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Abstract

The microfilaria of Brugia pahangi obtained from an experimentally infected dog were observed with the electron microscope. The sheath was composed of small granules and was covered with electron-dense particles on the outer surface and with small granules on the inner surface. The cuticle was composed of an outermost layer, a trilaminate membrane and an inner layer. The hypodermis was composed of four components as in the adult stage (two small ones on the lateral sides, two large ones on the dorsal and ventral sides). The muscle cells comprised a single layer under the hypodermis on the dorsal and ventral sides. On each side, two muscle cells usually appeared in a transverse section. The thick myofilament was surrounded with 8 to 12 thin myofilaments. Dense bodies were present around the cephalic space. In the cells of the nuclei column, the cytoplasm was very narrow, and the electron-dense nucleus close to each other. The cuticular central canal was connected to the buccal cavity and to the inner body. A sponge-like structure was seen at the junctional part of the canal and the inner body. The inner body showed a homogeneous granular appearance. Eight cephalic papillae were observed at the head tip. Two amphids, each having more than eight cilium-like structures, were connected with the nerve elements and open in the head part. Two phasmids, each having one ciliumlike structure, opened in the caudal part. Two types of neurosecretory granules were observed in the nerve ring and the dorsal and ventral longitudinal nerves were clear except in the anterior and the posterior part of the worm. The excretory and the anal vesicles had contacts with thin and thick cytoplasmic processes respectively, and these vesicles opened to the exterior. The nuclei of the G cell and R cells showed similar electron-density. Lamellate structures were present in the muscle and the hypodermis.

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ULTRASTRUCTURE OF THE MICROFILARIA OF *BRUGIA PAHANGI* (BUCKLEY AND EDESON, 1956) BUCKLEY, 1958

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Abstract. The microfilaria of *Brugia pahangi* obtained from an experimentally infected dog were observed with the electron microscope. The sheath was composed of small granules and was covered with electron-dense particles on the outer surface and with small granules on the inner surface. The cuticle was composed of an outermost layer, a trilaminate membrane and an inner layer. The hypodermis was composed of four components as in the adult stage (two small ones on the lateral sides, two large ones on the dorsal and ventral sides). The muscle cells comprised a single layer under the hypodermis on the dorsal and ventral sides. On each side, two muscle cells usually appeared in a transverse section. The thick myofilament was surrounded with 8 to 12 thin myofilaments. Dense bodies were present around the cephalic space. In the cells of the nuclei column, the cytoplasm was very narrow, and the electron-dense nucleus close to each other. The cuticular central canal was connected to the buccal cavity and to the inner body. A sponge-like structure was seen at the junctional part of the canal and the inner body. The inner body showed a homogeneous granular appearance. Eight cephalic papillae were observed at the head tip. Two amphids, each having more than eight cilium-like structures, were connected with the nerve elements and open in the head part. Two phasmids, each having one cilium-like structure, opened in the caudal part. Two types of neurosecretory granules were observed in the nerve ring and the dorsal and ventral longitudinal nerves were clear except in the anterior and the posterior part of the worm. The excretory and the anal vesicles had contacts with thin and thick cytoplasmic processes respectively, and these vesicles opened to the exterior. The nuclei of the G cell and R cells showed similar electron-density. Lamellate structures were present in the muscle and the hypodermis.

Ultrastructures of microfilaria have been studied by some workers; e.g., ciliary structures in the cephalic and the caudal channels of *Dirofilaria immitis* (1) and *Loa loa* (McLaren, (2)), cuticle and muscle cells of *D. immitis* (Johnson & Bemrick, (3)), the sheath and body wall of *Cordianema* sp. (Johnston & Stehbens, (4)) and some other organs of *D. immitis* (Kozek, (5)), *D. immitis*, *Dipetalonema viteae*, *Dipetalonema setairosum*, *Loa loa* and *Litomosoides carinii* (McLaren, (6)),

Brugia malayi (Tongu, (7)), *Breinlia sergenti* (Kanagasuntheram *et al.* (8) and Singh *et al.* (9)) and *Brugia pahangi* (Laurence & Simpson (10)). In the present paper the ultrastructure of *Brugia pahangi* will be reported and compared with the structures reported in other publications.

MATERIALS AND METHODS

Microfilariae of *Brugia pahangi* were obtained from the blood of a dog which had been infected with *Brugia pahangi*. The heparinised blood was transferred to 2% saponin in 0.85% NaCl solution buffered with M/15 phosphate to pH 7.2. After centrifugation (2,000 R.P.M., 5 min) the materials were washed with the buffered salt solution and then fixed with 2% glutaraldehyde (buffered with M/10 phosphate at pH 7.2) for 1.5 hr at ice-water temperature. The materials were washed with the phosphate buffer and post-fixed with 1% Osmium tetroxide (buffered with phosphate at pH 7.4) for 2 hr. The fixed materials were dehydrated with an ethanol series and embedded in Epon or stylene metacrylate. The sections cut with an ultramicrotome were stained with saturated aqueous solution of uranyl acetate and then with lead citrate.

RESULTS

The sheath. The sheath is about 150 Å in thickness and shows a homogeneous structure composed of small granules (Fig. 1, Sh). The outer surface of the sheath is covered with an electron-dense particulate layer of variable thickness. Each particle appears both in a transverse and a longitudinal section of the microfilaria but may sometimes appear much elongated. The diameter of the particle varies from 430 Å to 860 Å. The inner surface of the sheath is also covered with electron-dense granules. But these particles (Fig. 1, broken arrow) are small (80 Å in diameter) and sparse compared with those of the outer surface.

The cuticle. The cuticle has transverse striations (Fig. 2, arrow) and the mean distance of the striations is 0.2 μm. The cuticle is about 500 Å in thickness and composed of three units (Fig. 2), *i. e.*; outermost layer (OL), trilaminated membrane (TM) and inner layer (IL). The outermost layer (80 Å in thickness) is electron-dense and mostly discontinuous at the deepest parts of the striations, especially on the stretched side of the microfilaria. The trilaminated membrane is composed of two electron-dense membranes (50 Å in thickness) separated by an interval of 40 Å; the outer membrane is discontinuous at the deepest part of the striation and the inner membrane is continuous throughout the body length. The inner layer has a fibrous structure and may be divided into outer less electron-dense (90 Å in thickness, (OLL)) and inner dense (170 Å in thickness, (IDL)) zones.

The hypodermis. The hypodermis situated beneath the cuticle layer (Figs. 2, 3 and 4) is broad at the lateral parts and very narrow at the other parts. Hence,

when cut transversely, four different components (two small and two large) can be distinguished, and each component is connected with its neighbouring partners by a desmosome (Fig. 4, D). The small components (Fig. 4 and 13, NH) seen at parts of the lateral broad area contain the nucleus, mitochondria, and many ribosomes. The large two are situated on the dorsal and ventral sides. They are slender in shape, but where they connect with the lateral small components, they become broader (Fig. 13, H).

The musculature. The muscle cells have an elongated fusiform shape and lie as a single layer in the periphery on the dorsal and the ventral sides of the body under the hypodermis (Fig. 13, M). In transverse sections, two to four muscle cells (Fig. 13) are often seen divided by infoldings of the hypodermis. This general arrangement changes in the cephalic space and around the nerve ring. In the cephalic space only two large muscle cells are found at the anterior part (Fig. 8, M). At the posterior part, four large cells exist, two of them being continuous with the anterior cells (Fig. 9, M). Near the nerve ring there are more than nine smaller cells (Fig. 10, M). In the ventral muscle band, only one muscle cell is observed at each side of the excretory and the anal vesicle (Fig. 27, M).

The muscle cells can be divided into contractile (Fig. 7, CP) and noncontractile (Fig. 7, NP) portions as in other nematodes (Fig. 7). The contractile portion is situated at the periphery. In the anterior region of the body, dense bodies are observed between the hypodermis and the contractile portion (Fig. 3, DB). The contractile portion is composed of thick and thin filaments. The thick filament is surrounded by 8 to 12 thin filaments (Fig. 5). The diameter of the thick filament is about 190 Å and that of the thin, about 40 Å. The noncontractile portion contains many elongated mitochondria, condensed glycogen particles and a large nucleus. The nucleus is less electron-dense than the cells in the nuclei column (Fig. 7, NM).

The cephalic space. The cephalic space occupies the anterior tip of the microfilaria and has no nucleus (Fig. 7). The space is about 10 μm in length and ends at the nuclei of the muscle cells which extend from the head tip. The space has four large muscle cells (Fig. 9, M), two amphidial channels (Figs. 7 and 8, Am), eight papillae (Fig. 8, CP) and the buccal cavity (Fig. 8, BC) connecting to the central canal (Figs. 9 and 10, CC). Two muscle cells start from the head tip and the other two, from the middle part of the cephalic space (Fig. 7).

The amphid. The amphidial channels run in parallel along the lateral sides of the cephalic space (Figs. 8, 9 and 10, Am). The longer one opens near the mouth at the head tip and the other, at the posterior part of the first annulation of the cuticle. These channels which are lined with a cuticular layer compos d of an outer trilaminated membrane and inner layer originate at the middle part of the cephalic space. The channels an enclose cilium-like structures, microtubules

of which do not show any consistent pattern. In the transverse section, more than eight cilium-like structures can be seen at the basal part of each channel (Fig. 10, Am), but as the channel approaches the head tip, the number of cilium-like structures becomes less and the shape of the channel becomes flat (Figs. 8 and 9, Am). At the oriface of the channel, a plug with a fibrous structure connects with the cilium. At the basal part, the cilium-like structure has no basal body. It is noteworthy that here the structure is clearly continuous with the nervous element (Fig. 6, Ne).

The cephalic papilla. Eight cephalic papillae (four dorsal, four ventral) are observed at the head tip of the microfilaria. Cilium-like structures connecting to the papillae are short and have irregular microtubule patterns (Fig. 8, CP).

Nuclei column. The nuclei column is the row of electron-dense nuclei of the nondifferentiated cells (Fig. 11, N). The column runs from the end of the cephalic space to the tail part but is intercepted by other structures such as the nerve ring, the excretory apparatus, the inner body, etc. The squarish nucleus occupies almost the whole cell, with only a small space for the cytoplasm. The nuclei thus become very close to each other.

The nerve ring and the nervous system. The nerve ring is composed of many nerve processes (Fig. 12). The processes contain microtubules, mitochondria, glycogen granules and large and small secretory granules; the large ones, 500 to 800 Å in diameter (Fig. 12, LG) and the small ones, about 350 Å (Fig. 12, SG). The nerve processes are clearly seen along the dorsal and the ventral sides throughout the body length except at both extremes in the cephalic and anal regions (Fig. 13, LNe). These nerves run between the two muscle cells but when they meet the excretory and the anal vesicles, they deviate running along the side of each vesicle (Fig. 27, LNe). In addition, short nerves run posteriorly from the nerve ring along the inside surface of the hypodermal chord.

A lamellate structure. Lamellate structures, 0.1 to 0.2 μm in diameter and 0.2 to 0.57 μm in length (Fig. 15), are observed in the muscle cells and the hypodermis throughout the microfilaria.

The excretory apparatus. The excretory apparatus is composed of (a) a nucleus, (b) a cytoplasmic bridge and (c) a vesicular portion. The nuclear region corresponds to the so-called excretory cell and is larger than the cells of the nuclei column (Fig. 16, EC). The nucleus is less electron-dense and the chromatin particles are evenly distributed. The cytoplasm of the excretory cell contains free ribosomes, rough endoplasmic reticula and mitochondria. The narrow cytoplasmic bridge (Fig. 16, CB) extending to the vesicular portion is filled with many free ribosomes which are known to be the characteristic structure of the excretory cell. The vesicular portion makes contact with many cytoplasmic processes of 300 Å in thickness (Fig. 17, CP) from the bridge. The vesicule is filled with fine

electron-dense granules. The excretory pore is 0.42 μm in diameter and the cuticle becomes thick around the pore and continues to the inner part of the vesicle (Fig. 17, EP).

The buccal cavity, the central canal and the inner body. The cylindrical buccal cavity has a small round-shaped opening at the anterior tip of the microfilaria (Fig. 8, VC), and the surface of the cavity is covered with a cuticular layer which is extended over to the body surface of the microfilaria through the opening.

The central canal is a cuticular canal, 0.14 μm in diameter, and extends from the posterior part of the buccal cavity to the anterior part of the inner body. The wall of the canal is covered with a cuticular layer (140 Å in thickness) and small granular materials exist in the lumen. In the transverse section, the canal appears at the central part of the worm and is surrounded by five to eight radially arranged desmosomes (Figs. 13 and 14, PT). The desmosome could not be observed at the anterior part of the microfilaria (Figs. 9 and 10, PT).

The inner body is situated between the excretory cell and the G cell. The structure is elongated, uniformly electron-dense, and surrounded by a thin membrane (Figs. 19 and 20, arrow). In the transverse section, thin cytoplasm and one or two long desmosomes are observed around the inner body (Figs. 19 and 20).

At the junctional part of the inner body with the central canal, a sponge-like structure is found (Fig. 18, SS), in addition to the desmosome which connects the central canal with the cell containing the inner body (Fig. 18, D). The sponge-like structure is 1.6 μm in length and 0.3 to 0.5 μm in diameter. In the transverse section the structure appears coil-like (Fig. 19, SS), whereas the longitudinal section shows that the structure starts from the cuticle layer of the central canal. The granular contents in the anal and in the inner body which is bridged by the sponge-like structure resemble each other (Fig. 18).

The G cell. The G cell is the largest cell in the microfilaria and has a large nucleus occupying almost the whole breadth of the cell (Fig. 22, NG). The cytoplasm of the cell is filled with free ribosomes. The nucleus shows a homogeneous structure and at the central part of the nucleus the chromatin is observed in the longitudinal section as a band running in a direction vertical to the worm.

The R cells and the anal vesicle. R2, R3 and R4 cells (Figs. 23 and 24) situated at anterior parts of the vesicle are elongated. At the end of the elongation, cytoplasmic processes being 800 Å in diameter (Figs. 25 and 27, CP) are formed over the basal part of the vesicle. The cells are relatively large and are filled with free ribosomes. The nucleus is similar to that of the G cell and is paler than those of the cells in the nuclei column (Figs. 23 and 24, NR).

The anal vesicle occupies one-third of the width of the microfilaria and has electron-dense granules. The pore of the vesicle opens at the ventral side and is 0.16 μm in diameter. The cuticle becomes thick around the pore and continues to

the inside of the vesicle as in the excretory pore (Fig. 26).

The phasmid. Two phasmidial channels run posteriorly along the lateral sides in the caudal part, and open to the outside. The opening sites of the two are shifted anteroposteriorly. Each channel contains a cilium-like structure, whose microtubules are arranged irregularly (Fig. 30, Ph). The structure connects with the nerve element on one side (Fig. 28) and on the other side with the plug in the pore (Fig. 29).

DISCUSSION

McLaren (6) has described the structure of the sheath of the blood microfilaria of the *Loa loa* and *Litomosoides carinii* and Tongu (7), that of *Brugia malayi.* The present observation on *Brugia pahangi* has confirmed the essential feature of the sheath structure described by them. McLaren (6) suggested that the electron-dense particulate layer covering the sheath surface was derived from the host, since it was absent from the sheath of *in utero* forms. McLaren (6) and Tongu (7) noted the fibrous materials in the space between the sheath and the cuticle. These materials could not be distinctly demonstrated in the present observation except around the pore of the excretory and the anal vesicles.

The thickness of the cuticle is thicker in a microfilaria of *Dirofilaria immitis* than that of others; 1000 Å in *Dirofilaria immitis* of the former type and about 500 Å in *Brugia pahangi*, 410 Å in *Loa loa* and 420 Å in *Litomosoides carinii* (6) of the latter type. In *Dirofilaria immitis* microfilaria, Johnson & Bemric (3) and Kozek (5) have reported that the cuticle is composed of four layers, *i. e.*; the external cortical layer, the internal cortical layer, the fibrillar layer and the homogeneous layer. McLaren (6) has stated in microfilariae of *Dipetalonema*, *Loa* and *Litomosoides* that the cuticle is composed of the dense surface layer, the trilaminate membrane, the outer layer and the fibrous layer, that the trilaminate membrane covers the cephalic channel, the caudal channel, the excretory vesicle and the anal vesicle and also that the external cortical layer is lost at the first molt. Based on observations of *Brugia pahangi*, Laurence & Simpson (10) interpreted the electron-dense membranes as the equivalent of the external cuticular layer of other nematodes (Bird, (11)). In the present observation, the cuticle was divided into three units, lacking McLaren's outer cortical layer (6).

Bird (11) divided the hypodermis of nematodes into three types, *i. e.*; the cellular, the partially cellular and the syncytial hypodermis. The hypodermis of *Brugia pahangi* microfilaria is a syncytial one as in both the infective larva and the adult worm. The hypodermis is not distinct in the microfilaria, since it is very slender at the ventral and dorsal parts, and at the lateral chord part it connects not with pseudocoel but with the cells of the nuclei column and other structures. The castellation observed by McLaren (6) was not found in the present study but

the dense bodies of muscle cells are sometimes scattered at the border with the hypodermis in the part anterior of the nerve ring.

Kozek (1) has reported that the number of the thin filaments which surround one thick filament is ten to twelve whereas McLaren (6) claimed that it is twelve. In the present observation, the number was variable, from eight to twelve. The muscle arrangement of the *Brugia pahangi* microfilaria resembles that of the other microfilariae, except that the present worm has four muscle cells in the cephalic space and more than nine cells around the nerve ring. The dimensions of the myofilaments of the present species, thick (180 Å) and thin (40 Å) were similar to those previously reported (for thick ones, 200 Å in *Dirofilaria immitis* by McLaren (6) and 190 Å in *Brugia malayi* by Tongu (7) and for thin ones, 60 Å in the former and 50 Å in the latter). McLaren (6) and Laurence & Simpson (10) stated that the muscle of the anterior tip may function to evert the hook. It is difficult to support such a notion, not only because muscle cells are too big to participate in the movement of the small hook, but also because no structural connection between the muscle cells and the hook was stated.

The squarish nucleus of the cells in the nuclei column is very electron-dense and occupies almost the whole cell resulting in the side-by-side appearance of the nuclei.

In the microfilaria of *Brugia pahangi*, three spines and one hook have been observed beneath the head disk at opposite sides to each other as regards the body axis by light (12) as well as scanning electron microscope (Aoki & Katamine, (13); Aoki & Ash, (14) and Suguri (unpublished)). As regards the lip described by Kozek (5) and Tongu (7) in the head region of *Dirofilaria immitis* and *Brugia malayi* microfilariae, Aoki & Katamine (12), Aoki and Ash (14) have claimed that the microfilaria of *Brugia pahangi* has no lip but is provided with the deep first annulation. It must be noted that the amphidial channel opens in this region. It is probable that the structure intervening between the deep annulation and the opening was mistaken as a lip when observed longitudinally.

The buccal cavity opens to the outside which may correspond to the opening at the head tip observed with a scanning electron microscope by Aoki & Katamine (13). The author agrees to changing the name of the pharyngeal thread to the central canal (Tongu (7)), since it is a cuticular canal. At the junction of the central canal and the inner body, a sponge-like structure is observed and the granular contents on both sides of the structure resemble each other closely. The inference is that the central canal may be connected structually to the inner body. Laurence & Simpson (10) have stated that the pharyngeal thread and the inner body of the microfilaria are situated at the position of the future digestive system of the larvae. McLaren (6) has suggested that the cells surrounding the inner body may become the larval intestine. Schacher (15) observed the development

of the larval stages and confirmed that the inner body disappeared when the microfilaria becomes the first-stage larva. He suggested that the inner body is a kind of reservoir for nutritive materials.

Concerning sensory organs, the cephalic papillae, the amphid and the phasmid were observed. Eight papillae, four on the dorsal and four on the ventral side of the head tip, have a short cilium-like structure. These cephalic papillae were not clear in the surface feature with a scanning electron-microscope (Suguri, unpublished). McLaren (6) has suggested that the cilia within the cephalic papillae do not open to the exterior but still function as a mechanoreceptor. Microtubules in the cilium-like structure found in the amphidial and the phasmidial channels did not conform to any fixed pattern, though Kozek (1) has reported five types in them; 1+11+4, 1+9+6, 10+5, 9+double+3 and 9+3 patterns.

The present study has shown that the amphidial channel connects to the nerve system at the basal part, implying a sensory function. Hawking (16) suggested a chemoreceptive function for these cilium-like structures because of their direct exposure to the external environment.

Using a light microscope Taylor (17) and Williams (18) reported that the nerve ring was composed of diamond-shaped cells. Ultrastructural studies by O'Leary *et al.* (19) indicated that the nerve ring is composed of extensions from at least three cells in *Dirofilaria immitis*, but the origin and the number of the cell somata could not be determined in the present study. Concerning the neurosecretory granules, O'Leary *et al.* (19) described two types, small (410–560 Å in diameter) and large (700–1,100 Å in diameter), both having cores. In the present species, the small granules are 350 Å in diameter and the large, 500–800 Å, both having no core but being composed of fine granules.

The excretory apparatus is composed of three portions as in other microfilariae. The excretory cells have many characteristic ribosomes. In the pore cavity fine granules were observed as excretory materials from the vesicle. The diameter of the cytoplasmic process is thinner (300 Å) than that of the anal vesicle. Taylor (17) showed by the method of vital staining that the supposedly excretory apparatus undergoes in fact an excretory function. Kanagasuntheram *et al.* (8, 20) have put forward the rather unusual suggestion that the structures of what have been called the excretory complex and the rectal cells are indeed nerve cells because of their having pale nucleus, rough endoplasmic reticula, and long cytoplasmic processes. The author is not fully in accord with their claim, however, since (a) the apparatus is provided with a pore where supposedly excretory materials are present, (b) the bridge of the excretory cell does not resemble the nerve fiber and (c) it is difficult to assume, as they did, that the cytoplasmic processes are analogous to the outer segments of rods or cones in

the vertebrate retinae. Developmental analyses will give an answer to this controversy.

Light microscopic study by Schacher (15) showed that the G cell divides and its daughter cell nuclei become very large when the microfilaria changes to the first-stage larva, but he could not establish what form the G cell changes into in the third-stage larva or in the adult worm. The characterirtics of the G cell revealed by the present study are the chromatin granules aligning vertically in the nucleus and nucleus being less electron-dense and larger than the surrounding cells.

Studying the larval development with light microscope Schacher (15) showed that R2-4 cells from the rectum in the larva. Ultrastructural studies on larval development by McLaren (6) showed that R2-4 cells elongate their cytoplasmic bridges to the anal vesicle. In the present study, the characteristic of R2-4 cells is that they have less electron-dense nuclei like the G cell. Laurence & Simpson (10) reported that the microfilaria of *Brugia pahangi* has no anal pore, but in the present study, a distict anal pore has been observed.

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REFERENCES

1. Kozek, W. J.: Unusual cilia in the microfilaria of *Dirofilaria immitis*. *J. Parasitol.* **54**, 838-844, 1968.
2. McLaren, D. J.: Ciliary structures in the microfilaria of *Loa loa*. *Trans. R. Soc. Trop. Med. Hyg.* **63**, 290-291, 1969.
3. Johnson, K. H. and Bemrick, W. J.: *Dirofilaria immitis* microfilariae: electron microscopic examination of cuticle and muscle cells. *Am. J. Vet. Res.* **30**, 1443-1450, 1969.
4. Johnston, M. R. L. and Stehbens, W. E.: Ultrastructural studies on the microfilaria of *Cardianema* sp. Alicata, 1933 (Nematoda; Onchocercidae). *Int. J. Parasitol.* **3**, 243-250, 1973.
5. Kozek, W. J.: Ultrastructure of the microfilaria of *Dirofilaria immitis*. *J. Parasitol.* **57**, 1052-1067, 1971.
6. McLaren, D. J.: Ultrastructural studies on microfilariae (Nematoda: Filarioidea). *Parasitology* **65**, 317-332, 1972.
7. Tongu, Y.: Ultrastructural studies on the microfilaria of *Brugia malayi*. *Acta Med. Okayama* **28**, 219-242, 1974.
8. Kanagasuntheram, R., Singh, M., Ho, B. C. and Chan, H. L.: Some ultrastructural observations on the microfilaria of *Breinlia sergenti*—the excretory complex, rectal cells and anal vesicle. *Int. J. Parasitol.* **4**, 7-15, 1974.
9. Singh, M., Kanagasuntheram, R., Ho, B. C., Yap, E. H. and Chan, H. L.: Some ultrastructural observations on the microfilaria of *Breinlia sergenti*—the pharyngeal thread and innenkorper. *Int. J. Parasitol.* **4**, 375-382, 1974.
10. Laurence, B. R. and Simpson, M. G.: The ultrastructure of the microfilaria of *Brugia*, Nematoda: Filarioidea. *Int. J. Parasitol.* 4, 523-536, 1974.

11. Bird, A.F.: *The structure of Nematodes.* Academic press, New York and London, pp. 45-77, 1971.
12. Laurence, B.R. and Simpson, M.G.: Cephalic and pharyngeal structures in microfilariae revealed by staining. *J. Helminthol.* **42**, 309-330, 1968.
13. Aoki, Y. and Katamine, D.: Scanning electron microscopic observations on *Dirofilaria immitis. Nettai Igaku* **17**, 27-34, 1975 (in Japanese).
14. Aoki, Y. and Ash, L.R.: Scanning electron microscopic study on adult worm of *Brugia pahangi. Joint Conference on Parasitic Disease, Japan-U.S. Coop. Med. Sci. Prog.* Nagasaki, pp. 5, 1974.
15. Schacher, J.F.: Morphology of the microfilaria of *Burgia pahangi* and of the larval stages in the mosquito. *J. Parasitol.* **48**, 679-692.
16. Hawking, F.: The 24-hour periodicity of microfilariae: biological mechanism responsible for its production and control. *Proc. Roy. Soc.* **169**, 59-76, 1967.
17. Taylor, A.E.R.: Studies on the microfilaria of *Loa loa, Wuchereria bancrofti, Brugia malayi, Dirofilaria immitis, D. repens* and *D. aethiops. J. Helminthol.* **34**, 13-26, 1960.
18. Williams, P.: Studies on Ethiopian *Chrysops* as possible vectors of Loiasis. I. Chrysops silacea Austen and Human Loiasis. *Ann. Trop. Med. Parasitol.* **54**, 439-459, 1960.
19. O'Leary, T.P., Bemrick, W.J. and Johnson, K.H.: Serial section analysis of cells in the microfilariae of *Dirofilaria immitis* containing neurosecretory-like granules. *J. Parasitol.* **59**, 701-705, 1973.
20. Kanagasuntheram, R., Singh, M., Ho, B.C., Yap, E.H. and Chan, H.L.: Some ultrastructural observations on the microfilaria of *Breinlia sergenti*—nervous system. *Int. J. Parasitol.* **4**, 489-495, 1974.

Legends to Figures

Fig. 1. Longitudinal section of the sheath. The sheath (Sh) is composed of small granules. The outer surface is covered with electron-dense particles (arrow), and the inner surface has small granules attached (broken arrow). ×88,000

Fig. 2. Longitudinal section of the cuticle. The cuticle is composed of outermost layer (OL), trilaminate membrane (TM) and inner layer (IL). The outermost layer and the outer memrbane of the trilaminate membrane are discontinuous at the deepest part of the transverse striations (arrow). The inner layer may be divided into outer less electron-dense (OLL) and inner dense (IDL) zones. M; muscle. ×86,000

Fig. 3. Longitudinal section of dense bodies (DB). The dense body is situated between the hypodermis (H) and the muscle (M). Cu; cuticle, Sh; sheath. ×56,000

Fig. 4. Transverse section at the lateral chord part. The hypodermal cell at the center connects with its neighboring cell by a desmosome (D). Cu; cuticle, NH; nucleus of hypodermal cell, M; muscle. ×81,000

Fig. 5. Transverse section of the muscle. A thick filament (TkF) is surrounded by 8 to 12 thin filaments (TnF). LS; lamellate structure. ×128,000

Fig. 6. Longitudinal section at the base of the amphid (Am). The amphid connects to the nerve elements (Ne) which contains neurosecretory granules (arrow). MT; microtubule. ×42,600

Fig. 7. Longitudinal section of the cephalic space. The cephalic space (CS) is the part anterior to the anteriormost nucleus (NM1). The muscle cell (M1) stretches into the anterior tip from NM1. Am; amphid, Mi; mitochondria, M; muscle, GG; glycogen granule, DB; dense body, CP; contractile portion, NP; noncontractile portion. ×15,000

Fig. 8. Transverse section of the anterior tip. The buccal cavity (BC) is situated at the center which is lined with cuticular layer. Four cephalic papillae (CP) are seen at each

of the dorsal and ventral sides. Two large muscles (M) are seen. The openings of the amphids (Am) are flattened. ×35,900

Fig. 9. Transverse section a little posterior to that of figure 8. Four muscles (M) are seen. The amphidial channels (Am) become round in shape. Longitudinal nerves (LNe) can be seen at the dorsal and ventral parts. CC; central canal. ×29,400

Fig. 10. Transverse section at the basal part of the amphid (Am). The amphid contains cilium-like structures having microtubules. Eight muscle cells (M) can be seen at the periphery. CC; central canal, Mi; mitochondria, GG; glycogen granule, LNe; longitudinal nerve. ×29,000

Fig. 11. Semi-transverse section at a part between the cephalic space and the nerve ring. The nucleus (N) of the cell in the nuclei column is electron-dense and squarish in shape. M; muscle, GG; glycogen granule. ×16,400

Fig. 12. Longitudinal section at the nerve ring. The nerve process contains two types of secretory granules. LG; large neurosecretory granule, SG; small neurosecretory granule, M; muscle. ×22,800

Fig. 13. Transverse section at a part between the nerve ring and the inner body. The central canal runs at an inner part. Two muscle cells are situated on each of the dorsal and the ventral sides. Between these muscle cells the longitudinal nerve (LNe) runs. Two desmosomes (D) can be seen at each lateral side. NH; nucleus of hypodermal cell, M; muscle, H; hypodermis. ×24,000

Fig. 14. Transverse section of the central canal. The central canal (CC) is lined with a cuticular layer and contains granular materials. Around the duct, many desmosomes (D) can be seen. ×66,000

Fig. 15. Transverse section of the lamellate structure (LS). The structure can be seen in a muscle cell. ×64,800

Fig. 16. Longitudinal section of the excretory apparatus. The excretory vesicle (EV) opens to the ventral side and connects to the excretory cell (EC) *via* a cytoplasmic bridge (CB). The excretory cell has many free ribosomes (R). M; muscle, Mi; mitochondria. ×16,000

Fig. 17. Longitudinal section at the excretory vesicle. The vesicle contains fine granules. At the basal part of the vesicle, many cytoplasmic processes (CP) can be seen. Around the opening (EP) the cuticle becomes thick and the trilaminate membrane continues to the inner part of the vesicle. CC; central canal, Mi; mitochondria, GG; glycogen granule. ×31,000

Fig. 18. Longitudinal section at the joint of the central canal (CC) with the inner body (IB). A sponge-like structure (SS) is found at the jointing part and a desmosome is situated around the joint. The contents of the central canal and the inner body are similar. ×36,000

Fig. 19. Transverse section at the anterior tip of the inner body (IB). A sponge-like structure (SS) can be seen at the center. The inner body is surrounded by a thin membrane (arrow). D; desmosome ×39,600

Fig. 20. Transverse section of the inner body. The inner body (IB) is composed of fine granules and bounded with a membrane (arrow). M; muscle, LNe; longitudinal nerve. ×23,500

Fig. 21. Longitudinal section at a middle part of the microfilaria. The cytoplasm of the inner body cell is very narrow (arrow). NM; nucleus of muscle cell, NI; nucleus of inner body cell. M; muscle, Mi; mitochondria. ×18,600

Fig. 22. Longitudinal section of the G cell. The nucleus (NG) is a homogeneous structure and chromatin granules (G) are observed as a vertical band. M; muscle, R; ribosome. ×18,000

Fig. 23. Longitudinal section of R2 cell. The nucleus of the R cell (NR) has the same

electron-density as that of the G cell. The cytoplasm is filled with many free ribosomes (R). ×27,300

Fig. 24. Longitudinal section of R4 cell. The cytoplasm is filled with many free ribosomes (R). NR; nucleus of the R cell, M; muscle. ×29,400

Fig. 25. Longitudinal section at the anal vesicle. The cytoplasmic processes (CP) are divided into two parts. Between these parts, a desmosome (D) can be seen. Mi; mitochondria. ×18,600

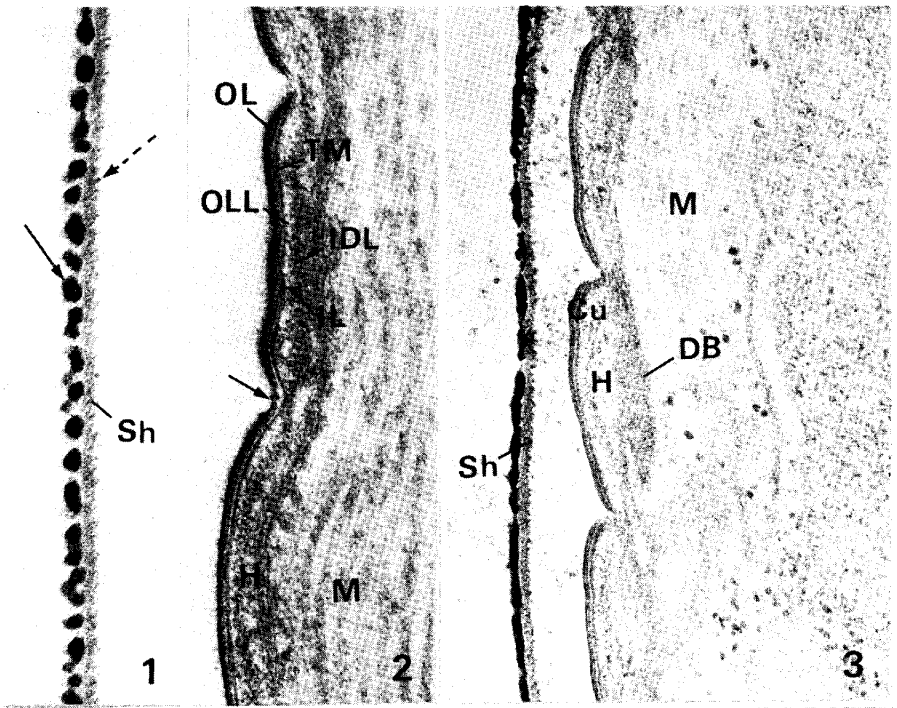
Fig. 26. Longitudinal section of the anal pore. Around the opening (AP) the cuticle becomes thick and the trilaminate membrane continues to the inner part. ×19,600

Fig. 27. Transverse section at the anal vesicle. At the base of the vesicle, cytoplasmic processes (CP) can be seen. The ventral longitudinal nerve (LNe) runs at both sides of the vesicle. M; muscle. ×29,000

Fig. 28. Transverse section at the basal part of the phasmid (Ph). Ne; nerve M; muscle. ×39,000

Fig. 29. Longitudinal section at the opening of the phasmid. A plug connecting with the cilium-like structure can be seen at the opening. ×19,000

Fig. 30. Transverse section of the phasmid (Ph). Each phasmid has a cilium-like structure whose microtuble pattern is not regular. LS: lamellate structure, M; muscle. ×39,800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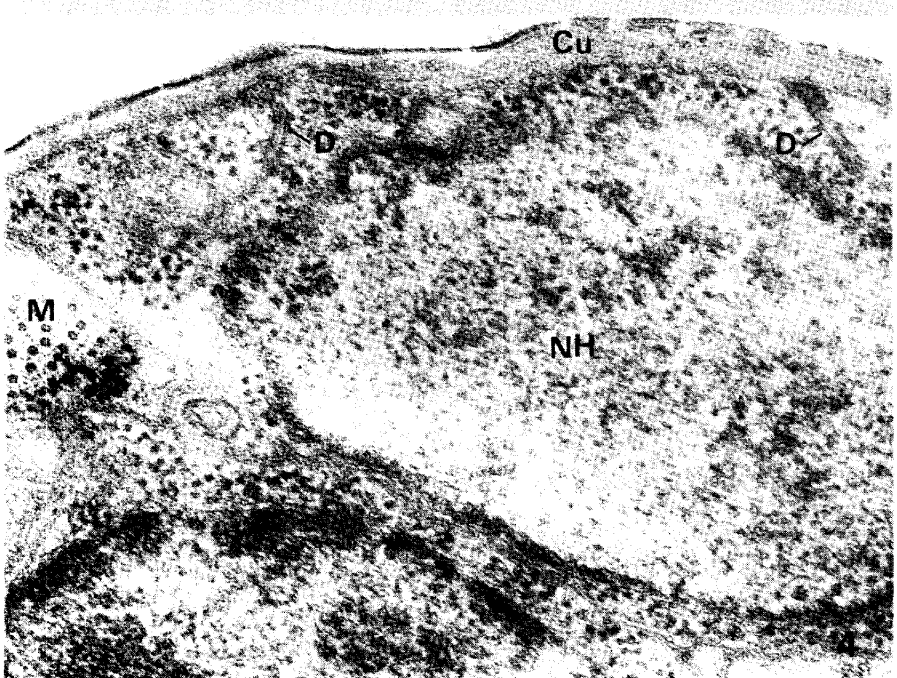
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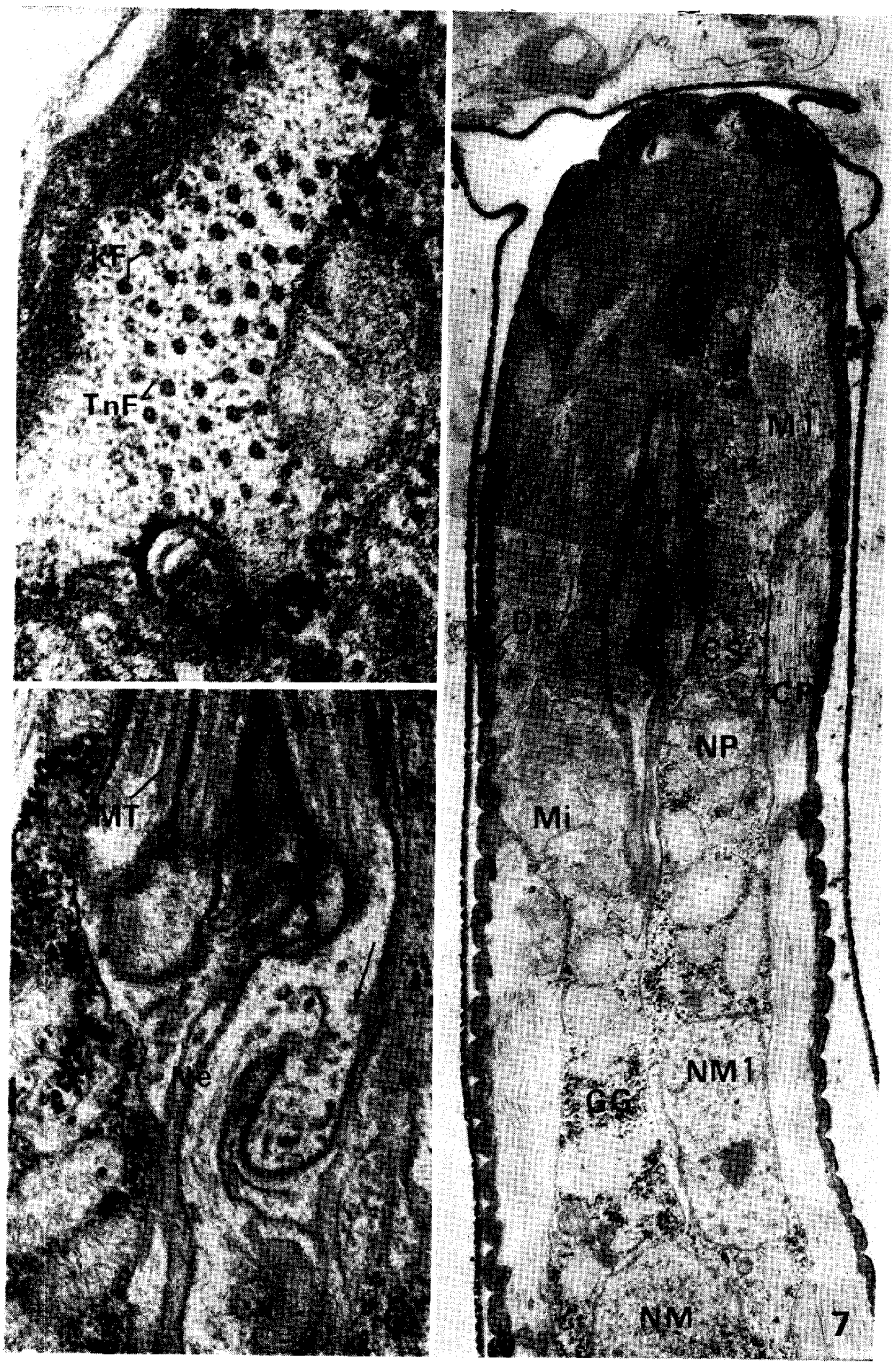
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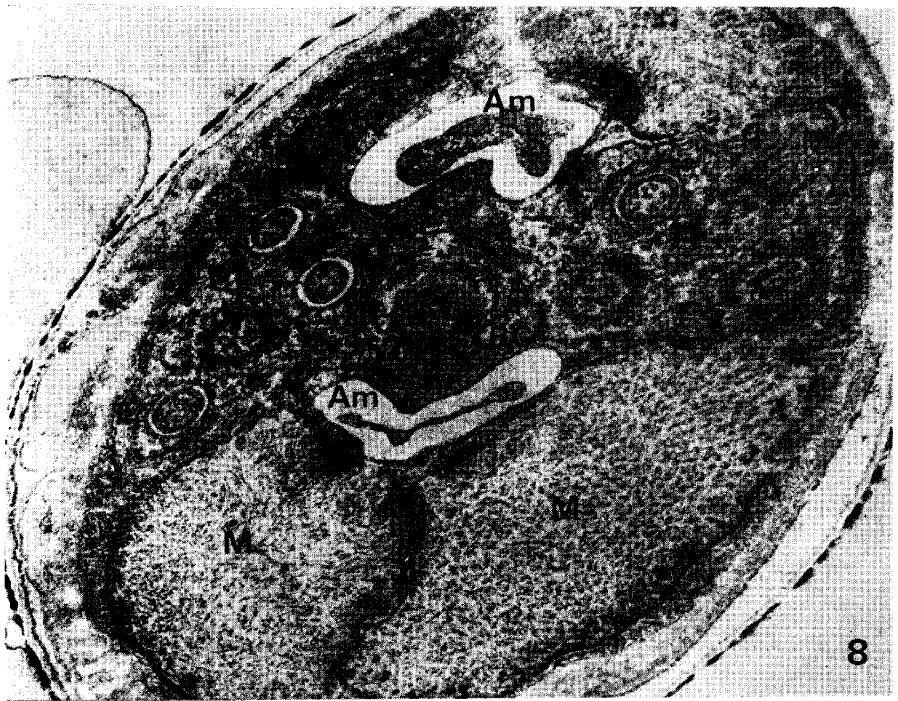
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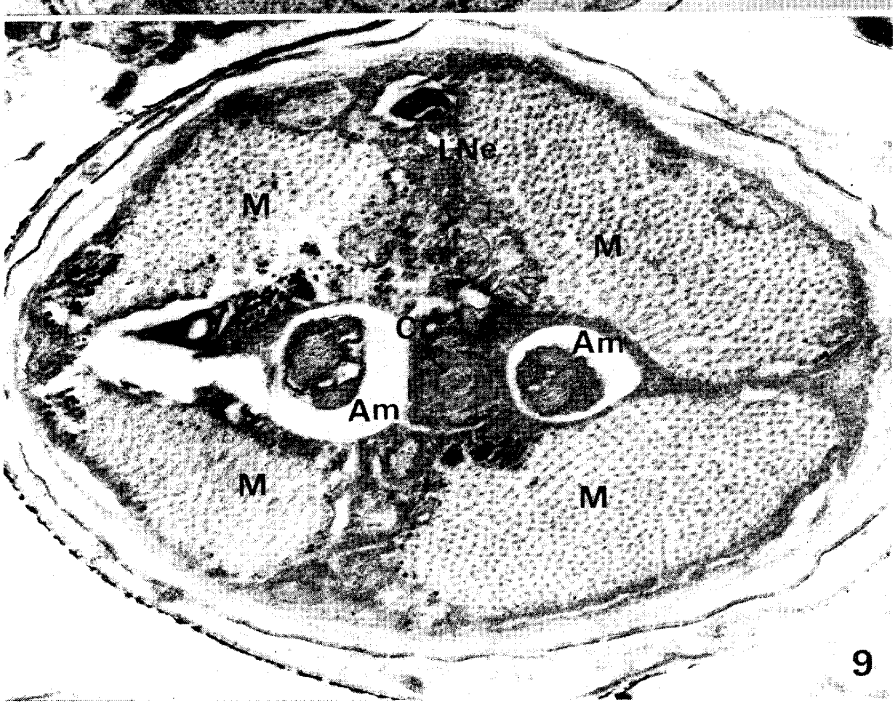
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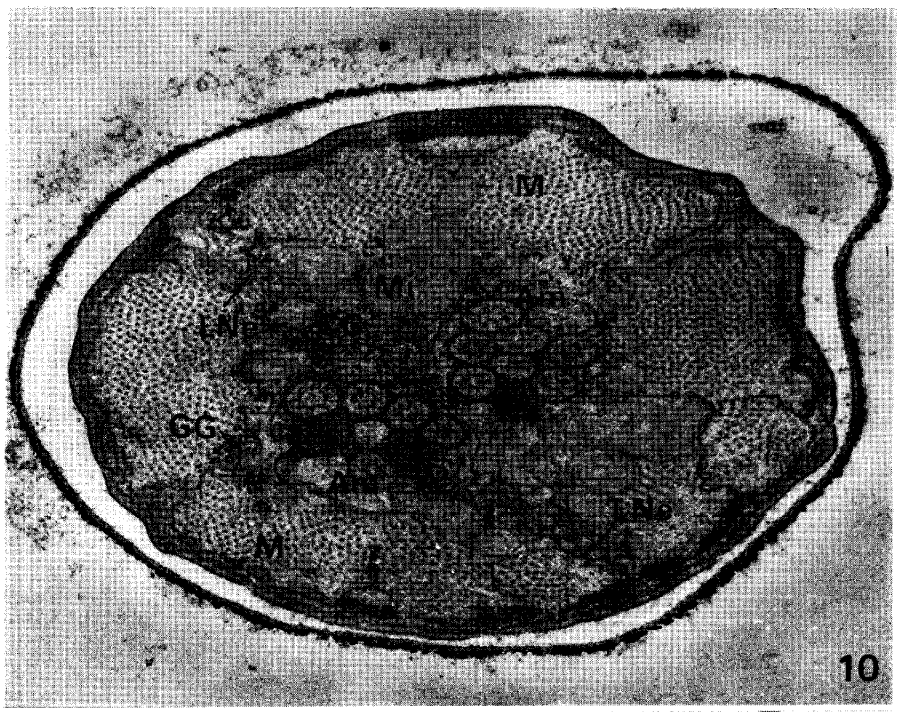
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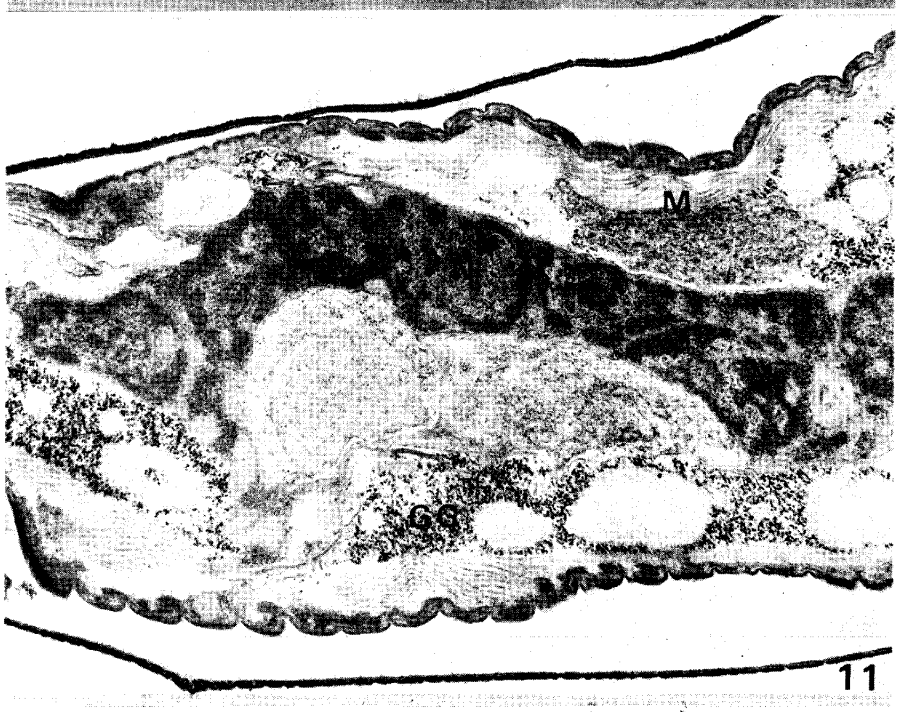
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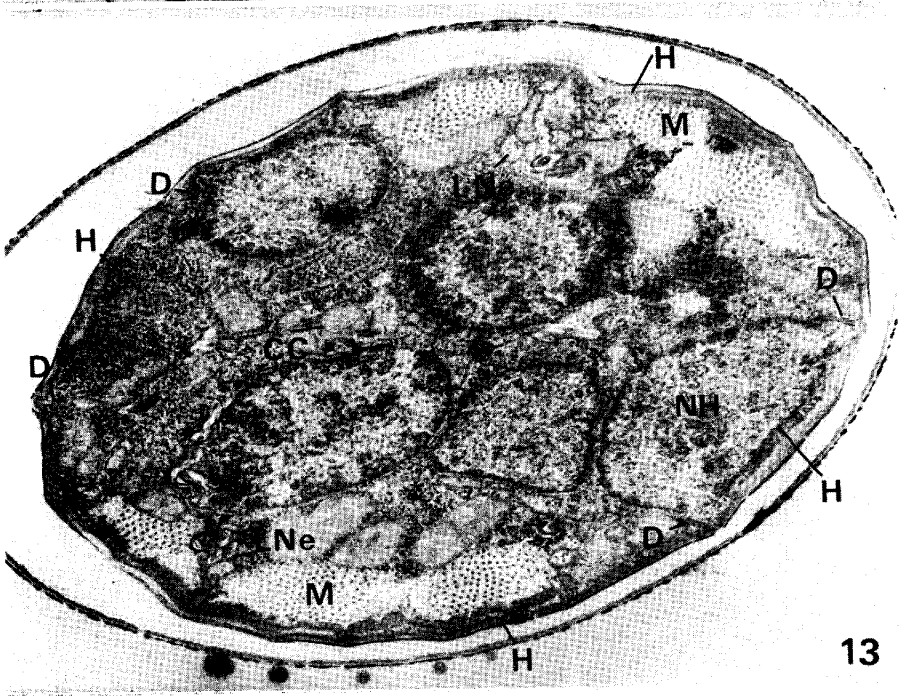
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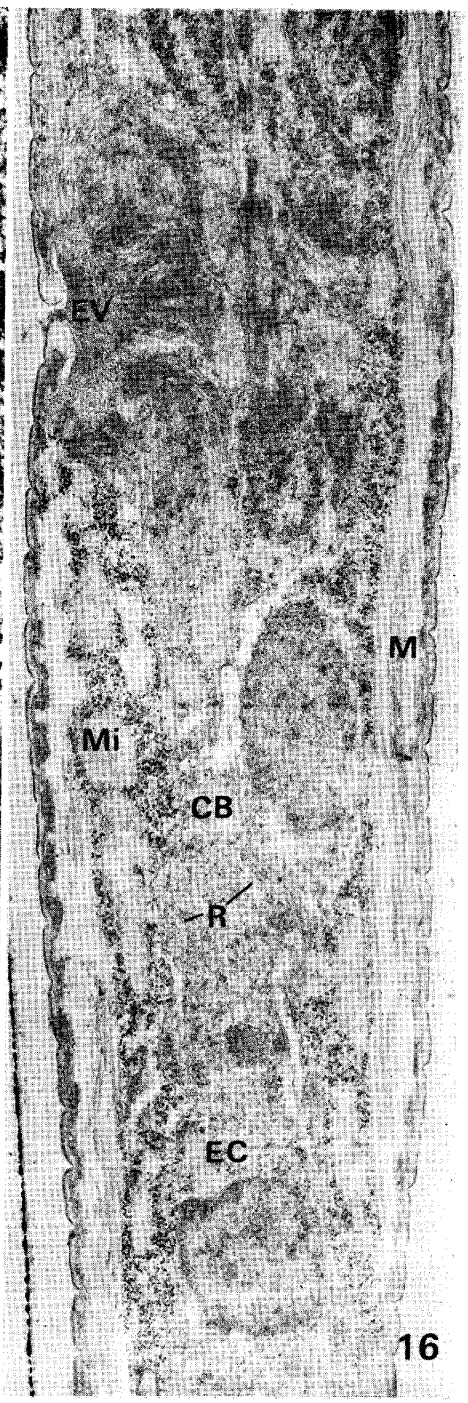
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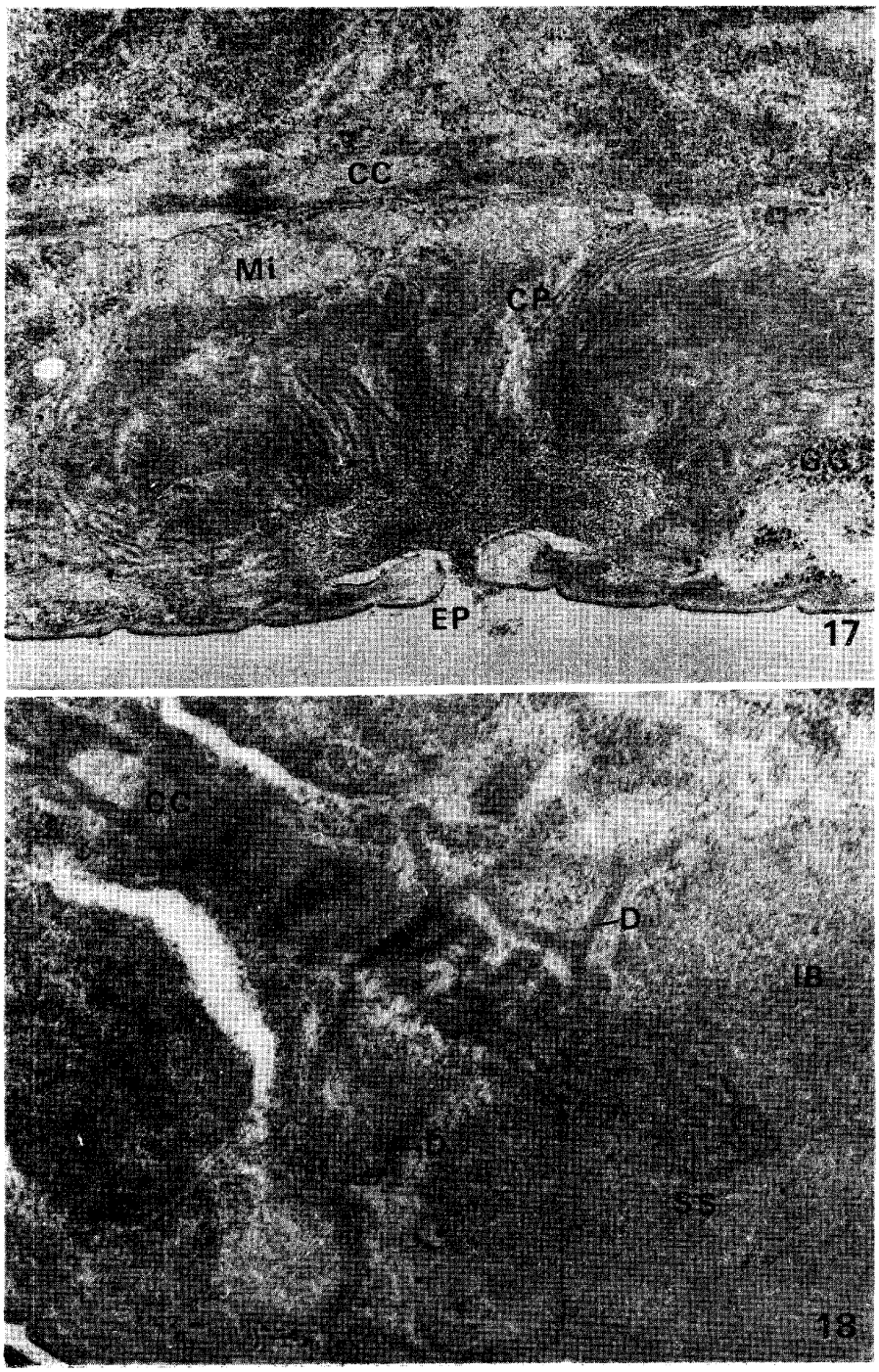
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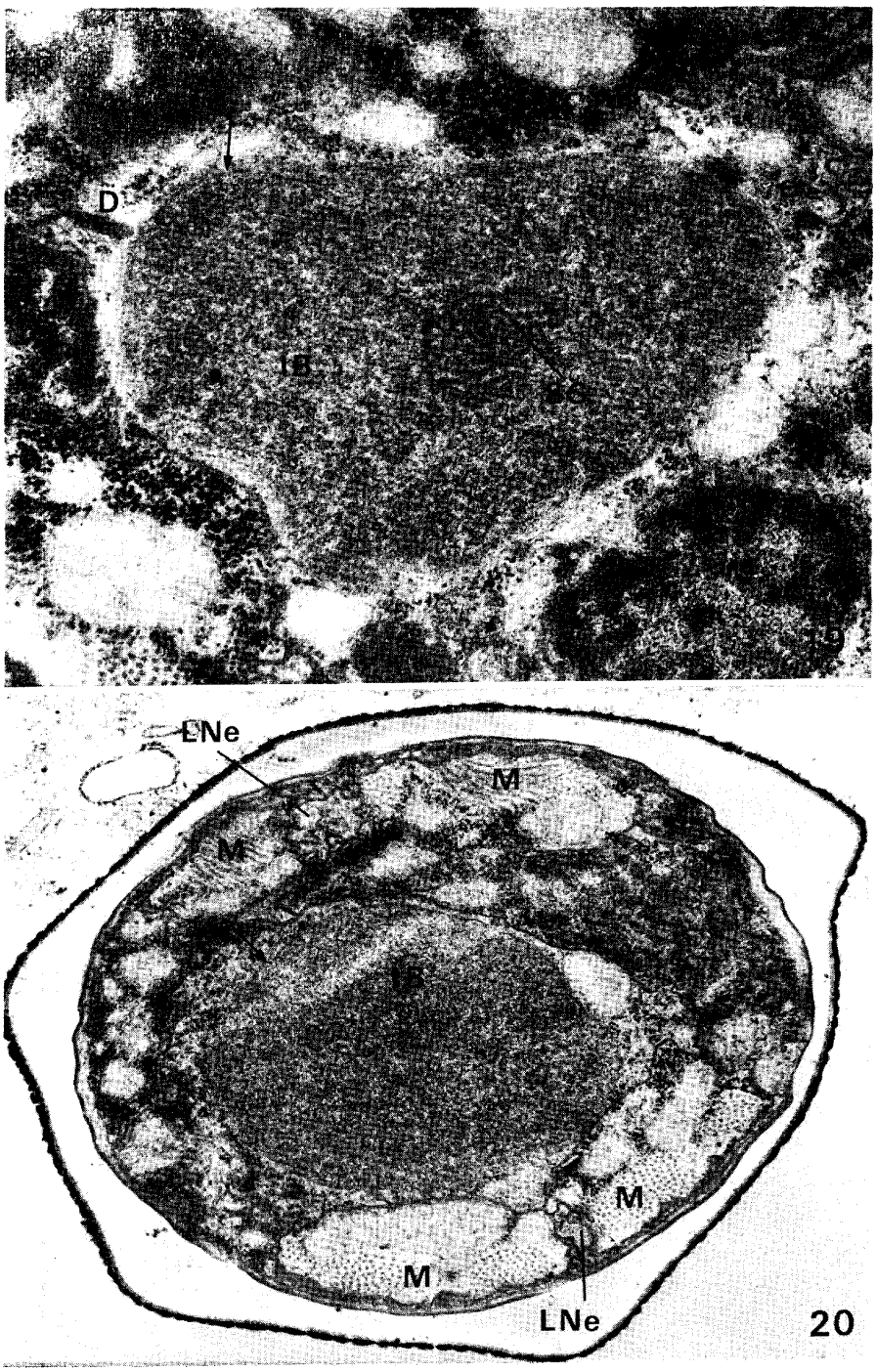
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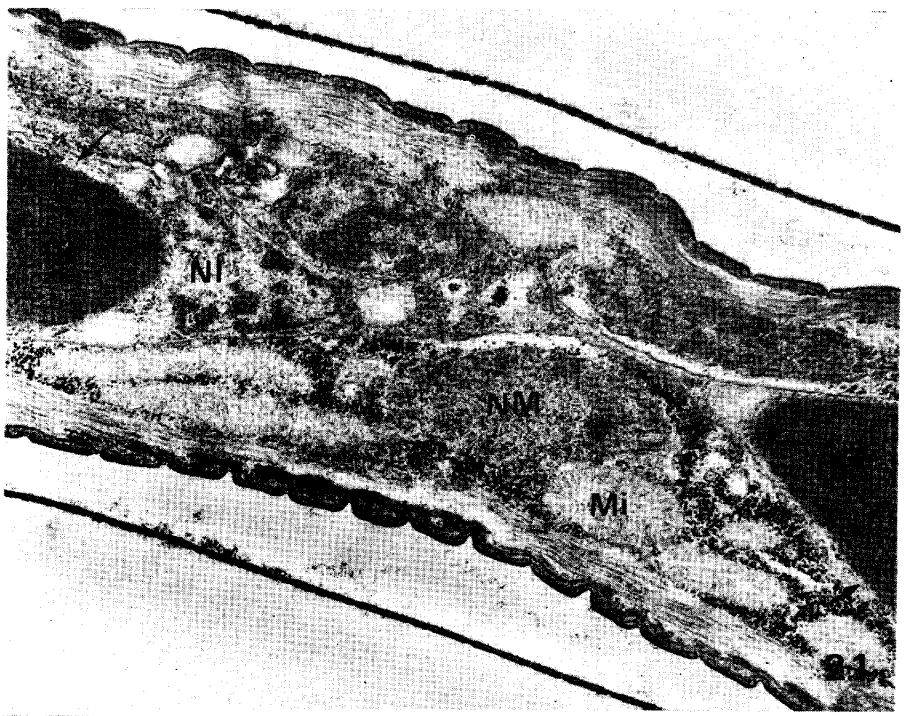
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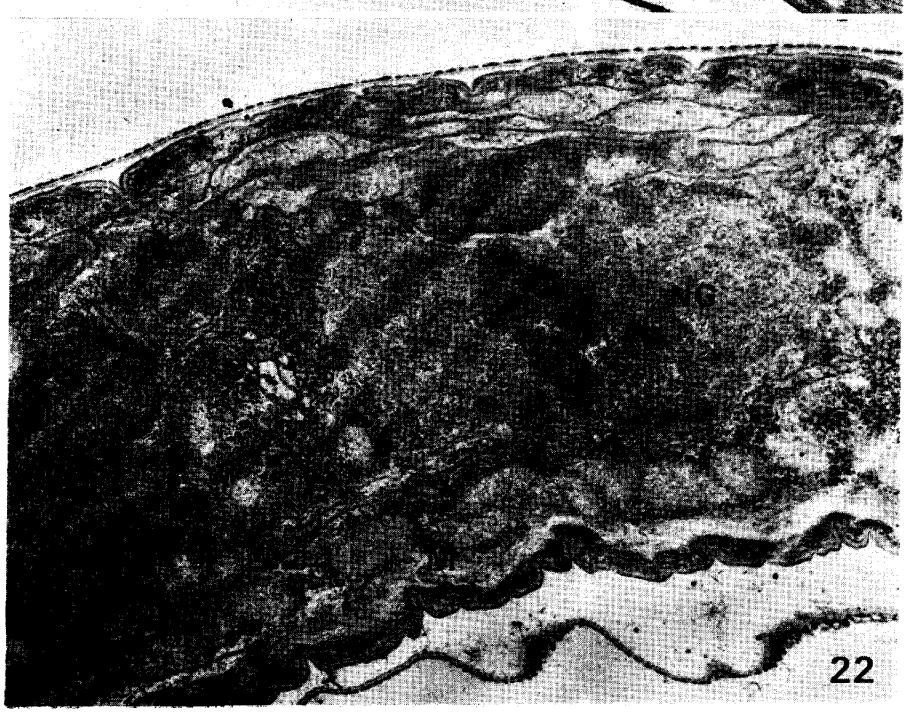
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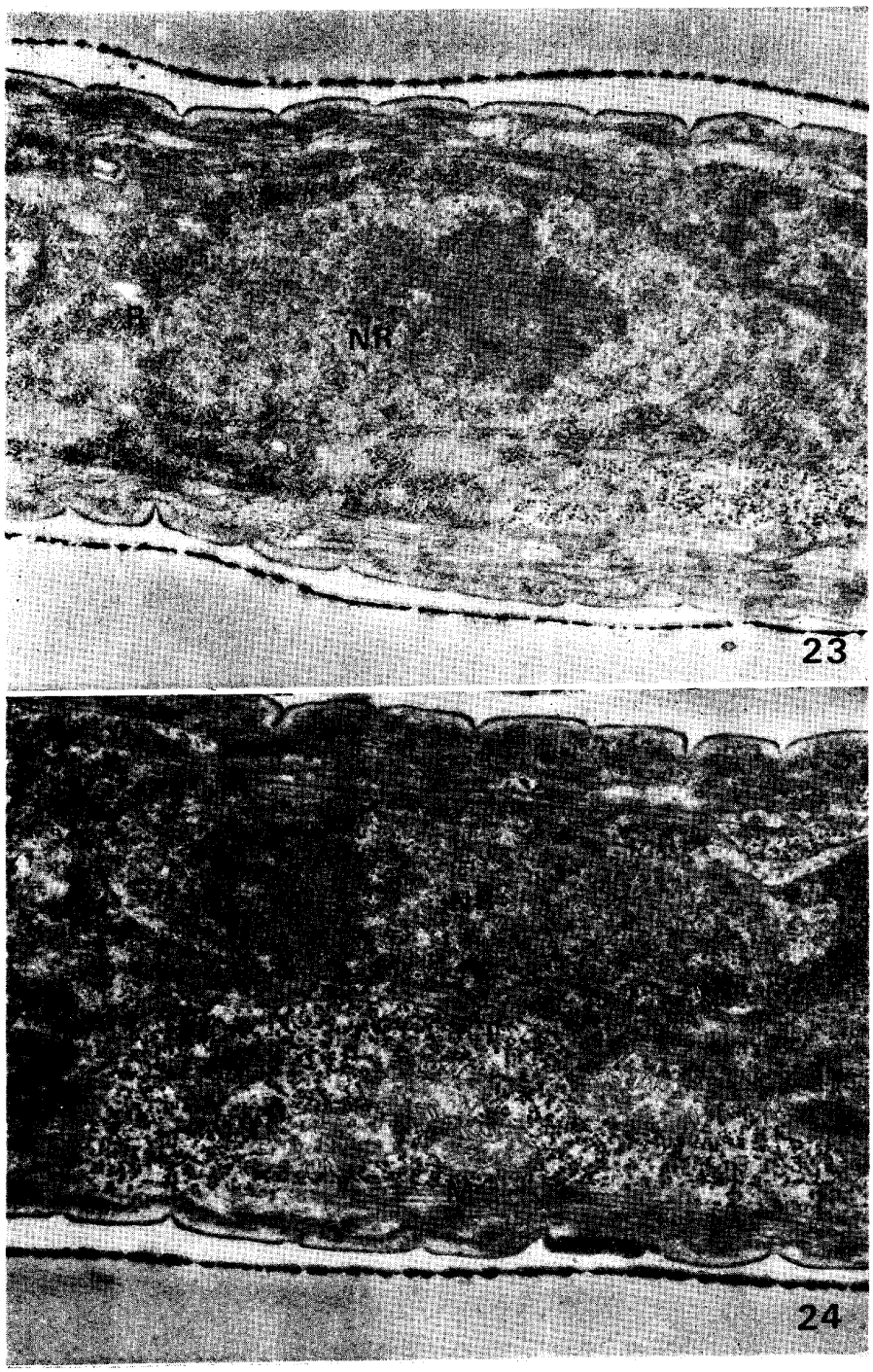
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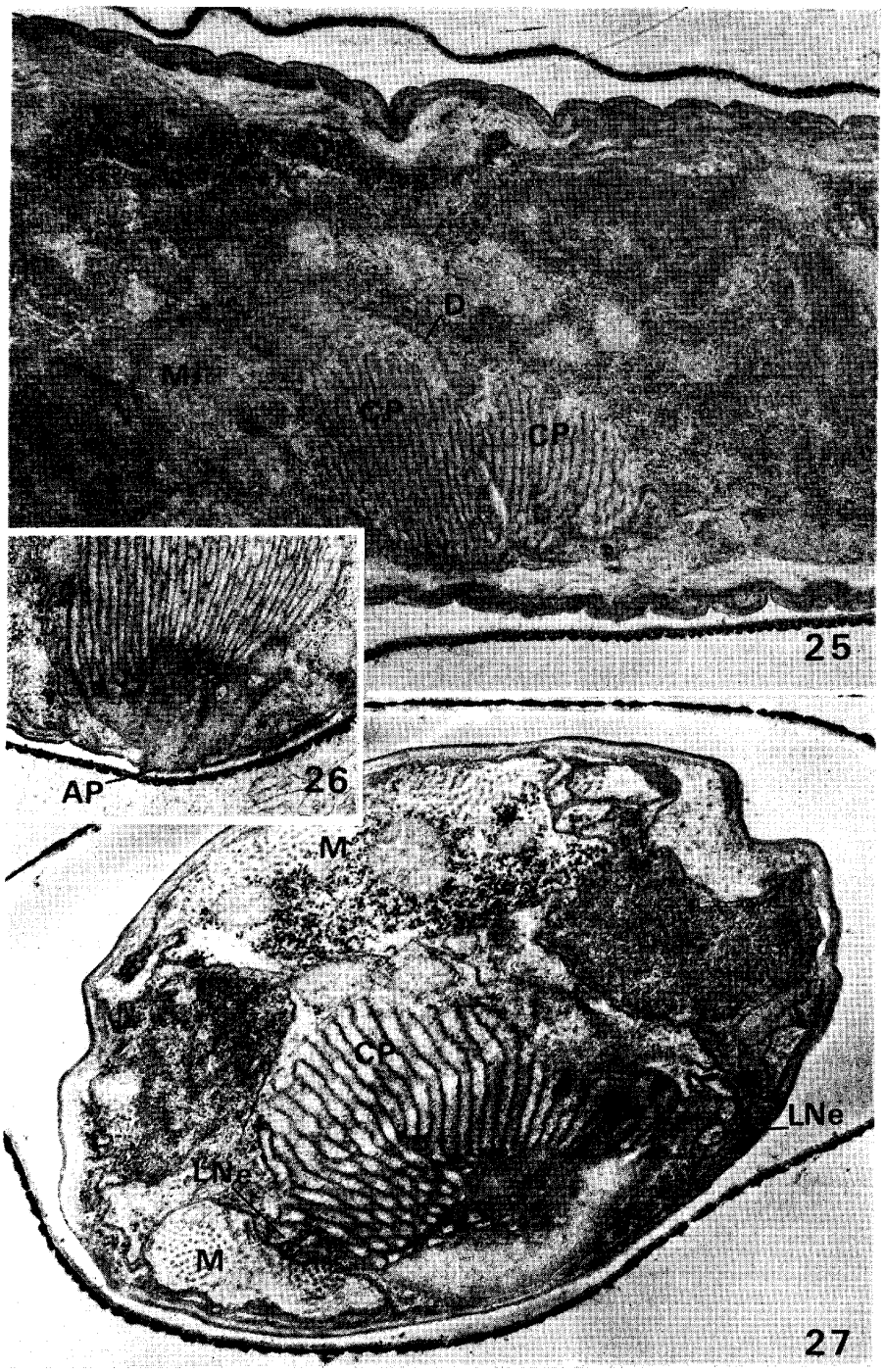
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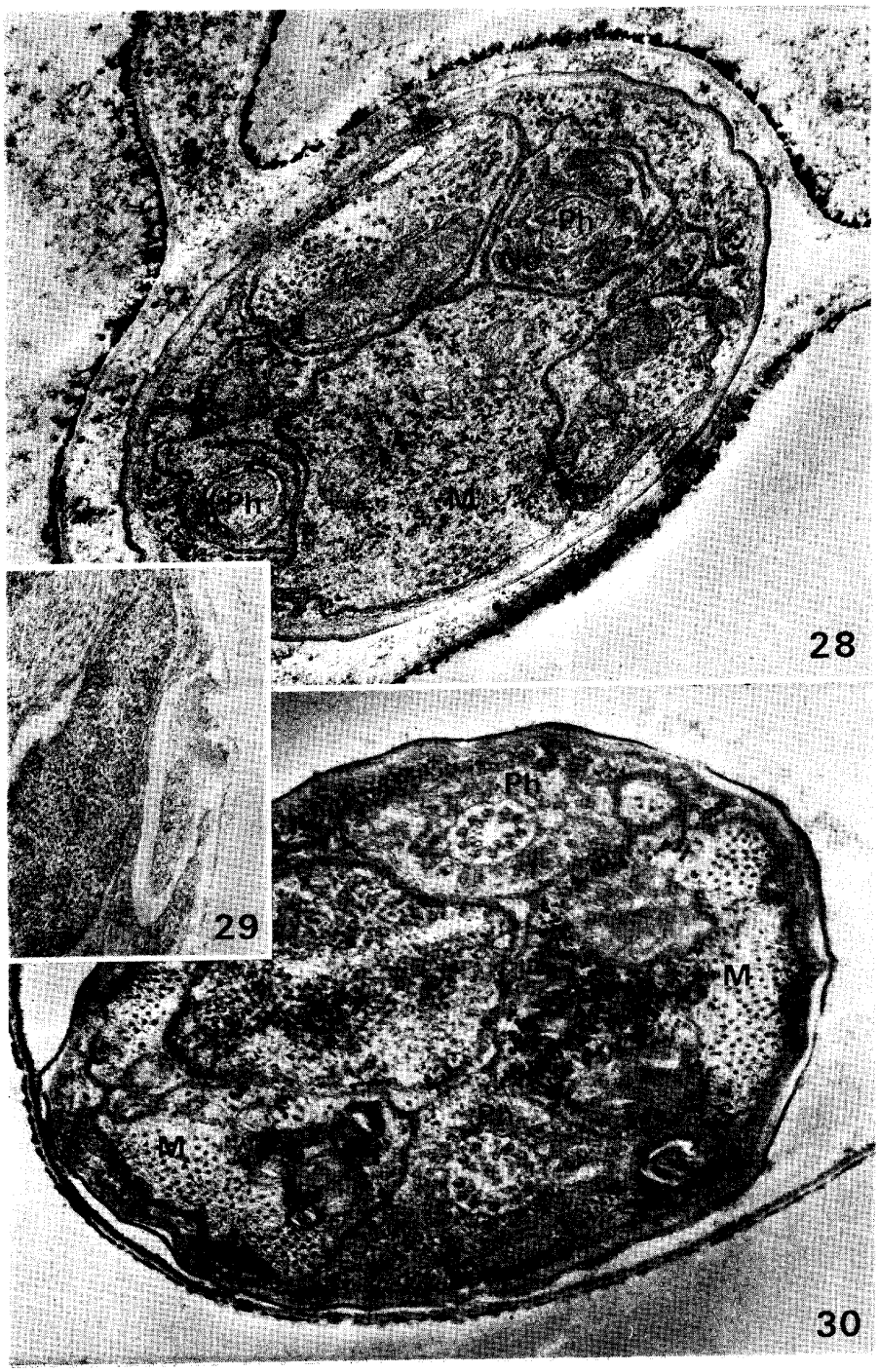
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