had differentiated among the R-cells. It seems unlikely, therefore, that intestinal wastes could be expelled through the anus.

The function of the excretory cell remains unclear, though it has been identified in a number of microfilariae (Kozek, 1971; McLaren, 1972; Kanagasuntheram *et al.*, 1974; Laurence and Simpson, 1974; Tongu, 1974; Gibson *et al.*, 1976; Suguri, 1977).

The ultrastructure of this cell resembles the osmoregulatory cell of pentastomids (Banaja *et al.*, 1977). The excretory pore persists in five-day larvae, but it appears to have lost the microvilli of the microfilariae. The shape also differed from that in the microfilariae, in which it possesses a cytoplasmic bridge (Suguri, 1977). These observations suggest a change or reduction in some functions such as osmoregulation or secretion.

Rickettsia-like bodies have been reported by McLaren *et al.* (1975) and Vincent *et al.* (1975) in the hypodermis of the microfilariae and adult filariae. Similar organisms observed in the adult and microfilariae of *Dirofilaria immitis* were not recognized as symbionts by Harada *et al.* (1970), Kozek (1971), or Lee (1975). Kozek (1977) later observed the fine structure of these microorganisms within the lateral chords, oogonia, oocytes and eggs of adult and larval stages of *Onchocerca volvulus*. They appeared to have a developmental cycle consisting of three morphologically distinct forms. Kozek suggested that these organisms are more similar to the chlamydiae than to the rickettsiae. In the present study, these bodies were found in the hypodermis of the five-day larva. The morphological features and size of these bodies resembled the microorganisms reported by McLaren *et al.* (1975) and Vincent *et al.* (1975), and the bacillary form of Chlamydia-like bodies observed by Kozek (1977). The significance of this finding with respect to the host-parasite relationship is in need of further study.

Summary

The authors have studied the ultrastructure of the early morphogenesis of *Brugia pahangi* by transmission electron microscopy. Larvae for study were collected from *Armigeres subalbatus* mosquitos at two, three, five, and seven days after a blood meal. Perhaps associated with a protected, intracellular environment, the cuticle was reduced during early larval morphogenesis. A thin, irregular cuticle covered part of the body between two and five days, whereas the seven-day larva showed only a thin sheath-like structure of double membranes. The apparent absence of a cuticle in seven-day larvae may be associated with the fact that this stage is passed entirely within the intracellular environment of the mosquito fiight muscle. The anterior hook and spines of the microfilaria were cast off at the first molt. The somatic musculature consisted usually of two cells per quadrant and was more weakly developed than that of the microfilaria or infective larva. Rickettsia-like bodies, similar to those reported from other filariae, were encountered in the hypodermal cell of several five-day specimens. The central canal (pharyngeal thread) of the microfilaria persisted for five days, but was replaced afterwards by a recognizable triradiate esophagus. Transverse sections showed that the inner body breaks down and is replaced by two cells which later appear to form the intestinal epithelium. At seven days a spacious intestinal lumen showed ingested mosquito flight muscle mitochondria, but microvilli were not evident. No lumen differentiated among the R cells as late as five days' development. The microfilarial excretory cell persisted throughout this period, but the loss of microvilli and other alterations suggested a change or reduction in function. No changes were noted in the amphids, phasmids, or nerve ring.

Acknowledgments

The authors wish to thank Mr. Max Lent for technical assistance, and Mrs. Virginia Hansen for her help in preparing the manuscript.

Literature cited

1) Aoki, Y. and Katamine, D. (1975) : Scanning electron microscopic observations on *Dirofilaria immitis*. Nettai Igaku, 17, 27–34.
2) Ash, L. R. and Schacher, J. F. (1971) : Early life cycle and larval morphogenesis of *Wuchereria bancrofti* in the jird, *Meriones unguiculatus*. J. Parasit., 57, 1043–1051.
3) Banaja, A. A., James, J. L. and Riley, J. (1977) : Observations on the osmoregulatory system of pentastomids : The tegumental chloride cells. Internat. J. Parasit., 7, 27–40.
4) Beckett, E. B. and Boothroyd, B. (1970) : Mode of nutrition of the larvae of the filarial nematode *Brugia pahangi*. Parasitol., 60, 21–26.
5) Collin, W. K. (1971) : Ultrastructural morphology of the esophageal region of the infective larva of *Brugia pahangi* (nematoda : filarioidea). J. Parasit., 57, 449–468.
6) Gibson, D. W., Conner, D. H., Brown, H. L., Fuglsang, H., Anderson, J., Duke, B. O. L. and Buck, A. A. (1976) : Onchocercal dermatitis : Ultrastructural studies of microfilariae and host tissues, before and after treatment with diethylcarbamazine (Hetrazan). Amer. J. Trop. Med. Hyg., 25, 74–87.
7) Harada, R., Maeda, T., Nakashima, A., Sadakata, Y., Ando, M., Yonamine, K., Otsuji, Y., and Sato, H. (1970) : Electronmicroscopical studies on the mechanism of oogenesis and fertilization in *Dirofilaria immitis*. Recent Advances in Researches on Filariasis and Schistosomiasis in Japan, 99–121. University of Tokyo Press, Tokyo.
8) Ho, B.-C., and Kan, S.-P. (1972) : Development of *Breinlia sergenti* (Dipetalonematidae) in the fat-body of mosquitoes. III. Mode of nutrition of the infective larvae. J. Med. Entomol., 9, 262–265.
9) Johnson, K. H. and Bemrick, W. J. (1969): *Dirofilaria immitis* microfilariae : Electron microscopic examination of cuticle and muscle. Amer. J. Vet. Res., 30, 1443–1450.
10) Johnston, M. R. L. and Stehbens, W. E. (1973) : Ultrastructural studies on the microfilaria of *Cardianema* sp. Alicata, 1933 (Nematoda ; Onchocercidae). Internat. J. Parasit., 3, 243–250.
11) Kanagasuntheram, R., Singh, M., Ho, B.-C.,

and Chan, H.-L. (1974a) : Some ultrastructural observations on the microfilaria of *Breinlia sergenti*— the excretory complex, rectal cells and anal vesicle. Internat. J. Parasit., 4, 7–15.

12) Kanagasuntheram, R., Singh, M., Ho, B.-C., Yap, E.-H., and Chan, H.-L. (1974b) : Some ultrastructural observations on the microfilaria of *Breinlia sergenti*— nervous system. Internat. J. Parasit., 4, 489–495.

13) Kobayashi, H. (1940) : On the development of *Microfilaria bancrofti* in the body of the mosquito, (*Culex fatigans*). Acta Japonica Medicinae Tropicalis, 2, 63–88.

14) Kozek, W. J. (1971) : Ultrastructure of the microfilaria of *Dirofilaria immitis*. J. Parasit., 57, 1052–1067.

15) Kozek, W. J. and Marroquin, H. F. (1977) : Intracytoplasmic bacteria in *Onchocerca volvulus*. Amer. J. Trop. Med. Hyg., 26, 663–678.

16) Laurence, B. R. and Simpson, M. G. (1968): Cephalic and pharyngeal Structures in microfilariae revealed by staining. J. Helminth., 42, 309–330.

17) Laurence, B. R. and Simpson. M. G. (1974): The ultrastructure of the microfilaria of *Brugia*, Nematoda: Filarioidea. Internat. J. Parasit., 4, 523–536.

18) Lee, C.-C., and Miller, J. H. (1967) : Fine structure of *Dirofilaria immitis* body wall musculature. Exp. Parasit., 20, 334–344.

19) Lee, C.-C. and Miller, J. H. (1969) : Fine structure of the intestinal epitherium of *Dirofilaria immitis* and changes occurring after vermicidal treatment with caparsolate sodium. J. Parasit., 55, 1035–1045.

20) Lee, C.-C. (1975) : *Dirofilaria immitis:* Ultrastructural aspects of oocyte development and zygote formation. Exp. Parasit., 37, 449–468.

21) Maeda, T., Harada, R., Nakashima, A., Sadakata, Y., Ando, M., Yonamine, K., Otsuji, Y., and Sato, H. (1970) : Electronmicroscopic studies on spermatogenesis in *Dirofilaria immitis*. Recent Advances in Researches on Filariasis and Schistosomiasis in Japan, 73–97. University of Tokyo Press, Tokyo.

22) McLaren, D. J. (1969) : Ciliary structures in the microfilaria of *Loa loa*. Trans. Roy. Soc. Trop. Med. Hyg., 63, 290–291.

23) McLaren, D. J. (1972) : Ultrastructural studies on microfilariae (Nematoda : Filarioidea). Parasitol., 65. 317–332.

24) McLaren, D. J., Worms, M. J., Laurence, B. R., and Simpson, M. G. (1975) : Microorganisms in filarial larvae (Nematoda). Trans. Roy. Soc. Trop. Med. Hyg., 69, 509–514.

25) Mollenhauer, H. H. (1964) : Plastic embedding mixtures for use in electron microscopy. Stain Technology, 39, 11.

26) Schacher, J. F. (1962) : Morphology of the microfilaria of *Brugia pahangi* and of the larval stages in the mosquito. J. Parasit., 48, 679–692.

27) Schacher, J. F. and Khalil, G. M. (1968): Development of *Foleyella philistinae* Schacher and Khalil, 1967 (nematoda : filarioidea) in *Culex pipiens* molestus with notes on pathology in the arthropod. J. Parasit., 54, 869–878.

28) Singh, M., Kanagasuntheram, R., Ho, B.-C., Yap, E.-H., and Chan, H.-L. (1974) : Some ultrastructural observations on the microfilaria of *Breinlia sergenti*— the pharyngeal thread and innen körper. Internat. J. Parasit., 4, 375–382.

29) Singh, M., Kanagasuntheram, R., Ho, B.-C., Yap, E.-H., and Chan, H.-L. (1975a) : Some aspects of the fine structure of the nervous system of the infective larva of *Breinlia sergenti* (Nematoda : Filarioidea). Internat. J. Parasit., 5, 165–170.

30) Singh, M., Kanagasuntheram, R., Ho, B.-C., Yap, E.-H., and Chan, H.-L. (1975b) : Some aspects of the fine structure of the alimentary tract of the infective larva of *Breinlia sergenti* (Nematoda : Filarioidea). Internat. J. Parasit., 5, 171–176.

31) Suguri, S. (1977) : Ultrastructure of the microfilaria of *Brugia pahangi* (Buckley and Edeson, 1956) Buckley, 1958. Acta Medica Okayama, 31, 295–318.

32) Tongu, Y. (1974) : Ultrastructural studies on the microfilaria of *Brugia malayi*. Acta Medica Okayama, 28, 219–242.

33) Vincent, A. L., Ash, L. R. and Frommes, S. P. (1975a) : The ultrastructure of adult *Brugia malayi* (Brug, 1927) (Nematoda : Filarioidea). J. Parasit., 61, 499–512.

34) Vincent, A. L., Portaro, J. K., and Ash, L. R. (1975b) : A comparison of the body wall ultrastructure of *Brugia pahangi* with that of *Brugia malayi*. J. Parasit., 61, 567–570.

Brugia pahangi 幼虫の電顕的観察

頓宮廉正
(岡山大学医学部寄生虫学教室)
ALBERT L. VINCENT AND LAWRENCE R. ASH
(カリフォルニア大学)

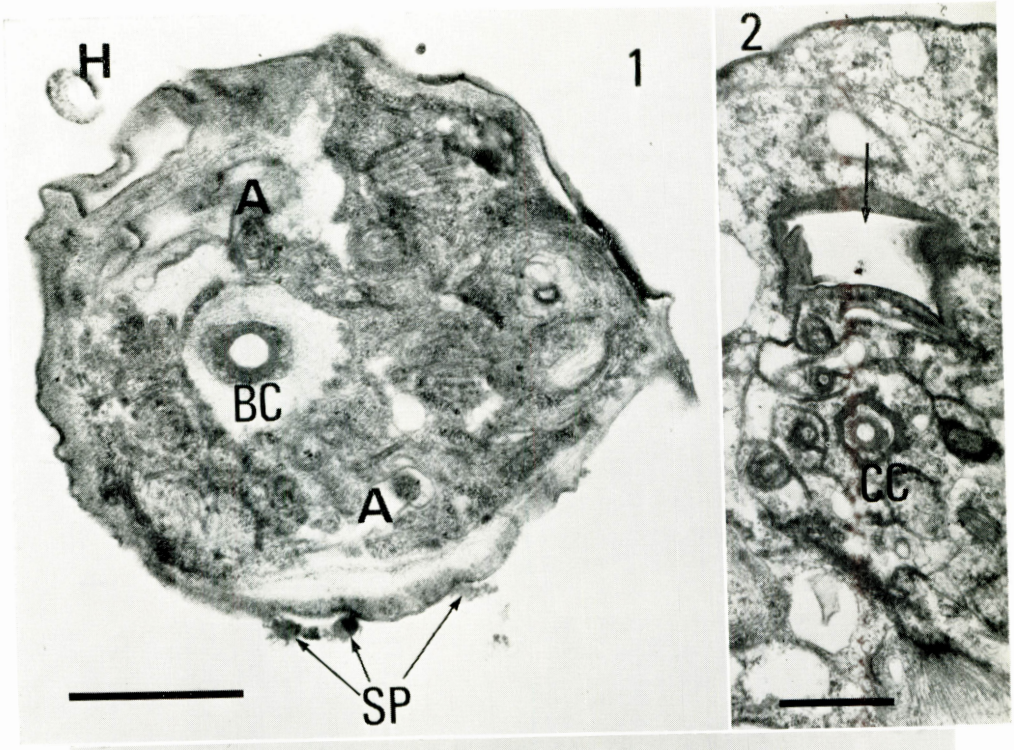
スナネズミ寄生の *Brugia pahangi* のミクロフィラリアを *Armigeres subalbatus* に感染させ，2日，3日，5日，7日後に得た幼虫を電顕的に観察し各種器官の形成状況を比較した．第1回目の脱皮が行なわれていない2日と3日目の幼虫ではミクロフィラリアにあるような1本の hook と3本の spine が頭部前端に存在するが，脱皮後の5日目の幼虫には存在しない．角皮は2日から5日目の幼虫に於ては種々の厚さの fibrous layer が存在しているが，部分的には細胞膜だけで角皮はみられない．7日の幼虫では全くみられず，しばしば脱皮のためと考えられる2層の膜によつて被われている．上皮細胞を横断像でみると5日及び7日目の幼虫では7ないし8個の細胞よりなり，側線部以外でも核を有する．5日目の幼虫の上皮細胞にはリケッチア様の微生物がみられた．筋層は横断像でみると4群に分れて存在するが各々細胞数は普通2個と少なく，又フィラメントの数も非常に少数である．central canal は5日目の幼虫には存在するが7日目のものでは既に食道が形成されている．R-cell は5日目の幼虫では未だ管腔は形成されていないが，inner body に於ては5日目になると小管腔を有している．7日目になると腸管腔が形成され腔内には蚊より取込んだミトコンドリアが多数存在する．従つて7日目の幼虫になると少なくとも腸管までは食物摂取が可能である．同時に体表に角皮層が存在しない事は体表からの栄養吸収も行なつていると考えられる．

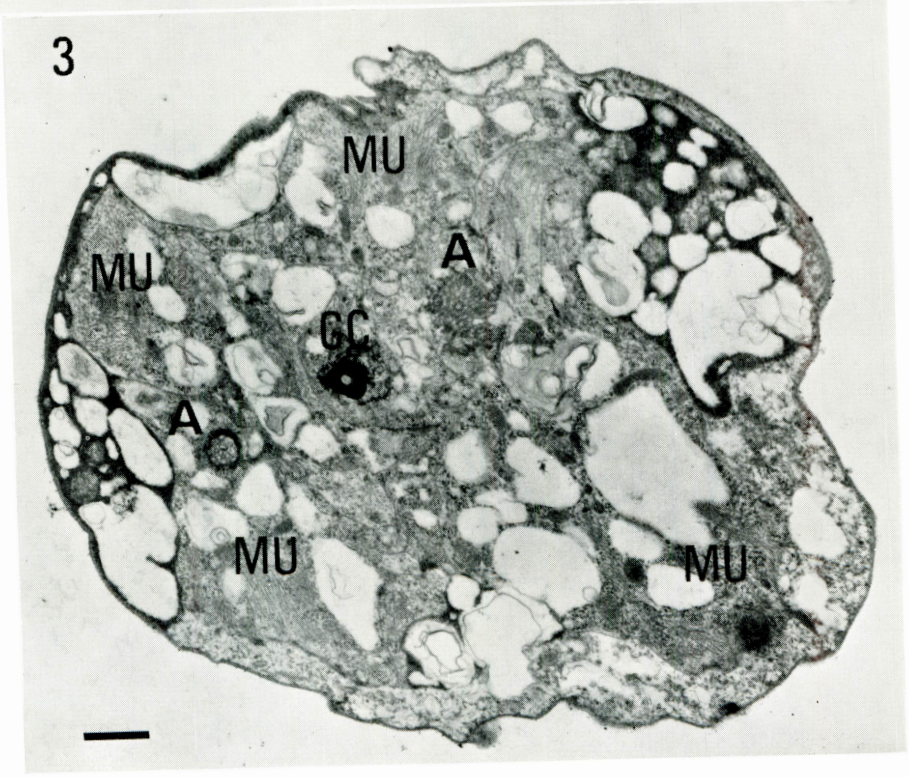
肛門，排泄腔は5日目の幼虫にも観察された．排泄細胞はミクロフィラリアに較べると微絨毛がなくなつてをり，核は排泄口近くにあって細胞全体もより円形に近くなつている．

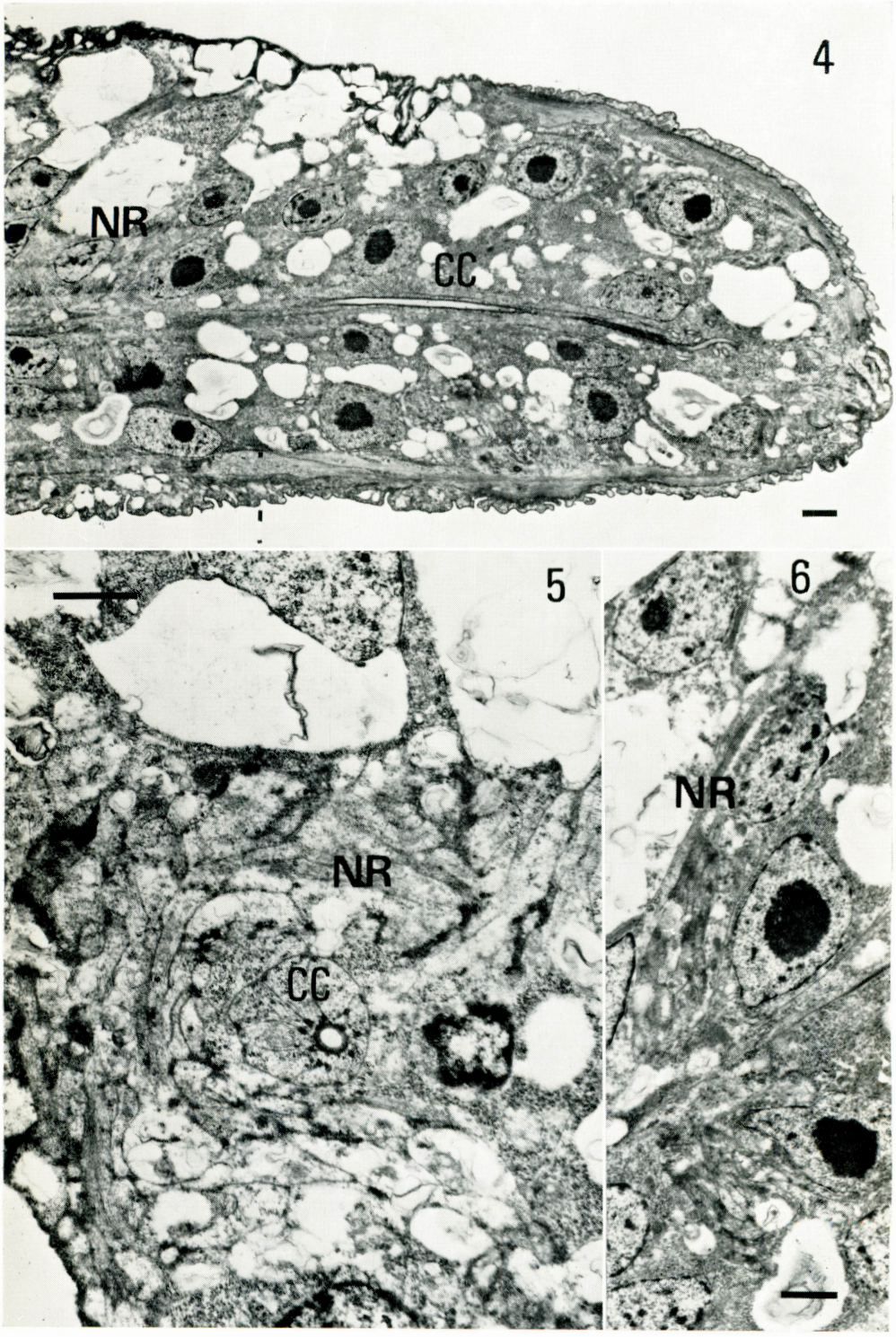
Figure Legends

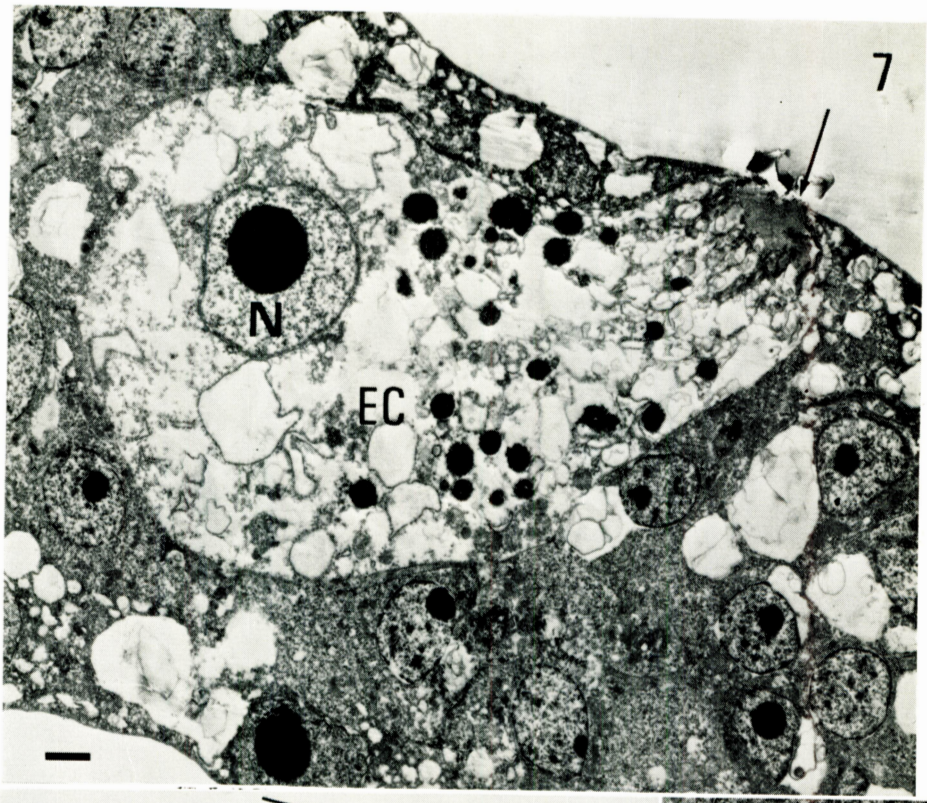
Fig. 1 Cross-section of the head of two-day larva having a hook (H) and three spines (SP). A: amphid; BC: buccal cavity.
Fig. 2 Cross-section of the head of five-day larva showing an invagination of the cuticle (arrow). CC: central canal.
Fig. 3 Cross-section of the head region of five-day larva near the nerve ring. Muscle cells (MU) are arranged into four groups. A: amphid; CC: central canal.
Fig. 4 Longitudinal section of the head region of five-day larva, showing a central canal (CC) running through the nerve ring (NR).
Fig. 5 Cross-section of the nerve ring (NR) of five-day larva showing a central canal (CC) surrounded by many axons.
Fig. 6 Longitudinal section of the nerve ring (NR) of five-day larva at higher magnification.
Fig. 7 Longitudinal section of the excretory cell (EC) with a nucleus (N) and a pore orifice (arrow) in five-day larva.
Fig. 8 Cross-section of the excretory cell (EC) of five-day larva showing a pore orifice (arrow) and a central canal (CC) located on one side of the body.
Fig. 9 Higher magnification at the cytoplasm of excretory cell of fiive-day larva, showing the vesicles with granules of various shapes and densities.
Fig. 10 Cross-section of the region formerly occupied by the inner body (I) in five-day larva. There is a small lumen (arrow) surrounded by two large cells.
Fig. 11 Cross-section of intestinal lumen (arrow) of five-day larva.
Fig. 12 Cross-section of the region formerly occupied by the inner body (I) in five-day larva. Dense material is seen in the lumen (arrow). MU: muscle.
Fig. 13 Cross-section of a phasmid (P) behind the anal pore region of five-day larva.
Fig. 14 Cross-section of the region formerly occupied by R-cells. There are two large cells (R) containing many vacuoles in flve-day larva.
Fig. 15 Higher magnification of the R-cell region of five-day larva. There are two different types of cells in electron density.
Fig. 16 Cross-section of the region behind the anal pore of five-day larva. A large pseudocoal (arrow) is seen behind the anal pore. P: phasmid.
Fig. 17 Cuticle in five-day; note dense materials on the outer surface.
Fig. 18 Longitudinal section of the anal apparatus (AP) of five-day larva, showing a cup, a nucleus (N), and many microvilli at the bottom of the pore.
Fig. 19 Higher magnification of the anal pore (AP) of Figure 18, showing many microvilli (MV) at the pore bottom.
Fig. 20 Higher magnification of the anal pore lumen of five-day larva.
Fig. 21 Cross-section of the microvilli of anal pore in five-day larva.
Fig. 22 Longitudinal section of the caudal end of five-day larva, showing a small tail with many striations and a chromatin-like dot (arrow). MU: muscle.
Fig. 23 Logitudinal section of the hypodermal cells of five-day larva, showing several rickettsia-like bodies (arrows) near the nucleus (N).
Fig. 24 Cross-section of seven-day larva having a large intestinal lumen (LU).
Fig. 25 Higher magnification of the intestinal wall and lumen (LU) containing many mitochondria (M) from the mosquito.
Fig. 26 Cross-section of the esophagus of seven-day larva.

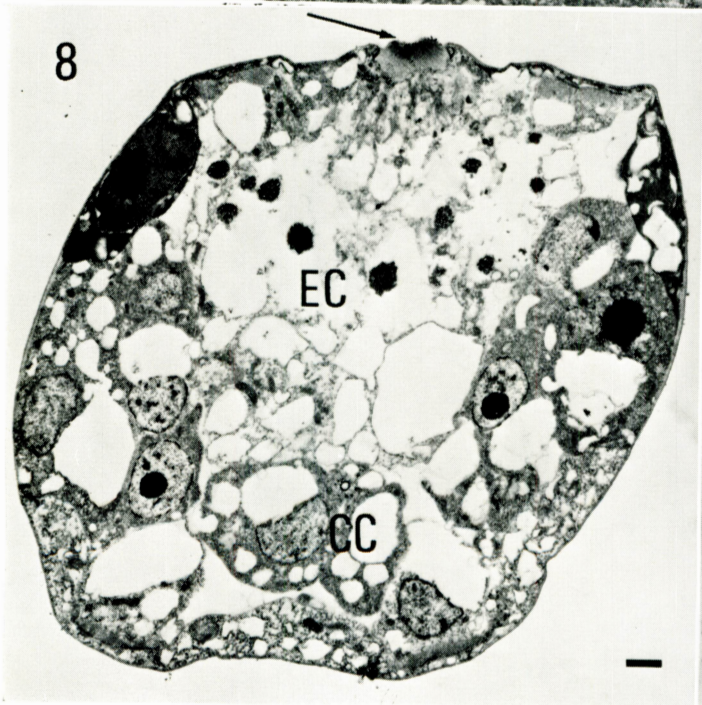
NOTE: Scale is one micron in each figure.

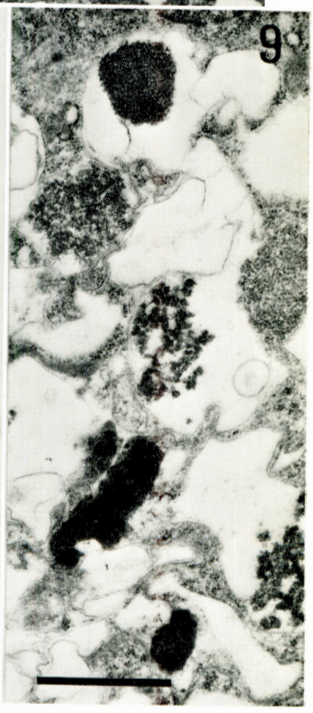

H
1
A
BC
A
SP
2
CC

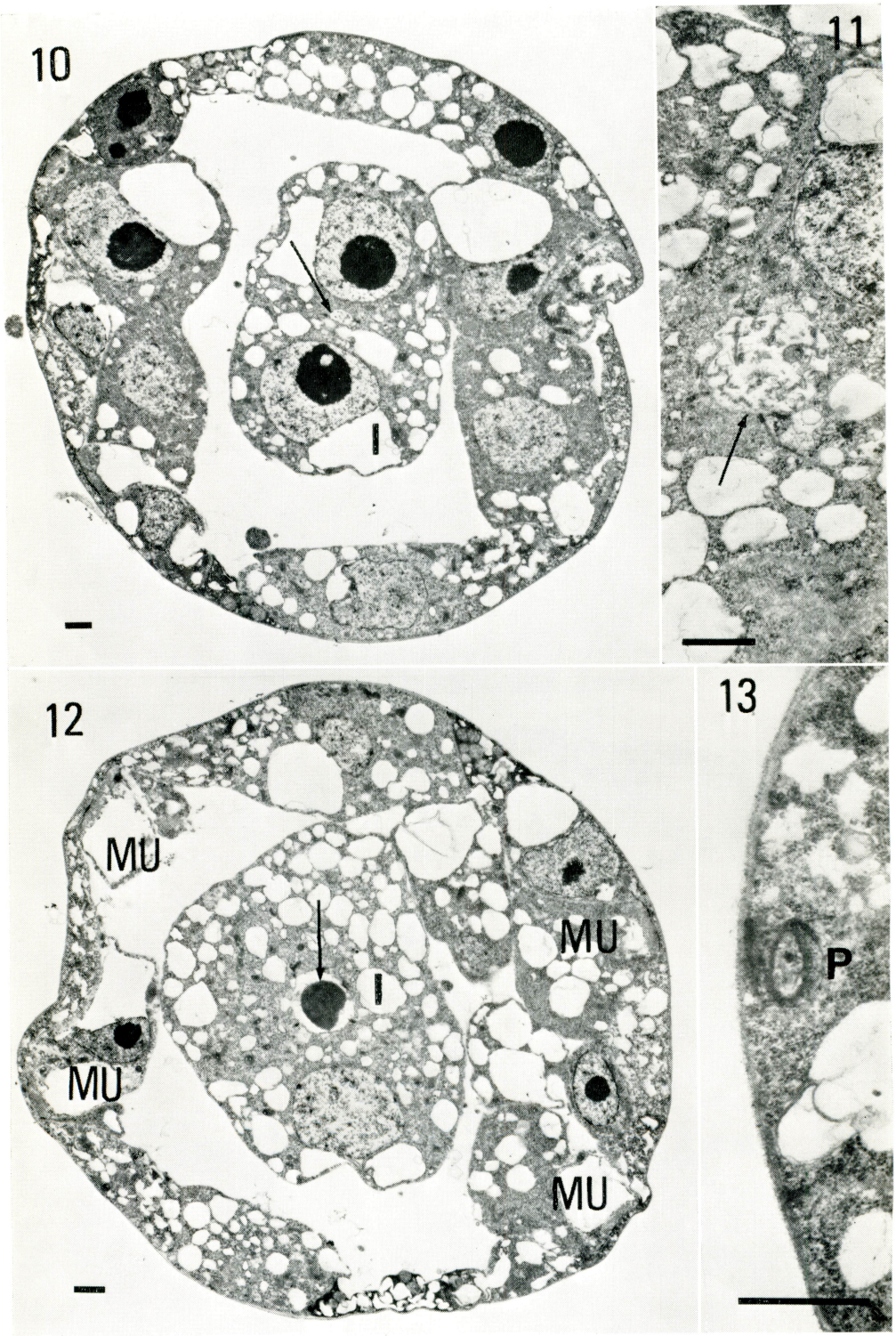

3
MU
A
MU
CC
A
MU
MU

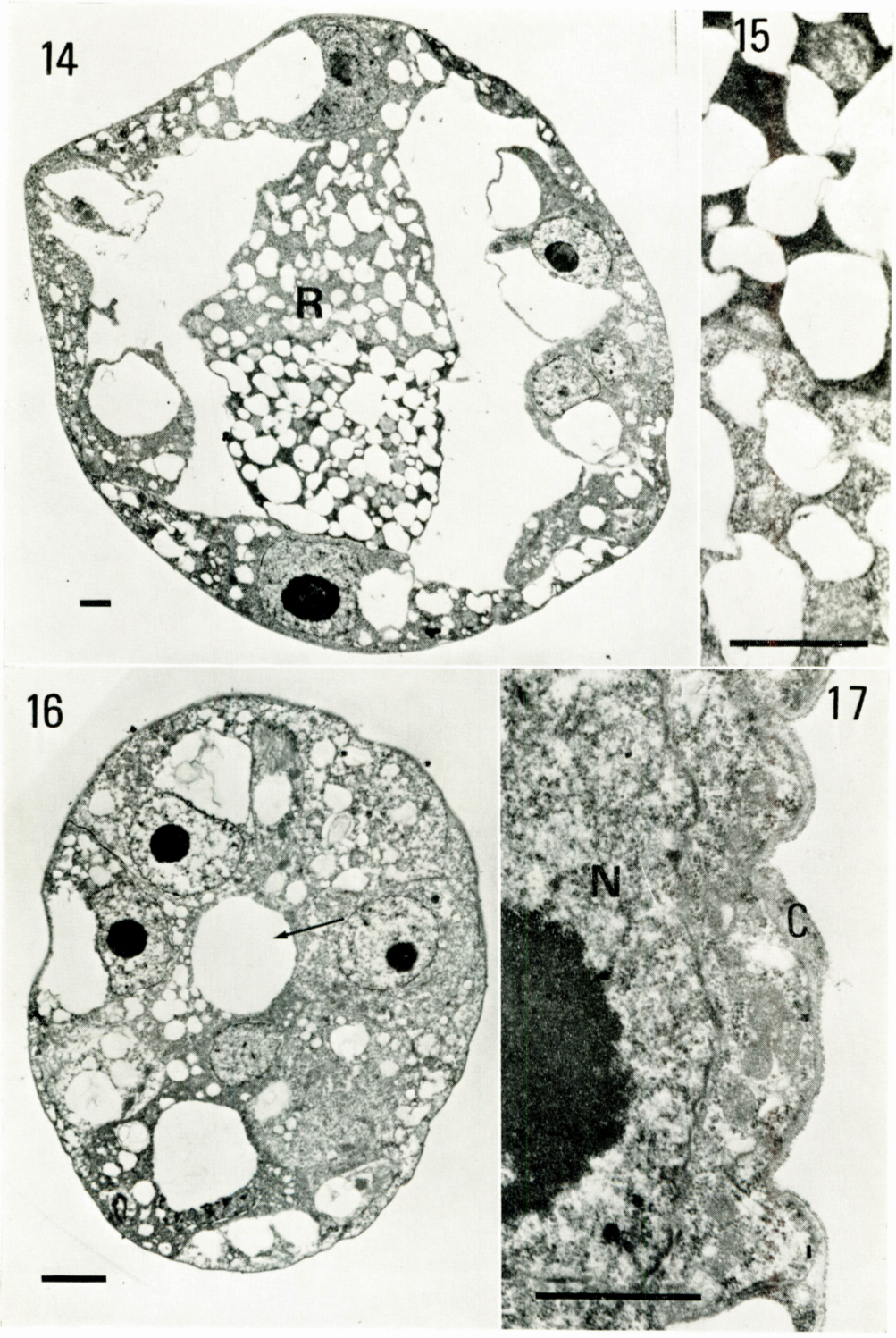

4
NR
CC
5
NR
CC
6
NR

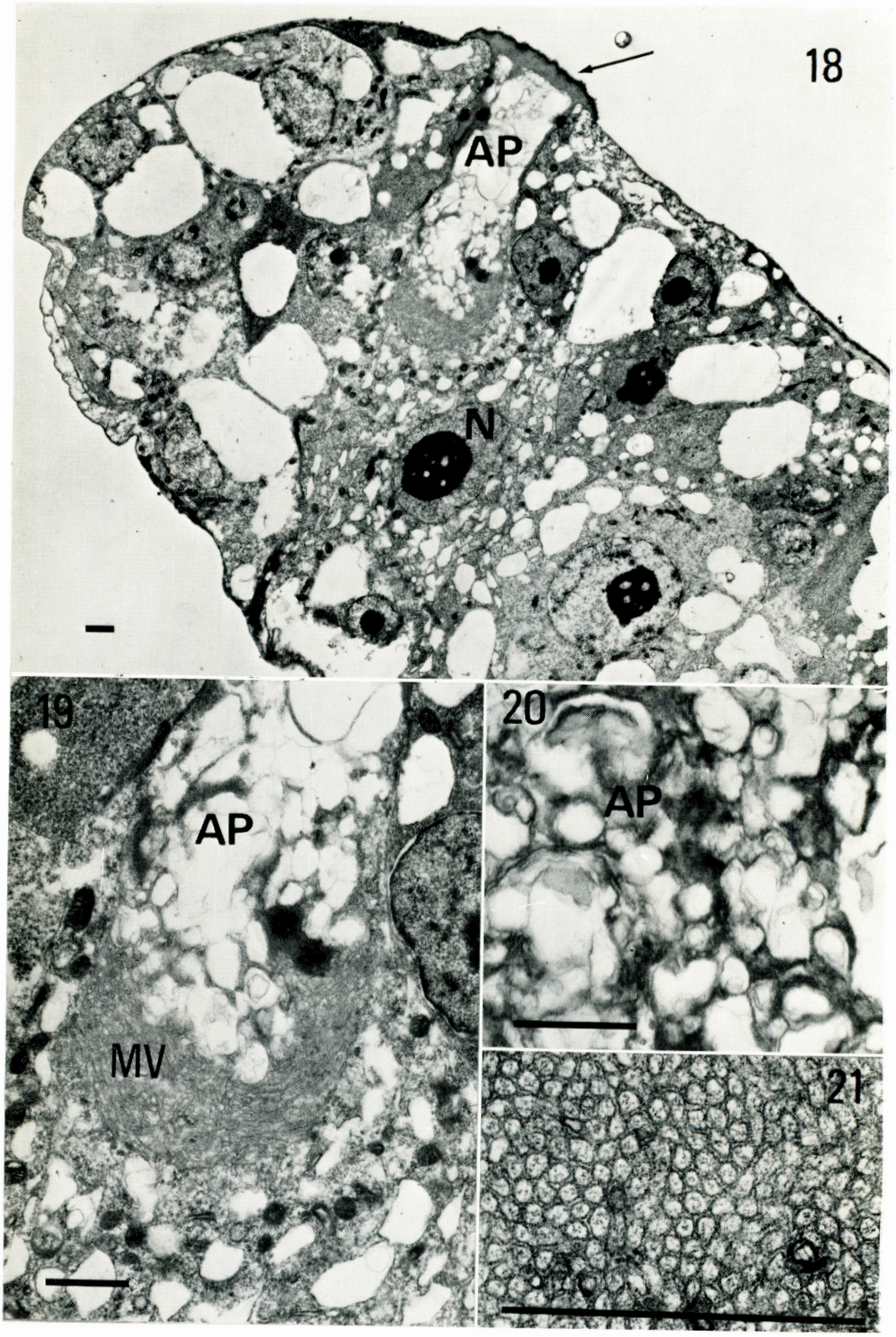

7
N
EC

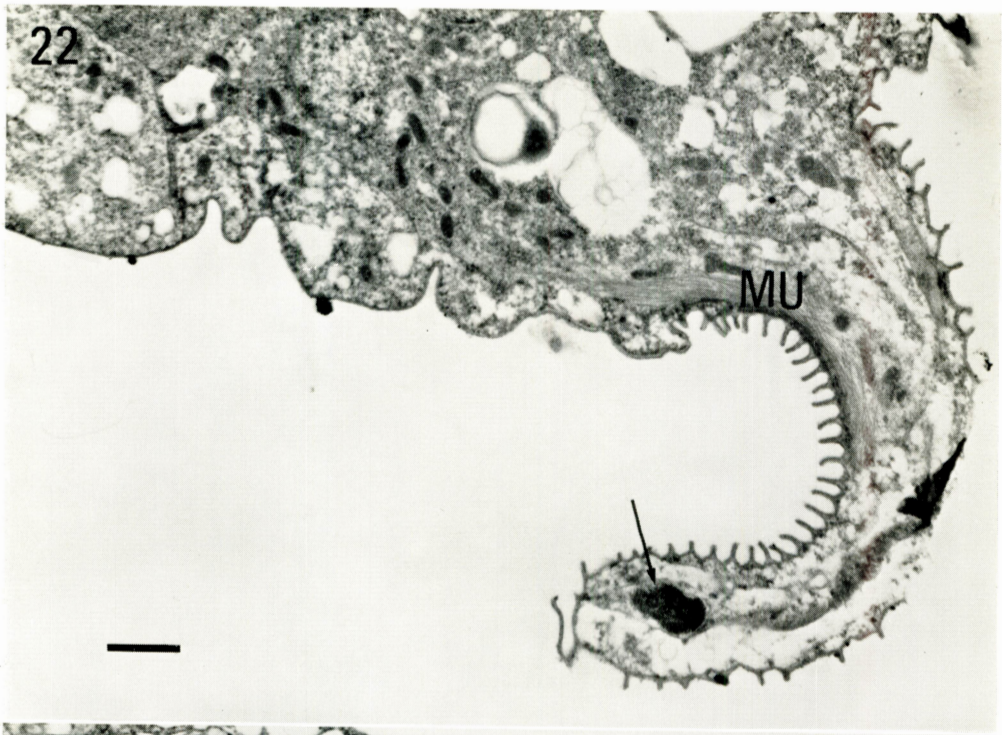

8
EC
CC

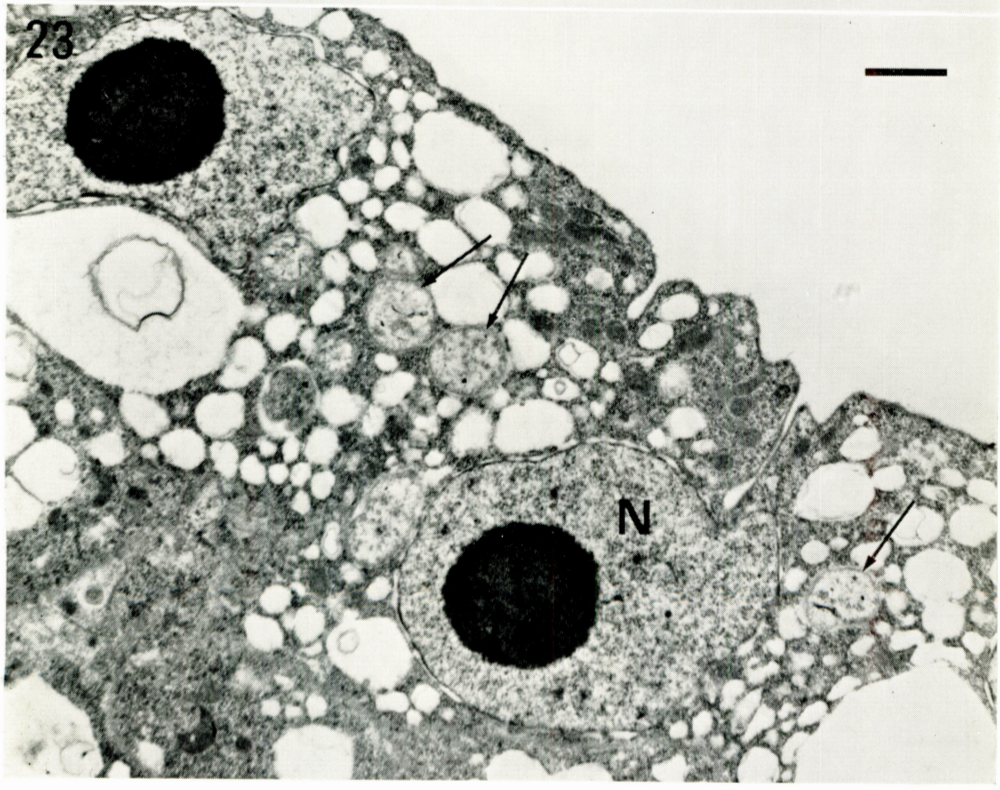

9

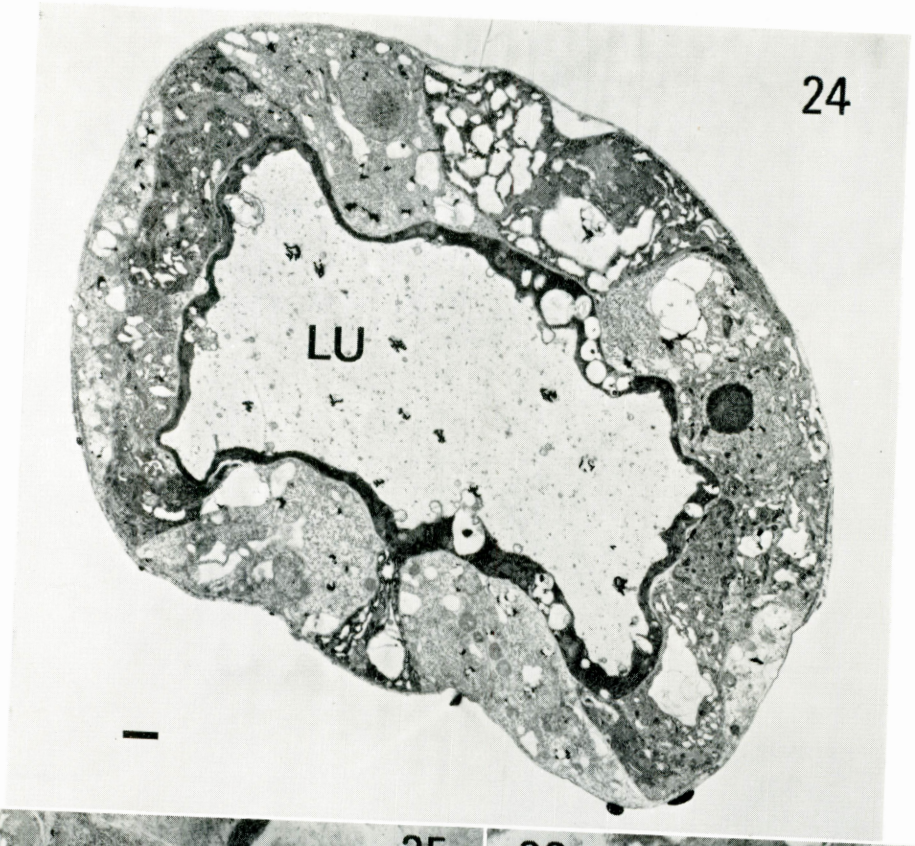

10
I
11
12
MU
MU
MU
MU
I
13
P

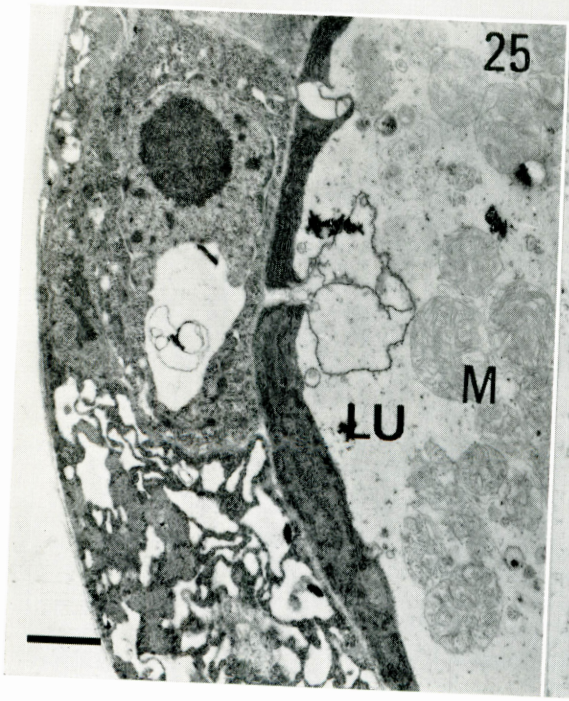

14
R
15
16
17
N
C

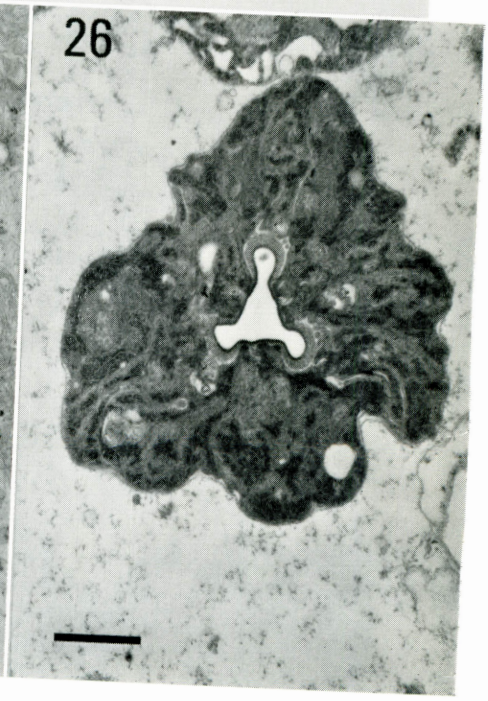

18
AP
N
19
AP
MV
20
AP
21


22
MU


23
N


24
LU


25
LU
M


26