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ULTRASTRUCTURAL STUDIES ON THE MICROFILARIA OF *BRUGIA MALAYI*

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Abstract: Ultrastructure of microfilaria *Brugia malayi* was investigated with electron microscope. Microfilariae are covered by a sheath membrane with dense materials on its outer surfaces. The cuticle consists of 3 layers; namely, external cortical, internal cortical and fibrous layer. Beneath these cuticular layers, thin hypodermis is present and the muscle cells are arranged of 4 groups in a cross-section except for the head and tail. A pair of cephalic channel containing several cilial rods opens at the anterior end of the worm. A hook is situated on the anterior edge of one channel orifice, and several spines grow on the opposite side to the hook. Caudal channels paired laterally opening into the both sides of the posterior region differ from cephalic channels by the presence of a single cilial rod. A central canal runs from the buccal cavity to the inner body, and opens into the inner body cell through the filamentous apparatus. The inner body appears to consist of several cells having storage substances and a flat nucleus located on the periphery of the cell. An excretory apparatus, *i.e.*, a cell, is composed of a nucleus and a large vesicle which has many microprojections on the luminal surfaces. The G1 cell which occupies the whole width in a cross-section is larger than the R cell. R2-R4 cells appear to be in a close contact with the anal apparatus having many microprojections on the luminal surfaces. These microprojections differ from those of the excretory vesicle in their thickness and length. The characteristic patterns of these organs are compared with other microfilariae.

The morphological, developmental, and histochemical characteristics on filarial worms have been described by numerous authors with light microscope, notably by FENG (1), SCOTT *et al.* (2), TAYLOR (3), SCHACHER (4, 5), SAWYER *et al.* (6), LAURENCE *et al.* (7, 8, 9) and SIMPSON *et al.* (10).

However, little is known about the morphological features in microfilaria. Recently an interest has been aroused on the fine structures of microfilaria, especially on the relationships between structure and function.

Several brief notes concerning the ultrastructure of microfilaria have been published by ARMSTORONG *et al.* (11), CHATTERJEE *et al.* (12), HOCKLEY *et al.* (13) and MCLAREN (14). Further observations on filarial worms have been carried electron microscopically; SONODA *et al.* (15) on external structure

of microfilaria, LEE *et al.* (16) on morphology of adult worms, SCHARDEIN *et al.* (17) on microfilaria treated with diethylcarbamazine, BECKETT *et al.* (18) on larvae in mosquito, COLLIN (19) on morphology of infective larvae, HO *et al.* (20) on mode of nutrition of the infective larvae, and JOHNSON *et al.* (21), KOZECK (22), MCLAREN (14, 23), JOHNSTON *et al.* (24) on morphology of microfilariae. However, there can be had only a few brief reports on the fine structures of microfilaria *Brugia mallayi*. The present report describes further observations on the ultrastructure of *Brugia malayi*.

MATERIALS AND METHODS

Microfilariae were obtained from the blood of patients infected by filariasis at Cheju island in Korea.

The specimens for light microscope were used to have an orientation in electron micrograph. Blood films containing microfilariae were allowed to dry at room temperature for at least several hours. Prior to fixation, the blood films were dehaemoglobinized by running tap water for 10-30 min, fixed in methanol, washed well in running water, and stained with Delafield haematoxiline or Giemsa's solution.

For the electron microscope, erythrocytes in the blood with microfilariae were lysed by adding two parts of distilled water to one part of blood for 5-10 min. Other specimens were directly fixed in glutaraldehyde solution without haemolysis.

Microfilariae were collected in sedimentation form after 5 min centrifugations at 2000 r. p. m. The sediments were fixed in 2.5% phosphate buffered glutaraldehyde solution (pH 7.4) for 30 min. The sediments were rinsed three times in phosphate buffer for 30 min, fixed for one hr in 1% osmium tetroxide buffered with phosphate at pH 7.4, and dehydrated in series of ethanol by routine methods. The sediments were interspersed in stylene methacrylate and epoxy resin mixture according to KUSHIDA (25, 26) after passing through N-butyle glycidyl ether, and embedded in flat plates. Individual worms were oriented vertically or tangentially, and sectioned serially for the most part.

Thin sections were cut with either a Porter-Blum or a Reichert ultramicrotome, collected on 100-mesh copper grids, and double-stained with uranyl acetate and lead citrate. The sections were examined with an electron microscope (Hitachi HS 8) at an accelerating voltage of 50 KV.

RESULTS

Sheath: The sheath of microfilaria *Brugia malayi* consists of a thin membranous layer, *i. e.* sheath membrane (Figs. 3, 8, SM), and dense granular materials (Figs. 3, 4, 7, DM) on its outer surface. This membrane has homogenous inner structure, uniform thickness of about 200 Å and is less dense than the external dense materials of outer surfaces. External dense materials

showing various thickness and grouval-like shape are distributed all over the surface of the sheath projecting beyond the head or tail. In some parts, the sheath membrane is not covered with these dense materials as in Fig. 3 (arrows). Almost all the specimens are surrounded by only dense thin granular materials on its outer surfaces, although they are sometimes covered by less dense and thicker fibrillar substances, *i. e.* outer coat (Fig. 4, OC). The space between body and sheath is often filled with less dense fluid materials (Figs. 7, 8). The fluid is occasionally aggregated along the inner margin of the sheath (Fig. 8, arrow). And there are a few cases containing no fluid materials.

Cuticle: The outer surface of the cuticle of microfilaria *B. malayi* has transverse striations, regularly spaced at intervals through tip to tail as in the case of other nematodes. The cuticular structure is clearly seen as external cortical (Fig. 7, EL), internal cortical (Fig. 7, IL), and fibrous layers (Fig. 7, FL) in longitudinal section. The outermost external cortical layer is most electron-dense and is composed of two electron-dense membranes of 45 Å thick which are separated by intervals of about 30 Å in distance. This layer is often discontinuous at the deepest transverse striations (Fig. 7, arrow). Below this outermost layer, the internal cortical layer being electron-dense and 90 Å in thickness is located. This layer is divided from the external cortical layer by a distance of approximately 30 Å. The innermost layer which is the thickest in cuticle is composed of less dense fibrous structure. Occasionally this fibrous layer is separated into two layers, *i. e.* the outer less dense and inner dense (Fig. 7). However, the basement membrane under the cuticle was not identified in the present observations.

Hypodermis and muscle cells: The hypodermis (Fig. 7, Hy) is situated beneath the fibrous layer of the cuticle, which is generally very narrow and is difficult to recognize in some part. But it bulges into the center of the worm at lateral cords as in the case of other nematodes. The organelles such as nucleus and mitochondria are not visible except the bulged part of the hypodermis. However, a dense amorphous body (Fig. 8, DB) is sometimes seen in each castellation of hypodermis. The muscle cells are elongated, spindle-shaped and lying along the long axis of the microfilariae. In transverse sections, the muscle cells are arranged in four groups (Fig. 15). Variations of this arrangement occur only in the head and tail. Muscle cells are not so well developed in the tail, and are located in two groups (Fig. 21). Two conspicuous groups of muscle cells are situated in the anterior end of the cephalic space (Figs. 9, 10, 11). Each cell is composed of a contractile and a noncontractile portion. The contractile portion adjacent to the hypodermis contains many thick and thin myofilaments (Fig. 5). In some sections

only thin filaments are localized in the contractile portion. The thick filaments measure approximately 190 Å in diameter, while the thin filaments about 50 Å. The arrangement of myofilaments shows an irregular pattern. Noncontractile porrtion, which has mitochondria, nuclei, a number of glycogen particles and other organelles, is located in the inner part of the cell.

External organs of anterior end: The cephalic space, which has no nucleus, occupies the anterior region of the body. There are a buccal cavity, openings of cephalic channels (Fig. 9, arrows), a hook (Fig. 10, H) and several spines (Figs. 11, 12, Sp). A small cylindrical buccal cavity is situated at the anterior end of the head and is lined with cuticle continuous with the body surface. The buccal cavity is connected to a long, narrow tube or central canal (Fig. 13, CC); other workers called it the pharyngeal thread. On one side of the body, there are two deep invaginations (Figs. 9, 10, 11) of the body surface. The anterior one is deeper than the posterior, and a cephalic channel opens in the bottom of the anterior one. The hook consisting of the dense materials is situated on the anterior edge of a deeper invagination backward to the long axis. It is approximately 0.8 μ long. Another spine which grows on the opposite portion near the anterior end is electron-dense and about 0.3 μ long. Details of the number of these spines are not fully clarified in the present report.

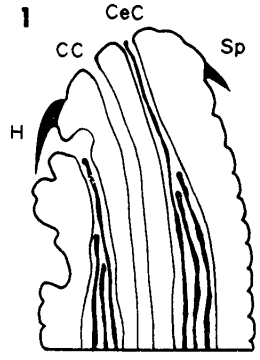

Fig. 1. Diagram showing a schematic longitudinal section of the head part. CC: central canal, CeC: cephalic channel, H: hook, Sp: spine

Central canal: A long cylindrical tube, *i. e.* the central canal (Figs. 17, 18, CC), which is lined by cuticle of about 500 Å thickness, runs through the center of the worm from anterior end to inner body diverging around the excretory pore. The cuticles differ from those of body surface consisting of three layers. The canal is connected to the buccal cavity, and opens into the anterior part of the inner body (Fig. 28). In this opening the canal adhesces to electron-dense fibers extending into the inner body (Fig. 28, arrow) and this filamentous structure is composed of an aggregation of the filaments. It does not extend over the entrance of canal. The lumen of this canal is often filled with less dense materials. Although the diameter of the canal is evenly 0.2 μ, it shows the maximum diameter in the area near the buccal cavity. The outside of the canal is surrounded by several small cells in the cross-section, and electron dense desmosomes are observed along the boundaries between these cells (Fig. 18, D). Details of the number and

shape of these cells are not identified in these present observations.

Nerve ring: The nerve ring is a space which appears to have no nucleus portion in light microscope. It is about 28 μ in length and occupies the whole width from end to end. In electron microscopic observations, this part is composed of tightly packed nerve fibers with mitochondria and microtubules (Fig. 16, NR). However, the nerve cells connected to the nerve fibers near the nerve ring were not observed in the present observations.

Cephalic channels: The cephalic channels are paired organs situated in the cephalic space. The one opens into the cuticle at the very tip of the worm. The other opens at the deeper invagination of the body. Two lateral channels originating in the posterior part of the cephalic space appear to have different lengths. The maximum diameter of each channel is approximately 0.8 μ in the base. And it is tapered distally. Tight junctions of nerve axons can often be recognized between the base of channels and nerve ring (Fig. 13, CeC). Each channel is usually filled with a plug (Fig. 9, P) consisting of dense materials at the outlet of the opening. The inner surface of the channel is covered with a thin membrane differing from body cuticle. The channel encloses a bandle of cilia-like structure, cilial rod (Figs. 9, 11, 13. 14, CR), which shows an irregular pattern of many microtubles. There are about ten or more cilial rods at the base of each channel. One to three rods reach the channel orifice and have usually unequal length. A cross-section of each cilial rod shows a dense material enclosing numerous microtubules. But there is no limiting membrane in outermost. In a cross-section of the head, several cilial rods are situated outside the channels (Fig. 12, CR). These cilial rods are probably other sensory organs.

Caudal channels: The caudal channels, consisting of a paired lateral organs, are located in the caudal region of the microfilaria (Figs. 20, 21, CaC); they are structurally similar to the cephalic channels except that each channel contains only one cilial rod. The channels surrounded by a membrane-like structure are covered with a thin cytoplasmic band in the cross-section (Fig. 21), and the granular materials scatter around the cilial rod. The maximum diameter of the channel is approximately 0.3 μ, and its length appears to be shorter than that of cephalic channel. The channel has an opening (Fig. 20, arrow) which is 0.1 μ in diameter. The body cuticle of the microfilaria forms a thickened rim around the opening of each channel as in the case of the opening of excretory pore. And the cuticle enters narrowly into the channel. In the opening, there is a dense material like a plug (Fig. 20, P). And a cilial rod is not located out of the opening. This rod surrounded by a limiting membrane is very electrone-dense and contains many microtubules which show an irregular pattern.

Excretory apparatus: The excretory apparatus (Fig. 19) consists of a large vesicle (Fig. 23, EV) which opens into the cuticle and a large excretory cell (Fig. 22, EC) which is connected to the vesicle by a cytoplasmic bridge. The vesicle usually flask-shaped with the maximum diameter of about 3 μ at its bottom. The diameter of excretory pore orifice is about 0.8 μ. Body cuticle covers the surface of pore entrance and extends into the upper half of vesicle (Fig. 26). This cuticle does not extend to the bottom of the vesicle. The vesicular lumen is filled with less dense fluid materials like those seen in the space between body and sheath. A plug (Fig. 23, P) which consists of dense fluid materials are often situated at the entrance. The limiting membrane in the base of the vesicle invaginates deeply to form long, fine microprojection, radially projecting into the lumen (Figs. 23, 26, MP). These projections have irregular shape, length and thickness. The mitochondria are located under the base of projections. The excretory apparatus with a cytoplasmic bridge between excretory vesicle and the cell is very large and long. A large nucleus is usually situated at the posterior part in the cell. The cell is characterized by well-developed rough-endoplasmic reticulum found even in the part of cytoplasmic bridge; it also contains mitochondria, Golgi bodies, free ribosomes and vesicles. The rough-surfaced endoplasmic reticulum often show a volute shape.

Inner body: The inner body seems to have a dense, elongated and amorphous structure situated between excretory cell and G1 cell (Fig. 24, IB); it is composed of several cells with a nucleus (Fig. 27, IB) and furthermore shows varying electron densities or shape without limiting membrane (Fig. 28). The number of inner body cell is not fully known in the present observations. A storage substances consisting of fine granular materials form the bulk of the inner body cells. Cytoplasm, nucleus and mitochondria are peripherally located. The lumen of central canal opens through the filamentous structures into the anterior part of inner body (Fig. 28).

G1 cell and R2-4 cells: G1 cell which is located in the posterior part of the inner body occupies nearly the whole width of the worm (Fig. 25, G1); therefore, the thin hypodermis is only located around the cell in cross-section. The cell measures about 8 μ in length and 4 μ in width. It contains a large nucleus, mitochondria, free ribosomes and Golgi bodies, whereas there cannot be seen the rough-endoplasmic reticulum as in the cytoplasm of other R cells. And this cytoplasm is less dense than that of other nuclear collum cells. R2-4 cells lie compact and near the anal vesicle. R4 cell appears to be connected to the anal apparatus (Fig. 29). R2 and R3 cells appear to join partially the anal apparatus. The relationship between R cells and anal vesicle are not distinct in these observations. R cells have a

large nucleus with a distinct nucleolus in the center, mitochondria, many rough-endoplasmic reticulum and sometimes several vacuoles.

Anal apparatus: The pore of anal vesicle is of a flask-shape and has an entrance of approximately 0.4 μ in diameter (Fig. 31, arrow). There are many microvilli-like projections on the luminal surface as in the case of excretory vesicles (Figs. 30, 31, MP). The cuticle of body surface enters the rim of the vesicle, but it does not extend beyond the rim. The lumen of the vesicle is usually filled with less dense materials which are continuous to the materials of the space between sheath and body. The microprojections of irregular and various thickness are extending into the lumen from base of the vesicle to a half of the total depth. These projections differ from those of excretory vesicle as they are thicker and shorter in size. The cytoplasm of anal vesicle contains mitochondria, glycogen granules and rough-endoplasmic reticulum. In some specimense, the cell forming the vesicle is composed of two or three and seems to be connected into R4 cell. But the connections between anal vesicle and R2 to R3 cell are not distinct in the present study.

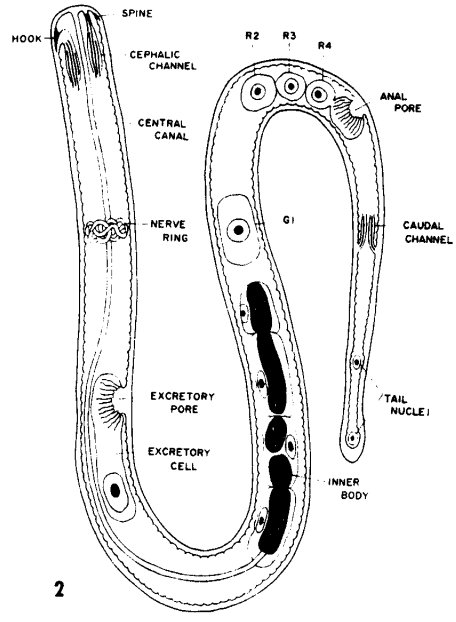

Fig. 2. Diagrammatic drawing of a microfilaria.

DISCUSSION

The sheath of microfilariae which appears as a delicate thin membrane by light microscopic observations has been studied electron microscopically (12, 13, 17, 23, 24). In microfilaria *Loa loa* and *Wuchereria bancrofti*, the sheath consisting of double membrane is covered with fibrous materials according to HOCKLEY (13) and CHATTERJEE (12). MCLAREN (23) has shown that the sheath of microfilaria *Loa loa* and *Litomosoides carinii* is composed of a uniform layer of electron-dense material and not limited by membranes. And the outer surface of the sheath is covered with a thick layer of particulate material. There is the same pattern as the sheath of microfilaria *Brugia malayi*. In microfilaria *Cardianema sp.* from the blood, JOHNSTON (24) has shown that microfilaria has the trilaminar sheath with external coat around

sheath, and no distinct laminar substructure was observed in any layer within the sheath. This trilaminar sheath has the same basic structures as those described here, and fibrous external coat is sometimes seen on the dense granular materials in microfilaria *B. malayi*. HOCKLEY (13) suggested that the fibrous materials on the sheath in microfilaria *L. loa* and *L. carinii* might possibly have come from the host. According to MCLAREN (23), a dense particulate layer of variable thickness on the outer margin of the sheath is thought to represent host material adhered to the sheath during the preparatory techniques. In the present investigation on microfilaria *B. malayi*, the presence of fibrous coat outside the dense granular materials is presumably the result of the preparatory techniques.

Granular materials like those of the external surface of the sheath have been shown in the space between body and cuticle of microfilaria *L. loa* (23) and *Cardianema* sp. (24). The microfilaria of *B. malayi* is filled with much granular materials in the space between sheath and cuticle as in the case of the excretory vesicle. This differentiation on the granular materials may have resulted from the preparatory techniques.

The cuticle of microfilaria *B. malayi* consists of three layers, namely, external cortical, internal cortical and fibrous layer. MCLAREN (23) reported similar cuticular structures which appear to be similar regardless of whether the microfilaria are sheathed or unsheathed, *in utero* or in the blood on five species. The outer cuticle of microfilaria *Dirofilaria immitis* is composed of trilayered, and homogenous layer is separated into fibrillar and homogenous zone (KOZECK) (22) or electron-dense and less dense layer (JOHNSON *et al.*) (21). Furthermore there is a basal lamella beneath the homogenous layer (KOZECK) (22). In the present investigation, the two zones of homogenous layer have been distinguished in some specimens. However, the basement membrane could not be identified in any specimens investigated. JOHNSTON *et al.* (24) also observed that the homogenous layer was consisted of fibrilar and subfibrilar layers, in addition to the outer and inner aspects of the cuticle. These membranous structures have not been observed in other microfilariae. The outer-most layer, external cortical of microfilaria *B. malayi*, is often discontinuous at the deepest point of the transverse striations. JOHNSON *et al.* (21) and MCLAREN (23) have reported the similar discontinuous cuticular indentation. Furthermore vesicular structures of variable sizes and shapes were occasionally seen in the inner membranous zone of the cuticle (JOHNSON *et al.*) (21). MCLAREN (23) reported the similar vesicles seen in the hypodermis. There is no vesicular structure in the cuticle of microfilaria *B. malayi*, whereas the dense body can be seen in the hypodermis. The fine structure of the cuticle of infective larva and adult worm has been reported

by several authors, for example, the cuticle of adult *Nippostrongylus brasiliensis* by LEE (27) or the larvae of *Ancylostoma* by SAKUMOTO *et al.* (28) having striped layers in the cuticle and the distinct hypodermis layer beneath the cuticle. It is difficult to compare the fine structures of the cuticular layers of microfilaria *B. malayi* with those of other Nematodes, because other reports were about infective larvae none of which are filarial worms.

Microfilarial hypodermis is very thin and indistinct layer except for lateral, ventral and dorsal lines. JOHNSON *et al.* (21) considered that the muscle cells were in direct contact with the cuticle. Many electron microscopists have been unable to identify the hypodermis of the microfilaria. MCLAREN (23) has demonstrated conclusively that the hypodermis exists. In the present study on microfilaria *B. malayi*, it appears to have no hypodermis in some part. But in view of the necessity to form the cuticle, the hypodermis will be situated beneath the whole cuticle.

LAURENCE *et al.* (9) has demonstrated that the small nuclei with larger nucleoli lying in a few very elongated, spindle-shaped cells lie joined end to end along the axis of the microfilaria of *Brugia* by a light microscope. TAYLOR (3) has also shown by a light microscope that the muscle cells have oval nuclei which are somewhat flattened against the cuticle of microfilaria *B. malayi* and others. The muscle cell usually contains an oval-shaped nucleus. The spindle-shaped nucleus which may belong to the muscle cell is rarely seen in *B. malayi*. Thick and thin myofilaments are visible as in the case of other nematodes.

The structural features of anterior end of the cephalic space were described by LAURENCE *et al.* (7) and SAWYER *et al.* (6) with light microscopy. Three tooth-like structures lie in a row across one side of the tip of the cephalic space. Furthermore, opposite and somewhat more anteriorly, there is a layer of single tooth-like structure (LAURENCE *et al.*) (7). MCLAREN (23) has shown a hook at the tip of cephalic channel by electron microscopy, and suggested that anterior muscle cells are probably involved in the movement of the hook, while the spine was not shown. Microfilaria *B. malayi* has one hook at near the opening of a cephalic channel and several spines at the opposite side. But it has been not possible to determine as to whether or not spines are composed of three in the numbers. The well developed muscle cells are situated in the anterior region of microfilaria *B. malayi*. However, it is not confirmed that these muscle cells are directly connected to the hook which is probably controlled by these muscle cells.

Concerning the hook and spine of microfilaria *D. immitis*, KOZECK (22) has reported that the internal composition of the hook seems to have mostly muscular and tubular attachments while the stylet appears to be composed of

cuticular material. He seems to interpret errorneously the undulation of the body surface as a hook. The buccal capsule and pharyngeal thread (central canal) of the microfilaria have been exactly demonstrated with light microscopy by LAURENCE *et al.* (7, 8, 9). According to his reports, the pharyngeal thread extends from tip of the cephalic space through the nuclear column back to the inner body, and joins the inner body. The central canal with cuticle-like wall shows the desmosome structure in its cross section in microfilaria of *B. malayi*, like the star-shaped structures observed by HOCKLEY (13). These desmosomes belong to the small cell surrounding the canal as McLAREN (23) referred to that structure. Furthermore she reported that the pharyngeal thread of the microfilariae of *D. viteae*, *D. setariosum*, *D. immitis*, and *L. loa* can be traced back to the region occupied by the inner body, except for the fact that the thread of *L. carinii* can only be traced back for a short distance behind the cephalic space. And in the microfilaria *D. setariosum* there is some evidence that the thread actually enters the inner body. The canal of microfilaria *D. immitis* is observed as a smaller indistinct central canal (unpublished observations). However, the central canal of microfilaria *D. immitis* by KOZECK (22) was not identifted. In the present observations on *B. malayi*, the canal is connected to the inner body, and its lumen opens into the inner body materials through the filter-like structures between canal lumen and inner body. But the function of the inner body and filter-like structures is not yet known in the microfilaria. The contents of inner body vary in shape or density according to present electron microscopy. In view of the above facts it seems probable that the materials in the inner body come into or go out through the filter-like structures.

TAYLOR (3) indicated that the nerve ring of the microfilaria was composed of two small cells by light microscopy; these cannot be identified by the present investigation. The nerve ring consists of tangled nerve axons. The nerve cells connecting to the axon are not identified in the present study.

FÜLLEBORN (29) described the "Mundgebilde and Schwanzgebilde" as paired eosinophillic organs located in the cephalic and caudal regions with light microscopy. Since then, these structures have been demonstrated to be ciliary organs (KOZECK) (30) (McLAREN) (14). Furthermore the study on microfilaria and adult worm by McLAREN (23, 31) has shown that these structures are amphids and phasmids as compared with the adult filarial worm. The cilia containing these structures in microfilaria *B. malayi* differ from normal cilia pattern of usual 9+2 arrangement, and these cilia are directly exposed to the matrix between the body and sheath. HAWKING (32) suggested that these organelles influence the periodic behaviour of the microfilaria. McLAREN (23) also suggested that these organs have the function as mechanoreceptor.

In microfilaria *B. malayi*, cephalic and caudal channels have a morphologic features as a chemoreceptor.

TAYLOR (3) observed a delicate, semipermeable membrane at the exit of the excretory pore with light microscopy; this membrane could not at all be identified in the present study with electron microscopy. However, in microfilaria *B. malayi*, there is a dense plug condensed from the fluid materials in the pore at the orifice of the pore. This plug is probably produced as the result of preparatory techniques. TAYLOR (3) has suggested by histochemical study that the excretory vesicle is a highly active region of the excretory cell.

Abundance of rough-surfaced endoplasmic reticulum in the excretory cells suggests an active synthesis of protein; this protein might be either a specific secretion or enzymes for breaking down waste materials (McLAREN) (23). SIMPSON *et al.* (10) observed that the excretory cells appear to be rich in RNA. In microfilaria *B. malayi*, the rough-surfaced endoplasmic reticula are located everywhere in excretory cells. This fact suggests that the excretory cell has a highly active region in the microfilaria. Most authors now agree with the view that the posterior three, R2 to R4, form the rectum in larval stage. But the destiny of G1 cell remains unknown. The three R cells are a part of the anal apparatus of the microfilaria according to McLAREN (23).

The present investigation has shown that in microfilaria *B. malayi*, the three R cells appear in close contact with anal apparatus. Therefore, the anal apparatus becomes the anus and rectum in larvae, and probably has a function for absorption or secretion.

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REFERENCES

1. FENG, L.: A comparative study of the anatomy of *Microfilaria malayi* Brug, 1927 and *Microfilaria bancrofti* Cobbold, 1877. *Chin. Med. J.* **47**, 1214-1246, 1933
2. SCOTT, J. A., MACDONALD, E. M. and TERMAN, B.: A description of the stages in the life cycle of the filarial worm *Litomosoides carinii*. *J. Parasitology* **37**, 425-432, 1951
3. TAYLOR, A. E. R.: Studies on the microfilaria of *Loa loa*, *Wuchereria bancrofti*, *Brugia malayi*, *Dirofilaria immitis*, *D. repens* and *D. aethiops*. *J. Helminthology* **34**, 13-26, 1960
4. SCHACHER, J. F.: Developmental stages of *Brugia pahangi* in the final host. *J. Parasitology* **48**, 693-706, 1962
5. SCHACHER, J. F. and KHALIL, G. M.: Development of *Foleyella philistinae* Schacher and Khalil, 1967 (Nematoda: Filarioidea) in *Culex pipiens molestus* with notes on pathology in the Arthropod. *J. Parasitology* **54**, **869-878**, 1968

6. SAWYER, T.K., RUBIN, E.F. and JACKSON, R.F.: The cephalic hook in microfilariae of *Dipetalonema reconditum* in the differentiation of canine microfilariae. *Proc. Helminthol. Soc.* **32**, 15-20, 1965
7. LAURENCE, B.R. and SIMPSON, M.G.: Cephalic and pharyngeal structures in microfilariae revealed by staining. *J. Helminthology* **42**, 309-330, 1968
8. LAURENCE, B.R. and SIMPSON, M.G.: Staining microfilariae for structures other than nuclei. *Tr. Roy. Soc. Trop. Med. Hyg.* **63**, 801-810, 1969
9. LAURENCE, B.R. and SIMPSON, M.G.: The microfilaria of *Brugia*: A first stage nematode larva. *J. Helminthology* **45**, 23-40, 1971
10. SIMPSON, M.G. and LAURENCE, B.R.: Histochemical studies on microfilariae. *Parasitology* **64**, 61-88, 1972
11. ARMSTRONG, J.A. and HAWKING, F.: Electron microscope sections of filarial worms. *Tr. Roy. Soc. Trop. Med. Hyg.* **58**, 9, 1964
12. CHATTERJEE, S.N. and CHOWDHURY, A.B.: Electron microscopic studies on the ultrastracture of microfilaria (*Wuchereria bancrofti*). *Bull. Cal. S.T.M.* **12**, 63, 1964
13. HOCKLEY, D.J., WORMS, M.J. and EBDEN, L.M.: Electron micrographs of microfilariae. *Tr. Roy. Soc. Trop. Med. Hyg.* **62**, 468-469, 1968
14. MCLAREN, D.J.: Ciliary structures in the microfilaria of *Loa loa*. *Tr. Roy. Soc. Trop. Med. Hyg.* **63**, 290-291, 1969
15. SONODA, M. and KOBAYASHI, K.: Electron microscopic studies on the morphology of microfilariae of *Dirofilaria immitis*. 1. Observations of the whole body. *Jap. J. Vet. Res.* **13**, 67-71, 1965
16. LEE, C. and MILLER, J.H.: Fine structure of *Dirofilaria immitis* body-wall musculature. *Expl. Parasitology* **20**, 334-344, 1967
17. SCHARDEIN, J.L., LUCAS, J.A. and DICKERSON, C.W.: Ultrastructural changes in *Litomosoides carinii* microfilariae in gerbils treated with Diethylcarbamazine. *J. Parasitology* **54**, 351-358, 1968
18. BECKETT, E.B. and BOOTHROYD, B.: Mode of nutrition of the larvae of the filarial nematode *Brugia pahangi*. *Parasitology* **60**, 21-26, 1970
19. COLLIN, W.K.: Ultrastructural morphology of the esophageal region of the infective larva of *Brugia pahangi* (Nematoda: Filarioidea). *J. Parasitology* **57**, 449-468, 1971
20. HO, B. and KAN, S.: Development of *Breinlia sergenti* (Dipetalonematidae) in the fat-body of mosquitoes. 3. Mode of nutrition of the infective larvae. *J. Med. Ent.* **9**, 262-265, 1972
21. JOHNSON, K.H. and BEMRICK, W.J.: *Dirofilaria immitis* microfilariae: Electron microscopic examination of cuticle and muscle. *Am. J. Vet. Res.* **30**, 1443-1450, 1969
22. KOZECK, W.J.: Ultrastructure of the microfilaria of *Dirofilaria immitis*. *J. Parasitology* **57**, 1052-1067, 1971
23. MCLAREN, D.J.: Ultrastructural studies on microfilariae (Nematoda: Filarioidea). *Parasitology* **65**, 317-332, 1972
24. JOHNSTON, M.R.L. and STEHBENS, W.E.: Ultrastructural studies on the microfilaria of *Cardianema sp. Alicata*, 1933 (Nematoda: Onchocercidae). *Int. J. Parasitology* **3**, 243-250, 1973
25. KUSHIDA, H.: On an epoxy resin embedding method for ultrathin sectioning. *Electron-Microscopy, Japan* **8**, 72-75, 1959
26. KUSHIDA, H.: A styrene-methacrylate resin embedding method for ultrathin sectioning. *J. Electronmicroscopy* **10**, 16-19, 1961
27. LEE, D.L.: The cuticle of adult *Nippostrongylus brasiliensis*. *Parasitology* **55**, 173-181, 1965
28. SAKUMOTO, D., TONGU, Y., SUGURI, S., ITANO, K. and INATOMI, S.: Ultrastructural observations on the transverse striation and lateral ala of the sheath and cuticle of

matured larvae of *Ancylostoma ceylanicum* and *A. braziliense*. *Jap. J. Parasitology* **20**, 410-424, 1971 (in Japanese)

29. FÜLLEBORN, F.: Beitr"ge zur Morphologie und Differentialdiagnose der Mikrofilarien. *Beihefte zum Archiv für Schiffs-und Tropenhygiene* **17**, 7-72, 1913
30. KOZECK, W. J.: Unusual cilia in the microfilaria of *Dirofilaria immitis*. *J. Parasitology* **54**, 838-844, 1968
31. MCLAREN, D. J.: Preliminary observations on the sensory structures in adult *Filariae*. *Tr. Roy. Soc. Trop. Med. Hyg.* **64**, 191-192, 1970
32. HAWKING, F.: The 24-hour periodicity of microfilariae: biological mechanisms responsible for its production and control. *Proc. R. Soc.* **169**, 59-76, 1967

Fig. 3. Longitudinal section of the sheath consisting of the sheath membrane (SM) and dense materials (DM).

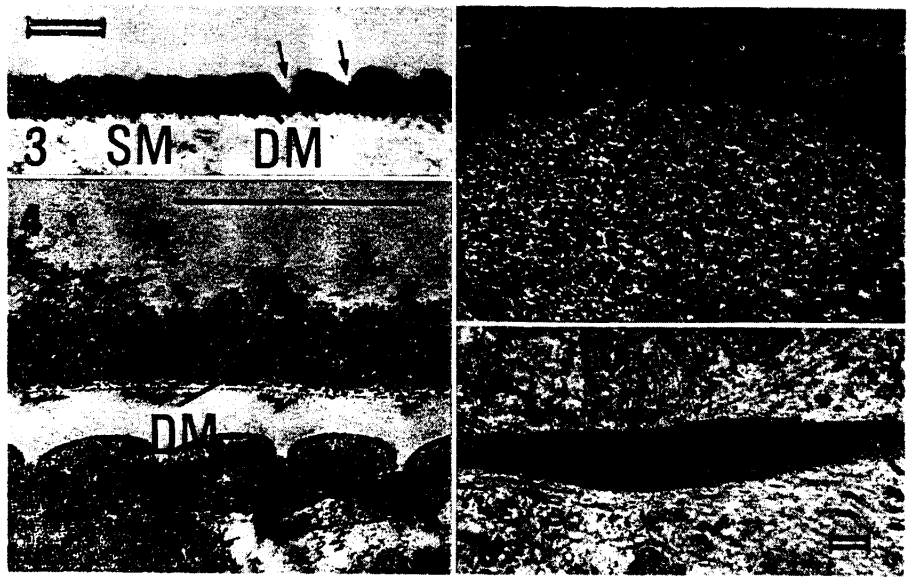
Fig. 4. Longitudinal section of the cuticle and sheath with outer coat (OC) on the granular dense materials (DM).

Fig. 5. Cross section of a contractile portion of muscle cells showing thick and thin myofilaments.

Fig. 6. Collagenous fibers in muscle tissue.

Fig. 7. Longitudinal section of the cuticle composed of three layers; external cortical (EL), internal cortical (IL) and fibrous layer (FL). Myofilaments (Mu) are located beneath the hypodermis (Hy).

Fig. 8. Showing dense bodies (DB) in the hypodermis. Mu: muscle, SM: sheath membrane.


3
SM
DM
DM


DM
FL
FL
FL


SM
8

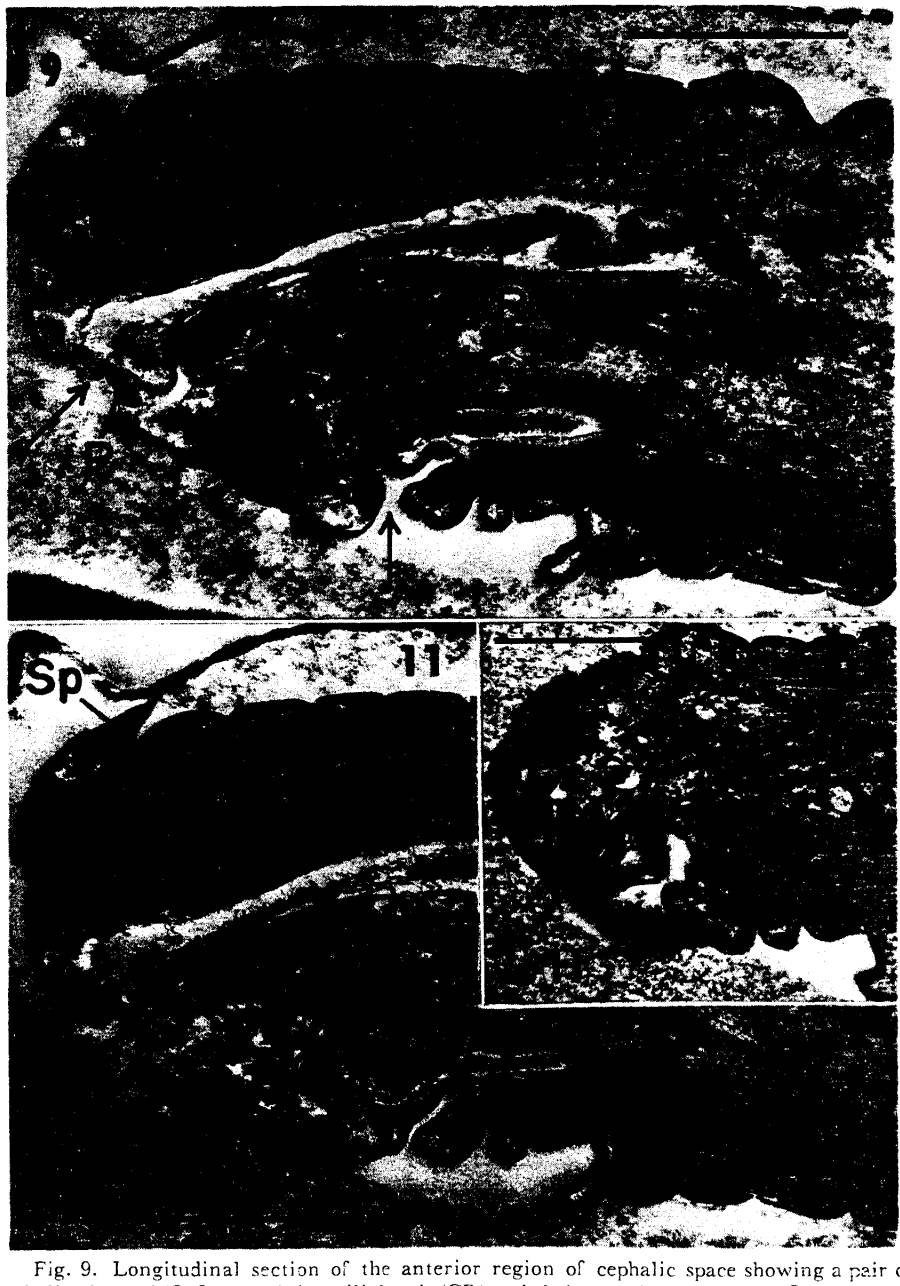


Fig. 9. Longitudinal section of the anterior region of cephalic space showing a pair of cephalic channel (CeC) containing cilial rods (CR) and their openings (arrows). One channel has a plug (P) at the orifice. Mu : muscle

Fig. 10. Longitudinal section through the anterior region of cephalic space showing a hook (H) at the entrance of the deeper invagination.

Fig. 11. Continuous section of Fig. 9 showing a spine (Sp) at the head. CC : central canal, CeC : cephalic channel

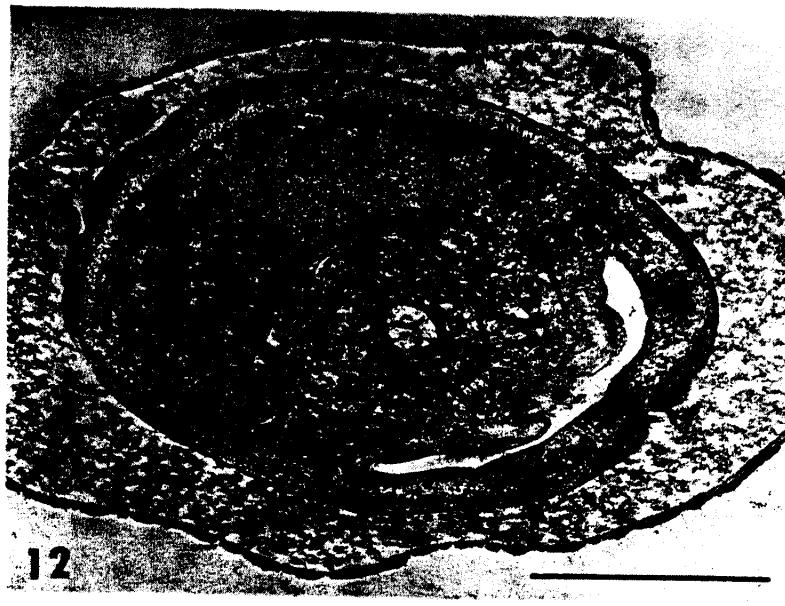
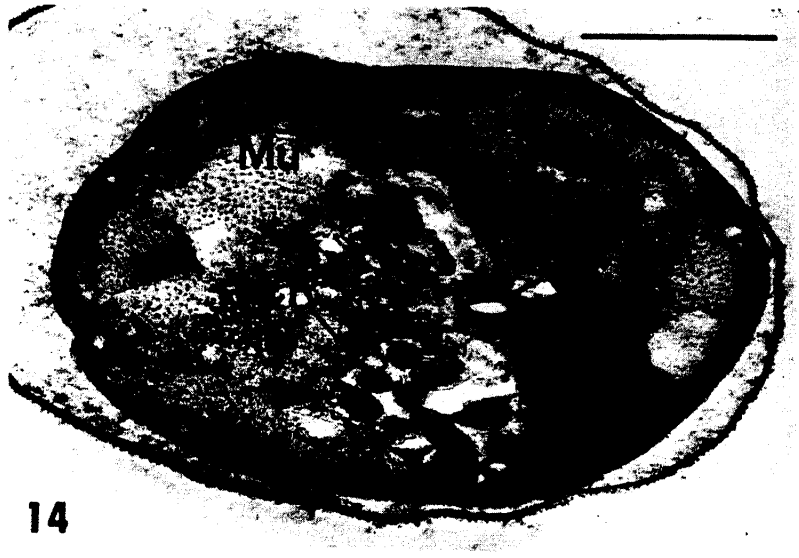


Fig. 12. Cross-section of the head part. CC: central canal, CeC: cephalic channel, CR: cilial rod, Mu:muscle, Sp: spine

Fig. 13. Longitudinal section of a cephalic channel (CeC) having many cilial rods (CR). CC: central canal



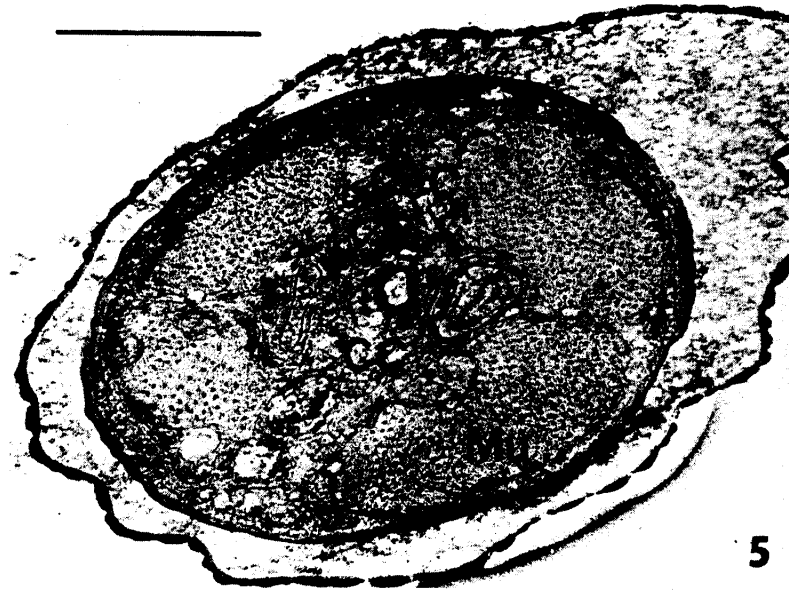
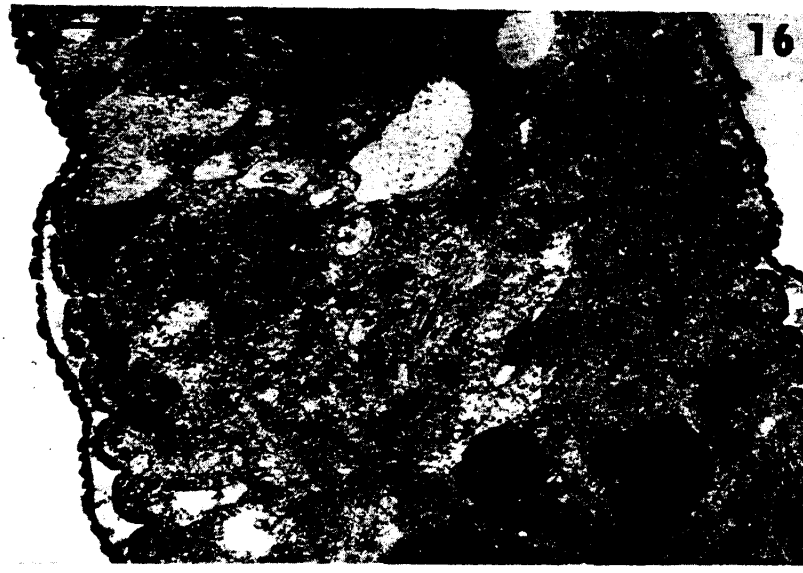


Fig. 14. Cross-section at the base of cephalic channels. CC: central canal, CR: cilial rod, Mu: muscle

Fig. 15. Muscle cells (Mu) are situated in four groups at the posterior region of cephalic space in cross-section. A pair of cephalic channel (CeC) and a central canal (CC) are seen in the center.



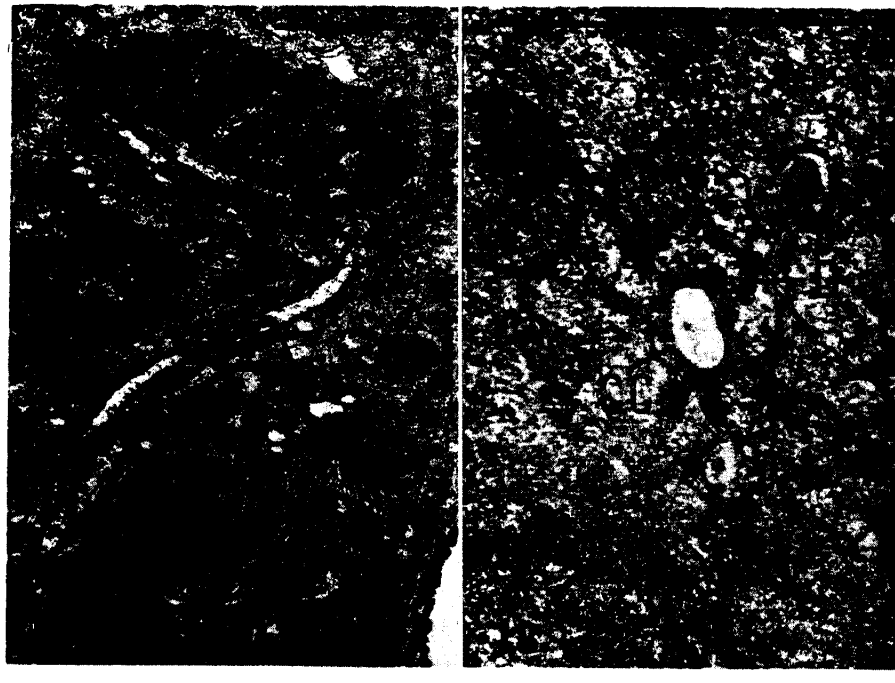


Fig. 16. Longitudinal section of the nerve ring (NR) containing nerve fibers.

Fig. 17. Longitudinal section of the central canal (CC) running through near the micro-projections (MP).

Fig. 18. Cross-section of central canal (CC). Desmosomes (D) are seen arround the canal.

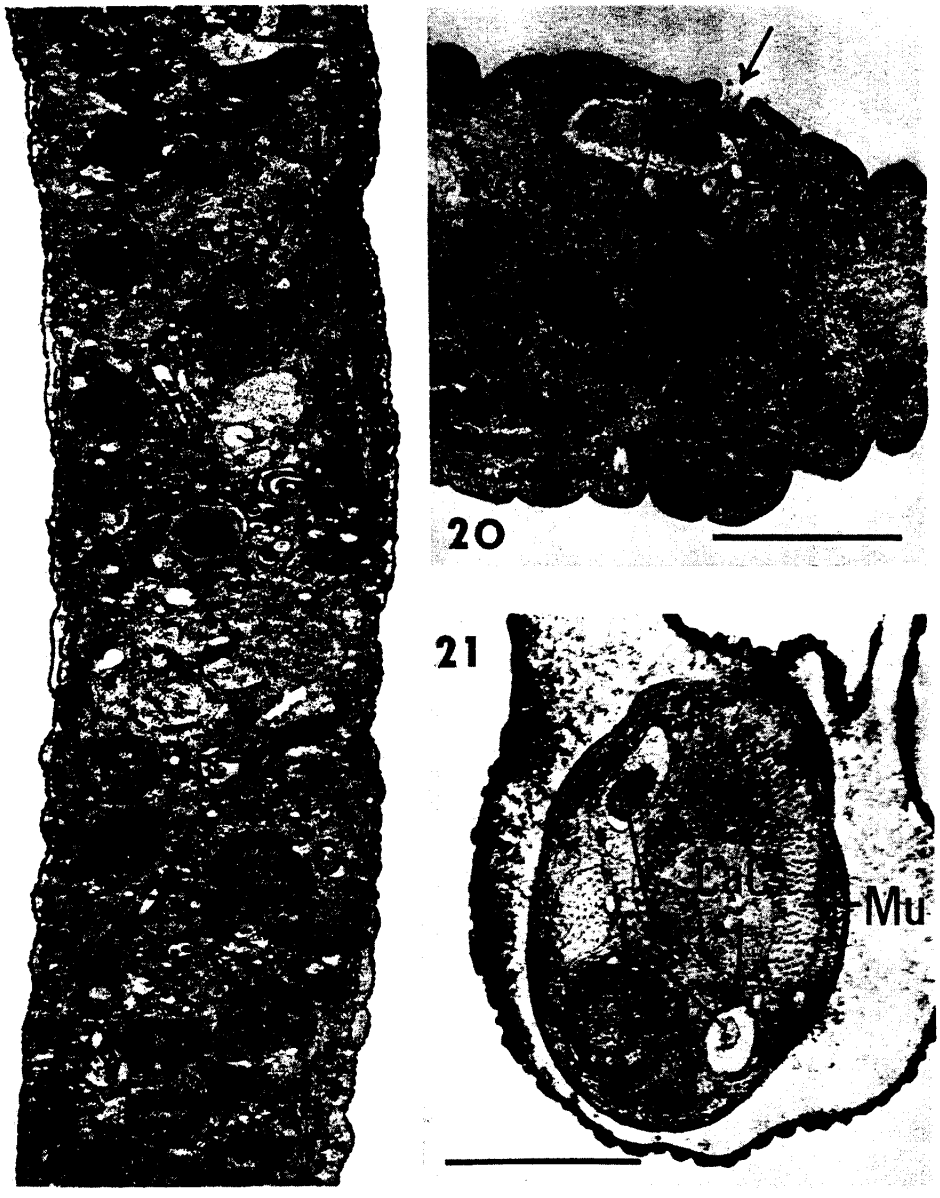


Fig. 19. Longitudinal section through the excretory apparatus. EC: excretory cell, EV: excretory vesicle

Fig. 20. Longitudinal section through the caudal channels (CaC) with a cilial rod (CR). A plug (P) can be seen at the orifice (arrow).

Fig. 21. Cross-section of the posterior region showing a pair of caudal channel (CaC) including a cilial rod (CR). Muscle cells (Mu) are arranged into two groups.

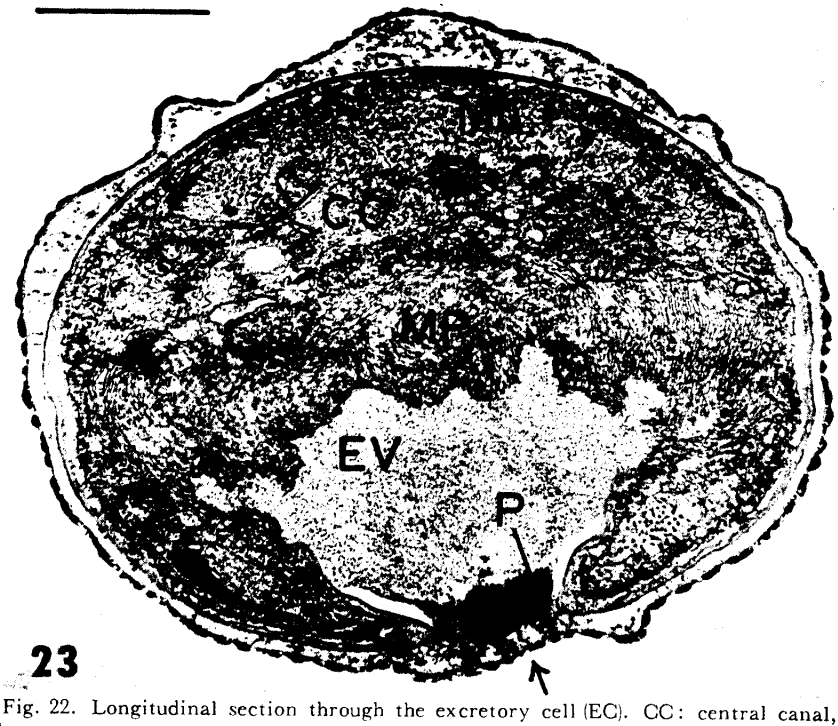


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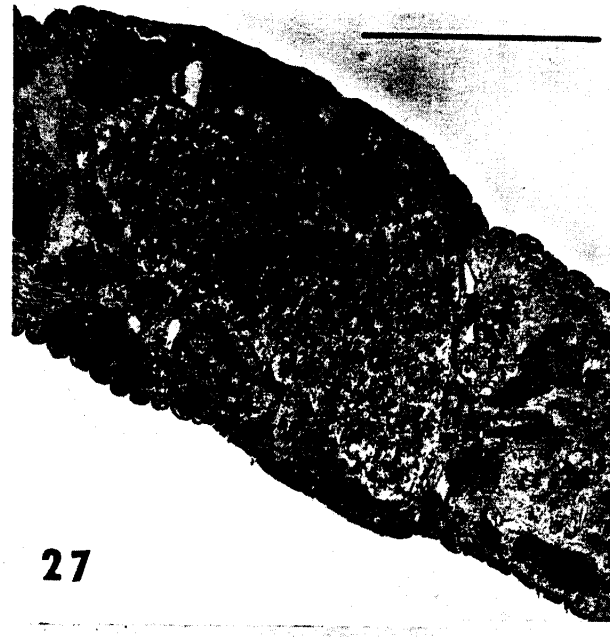
Fig. 22. Longitudinal section through the excretory cell (EC). CC: central canal, N: nucleus

Fig. 23. Cross-section at the excretory vesicle (EV) with a dense plug (P) at the opening (arrow). CC: central canal, MP: microprojection, Mu: muscle

Fig. 24. Longitudinal section through the inner body (IB).
Fig. 25. Longitudinal section through the GI cell (GI). N: nucleus
Fig. 26. Higher magnification at the orifice of the excretory vesicle (EV). CC:central canal, MP: microprojection



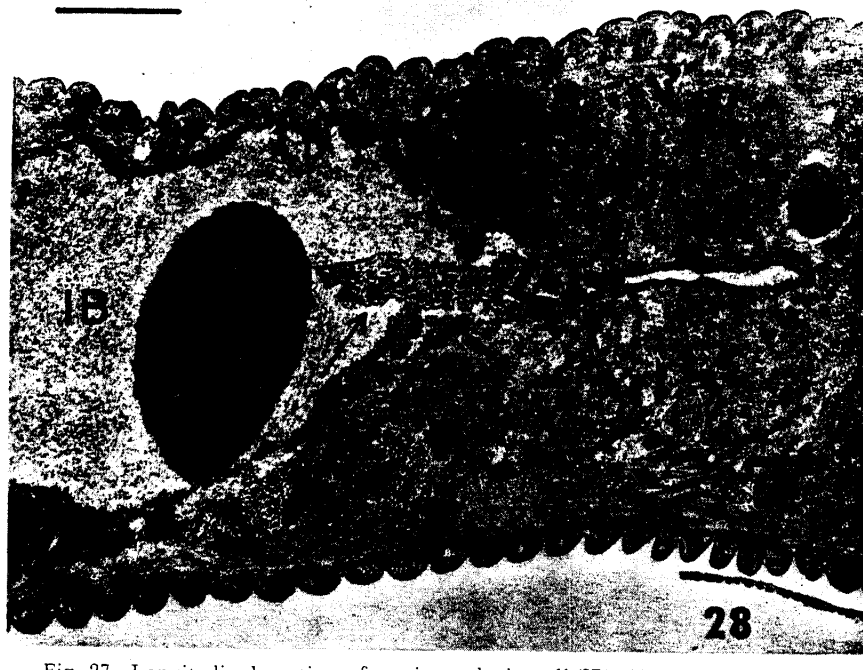


Fig. 27. Longitudinal section of an inner body cell (IB). N: nucleus

Fig. 28. Longitudinal section of the anterior part of the inner body (IB). A central canal (CC) connected to the filamentous structure (arrow) opens into the inner body.

Fig. 29. Longitudinal section through the anal apparatus.
MP: microprojection, RC: R cell

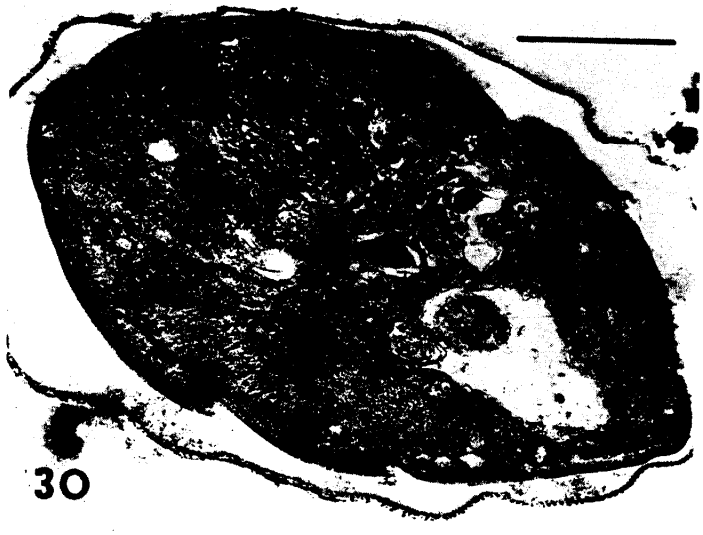


Fig. 30. Cross-section at the anal vesicle. MP: microprojection

Fig. 31. Longitudinal section at the anal vesicle with an opening (arrow). MP: microprojection

A single scale indicates 1 μ in each figure and double scales 0.1 μ