THE PROBOSCIS APPARATUS OF THE NEMERTINE *LINEUS RUBER*

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CONTENTS

The proboscis apparatus of the nemertine *Lineus ruber* is divided into three segments: the anterior, middle and posterior proboscis, and in addition to these, the retractor muscle. The latter connects the caudal end of the posterior proboscis to the posterior dorsal wall of the rhynchocoel.

The general arrangement of the constituent layers of the three segments of the proboscis is more or less similar: (1) inner epithelium, (2) basement membrane, (3) nerve plexus, and (4) one or two layers of muscle which are covered exteriorly by (5) basement membrane and (6) the endothelial cells. The endothelial cells are freely exposed to the rhynchocoel fluid.

The inner epithelium of the anterior and posterior proboscis consists of only one type of lining cells, whereas the epithelium of the middle proboscis has many different types of cells, among which may be mentioned (1) 'rhabdite'-forming cells, (2) 'sensory' cells, (3) cells with long microvilli, (4) mucus-secreting cells, and (5) cells with acidophilic granules.

The rhabdites of the rhabdite-forming cells are very characteristic. Two stages of the rhabdites have been seen: newly developed and mature rhabdites. In the former, the central tubular core of the structure is small and the 'pool' in which the rhabdite is embedded is large. In the mature rhabdite the reverse is true, i.e. the central tubular core is distended with electron-translucent secretion probably derived from the 'pool', since the latter is greatly reduced in size. The rhabdites are discharged in clusters into the lumen of the 'resting' proboscis and presumably over 'prey' when the proboscis is ejected.

The muscles of the proboscis have 'dual' innervation. Aminergic and cholinergic nerve fibres, which arise from the dorsal cerebral ganglia, enter the proboscis at its anterior connexion ('hinge'). In the aminergic nerve terminals two types of 'synaptic vesicles' have been resolved: vesicles of moderate density (20 to 50 nm) and dense-core vesicles (50 to 80 nm). Cholinergic terminals show typical vesicles of size 20 to 50 nm.

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The retractor muscle is apparently trebly innervated. 'Synaptic contacts' (mostly aminergic) occur at the junction of the proboscis and the retractor muscle. In addition, the retractor muscle has a probable peptidergic type of innervation. Neural terminals loaded with granules of size 140 nm, and thus comparable with other neurosecretory endings, are seen in the close vicinity of the retractor muscle. This histological evidence is supported by the observation that the muscle contracts vigorously when stimulated with oxytocin at a concentration of 0.01 unit/ml. The fluid relationships between the rhynchocoel and the vascular system, that allow the proboscis to be freely ejected and withdrawn, are discussed.

Introduction

This structure, which is peculiar to the phylum Rhynchocoela, consists of rhynchodaeum, proboscis, and retractor muscle. In *Lineus ruber* the proboscis is a tubular structure which lies free in a fluid filled tubular cavity, the rhynchocoel (figure 1). Its anterior region is attached to the junction between the rhynchodaeum and the rhynchocoel, while posteriorly it is fastened by the retractor muscle to the wall of the rhynchocoel cavity (figure 1). The rhynchocoel is therefore closed at both ends.

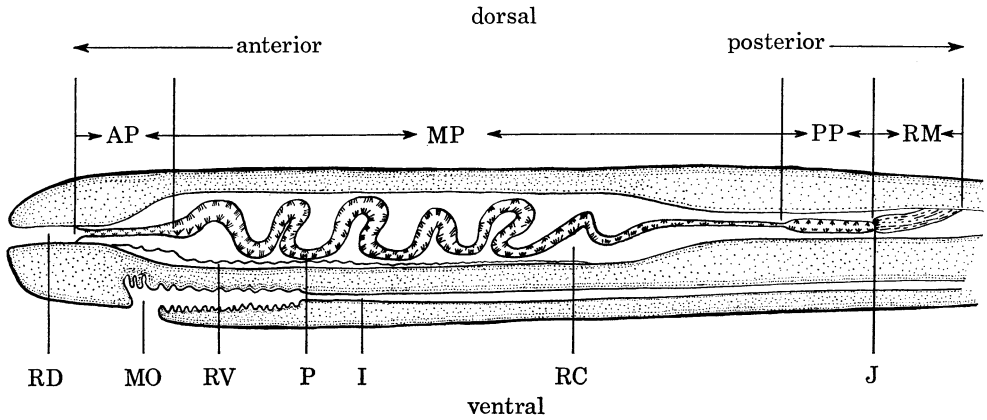

Figure 1. Diagram showing longitudinal section of *Lineus ruber*.

During development the proboscis is formed by a direct invagination of the primary epiblastic layer (Bürger 1895; Coe 1904; Hyman 1951). The invagination becomes the rhynchodaeum. A posterior outgrowth, which will become the proboscis, ensues and this grows backward as a solid mass of cells which soon arrange themselves into a narrow tube lined with cuboidal or low columnar cells. Later, the adjacent mesodermic cells accumulate around the tube and form the muscular walls of the proboscis. The mesoderm immediately surrounding the proboscis becomes separated from the adjacent mesoderm, the cleft forming the rhynchocoel. With regard to the retractor muscle, Reutter (1967) observed that it is regenerated from the aggregation of numerous mesenchymal cells in the preformed rhynchocoel at its posterior region.

The proboscis apparatus has been described as serving many functions; for example, it is employed in the capture of prey and in defence (Hyman 1951), for which purpose it is shot out with explosive force through muscular contraction exerting pressure on the rhynchocoel fluid (Dakin & Fordham 1936). The proboscis may also help in burrowing (Wilson 1900; Dakin & Fordham 1936), in locomotion (Pantin 1950), and in escape reaction in *Geonemertes hillii* (Hett 1924). Wilson (1900) also described that during locomotion of the worm the proboscis is used chiefly as an organ of feeling or touch (exploratory tactile organ), being thrust forward to determine the direction in which the burrow is to be made. That the proboscis is used as an organ of prehension was also noted several times by him.

It was thought that since the eversion and introversion mechanisms of the proboscis involves a rather elaborate and highly dynamic system, a thorough study of the structural organization of the proboscis with the aid of the electron microscope might provide some useful information and might be important to the behavioural studies of the creature.

Materials and Methods

The worms, obtained from the Marine Biological Laboratory, Plymouth, were sent to the aquarium in Cambridge. They were kept in open glass bowls with sand and stones on the bottom and in which the sea water was in continuous circulation with the rest of the aquarium. It is important to keep these worms fairly cool (about 15 °C) and in a rather dim light. For most histological purposes the worms, after they have been cleared of adherent sand, were fixed by dropping them into Heidenhain's Susa and leaving them for about 4 h. After paraffin-embedding and sectioning at 4 μm, Masson's trichrom stain (Haemalum, ponceau fuchsin, and light green) was found to be the most satisfactory method for routine purposes.

For the demonstration of acetylcholinesterase (AChE) the method of Koelle, modified by Lewis & Shute (1966), was followed. The worms were either fixed in 10 % neutral formalin or directly transferred into the incubation medium. Acetylthiocholine iodide was used as substrate. Ethopropazine at a final concentration of 2×10^{-4} mol/l was used as an inhibitor of pseudocholinesterase. After incubation the tissues were treated with sodium sulphide solution. They were then dehydrated, cleared, and sectioned in the usual manner.

For the demonstration of aminergic nerve fibres the method of Falck & Owman (1965) was employed.

For electron microscopy, tissues were generally fixed in Dalton's fixative (Pease 1964) for 2 to 4 h at 1 to 4 °C. They were dehydrated rapidly in graded concentrations of ethanol and embedded in Araldite mixture. Silver-white sections were cut on a Reichert Om U 2 ultramicrotome and picked up with either 400- or 200-mesh copper grids. All the sections were double stained–first in uranyl acetate saturated in 50 % ethanol and then in lead citrate. They were examined in a Siemens Elmiskop I electron microscope operating at 60 kV.

Observations

In *Lineus ruber* the proboscis can be divided visibly into three segments: the anterior, the middle, and the posterior segments, and, in addition, the retractor muscle (figure 1).

Anterior or proximal† segment of the proboscis

This is about one-tenth of the whole length of the proboscis and has a diameter of approximately 0.2–0.5 mm. At its anterior extremity, the proboscis becomes continuous all around with the muscular wall of the proboscis sheath. In a transverse section this part of the proboscis has the following layers (figure 2):

1. The outer‡ endothelium
2. The subendothelial layer of circular muscle fibrils
3. The outer basement membrane layer

† The term 'proximal' refers to the end of the proboscis that is connected to the posterior wall of the rhynchodaeum.

‡ In the following description the nomenclature of Bürger (1895) will be followed, by which in the invaginated proboscis, the layers nearest the lumen are termed the 'inner layers', those toward the periphery the 'outer'.

4. The longitudinal muscle layer
5. The inner basement membrane
6. The inner epithelium

The outer endothelium consists of low cuboidal cells covering exteriorly the anterior segment of the proboscis (anterior proboscis) (figure 2). The shape of the cells varies with the stretching of the proboscis, and the cells sometimes appear flattened. The endothelial cells have a very well developed endoplasmic reticulum which is often dilated and filled with electron-dense secretion (figure 7, plate 1). Sometimes the endoplasmic reticulum appears to have round profile and this is often in close contact with the apical cell membrane. Discharge of the secretion of the dilated endoplasmic reticulum is through the process of 'exocytosis', in which the secretion-filled endoplasmic reticulum has direct communication with the extracellular space (rhynchocoel). The mitochondria of the cells are enormous in size (figure 7)—very much larger than that of other cell types, suggesting that the cells are metabolically very active.

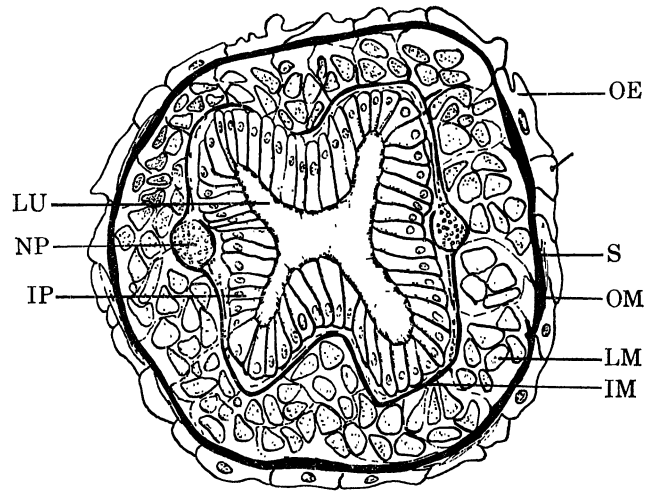

FIGURE 2. Diagram showing transverse section of the anterior proboscis.

On one occasion an endothelial cell was seen to bear a single cilium projecting into the rhynchocoel fluid (figure 7). The cilium possesses a distinct basal body which is in continuity with the peripheral fibrils of the axoneme. The cilium has no central fibrils. Clusters of electron-dense particles, probably free ribosomes, appear to form an aggregation around the ciliary basal body.

Except for some regions where circular muscle fibrils intervene, all the endothelial cells rest on a distinct basement membrane (the outer basement membrane) (figures 2, 7). The basement membrane is of typical structure, formed by the interlacing of collagen fibrils embedded in a ground substance of very low electron-density.

The discrete but circularly arranged muscle fibres between the endothelial cells and the outer basement membrane were also noted by Thompson (1901) in *Zygeupolia litoralis*. The layer is only one cell thick. The present study revealed the presence in the cytoplasm of these cells of two types of myofilaments, of thickness 30 and 5 nm respectively, and thus similar to those found in the smooth muscle cells of other animals (Shoenberg 1958; MacRae 1963) (figure 7).

Immediately beneath the outer basement membrane is the longitudinal muscle layer. These

muscles are separated into bundles (4 to 10 fibres in each) by connective tissue from both the outer and inner basement membranes. Transverse sections show that these muscle cells also contain two types of myofilaments similar to those found in the circular muscle fibres (figures 7 and 8, plates 1 and 2). The muscle cells have smooth boundaries and they do not interdigitate. Desmosome-like junctions are frequently observed between the cells. Between these muscle bundles and the inner basement membrane there often exist many nerve fibres containing a mixture of agranular (50 nm) and granular vesicles (50 to 100 nm) typical of an aminergic nerve fibre ending (Richardson 1962, 1964). In some sections it may be seen that a nerve terminal comes into close juxtaposition with three or more muscle cells (figure 8). The connective tissue layer (basement membrane) between these elements is usually greatly reduced or diminished. This finding is in substantial agreement with the finding of Reutter (1969) using the fluorescence method as developed by Falck & Owman (1965), in which he described adrenergic nerve fibres, which gave off a yellowish fluorescence, entering the proboscis from the cerebral ganglia. This has been confirmed in the present investigation with the same technique. The present study also demonstrated high activity of acetylcholinesterase between the muscle bundles, indicating that the proboscis has a dual innervation. However, with the electron microscope, cholinergic nerve endings have so far not been encountered.

The inner epithelium, which lines the lumen of the anterior proboscis, is a single layer of cells (figures 2, 9). The cells are essentially columnar and have basal nuclei. Electron-microscopy shows that the cells have very well developed microvilli extending into the cavity. Each of the lining cells is seen also to bear a single normal cilium at its apical surface (figure 9). A peculiar feature of these cilia is that each of them is always accompanied by 7 to 10 'accessory rods' arranged in a circular manner (figure 10). The epithelium of the anterior proboscis in *Lineus* is built up of only one type of lining cell. This is different from the finding of Thompson (1901) in *Zygeupolia*, in which he described the presence of glandular cells in this segment of the proboscis.

Middle region of the proboscis

The transition from the anterior proboscis to the middle segment (middle proboscis) is characterized by the sudden increase in the diameter (e.g. in a worm of body diameter 2 mm the change of the diameter of the anterior proboscis to the middle proboscis would be 0.3 to 1 mm) and in the types of cellular elements. In a transverse section this part of the proboscis has the following layers (figure 3):

1. The outer endothelium
2. The outer basement membrane
3. The longitudinal muscle layer
4. The middle layer of connective tissue
5. The circular muscle layer
6. The nerve plexus
7. The inner basement membrane
8. The inner epithelium

Of these layers, the outer endothelium does not show any structural difference from that of the anterior proboscis. On the other hand, as distinct from the anterior proboscis, the subendothelial circular muscle fibrils are absent.

The longitudinal muscle layer of the middle proboscis is comparatively thicker than that of the anterior proboscis and forms many bundles. With the electron microscope, two types of myofilaments of width 20 to 25 nm and 2 to 3 nm can be resolved (figure 11, plate 3). Structures of increased electron-density which may be equivalent to the 'irregular dense bodies' in other animals (Caesar, Edwards & Ruska 1957; Merrillees, Burnstock & Holman 1963;

Panner & Honig 1967) are found frequently throughout the cytoplasm of the muscle cells in intimate relation with the myofilaments (figure 11).

The middle proboscis differs from the anterior in the establishment of a layer of circular muscle inner to the longitudinal muscle layer. This is only about half the thickness of the latter and may lose its continuity in some regions.

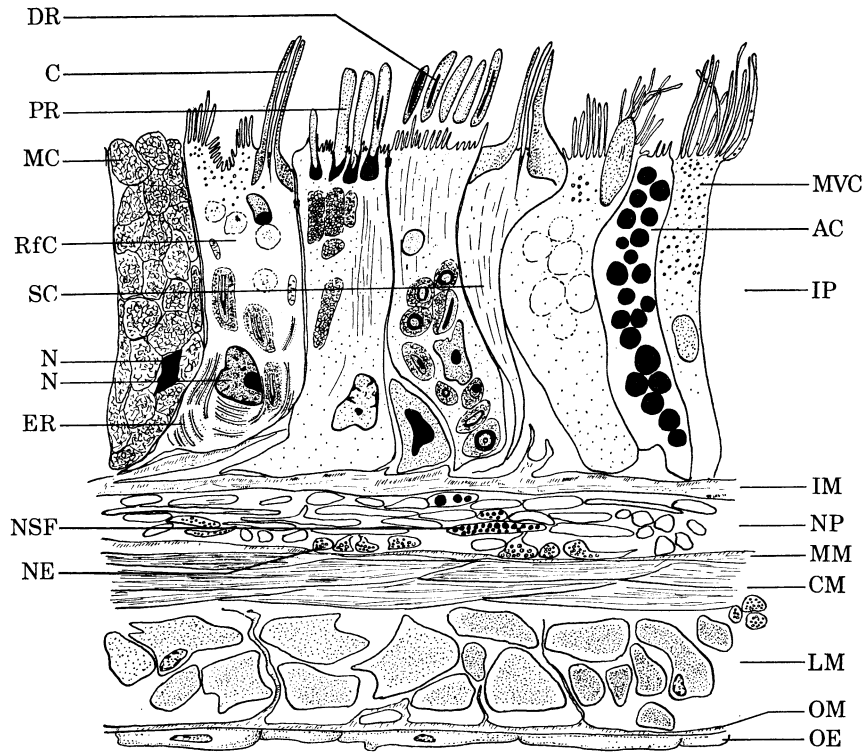

FIGURE 3. Diagram to show the different layers of the middle proboscis (transverse section).

It should be noted that the 'proboscidial nerves' which arise from the dorsal cerebral ganglia enter the proboscis as its anterior 'hinge' (Willmer 1970). As these two lateral nerves penetrate the middle segment from the anterior proboscis, they spread out into a circular nerve plexus between the circular muscle layer and the inner basement membrane and this runs along the whole length of the proboscis. Actual synaptic junctions have not been seen between the nerve endings and the longitudinal muscle cells, though in some sections nerve terminals or preterminals containing synaptic vesicles in the vicinity of the muscle fibres are clearly visible. Similar nerve endings are frequently seen in close proximity to the circular muscles, from which they are separated by a thin layer of basement membrane (figure 12, plate 3). The nerve endings consist of two types: cholinergic and aminergic. In the former there may be seen many vesicles (20 to 50 nm) of moderate density, whereas in the latter a few such vesicles, together with numerous dense-core vesicles (50 to 80 nm), are present (figure 12). This is compatible with the histochemical studies, since the nerve plexus is positive to AChE (figure 44, plate 21), and also gives off a yellowish fluorescence (figure 45).

Among the usual nerve processes there are also present one or two nerve fibres which are loaded with elementary neurosecretory granules of size 120 to 150 nm (figure 13, plate 3). The significance of this will be discussed later.

Another notable feature of this segment of the proboscis is the epithelium. The epithelium layer is elevated into many large circular folds (figure 49, plate 22) which are more or less broken up into many irregular ridges of papillae clothed with different types of cells: (*a*) 'rhabdite'-forming cells, (*b*) sensory cells, (*c*) cells with long microvilli, (*d*) mucus-secreting cells, and (*e*) cells with acidophil granules (figure 3).

The rhabdite cells

Among the above-mentioned cell types the most striking are the cells which contain masses of slender, rod-shaped acidophil bodies aggregated in clusters. Bürger (1895) described very similar structures in other nemertines and called them 'rhabdites'. Coe (1895) in *Cerebratulus lacteus* and Thompson (1901) in *Zygeupolia litoralis* also noted rod-like bodies and compared them with the rhabdites of the Rhabdocoela.

In *Lineus ruber* the 'rhabdite cells' are tall columnar cells containing numerous rhabdites which appear to be at different stages of development. The nuclei of the rhabdite cells are generally placed near to the inner basement membrane (figures 3; 14*A*, plate 4). They are small and highly electron-dense. Sometimes the cell is so full of rhabdites that practically no cytoplasm remains visible, except at the perinuclear zone which is often occupied by well-developed endoplasmic reticulum (figure 14*A*). The latter is probably directly concerned with the elaboration of the rhabdites. Other organelles, such as mitochondria, are very few in number; Golgi complexes have not been seen.

The intracellular rhabdites are of various shapes and sizes (figure 14*A*); those of the distal region of the cells have an approximate length of 7 μm and width 0.8 μm, whereas those near the nucleus are considerably smaller (2.5 μm long and 0.7 μm broad) and they could be the precursors of the fully differentiated rhabdites. The rhabdites of nemertines are clearly distinguishable in morphology from those that occur in Rhabdocoela and which have been described by Pedersen (1959) and Skaer (1961). According to Skaer in the triclad *Polycelis nigra* the rhabdite is a pale, cigar-shaped body 5 μm long and 0.8 μm broad, surrounded by a double membrane. The internal structure of the rhabdite consists of fibrils 12 nm wide spaced 30 nm apart. These are orientated longitudinally in the rhabdite and extend from one end of it to the other. Later, granular structures become deposited on the fibrils. The granules increase in number and size until the original fibrous structure is completely obscured.

In *Lineus*, electron-microscopic studies clearly defined the structural difference between what may be the newly developed rhabdites and the fully developed rhabdites. The newly developed rhabdites are normally embedded in a 'pool' of electron-translucent matrix (figure 14*b*) which is enclosed entirely within a membranous structure. The translucent matrix is probably derived from the endoplasmic reticulum, since a substance with similar characteristics has also been seen in some regions in the membranous cisternae (figure 14*A*). Sometimes a few rhabdites which are probably in the process of 'differentiation' are enveloped in a network of endoplasmic reticulum (figure 14*A*). A newly formed rhabdite when out longitudinally exhibits an elaborate pattern more complex in structure than was described by Gontcharoff (1957). Normally, a newly formed rhabdite consists of an outer layer which is blind at one end (proximal) but open at the other (distal) (figure 14*B*). The outer layer forms part of the lining (distal half) of the tubular core which opens distally. The proximal half of the tubular core is inserted into a highly electron-dense socket or pocket (figure 14*B*). The tubular core appears to contain a substance of very low electron-density. Sometimes, in a fully developed or discharged rhabdite,

the tube is dilated and filled with electron-translucent matrix, in which case the electron-lucent 'pool' around the rhabdite diminishes or decreases in size (figure 16, plate 5), suggesting that the translucent material forming the 'pool' is incorporated into the rhabdite during the migration of the latter towards the apical surface of the cell. In a fully developed rhabdite the size of the socket is greatly increased. This appears as a bulbous structure (figure 16), the upper part of which extends distally and may eventually appear to seal off the open end of the tubular core. The latter, which is now widely distended and filled with electron-translucent matrix, is therefore sequestered from the translucent 'pool'. As the rhabdite matures, the outer layer becomes diminished. At the base and on the inner surface of the socket there are usually seen a few filamentous processes of unknown significance extending into the tubular core. It should be noted that the structure of the discharged rhabdites is by no means uniform. In some, the central tubular core does not differ from that of a newly developed rhabdite. On the other hand, the outer layer is thickened. This contains electron-dense matrix and sometimes a few filamentous structures (figure 17, plate 6).

Covering the outer layer is an envelope which appears to have a fibrillar structure (figure 17). The fibrils have an average width of 2 to 3 nm. They form two layers: inner longitudinal and outer circular. Sometimes the inclusions of a discharged rhabdite appear to 'ooze out' into the surrounding medium so that the envelope becomes 'collapsed'; in this case the fibrils become more evident (figure 18).

It is probable that the rhabdites are liberated in clusters. Usually several of them are seen partially extruded, in which case the highly electron-dense sockets remain in the cell while the distal ends protrude into the lumen (figure 16). Transverse sections of these rhabdites show that sometimes they adhere by 'desmosome'-like structures (figure 17).

On one occasion it appears that the apical surface of a rhabdite-forming cell also bears a single cilium accompanied by seven accessory rods approximately 0.2 μm in diameter similar to those in the anterior proboscis (figure 15).

'Sensory' cells

These cells are rod-shaped (figure 3). The apical surface of each of the cells bears a single cilium (sensory receptors?) (figure 19, plate 7) enclosed by seven 'accessory rods' similar to those described previously in other cells (figure 20). The 'accessory rods' measured approximately 1 μm in diameter. The rods contain fibrillar structures which extend into the apical region of the cell and are anchored on an electron-dense 'cuticular plate' (figure 20). The cilium is a typical motile structure (figure 22, plate 7), i.e. it has the $9+2$ fibrillar pattern; each subfibril of the peripheral pairs bears arms (Gibbons 1961), though it may be difficult to understand how its beating activity, if any, could be achieved, since as mentioned above, the cilium is 'enclosed' by the accessory rods. A sensory function is therefore suggested. In one section it was found that the distal tip of the 'sensory cilium' was modified to form a bulb-like structure (figure 21). The bulb-like structure lay above the level where the accessory rods terminate.

Secretory cells

Between the rhabdite-forming cells are the mucus-secreting cells, which are usually filled with 'packets' of secretion (figures 3, 23). The secretion, when liberated, may occlude the lumen of the proboscis. It stained green with Masson's stain and showed β-metachromasia with toluidine blue at pH below 3.

Another type of secretory cell contains coarse, acidophil and electron-dense secretory granules about 500 nm in diameter (figure 3). The secretion is also fuchsinophil and picks up orange G.

Among the different cell types mentioned there are a few cells (other than the sensory cells) which do not contain any form of secretory products. These cells are columnar and have basal nuclei. A notable feature of the cells is the presence of well-developed microvilli at the luminal surface (figure 19). Immediately below the palisade of microvilli there are numerous microvesicles which, sometimes, may appear to 'invade' the microvilli. The relation of this cell type to the others is not certain.

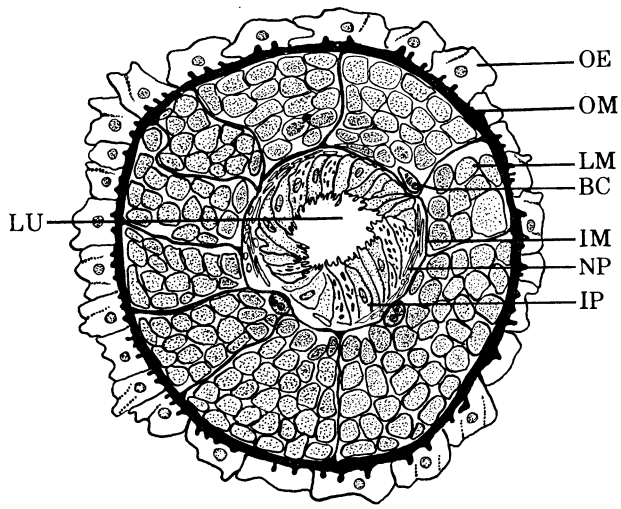

FIGURE 4. Diagram showing transverse section of the posterior proboscis.

Posterior region of the proboscis (posterior proboscis)

In *Lineus ruber* the diameter of the posterior region of the middle proboscis gradually decreases (from 1 to 0.03 mm), until, at the extreme tip, there is an abrupt change and an increase in size (diameter 0.15 mm) indicating the beginning of the posterior proboscis (figure 1). Transverse section shows that this region has the following layers (figure 4):

1. Outer endothelium
2. Outer basement membrane
3. Longitudinal muscle layer
4. Basal cells (zone of differentiation?)
5. Inner basement membrane
6. Nerve plexus
7. Inner epithelium

Unlike the endothelial cells of the anterior and middle proboscis, the outer endothelial cells are columnar and contain abundant cytoplasm (figure 24, plate 9). The nuclei of the cells are situated in the basal region. One of the peculiar characteristics displayed by these cells is their capacity for pinocytosis (figure 25). Chains of microvesicles, which appear either to be derived from the free surface of the cell or from pinocytotic channels deep inside the cell (figure 25), are arranged in rows in the cytoplasm, thus recalling the process of micropinocytosis as seen in the protozoan *Amoeba proteus* (Holter 1959). Sometimes in a chain of microvesicles (pinosomes) there may be present one or more larger vesicles, probably resulting from the coalescence of microvesicles.

The basal surface of the cell is irregular, forming a series of protuberances partially embedded or attached to the outer basement membrane. Between these 'basal feet' there are many processes, belonging to the underlying muscle cells (figure 24).

In fixed preparations the upper halves of the endothelial cells lie freely apart, but in the lower part the cells adhere to desmosome-like junctions (figure 24). The apical membrane of the cell often loses its continuity and is frequently 'torn away', though this appearance could be an artefact.

Immediately beneath the outer basement membrane is the longitudinal muscle layer. In electron-micrographs the surface of the muscle cells is very irregular, forming many interlocking cytoplasmic pouches each of which contains a few membranous structures (endoplasmic reticulum?) (figure 26, plate 10). The nucleus of the cell is often eccentrically located and contains finely granulated, homogeneous contents. Around the nucleus there are often seen numerous free ribosomes and smooth cisternae. Only one type of myofilament has been resolved (figure 26). The filaments have an average width of 18 nm and are slightly smaller than the large myofilaments of the anterior proboscis.

The muscle forms many longitudinal bundles separated from one another by connective tissue continuous with the outer and inner basement membranes. Within each of the muscle bundles, the cells cohere by the interlocking of cytoplasmic protrusions (figure 26). Desmosome-like junctions have not been seen.

Between the inner basement membrane and the longitudinal muscle layer there exist a few round or elliptical cells which appear to be undifferentiated. These undifferentiated cells have 'clear' cytoplasm which may contain a few small mitochondria (figure 27, plate 11). The nucleus of the cell has very fine, evenly dispersed chromatin; a single nucleolus is present. These cells resemble in morphology the undifferentiated interstitial cells of *Hydra* (Lentz 1965; Davis 1969). The latter have been described as contributing to the formation of many different cell types, including the myoepithelial and the neurosensory cells. In this study of the posterior proboscis, it has been possible to link up a series of different pictures which might illustrate the process of differentiation of the myofilaments within the muscle cells (figures 28, 29 and 30, plate 11). The 'differentiating' cells contain a number of membrane-limited globular bodies of various sizes which contain numerous ordered-microfilaments (14 nm) similar to the myofilaments of the muscle cells (figure 28). It seems that the limiting membranes of the larger globules gradually disappear so that the fibrillar contents of two or more globules may then appear to mingle. The larger globules could result from the enlargement of the smaller globules (figure 28). Major cytoplasmic changes during differentiation include not only the development of myofilaments but also the appearance and increase in number of both free ribosomes and endoplasmic reticulum (figure 28). Golgi lamellae have so far not been observed. On the other hand, randomly distributed microvesicles (Golgi vesicles?) are not infrequently seen.

The epithelial cells lining the lumen of the posterior proboscis consist of only one cell type (figure 32, plate 12). The cell bodies are irregular and contain the usual subcellular units.

Groups of non-myelinated nerve fibres are found between the basement membrane and the muscle cells. Many of the fibres contain both agranular and granular vesicles and may come into close contact with those muscle cells that lie closer to the lumen (figure 31). Within the nerve plexus and between the fibres there are seen a few nerve processes containing neurosecretory granules (120 to 140 nm) similar to those in the middle proboscis (figure 32 inset). Sections taken at different levels of the posterior proboscis show that the neurosecretory fibres travel towards the posterior tip of the proboscis, i.e. the junction of the proboscis and the retractor muscle. This suggests that discharge sites of these neurosecretory granules do not occur at the middle region of the posterior proboscis.

The retractor muscle

General properties

The retractor muscle is a cylindrical 'muscle cord' the anterior end of which is attached to the connective tissue (outer basement membrane of the posterior surface of the proboscis) (figures 1, 5, and 48, plate 22). The posterior end adheres to the dorsal wall of the rhynchocoel, to a layer of connective tissue, or in some sections it appears that the muscle fibres cross over the connective tissue layer and fuse with the longitudinal muscle bundles of the proboscidial sheath.

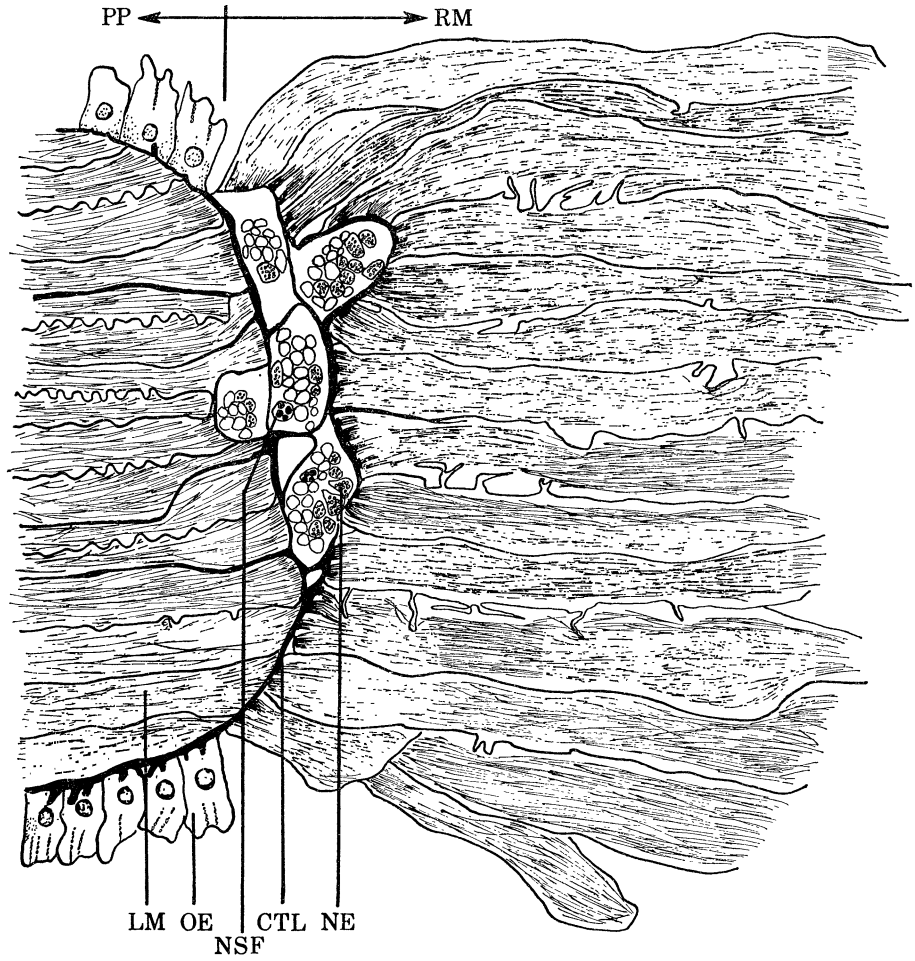

FIGURE 5. Diagram to show longitudinal section of the junction of the proboscis and the retractor muscle.

The functional organization of the muscle is of particular interest because of the enormous changes in its length as the proboscis is extruded or withdrawn. During ejection of the proboscis the muscle can be stretched to more than fifty times its original length, i.e. to approximately double the length of the animal body. Normally, the retractor muscle cord, obtained by carefully pulling the proboscis with its retractor muscle from the rhynchocoel cavity (the muscle generally becomes detached at its posterior end), measured 1 mm in length and 0.3 mm in width when fully contracted. However, these dimensions should be regarded with caution as the length of the muscle is extremely variable, especially when the measurement is done *in vitro*. It is found that both the length and the integrity of the muscle varies greatly with the surrounding

medium. Therefore to measure the length of the muscle in fixed preparations is unsatisfactory. Very often the muscle shortens freely in ordinary sea water, resulting in a balloon shape approximately 0.2 mm in length. This can be stretched mechanically, with the aid of very fine forceps or needles, to approximately 1 cm or more.

Fresh retractor muscle cord when observed under phase-contrast appears to contain many highly refractile cellular elements distributed throughout the whole length of the muscle but more prominent at the anterior region. The number of these 'dark cells' increases greatly when the muscle shortens or contracts. In a living state the muscle is more transparent than the longitudinal muscle of the proboscis. It is interesting to note that when the muscle was kept in sea water for a period of only a few minutes it started to disintegrate, especially at its 'detached end', the muscle cells (whole muscle cells or cell fragments?) simply floated away into the surrounding medium. This is apparent when the muscle is touched with a fine needle or when it is placed in an acetylcholine (ACh)-containing medium, though it should be noted that in an ACh-containing sea-water medium the length of the muscle cord remained unchanged. Attempts have also been made to stimulate the muscle with various chemical agents, e.g. oxytocin, vasopressin, lysine-vasopressin, adrenaline and atropine. Except in the case of vasopressin where the muscle did sometimes give a weak response, and oxytocin which evokes a large response, no other stimulants bring the muscle into contraction.

The muscle responds and contracts vigorously when treated with oxytocin. When oxytocin at a concentration of approximately 0.01 unit/ml was applied to the muscle or added into the surrounding medium the muscle shortens immediately, a phenomenon previously observed by Willmer (1970). Obviously the reaction of the muscle towards this peptide agent is 'reversible'. The muscle remains fully contracted as long as the 'stimulant' is present in the medium, but when the muscle is rinsed with or simply placed in clean sea water for about 5 minutes it becomes relaxed and returns to its original length. The muscle contracts and shortens again upon a second stimulation, though the response is slightly slower. The whole procedure could be repeated several times with decreasing effect until no stimulation can be effected, by this time it is likely that the muscle cells may have been poisoned or damaged.

The retractor muscle is a pure muscle cord. Unlike the longitudinal muscle of the proboscis, it is almost free from connective tissue and other cellular elements or structures. As seen in light-microscopy, the muscle lies freely in the rhynchocoel cavity. Thus, the outer muscle cells are exposed to the surrounding medium (rhynchocoel fluid), of which the ionic contents may not necessarily be similar to the blood of the worm. In some sections a few of the muscle cells are seen to have one end projecting into the surrounding fluid, though this may be artefact. All the muscle fibres are longitudinally arranged and intertwine with each other. Towards the proboscis end they are arranged in parallel. With Azan stain the anterior end of most, if not all, muscle cells are seen to anchor on a blue-staining connective tissue layer which is apparently continuous with the outer basement membrane of the proboscis. It is very difficult to estimate the approximate length of the individual muscle fibres because of their intertwining. However, there is some evidence, which will be described later, that at least some of the muscle cells have no structural relationship with the basement membrane.

Electron-microscopic structure

The relaxed muscle cells are generally cylindrical (3 to 5 μm width) and each of them contains an elongated nucleus containing finely granulated chromatin (figure 33, plate 13). The nucleus

is most often seen at the middle region of the cell body; it has a single nucleolus. Only one type of myofilament, 18 nm in diameter, has been resolved (figure 33). The filaments are not strictly parallel with each other, though they are generally longitudinally arranged. The structure and distribution of the filaments differ from those of the proboscidial muscle described previously but bear many resemblances to the retractor muscle of *Phascolosoma* (Hanson & Lowy 1960), the pharynx and penis retractors of *Helix* (Hanson & Lowy 1960), and the lantern retractor muscles of *Echinus esculentus* (L) (Cobb & Laverak 1966). There is no apparent connexion between the filaments and the cell membrane except at the anterior region of the cell, where the filaments merge with the cell membrane, forming very prominent 'attachment plaques' (figure 41, plate 18) similar to those described by Kelly & Rice (1969) in gizzard smooth muscle cells. In *Lineus*, however, attachment plaques of the myofilaments have not been found at any other regions of the cell. Again, because of the intertwining of the filaments it is not possible to assess their length nor is it clear whether all the filaments are attached to the attachment plaques. The cell membrane appears smooth, except for some regions where pinocytosis is taking place. In that case chains of pinosomes are arranged along the length of the cell beneath the cell membrane, or extend deep into the sarcoplasm and may come into close approximation with the fibril bundles or nucleus. Mitochondria are scarcely seen and Golgi complexes have so far not been encountered. At a higher magnification of electron-micrograph, free ribosomes are seen randomly distributed throughout the cell, particularly among the myofilaments. Large, circular vacuoles as big as 1 μm in diameter are often present in the cytoplasm (figure 34, plate 14). These vacuoles are electron-lucent and may resemble the potocytotic vacuoles in injured smooth muscle cells as described by Tapp (1969). The origin of these vacuoles is thus not clear, though their connexion with pinosomes is suggested. In some sections electron-lucent substances are seen in widely distended intercellular spaces (figure 34). Whether this is the product of discharged vacuoles or merely an artefact is again not known. Not infrequently, a few muscle cells appear to open into a common 'secretion pool' (figure 35), suggesting that liberation of the vacuolar product into the extracellular space is likely, though the significance of this is obscure.

The muscle cells probably adhere by means of some intercellular cementing substance, since no tight junctions nor special membrane contacts have so far been seen.

As has been stated before, freshly obtained retractor muscle often shortens freely in normal sea water or can be made to contract by stimulation. Contracted tissues could be fixed in position by applying Dalton's fixative (Pease 1964). As can be seen from thick Araldite sections stained with toluidine blue, a drastic change occurs to the arrangement of the muscle cells as the result of contraction. Some cells are tinted more intensely and these probably correspond to the dark elements as seen in the fresh tissue under phase contrast. They are thought to be the fully contracted muscle cells. The muscle cells do not contract in unison. Under the electron microscope cells can be observed that could represent either different stages or different types of contraction. These types or stages of contraction are as follows:

(1) Zig-zag or serpentine-like cells (diameter 3 to 5 μm) (figure 36, plate 15). These cells are probably in the state of passive shortening. They are packed in a more orderly fashion towards the proboscis end but show an undulating form in the posterior region. The diameter of the myofilaments (18 nm) and the distance from filaments to the cell membrane are not significantly different from those of the relaxed cells. No noticeable change occurs to the intercellular space, the density of the cytoplasm, or to the nuclear material.

(2) Concertina-like cells (the widest region of the cell is approximately 10 μm; the narrowest, about 2 μm); these are apparently fully contracted cells. Perhaps the most striking feature of these cells is that all the myofilaments clump together in a centrally placed mass in which they are randomly orientated (figure 37). The dark dense masses of the filaments probably account for the highly refractile elements as noted in fresh tissues. The filaments are so densely packed that except for occasional microvesicles (pinosomes?) a large peripheral space of the cytoplasm is completely devoid of all structure. In some sections one or two filaments may be observed to traverse towards the membrane, but no apparent connexions with the latter have been seen. Only one kind of myofilament has been resolved (figure 37) and these are about 17 to 20 nm in diameter, similar to those in the relaxed muscle cells. As the filaments are tangled together it is not possible to judge if there is any change in their length. The surface of the contracted cells is highly corrugated (figure 37) and numerous cytoplasmic projections interdigitate with those from the neighbouring cells.

Sometimes cells of an 'intermediate type' are also found; in these, the myofilaments have contracted to an elongated core of longitudinally orientated myofilaments (figure 38, plate 16). The core has a lower density than that of the concertina-like cells, apparently because the myofilaments pack less tightly. Also the surface of these cells is less infolded.

On one occasion a cell of yet another kind has been observed (figure 39). In this case the cell appears to be 'partially' contracted and therefore could also be considered as an 'intermediate type'. In this, the clumped appearance of myofilaments which suggests contraction is found at one end of the cell, while at the other end the myofilaments remain widely dispersed like those of a 'relaxed' cell. Also, numerous microvesicles, presumably pinosomes, are seen in the 'contracted end' but not at the 'relaxed end'.

It is likely that the proportion of 'contracted' to 'relaxed' muscle cells varies with the functional state of the whole muscle bundle. More and more muscle cells are brought into function when the muscle is excited. In oxytocin-treated tissue the contracted or concertina-like cells greatly increase in number, and these, unlike the freely contracted cells which show corrugation of the surface, are also extremely active in pinocytosis (figure 40, plate 17). At the same time, the zig-zag cells also increase in number, suggesting that the cells contract asynchronously. High concentrations of oxytocin, however, do not produce a 'complete' contraction but are deleterious to the cell structure. The outer membrane of the outer muscle cells directly in contact with the agent sloughs off, and part of the central fibrillar cores then become exposed.

Innervation

In this study attention has specially been placed on the problem of how the muscle is controlled. Willmer (1970) thought that the muscle might be regulated by some specific chemical transmitter (e.g. oxytocin-like substances) via the rhynchocoel fluid from the central nervous system or other sources. In the present investigation it was found that the retractor muscle gave a negative reaction in the test for AChE activity. Also, the muscle did not contract when stimulated with ACh (see p. 12). These observations suggest that the retractor muscle cells are not directly controlled by the cholinergic fibres. However, high activity of AChE was found at the posterior region of the proboscis, but anterior to the connective tissue layer which 'separates' the retractor muscle from the proboscis proper. The significance of this will be discussed later.

In electron-microscopy large bundles of non-myelinated nerve endings are found closely adjacent to the connective tissue layer (figure 41, plate 18) but these lie on the opposite side of the

connective tissue layer from the points of attachment of the retractor muscle cells. At some regions the connective tissue layer becomes flattened and thinned, or loses its continuity so that the nerve endings then can come into close contact with the muscle membrane, from which it is separated by a very narrow extracellular gap (figure 41). These nerve endings are probably aminergic since two types of synaptic vesicles—normal clusters of 20 to 50 nm vesicles and dense

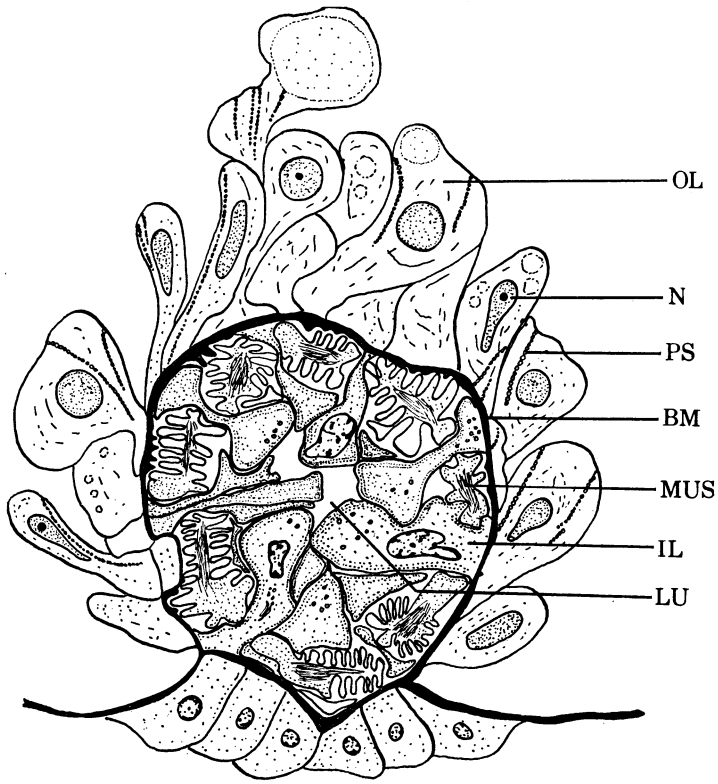

FIGURE 6. Diagram to show the 'rhynchocoel villus' on the floor of the rhynchocoel (squared area, figure 43, plate 20).

core vesicles (50–100 nm)—are present in the presynaptic knob (figure 41). An occasional dense body (diameter 320 nm) which is very characteristic in structure is present among the synaptic vesicles close to the synaptic junction (figure 41), though whether this has anything to do with the release of a neural transmitter is not clear. Among the more usual nerve fibres which contain typical synaptic vesicles there are sometimes seen one or two nerve fibres containing even larger granules (140 nm) (figure 42, plate 19). These are probably neurosecretory granules since they are morphologically similar in every respect to the neurosecretory granules found in other animals (Gabe 1966).

The rhynchocoel system

Rhynchocoel fluid

The rhynchocoel fluid is colourless and constitutes about one-quarter of the body volume of the creature. As much as 0.005 ml of the fluid can be obtained from a large worm. The Na^+ and K^+ contents of the fluid have been analysed. The result indicates that the fluid contains less sodium (about 300 mmol/l) than sea water (460 mmol/l). On the other hand, the potassium content of the fluid is more or less similar to that of sea water. The data, so far as they go, suggest that the composition of the rhynchocoel fluid is regulated.

The rhynchocoel villus

The rhynchocoel villus is a tubular structure on the floor of the rhynchocoel (figures 1, 6). The structure was noted as early as 1895 by Bürger and given the name 'dorsal blood vessel'. Pantin (1969) also noted a similar structure in *Geonemertes* and called it a 'vascular plug'. The structure has recently been described by Willmer (1970) and given the name 'rhynchocoel villus'.

The rhynchocoel villus in *Lineus ruber* is an extension of the mid-dorsal blood vessel, which penetrates dorsally through the proboscidial sheath and enters the rhynchocoel. After extending to almost one-third the whole length of the animal body the villus repenetrates the proboscidial sheath at the floor of the rhynchocoel and continues backward as the dorsal blood vessel above the intestine. The villus is lined inside and out by endothelial cells of different kinds on layer of basement membrane (Ling 1970; Willmer 1970) which is more evident on some occasions, e.g. when the villus is constricted.

In electron micrographs cells of another type have been revealed. These are muscle cells; they exist as isolated units, and are arranged in a circular manner between the basement membrane and inner lining cells around the lumen of the villus (figure 43, plate 20). The contraction and relaxation of the muscle cells apparently bring about the constriction and dilatation of the lumen. When contracted the muscle cells have an appearance similar to the contracted (concertina-like) cells in the retractor muscle cord (figure 43).

The lumen of the tubular organ is sometimes widely distended, whereas sometimes it is hardly discernible. An attempt was made to find out if there is any functional relation between the villus and the proboscis. Several specimens were examined for this purpose and it is possible to draw the conclusion that when the proboscis is extruded, the villus becomes widely distended (figure 46, plate 21). The villus becomes fully blown up and then appears as a balloon-shaped structure in transverse section of the worm. Conversely, it is flattened and contracted when the proboscis lies within the rhynchocoel cavity (figure 47). In this case the lumen of the villus may be completely obliterated.

Some problems in the regeneration of the proboscis

Coe (1934) found that during the regeneration of the proboscis the muscular and connective tissues are supplied by new mesenchyme cells which migrate in from the cephalic tissues at the time when the new nerves grow in from the brain. According to him, the basal stalk of a torn proboscis is capable of extraordinarily rapid growth. The tissues present in the basal stalk, supplemented by the proliferation of epithelium from the rhynchodaeum, form the new epithelium.

The present observations showed a few undifferentiated and differentiating cells between the epithelium and the muscle layer of the posterior proboscis. The origin of these undifferentiated cells is not clear, though they could be derived from the lining cells of the rhynchocoel, which are apparently also of mesenchymal origin, Indeed, it has been found in the present investigation that the lining cells of the rhynchocoel, especially those of the dorsal wall, are capable of reconstructing a new proboscis epithelium by rapid mitosis and proliferation (figure 50, plate 22). The mitotic cells appear to migrate into the rhynchocoel and accumulate there to form a tubular structure (proboscis). That a new proboscis could easily be restored by such a simple means is probably an adaptation to cope with the behaviour of the worm, since its proboscis is very liable to accidental detachment or loss.

Discussion

Accounts of the eversion mechanism of the proboscis given by the many classical as well as more recent investigators have usually stressed that the contraction of the muscles of the body wall exert pressure on the walls of the rhynchocoel cavity and thus would increase the hydrostatic pressure in the cavity and force the proboscis to evert. At first sight, this increase of pressure seems to be the most reasonable explanation for the ejection of the proboscis. However, the ejection of the proboscis is a rather subtle process by which the tubular organ turns completely inside out as the pressure in the rhynchocoel increases, and several vital problems are as yet unsolved. For example, how could the pressure in the rhynchocoel be maintained constant after the proboscis had been ejected and when the muscles of the body wall relax? When the muscles of the body relax, it is likely that the rhynchocoel volume might increase and there would certainly be a decrease of pressure in the rhynchocoel cavity. One would then expect, unless the resulting decrease in pressure in the rhynchocoel is compensated by some other mechanism, that the proboscis would be drawn back into the rhynchocoel immediately or have great difficulty in maintaining ejection. The rhynchocoel pressure might be maintained constant by continued contraction of the muscles of the body wall for as long as the proboscis remained outside the body. If this were so the animal would remain rigid and/or absolutely motionless. This, however, does not seem to be true (see pictures in Pantin 1950). It has been stated (Wilson 1900; Dakin & Fordham 1936) that the proboscis of the nemertine is, on some occasions, employed to help in burrowing, so that movement of the worm with an ejected proboscis is certainly feasible. Besides, it was noted in the present investigation that a nemertine with an everted proboscis does not become rigid, and it appears safe to assume that the muscles of the body wall, which help to force the proboscis out of the body cavity, are not required for the maintenance of the rhynchocoelic pressure once the proboscis is ejected. Pantin (1950) in *Geonemertes dendyi* noted that when the animal was stimulated to extrude the proboscis (this could be done by strong handling of the creature, e.g. tail stimulation), there was a sudden simultaneous contraction of the whole of the circular muscle of the body. The circular muscles became apparently relaxed when the proboscis had been ejected.

What then is the mechanism by which the pressure of an 'empty' rhynchocoel (worm with an ejected proboscis) is maintained unvaried when the muscles of the body wall become relaxed? In answer to this, attention is drawn to the rhynchocoel villus, the size of which, as was noted, varies according to the extent of ejection of the proboscis. The dilatation of the villus during the ejection of the proboscis could compensate for any decrease of pressure in the rhynchocoel when the muscles of the body wall reduce their tension (see p. 16). It may be important in this connexion to mention that when the villus is dilated its wall becomes thinned and flattened. The lining cells and the basement membrane are stretched considerably, so that they all appear tenuous in section. It is not known whether the dilatation of the villus results from the relaxation of the muscle cells of the villus, or whether there is a direct flow of fluid from the dorsal blood vessels into the villus, though both could play their part. It was thought (Ling 1970) earlier that the villus is a passage by which some hormonal substances from the glandular cells of the neuroendocrine cephalic organs may enter the rhynchocoel when the animal is subjected to osmotic stress. These neuroendocrine substances could be concerned with the permeability of the villus. This suggestion seems to gain immediate support from the fact that the cephalic organ of the nemertine *Geonemertes chalicophora* (J. Moore 1970, personal communication) does

not contain any glandular elements and that in this worm the 'vascular plug' (rhynchocoel villus) is apparently absent.

It is generally supposed that the return of the proboscis is initiated by the contraction of the retractor muscle which pulls the tip of the proboscis back into the body cavity. This obviously does not apply to some nemertines, e.g. *Cerebratulus lacteus* (Coe 1895) in which the retractor muscle is absent. The present observation suggests that two other mechanisms may participate in the introversion of the proboscis. First, a direct contraction of the anterior proboscis, which is composed mainly of longitudinal muscles, could according to the resultant force from the contraction of the muscles involved, drag the proboscis into the rhynchocoel cavity. The process could be assisted by the second mechanism, i.e. by lowering the pressure in the rhynchocoel, which could be accompanied by an outflow of the blood from the rhynchocoel villus into the dorsal and lateral blood vessels. The large capacity for dilatation shown by the vascular spaces round the dorsal surface of the foregut might be important in this connexion.

The morphology of the proboscis in *Amphiporus lactifloreus* resembles that of *Lineus ruber* (Ling, unpublished observations), and the common structural pattern probably facilitates the eversion and the introversion of the tubular organ with the least expenditure of energy. The anterior region of the proboscis has fewer cellular elements than any other region, almost complete absence of circular muscle fibres, and fewer connective tissue elements, e.g. collagen fibres. This organization would allow it to bulge out whenever there was a slight increase of hydrostatic pressure in the rhynchocoel cavity.

The occurrence of rhabdites in the proboscis was noted by Gontcharoff (1957) and Jennings & Gibson (1969), and their possible function has also been suggested. Jennings & Gibson suggested that the rhabdites may serve to increase the grip of the proboscis on the prey. This seems most likely, since especially the partially discharged rhabdites could serve this purpose rather effectively. Though, so far, it has not been possible to find any synaptic junctions between the rhabdite cells and the underlying nerve fibres, this does not rule out the possibility of a sensory function of the rhabdite cells. The presence of a single cilium with its specialized associated structures at their apical surface suggests this as a possbile site of sensory reception (mechanoreceptor, chemoreceptor, or tactile receptor). Moreover, the partially extruded rhabdites might also act as sensory 'levers'.

Electron-micrographs showed that the rhabdite cells have a well-developed endoplasmic reticulum confined to the basal region of the cells. Its close relation with the newly formed rhabdites suggests that this is the site concerned with some aspect of rhabdite formation. Skaer (1961) and Lentz (1967) in Rhabdocoela described that flattened vesicles (Golgi lamellae?) are also involved in rhabdite formation. In *Lineus*, newly formed rhabdites are transported towards the apical border of the cells and become fully 'differentiated' in the course of this migration.

Although the rhabdites are different structurally from those in Rhabdocoela (Torok & Röhlich 1959; Klima 1961; Pedersen 1963; Reisinger & Kelbertz 1964), their histochemical tests are more or less consistent: strongly acidophilic and stained intensely with orange G and eosin. Skaer (1961) mentioned that rhabdites are organelles of purine excretion. This being so, it is hard to understand why the rhabdites are built up in such an elaborate pattern. Though it may not be a normal process, on some occasions the contents of the discharged rhabdites in *Lineus* pass into the surrounding medium when the whole structure collapses. This may suggest that rhabdites also form part of mucus secretion. The secretion in some species is toxic and may

help to capture a prey. Jennings & Gibson (1969) described that a prey of nemertines could be paralysed soon after being entangled by the proboscis.

Except for one or two nerve endings containing granules of very large size, the retractor muscle appears to be innervated almost exclusively by aminergic nerve fibres from the dorsal cerebral ganglia via the proboscis proper. The presence of aganular vesicles mixed with dense core vesicles is apparently typical of an aminergic nerve ending (Richardson 1962, 1964; V. Navaratnam, personal communication 1970). This interpretation, however, has not been supported by the experimental work (see p. 12), since the muscle did not contract on stimulation. with adrenaline. It was noted that the junction of the proboscis and the retractor muscle also shows AChE activity. This strongly suggests that cholinergic nerve fibres are also present. Cytochemical methods for the demonstration of AChE at the ultrastructural level would help to elucidate the actual location of the AChE activity.

The apparent presence of neurosecretion in some of the nerve endings as far as the posterior proboscis and close to the retractor muscle suggests a peptidergic type of innervation. This would explain the muscle's specific sensitivity of octapeptides, e.g. oxytocin. It is by no means suggested that oxytocin or vasopressin is the actual product of this neurosecretion which functions as a retractor-muscle excitant. It is clear, however, that since oxytocin causes the muscle to contract, and since the neurosecretory fibres which emanate from the cerebral ganglia travel a long distance to the junction of the proboscis and the retractor muscle, then there is probably a relationship between the two systems. Moreover, the development of a suitable vascular connexion (rhynchocoel) around the neurosecretory endings suggests a neurosecretory release into the rhynchocoel fluid. If this neurosecretory product is a chemical messenger (oxytocin, vasopressin or one or their analogues), it could alter the activity (e.g. ion-pumping systems) of the lining cells of the rhynchocoel, i.e. outer endothelial cells of the proboscis, outer lining of the rhynchocoel villus, the lining cells of the rhynchocoel wall and the retractor muscle cells. The connexion between neurosecretory material and permeability changes of cell membranes has been fully established (Gabe 1966).

The whole system therefore is constructed in a manner rather similar to the neurovenous tissues of the cephalopods (Martin 1966, 1968; Young 1970). These are concerned somehow with regulation of the volume, composition and disposition of the body fluids.

Further information which may be important in this connexion is that the retractor muscle cord is very 'brittle', disintegrating when placed in a sea-water medium. This would suggest that the ionic composition of the rhynchocoel fluid, in which the retractor muscle is freely exposed, is different from that of the external medium. This is substantiated by such chemical analysis as has so far been possible, for this has shown that the sodium content of the rhynchocoel fluid is apparently lower than that of the external medium. It may be that the whole of the regulatory system functions constantly so that appropriate adjustments of the internal environment could be maintained. It is not known how this regulation could be achieved on occasions when the proboscis is discarded or lost. Vigorous contraction of the animal body under toxic stimuli can force the proboscis to extrude. In this case, the anterior 'hinge' of the proboscis and the posterior connexion of the retractor muscle are often broken. Under such conditions the 'barrier' between the rhynchocoel fluid and the external medium breaks down. The sudden change of environment probably 'activates' the lining cells of the rhynchocoel wall. The latter, as was noted earlier, are capable of reconstructing a new proboscis.

Looking at the muscular system of the proboscis apparatus, one is impressed by its diverse

structural patterns in the different segments of the proboscis. The muscles in the anterior and posterior proboscis show some similarity, i.e. normally two types of myofilaments of size about 5 and 20 nm, respectively, are present in these cells. In the posterior proboscis and the retractor muscle, however, only one type of myofilament (18 nm) is present. The muscle cells of the anterior, middle, and posterior proboscis are all separated into bundles and are enlcosed in connective tissue layers, whereas the retractor muscle is free from connective tissue. The calling of the different patterns or different types of myofilaments is not clear, nor is it known if these different sizes and types of myofilaments are important with regard to the part they play in the function of the whole proboscis, e.g. in determining the type of contraction of the various muscle cells.

A remarkable contraction mechanism has been observed in the retractor muscle. On the evidence of the special morphological appearance of the muscle cells, it can be postulated that the contraction mechanism is unique, and appears to be difficult to reconcile in some respects with a sliding-filament mechanism of contraction. Unlike striated muscle (Huxley & Hanson 1960), in which contraction and relaxation are reflected only in changes in the spatial arrangement of two different kinds of existing filaments, or the vertebrate smooth muscles (Kelly & Rice 1969) of which the contraction mechanism may involve aggregation and dispersal of the myosin filaments, the observations here suggest that the contraction of the retractor muscle involves not only the aggregation but also the contraction of myofilaments of a single type to form a compact mass at the centre of the cell followed by a 'deformation' of the entire cell resulting in the corrugation of the cell surface (concertina-like cells). The observations in this study also indicate the constant diameter of the myofilaments during contraction. With this in mind, together with the fact that a contracted muscle cell usually has a very low electron-density in its peripheral cytoplasm, it is suggested that during the process of contraction a 'centripetal' cytoplasmic flow occurs. This is probably coupled with the self-contraction of the myofilaments and the processes bring all the cytoplasmic structures together in a central mass. The whole of the contraction mechanism may thus seem unorthodox, but it is not impossible that a rather specific contraction process has evolved for this highly extensible and contractile retractor muscle.

Several of the above-mentioned problems, particularly the innervation of the retractor muscle, deserve further morphological and cytochemical studies. The present work has, however, laid down the first stepping-stone for further study of the behaviour of the proboscis and also of its very peculiar muscle.

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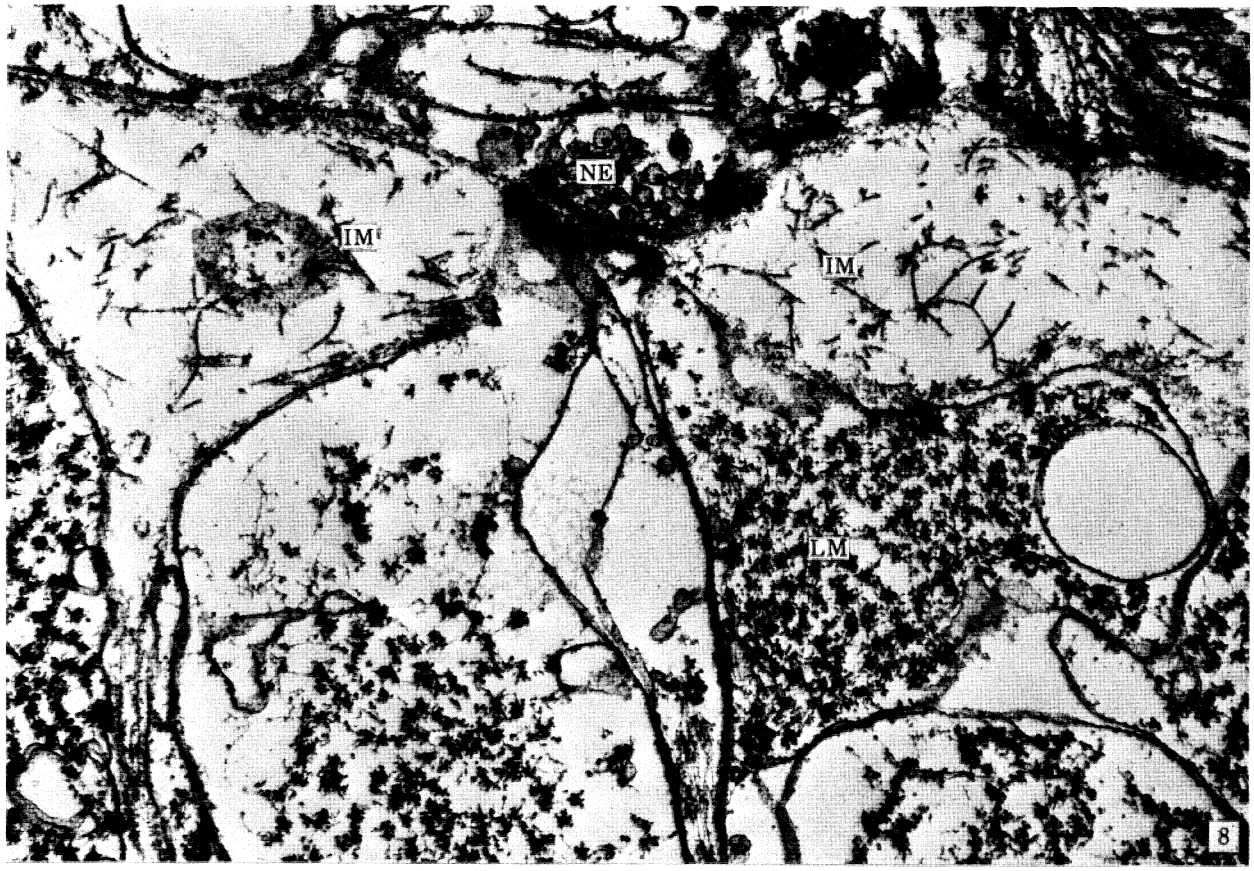
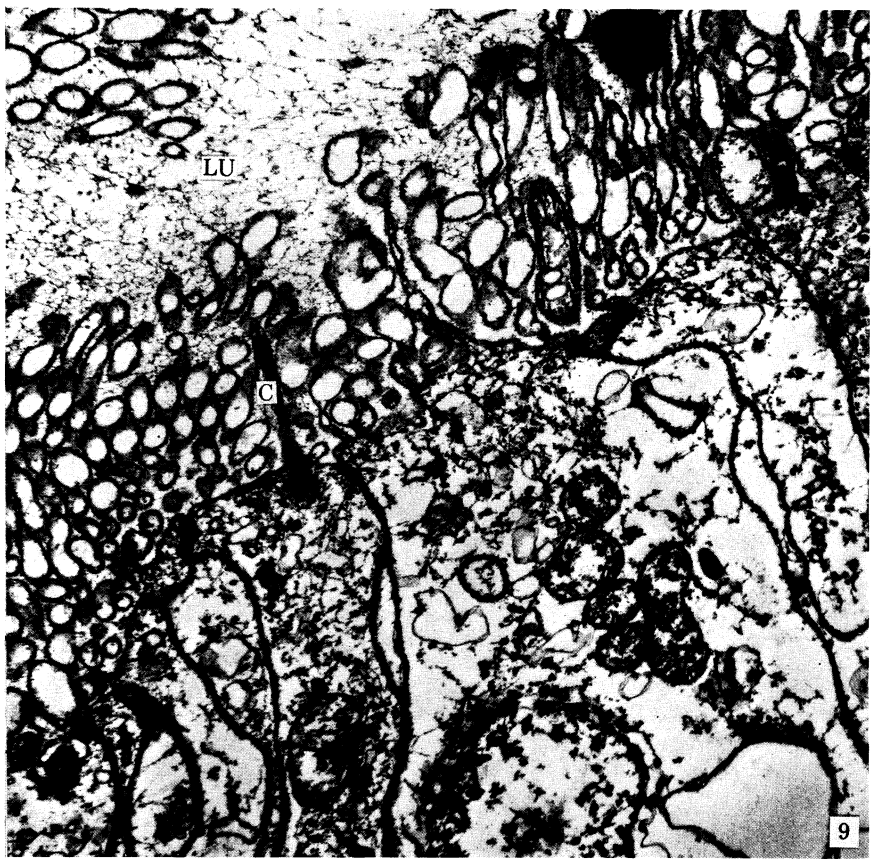
FIGURE 7. Transverse section of the anterior proboscis. An endothelial cell is seen to bear a single cilium projecting into the rhynchocoel. The figure (see inset) also shows that the circular muscle fibres contain two types of myofilaments. (Magn. × 15000.)

(*Facing p.* 22)





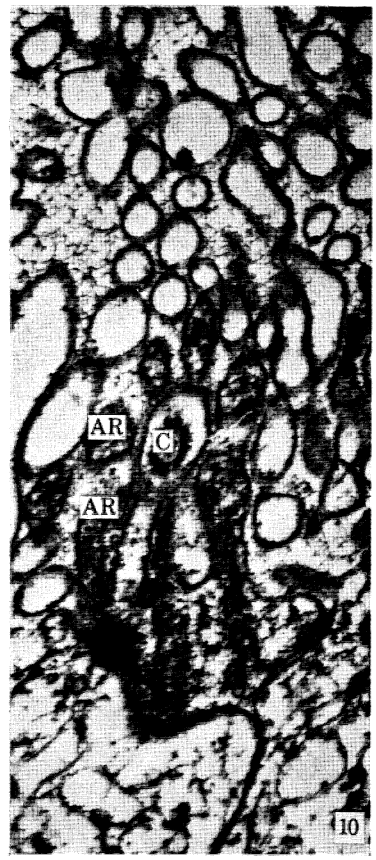
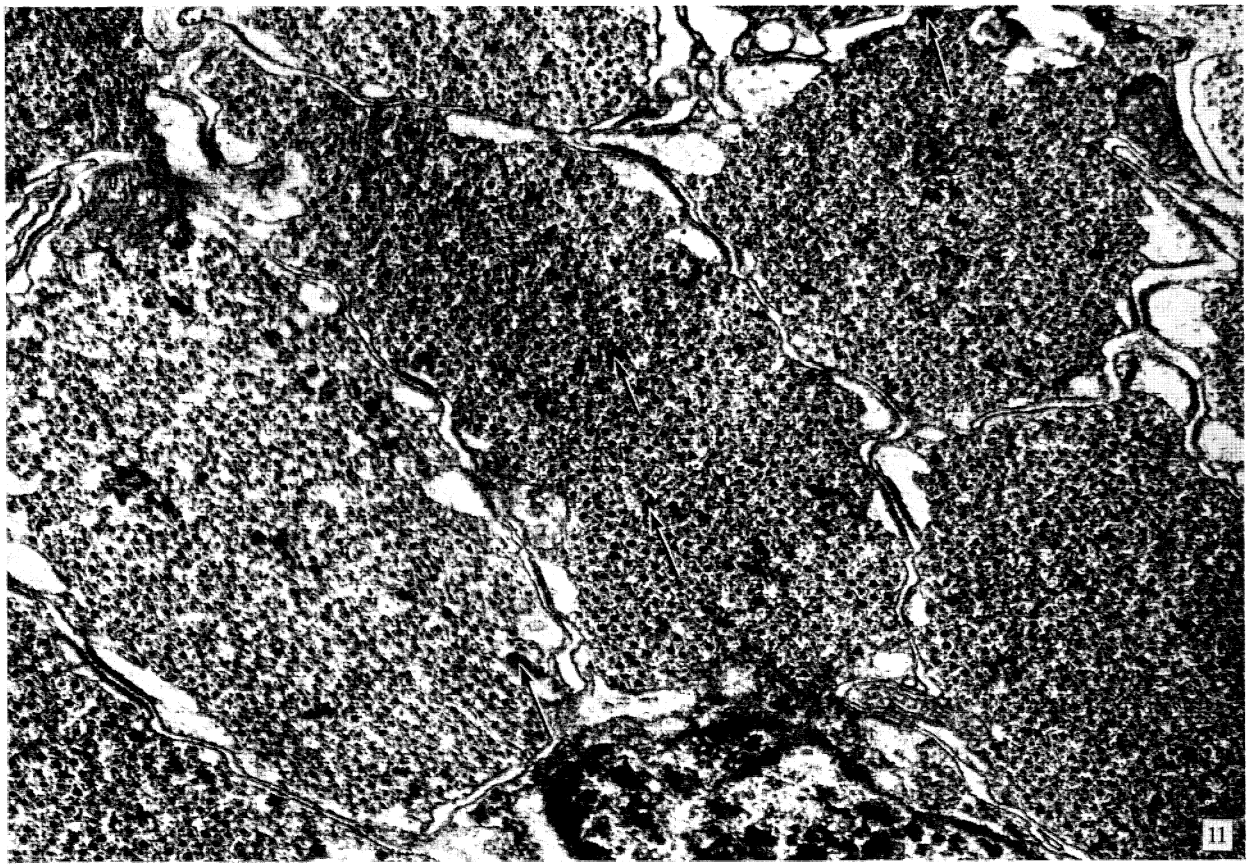
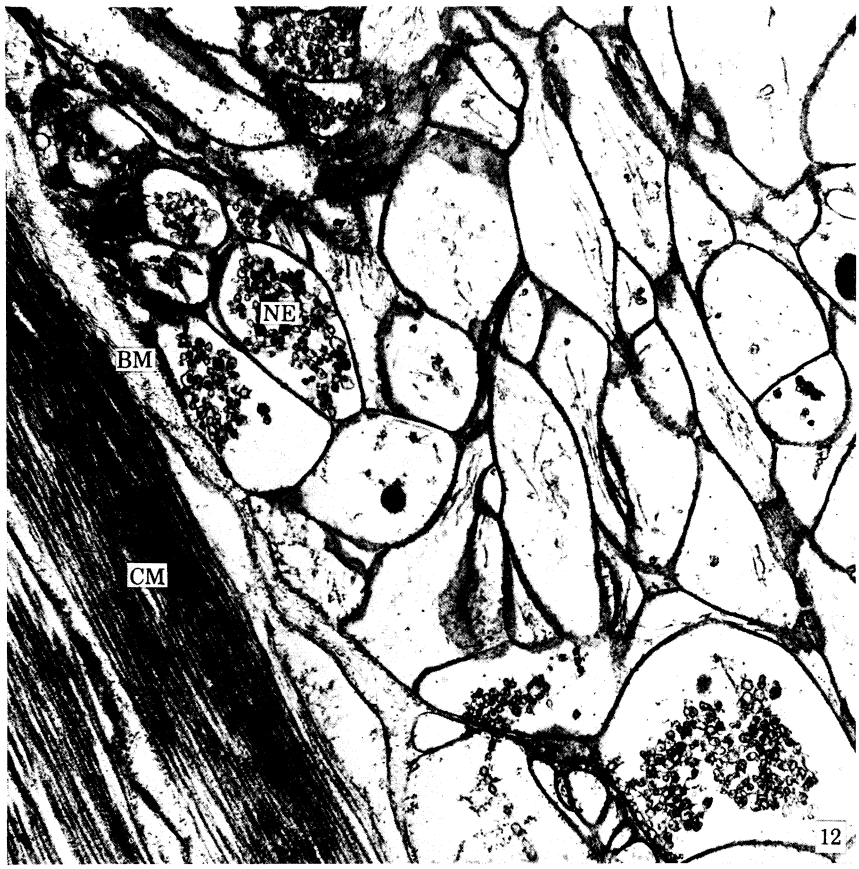


FIGURE 8. The 'innervation' of the longitudinal muscle of the anterior proboscis. The muscle cells contain two types of myofilaments. Note that two types of synaptic vesicles (granular and agranular) are present in the nerve endings. (Magn. × 27 500.)

FIGURE 9. The inner epithelium of the anterior proboscis. Each of the epithelial cells is seen to bear a single cilium. (Magn. × 14 000.)

FIGURE 10. Transverse section of the cilium on the apical surface of an epithelial cell of the anterior proboscis. (Magn. × 18 000.)





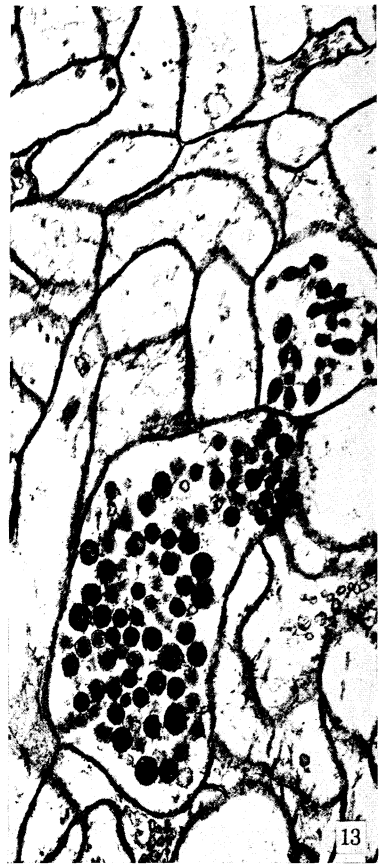
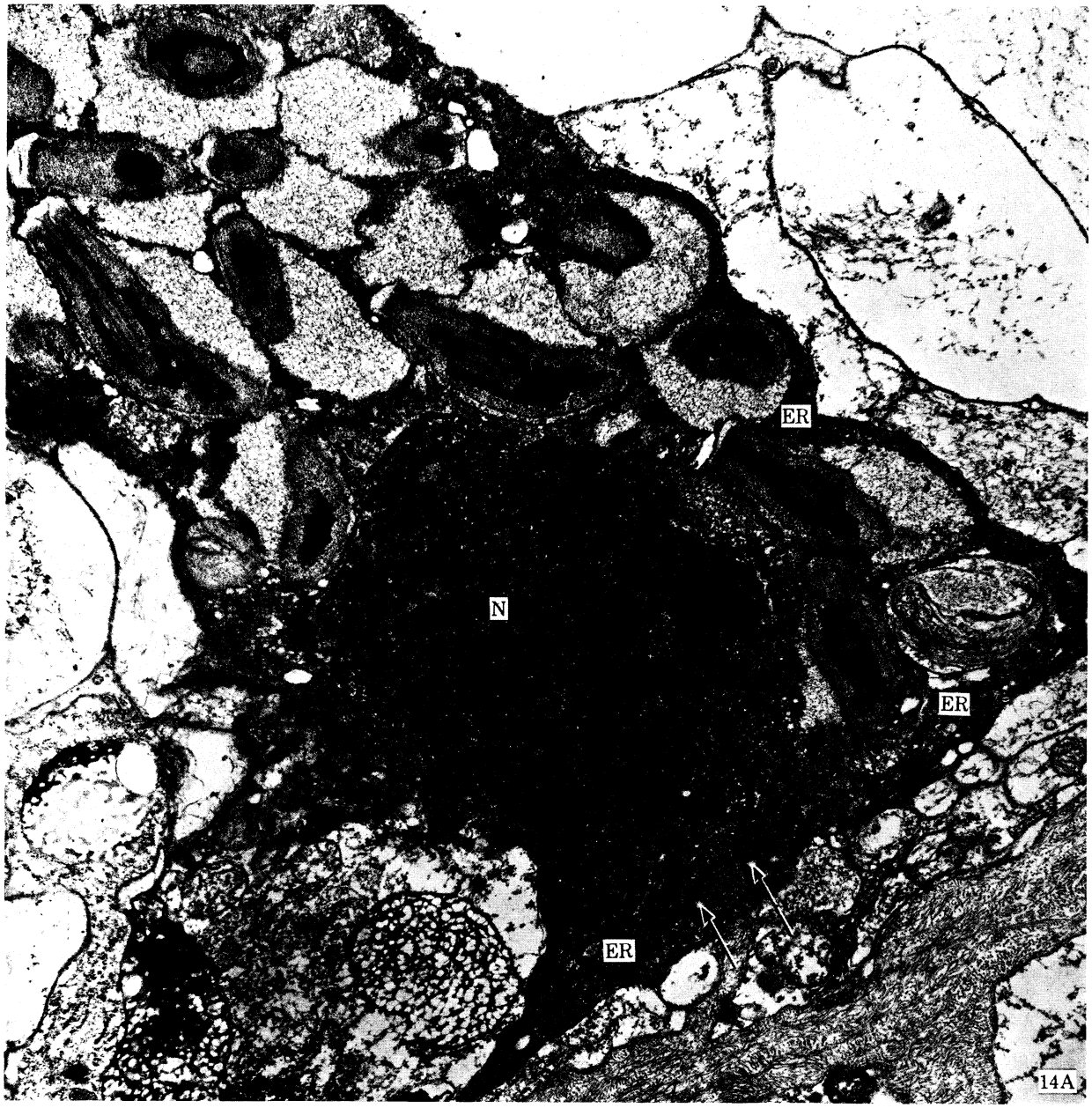


FIGURE 11. Transverse section of the longitudinal muscle of the middle proboscis. The muscle cell contain two types of myofilaments. Arrows indicate 'irregular dense bodies' (see text). (Magn. × 40 000.)

FIGURE 12. Transverse section of the middle proboscis. Note many nerve endings are seen closely applied to the surface of the circular muscle cell. (Magn. × 22 000.)

FIGURE 13. Two nerve fibres loaded with neurosecretory granules are present in the neuropile of the nerve at the middle proboscis. (Magn. × 22 000.)



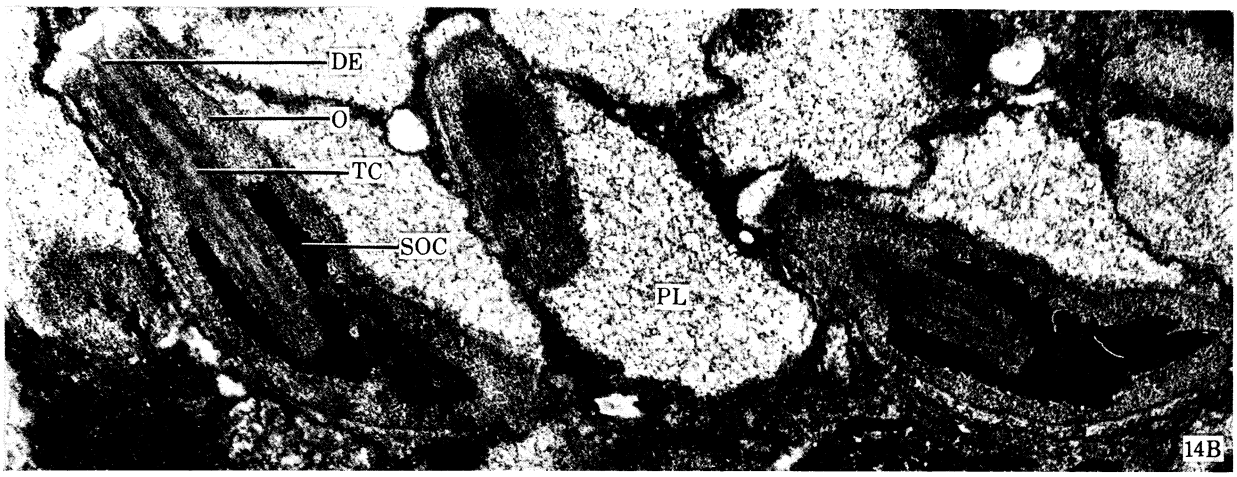
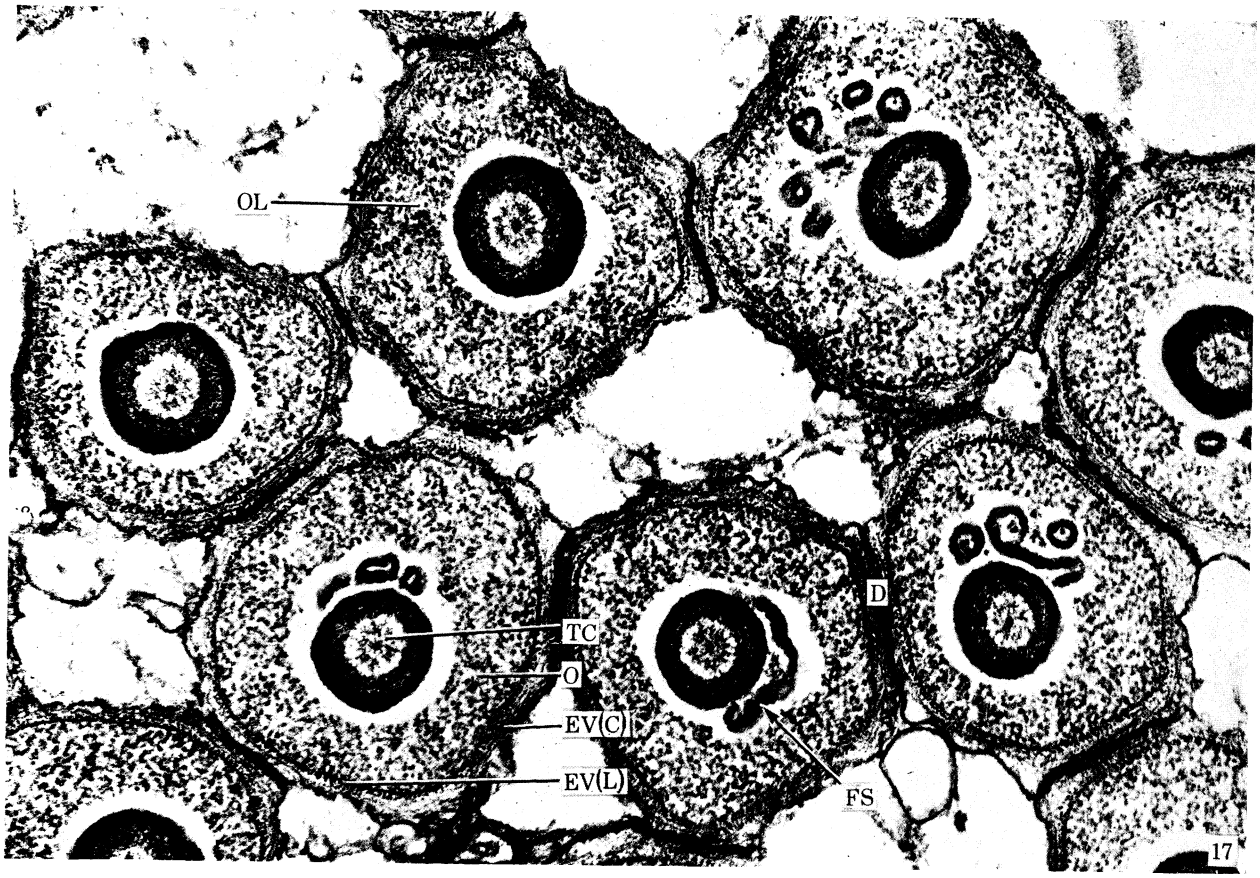


FIGURE 14. A rhabdite-forming cell. Arrows indicate electron-translucent secretion, similar to the material which builds up the electron-translucent 'pool', in the dilated cisternae of endoplasmic reticulum. (Magnifications: *A*, ×15000; *B*, ×26000.)

FIGURE 15. The apical surface of a rhabdite cell. A single cilium with its associated structures, is seen to project into the mucus-filled lumen. (Magn. × 6500.)

FIGURE 16. Fully differentiated and partially discharged rhabdites at the apical surface of the rhabdite cells. Note that the central core of the rhabdite is filled with electron-translucent substance (arrows). (Magn. × 24000.)



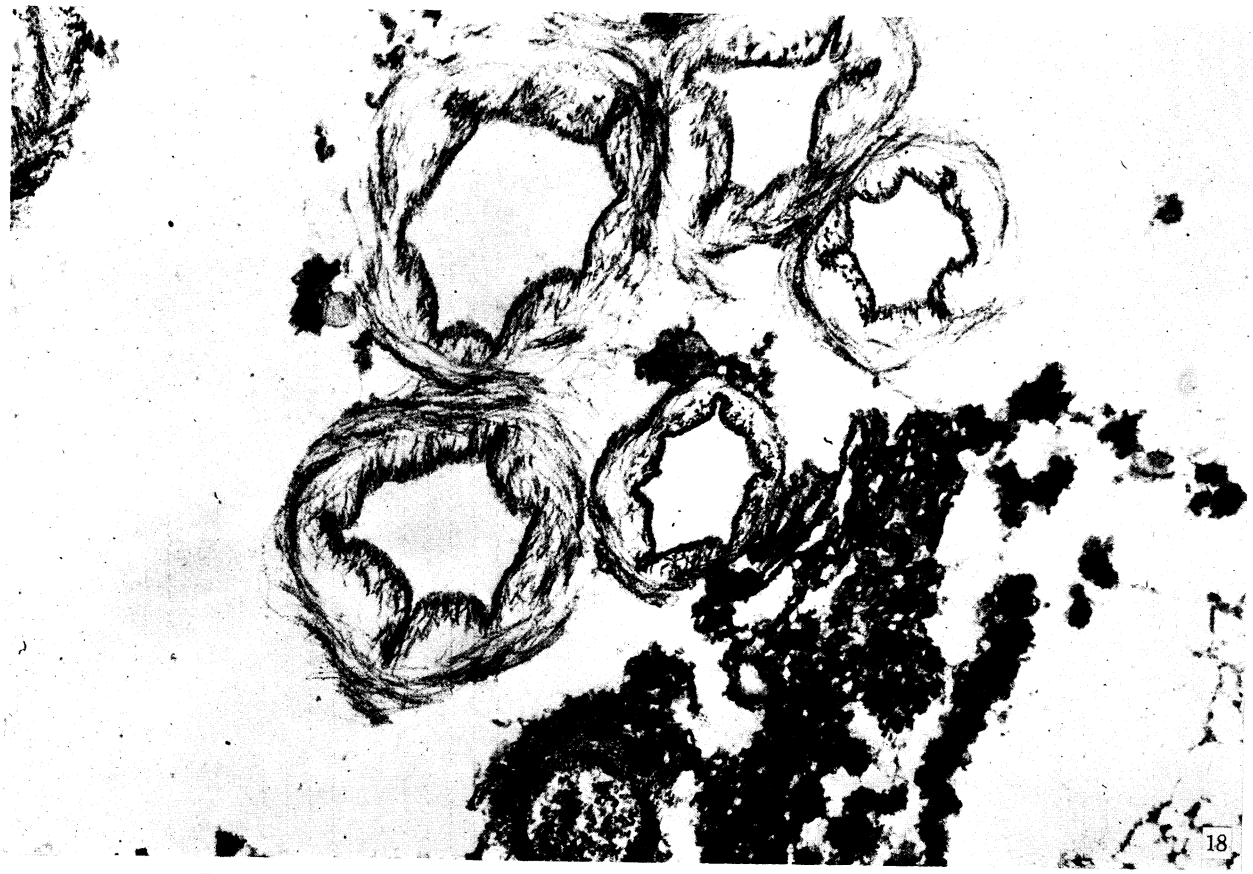
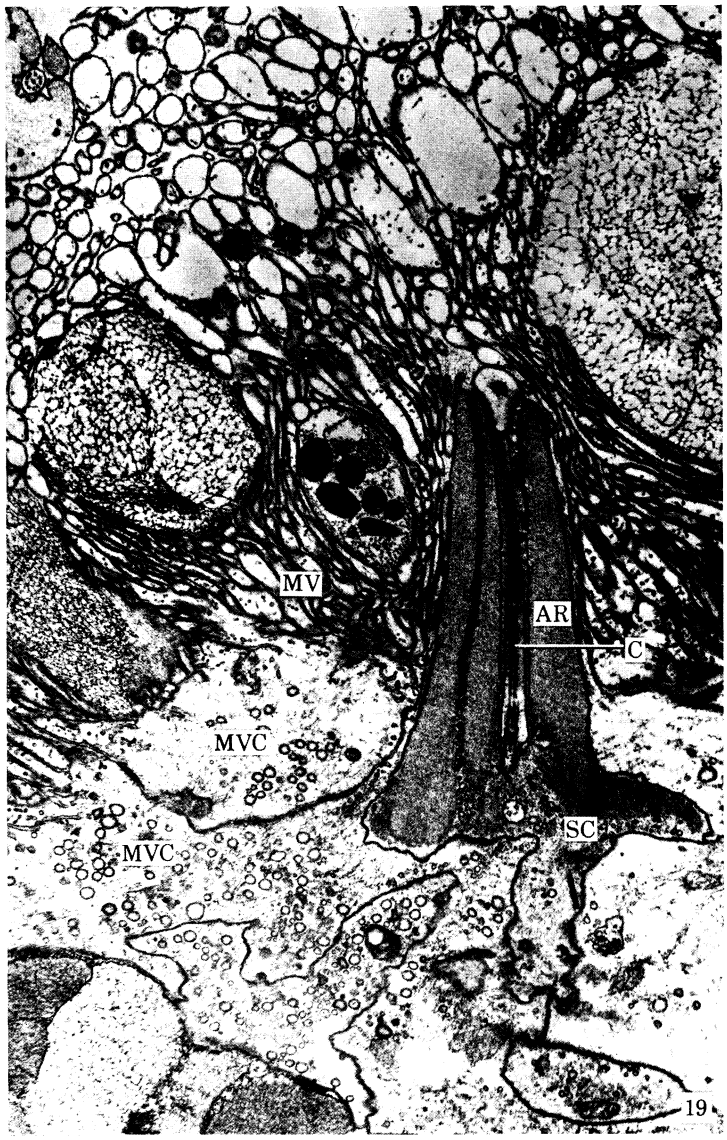


FIGURE 17. Transverse section of discharged rhabdites (see text). (Magn. ×40000.)

FIGURE 18. Transverse section of discharged rhabdites (see text). (Magn. ×40000.)



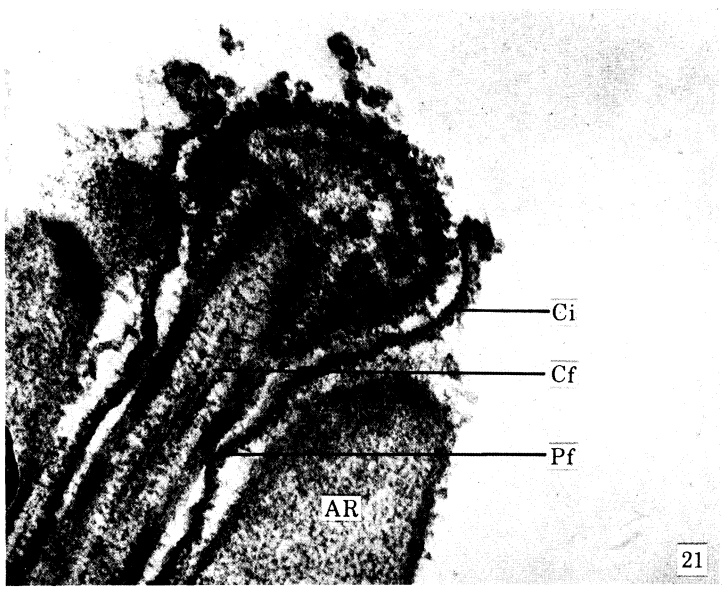


FIGURE 19. 'Sensory' cell and major lining cells (cells with well-developed microvilli). The sensory cell is seen to bear a single cilium. (Magn. × 8000).

FIGURE 20. The cilium of a sensory cell is enclosed by seven 'accessory rods'.

FIGURE 21. The distal tip of a cilium of a 'sensory cell'. (Magn. × 55000.)

FIGURE 22. The 9+2 fibrillar pattern of the cilium of a 'sensory' cell. (Magn. × 40000.)

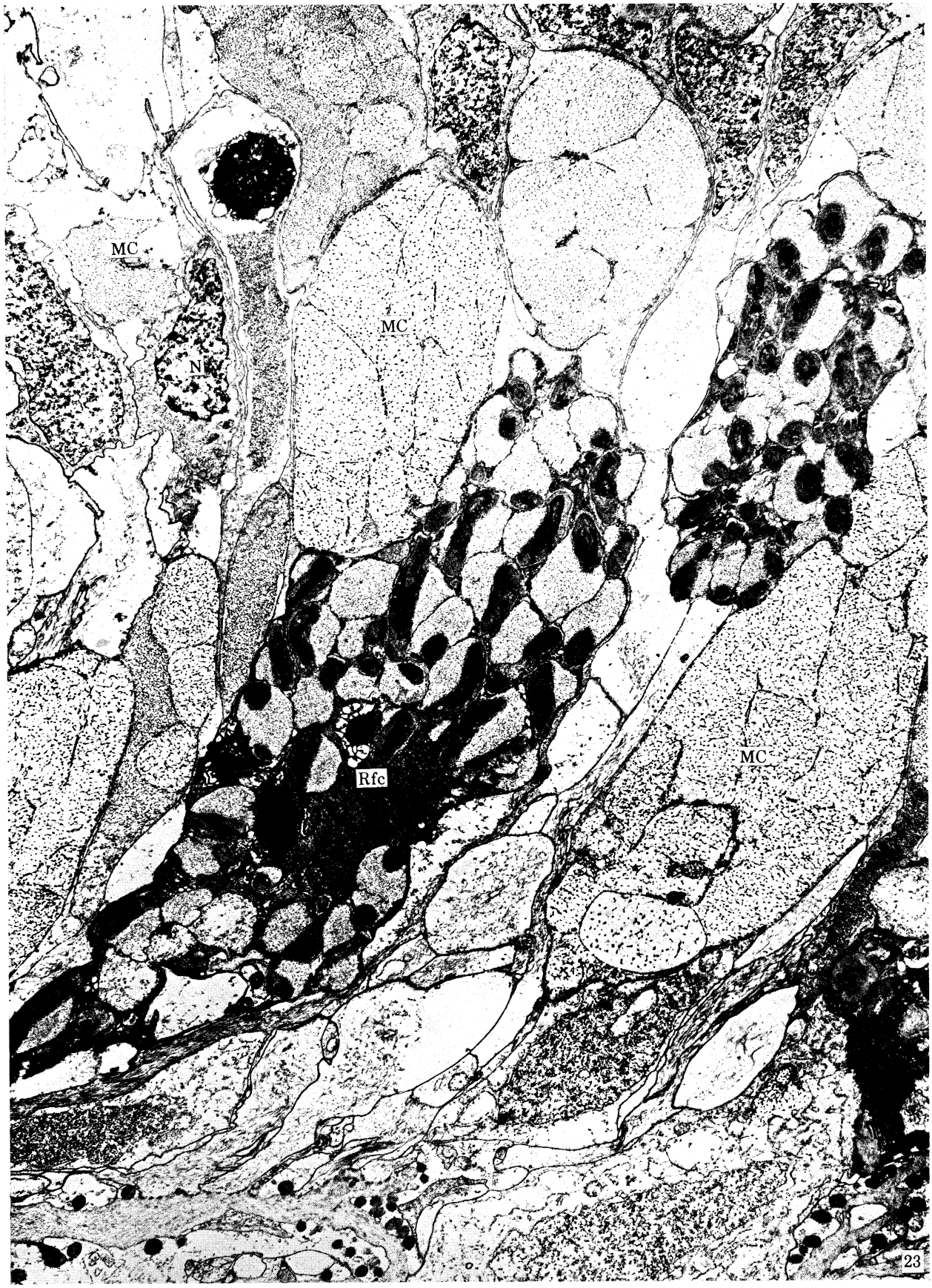
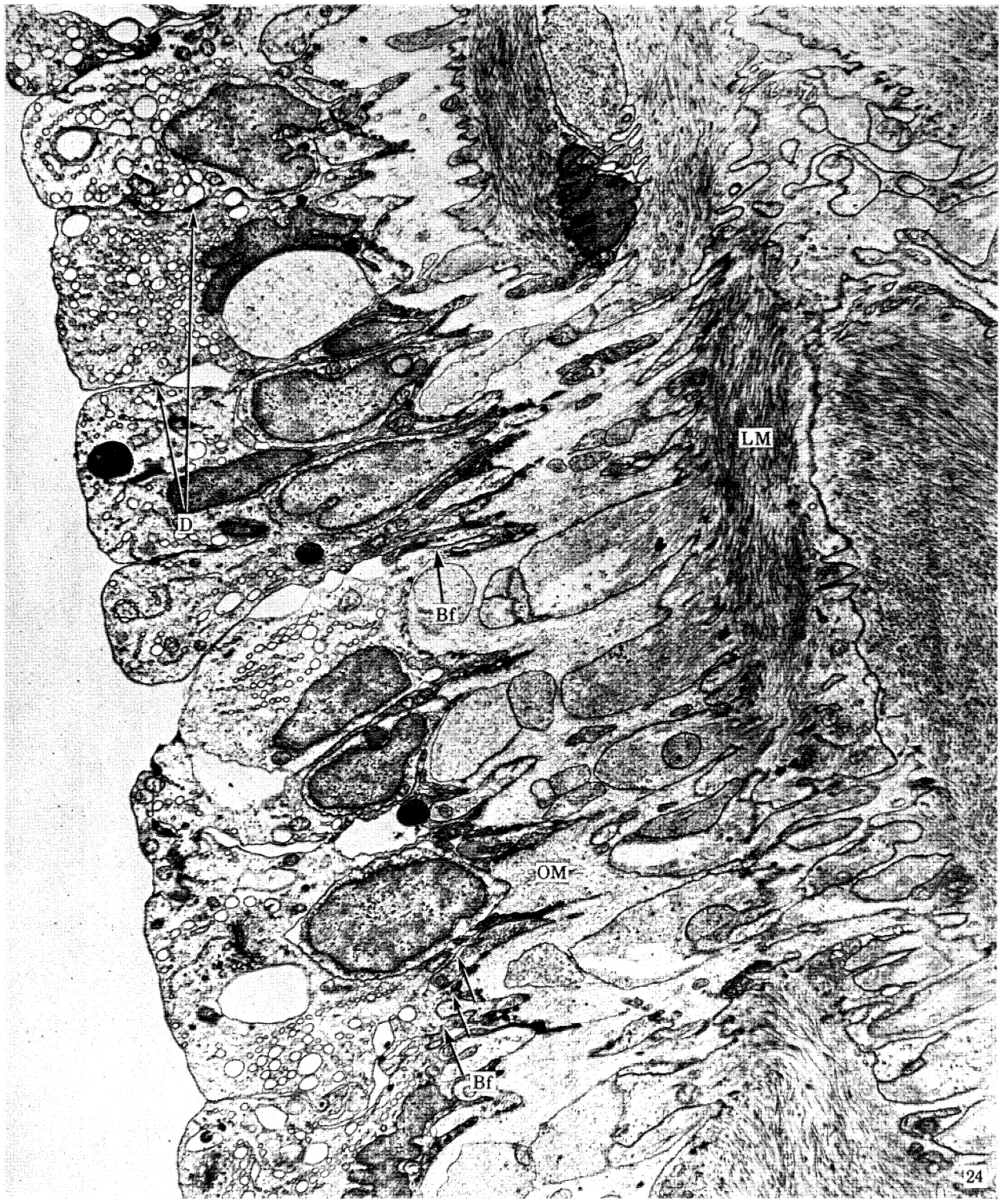


FIGURE 23. Mucus-secreting cells at the middle region of the proboscis. (Magn. × 5400.)



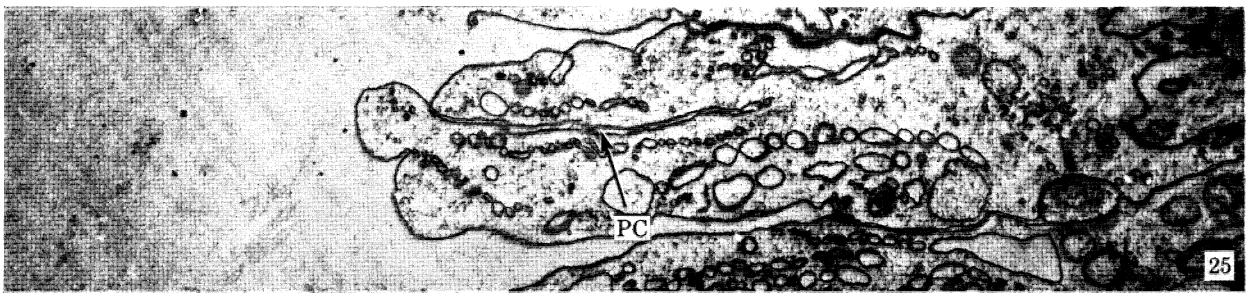
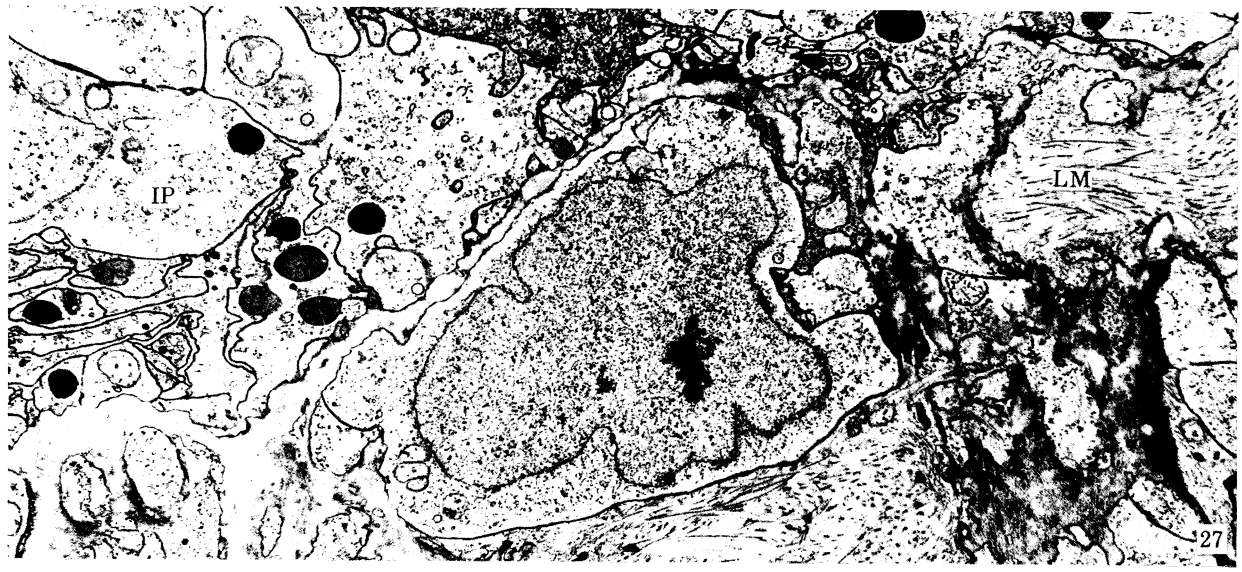
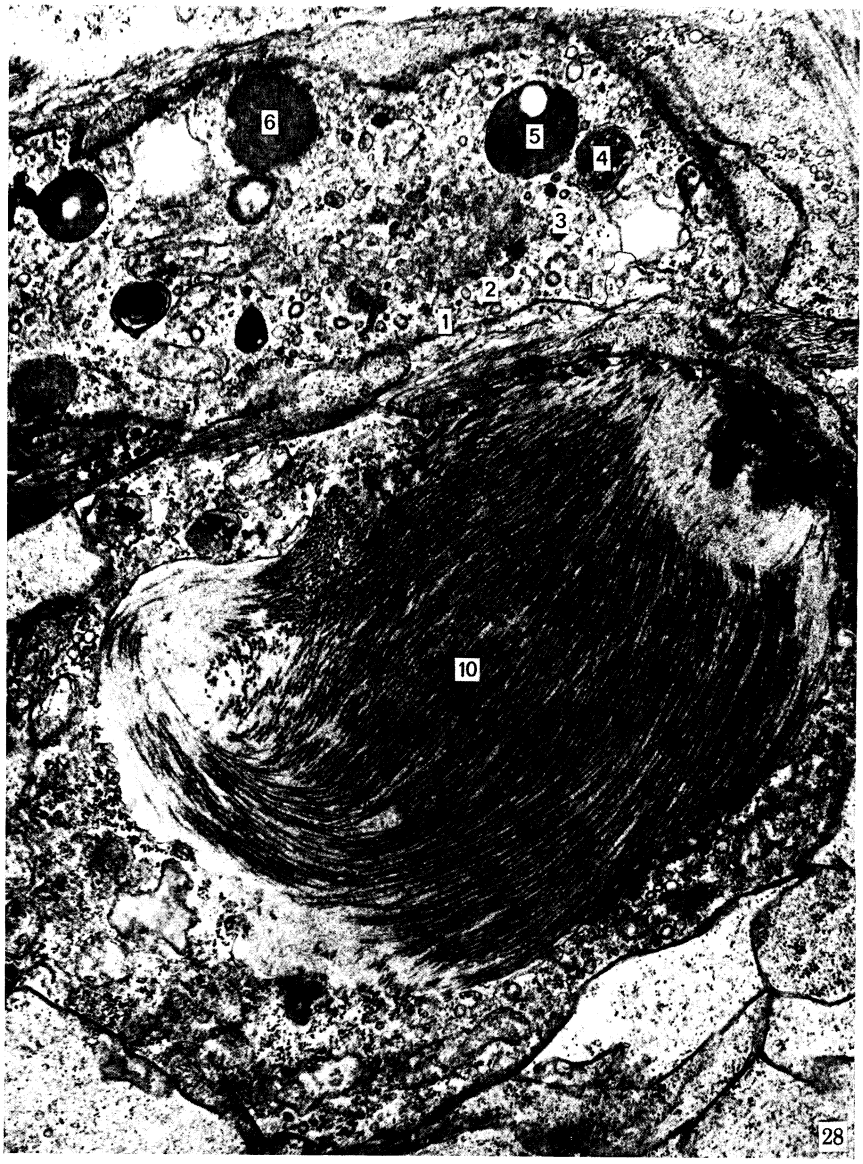


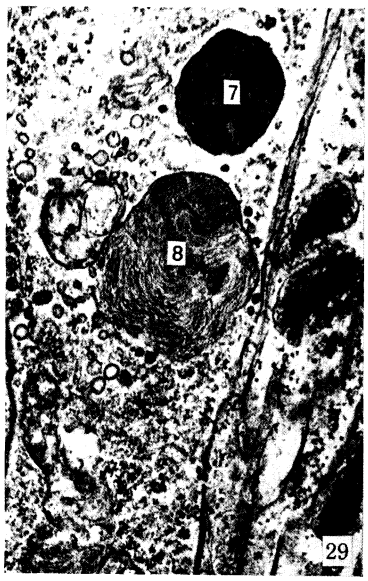
FIGURE 24. The outer endothelial cells of the posterior proboscis. The cells are supported basally by a layer of basement membrane. (Magn. × 6000.)

FIGURE 25. Active pinocytosis in the outer endothelial cells of the posterior proboscis. (Magn. × 10000.)

FIGURE 26. Longitudinal section of the longitudinal muscle of the posterior proboscis. (Magn. × 6500.)







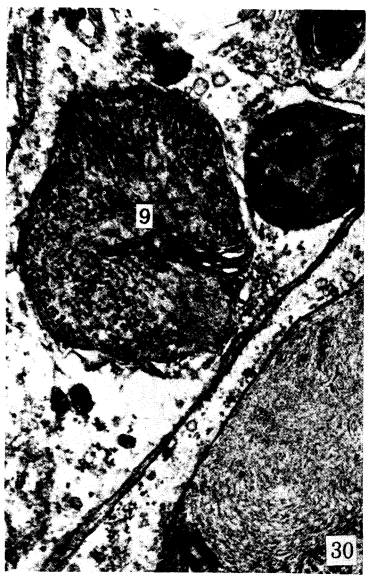
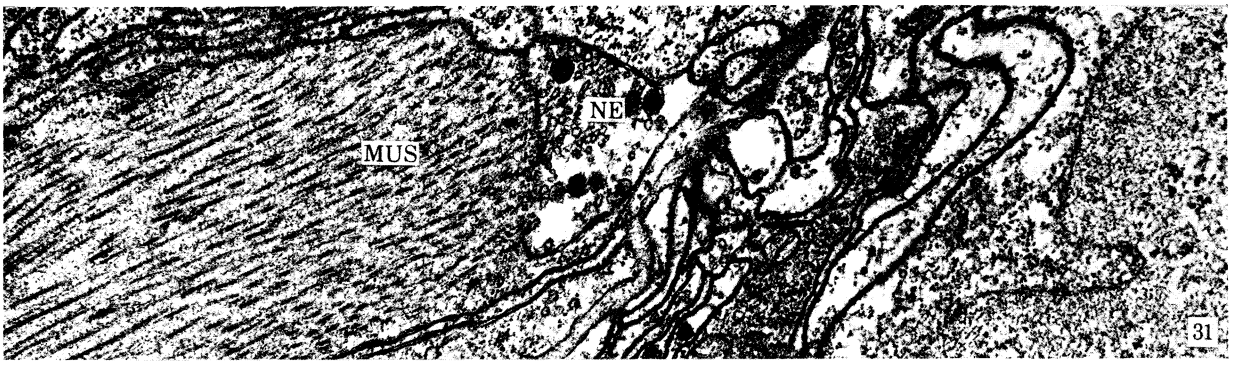


FIGURE 27. An undifferentiated cell found between the inner epithelium and the longitudinal muscle layer. (Magn. × 8000.)

FIGURES 28–30. 'Differentiating cells.' Numbers indicate a possible route by which the myofilaments are formed. Note the cells contain abundant free ribosomes. (Magnifications: figure 28, × 24 000; figure 29, × 16 000; figure 30, × 20 000.)



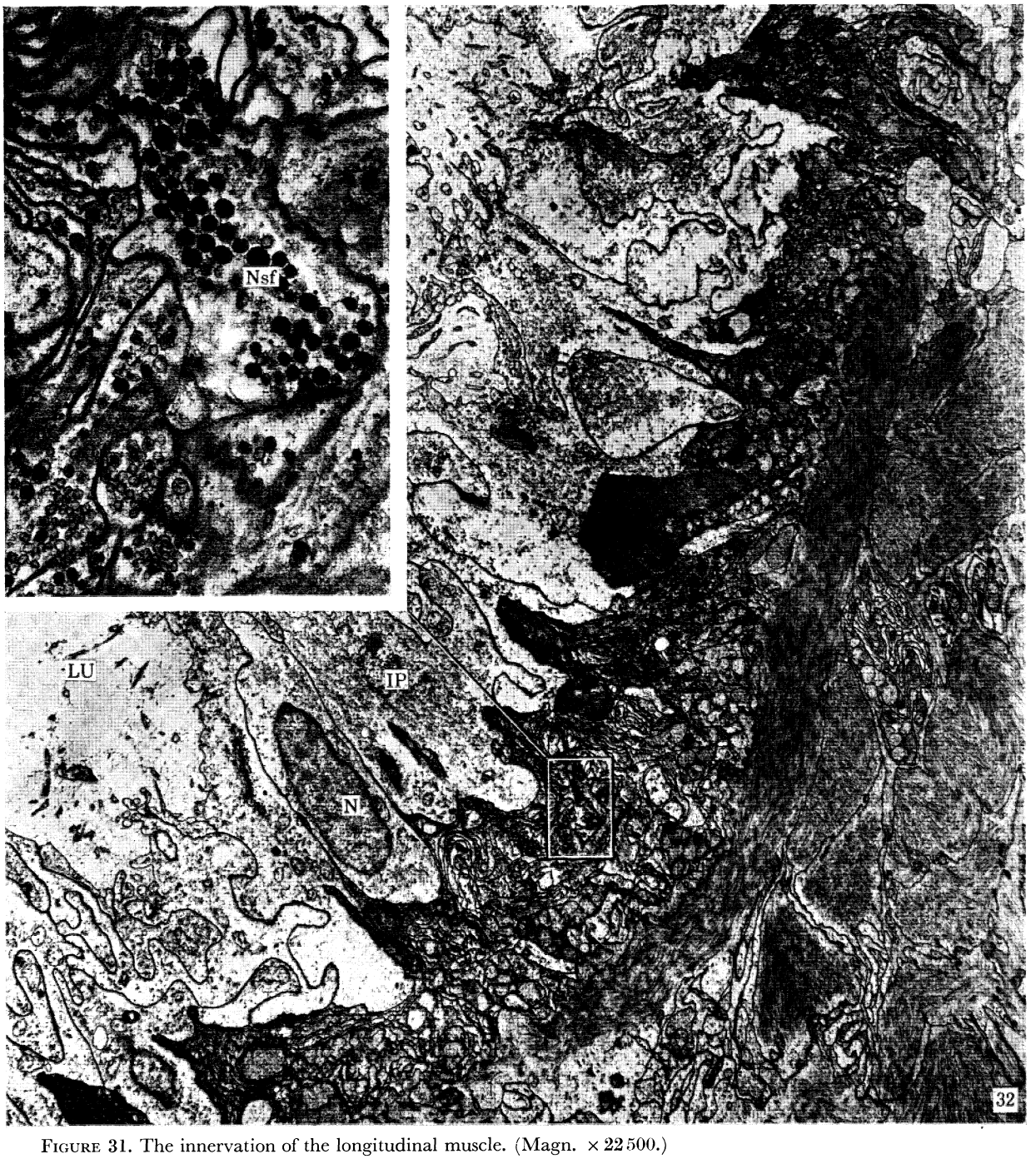


FIGURE 31. The innervation of the longitudinal muscle. (Magn. × 22 500.)

FIGURE 32. Transverse section of the posterior proboscis, showing the epithelial lining cells. Neurosecretory fibres together with the usual terminals are seen beneath the epithelium (see inset; magn. × 30 000). (Magn. × 3300.)

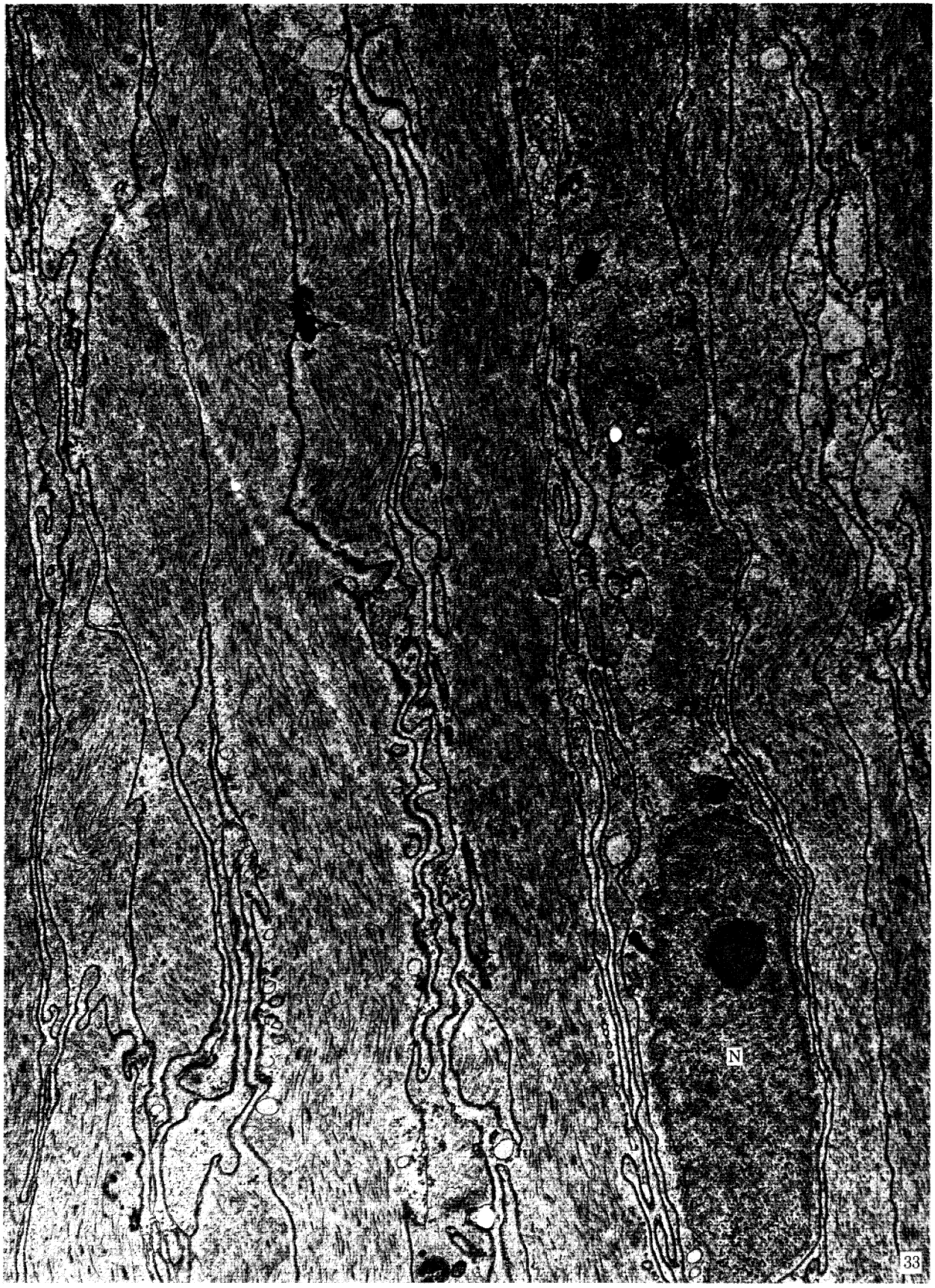
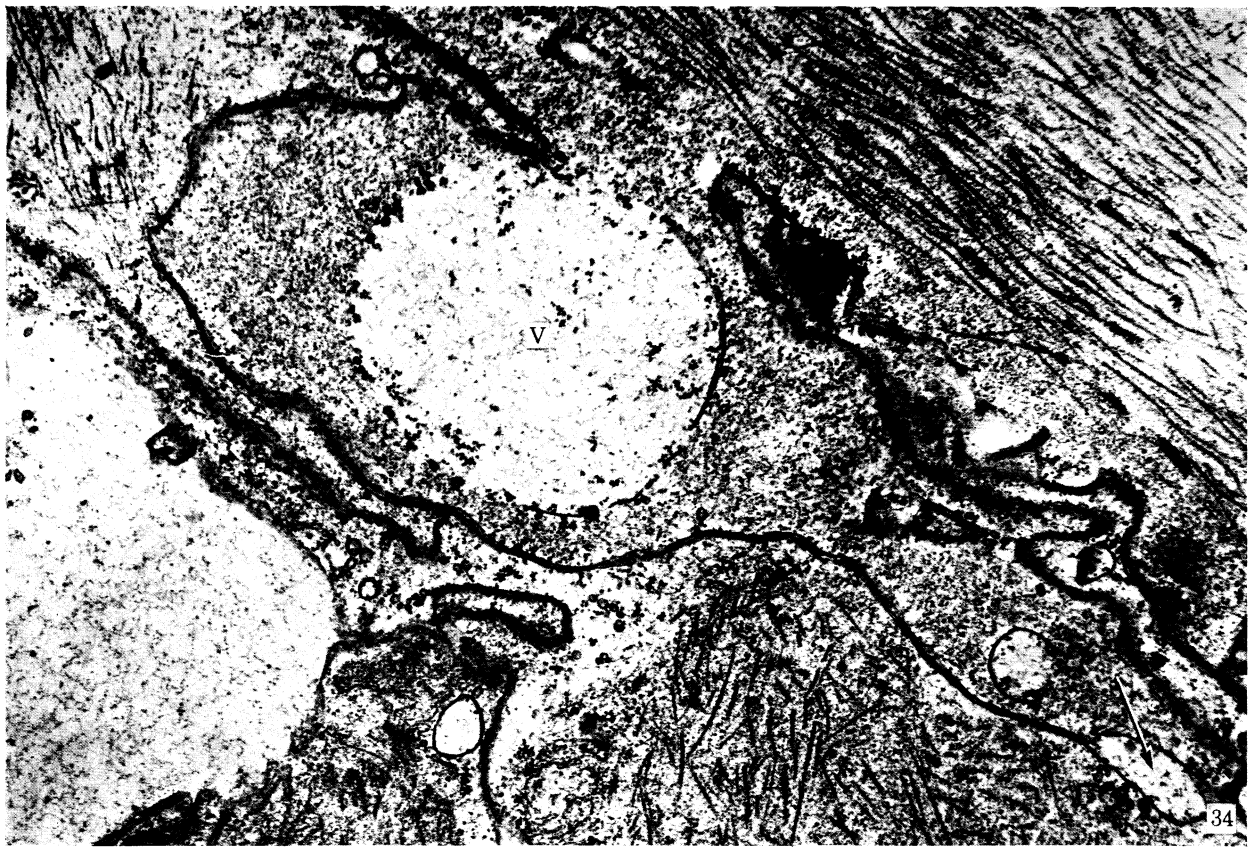


FIGURE 33. Longitudinal section of the retractor muscle. (Magn. × 8000.)



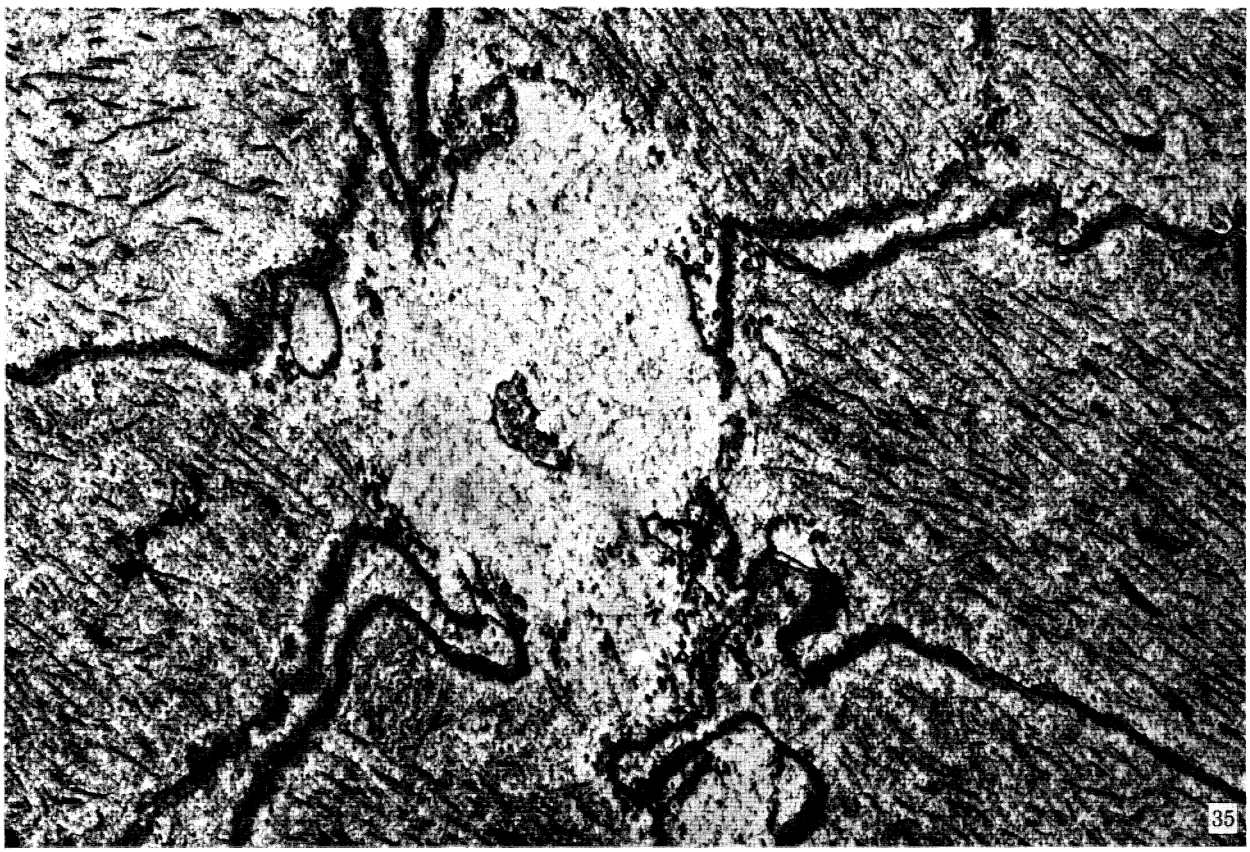
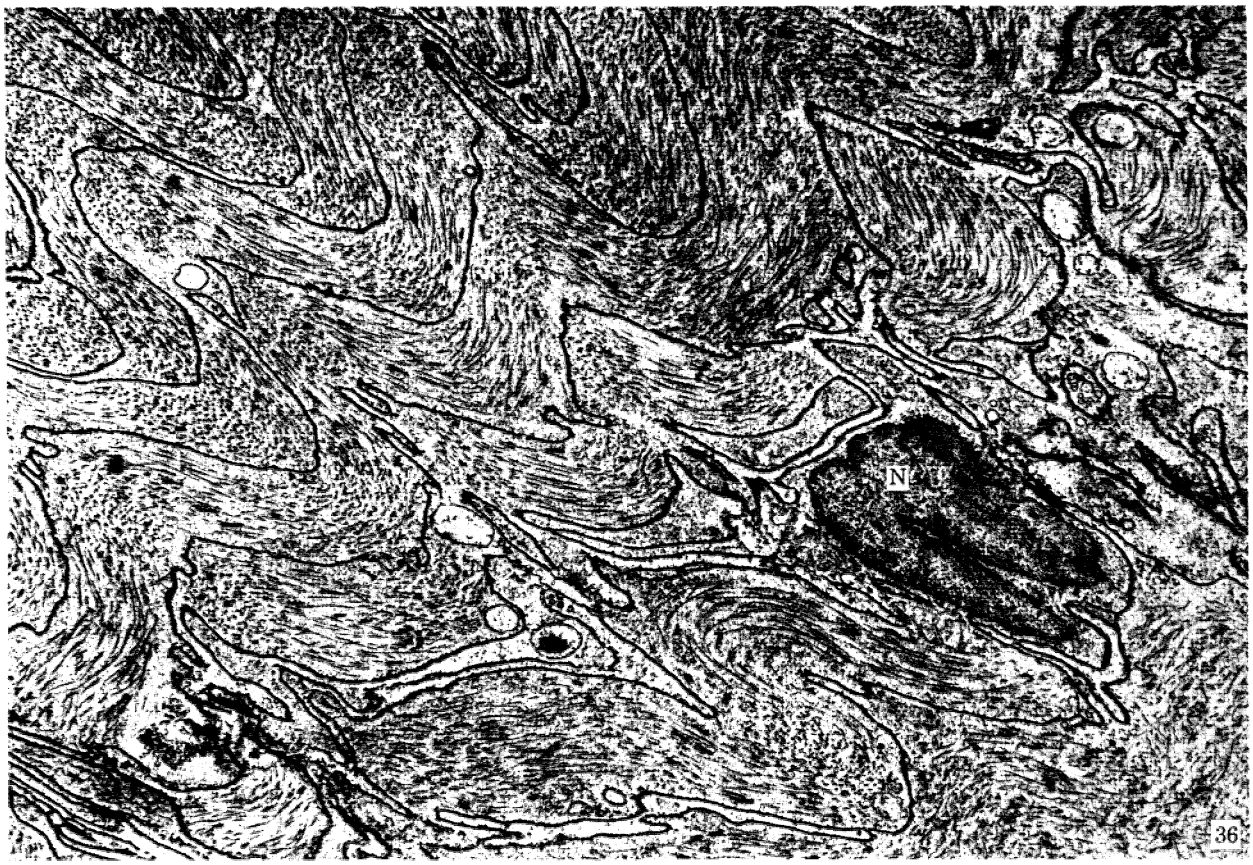


FIGURE 34. Vacuoles in the cytoplasm of the retractor muscle cells. Arrow indicates electron-lucent substance in intercellular space. (Magn. × 18000.)

FIGURE 35. Four muscle cells are seen to 'open' into a common 'secretion pool'. (Magn. × 27500.)



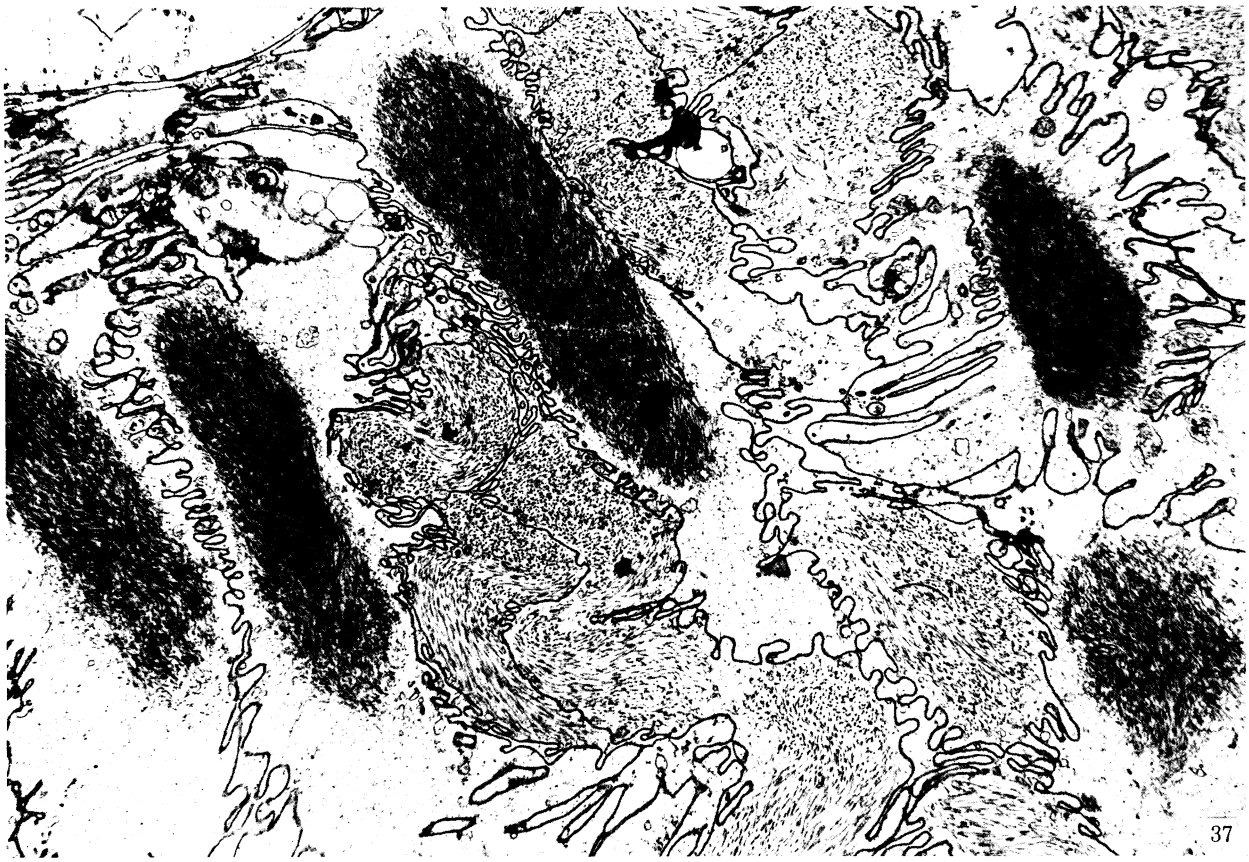
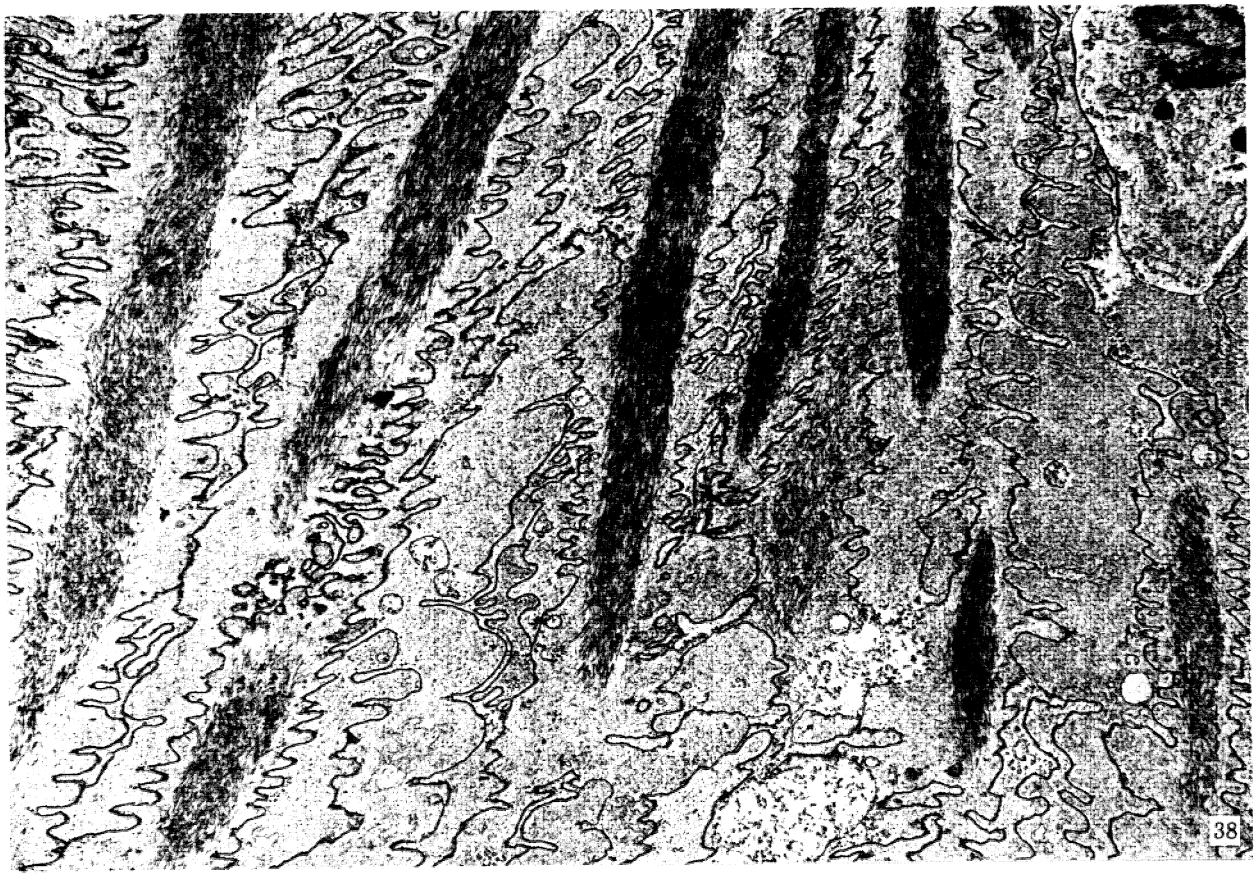


FIGURE 36. Zigzag pattern of serpentine-like muscle cells. Note the deeply infolded nucleus. (Magn. × 7500.)

FIGURE 37. Concertina-like or contracted muscle cells. (Magn. × 4500.)



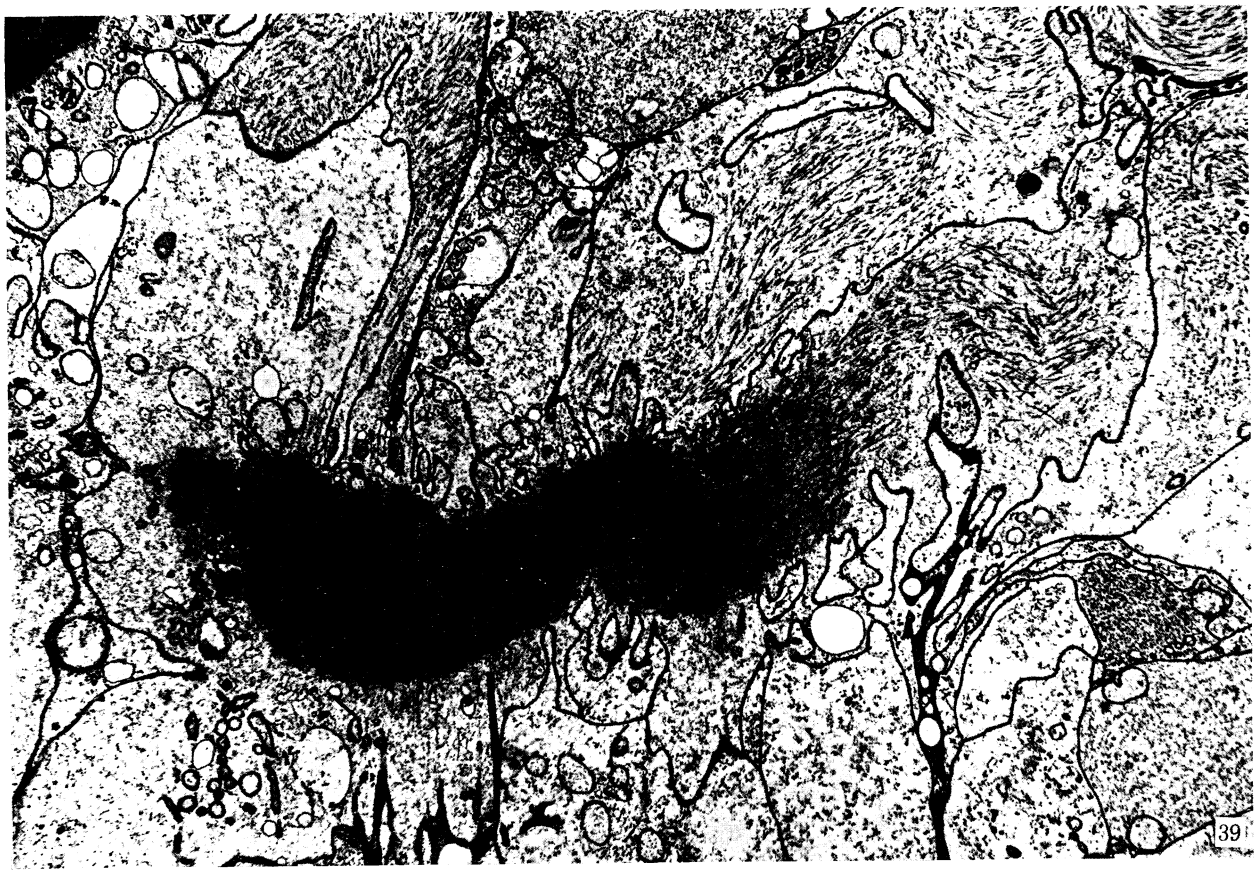


FIGURE 38. 'Intermediate' muscle cells. (Magn. ×4200.)

FIGURE 39. 'Intermediate' muscle cells. (Magn. ×6000.)

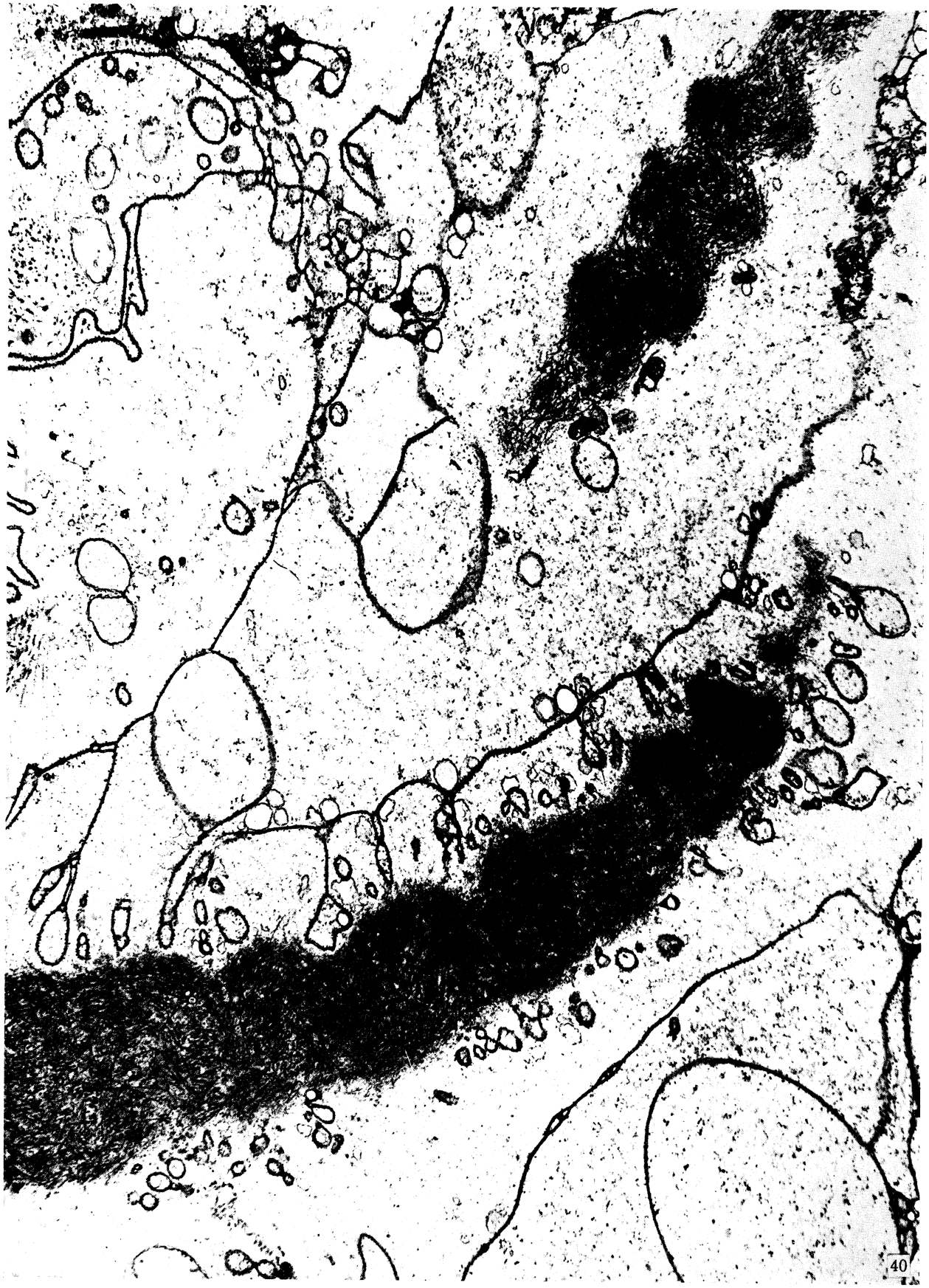


FIGURE 40. Oxytocin-treated. Contracted muscle cells. Note the presence of numerous microvesicles in the cytoplasm. (Magn. × 14000.)

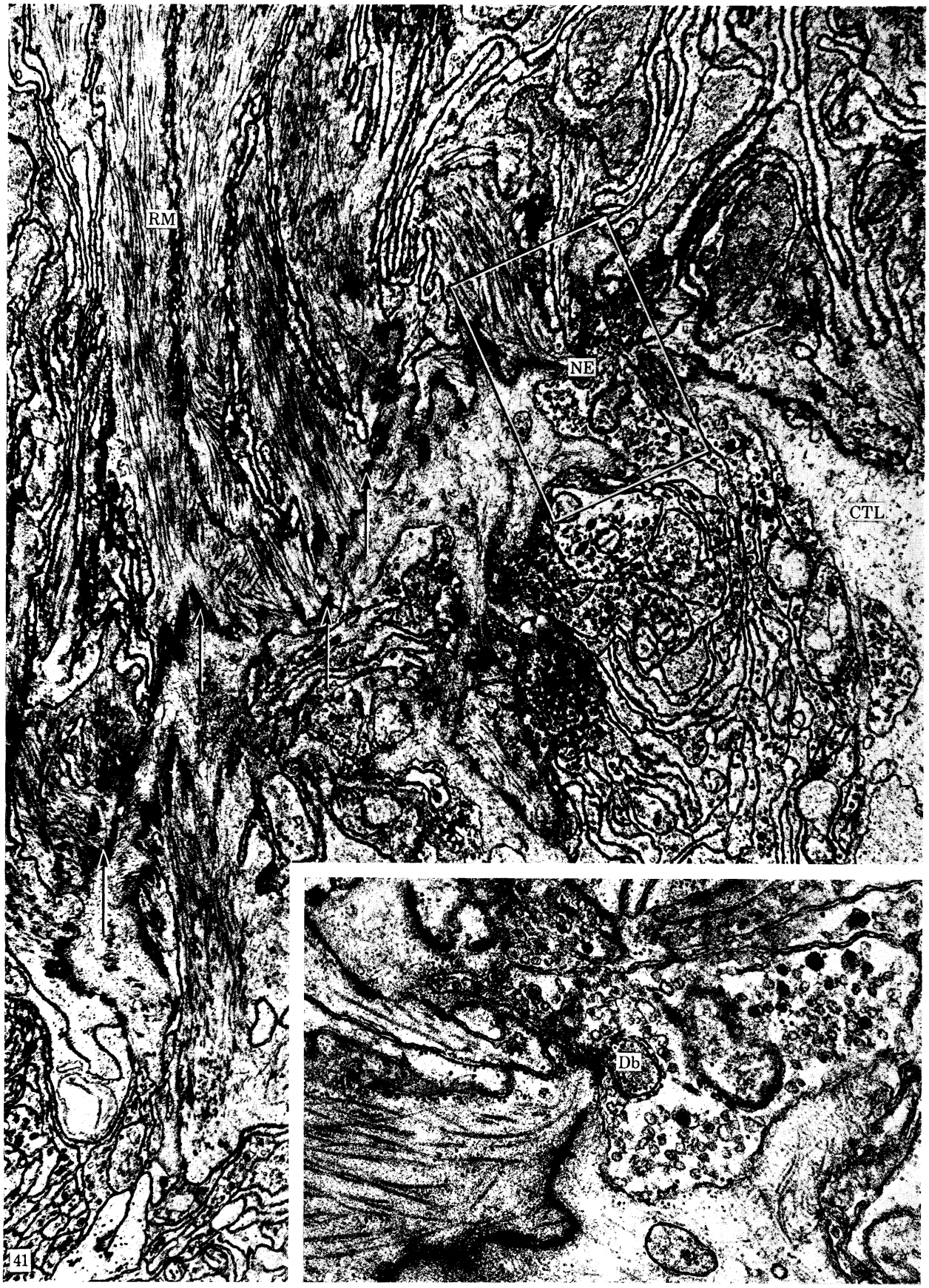


FIGURE 41. Large bundles of non-myelinated nerve endings are seen closely appressed to the connective tissue layer (CTL) separating the retractor muscle from the proboscis. Close contacts between the nerve endings and the retractor muscle cells are not infrequently seen (see inset; magn. × 30 000). Arrows indicate 'attachment plaques'—regions where the myofilaments fuse to the cell membranes. See also figure 5. (Magn. × 12 000.)

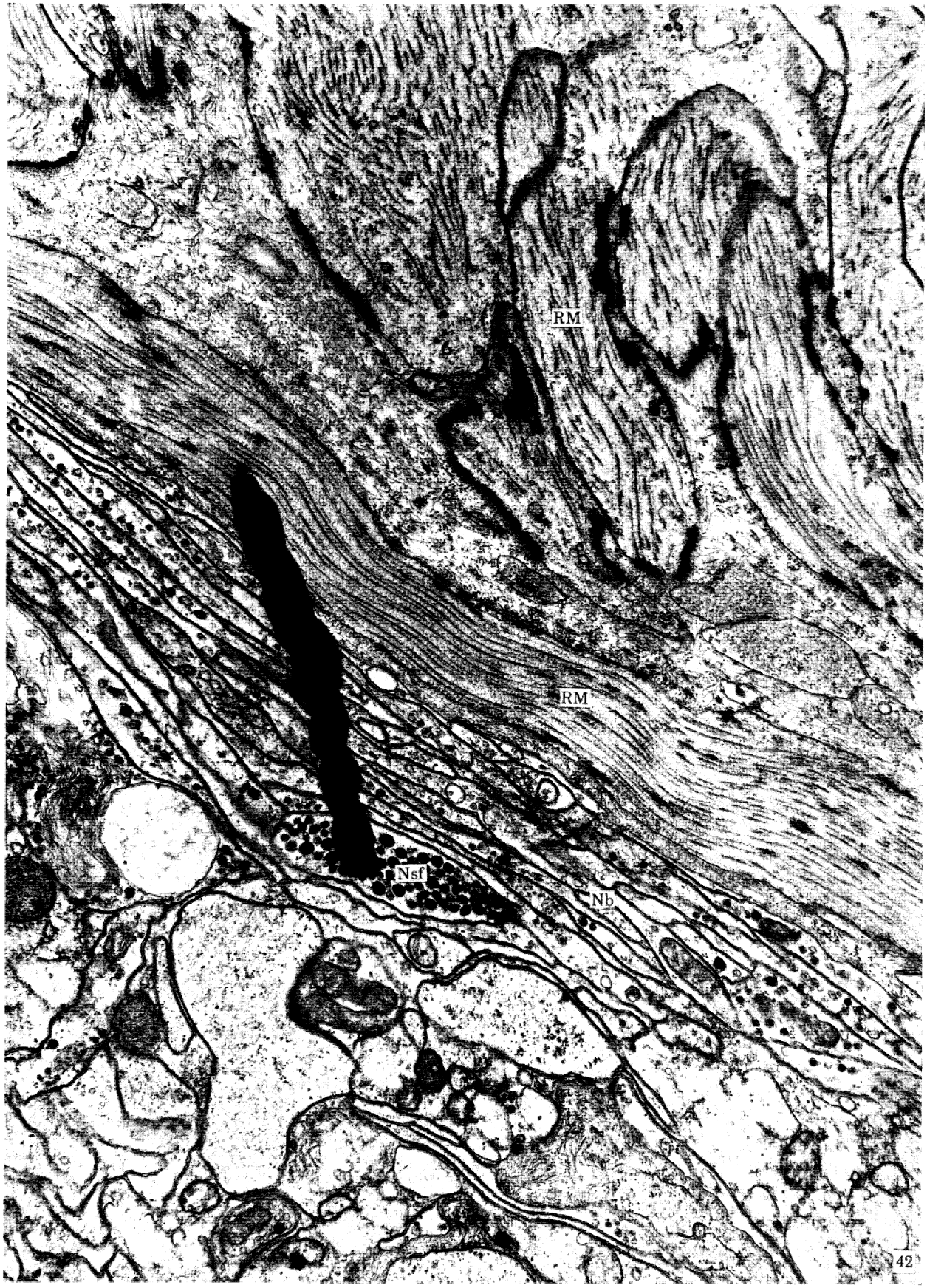


FIGURE 42. Neurosecretory fibres (terminals) are seen in the close vicinity of the retractor muscle. (Magn. × 18000.)

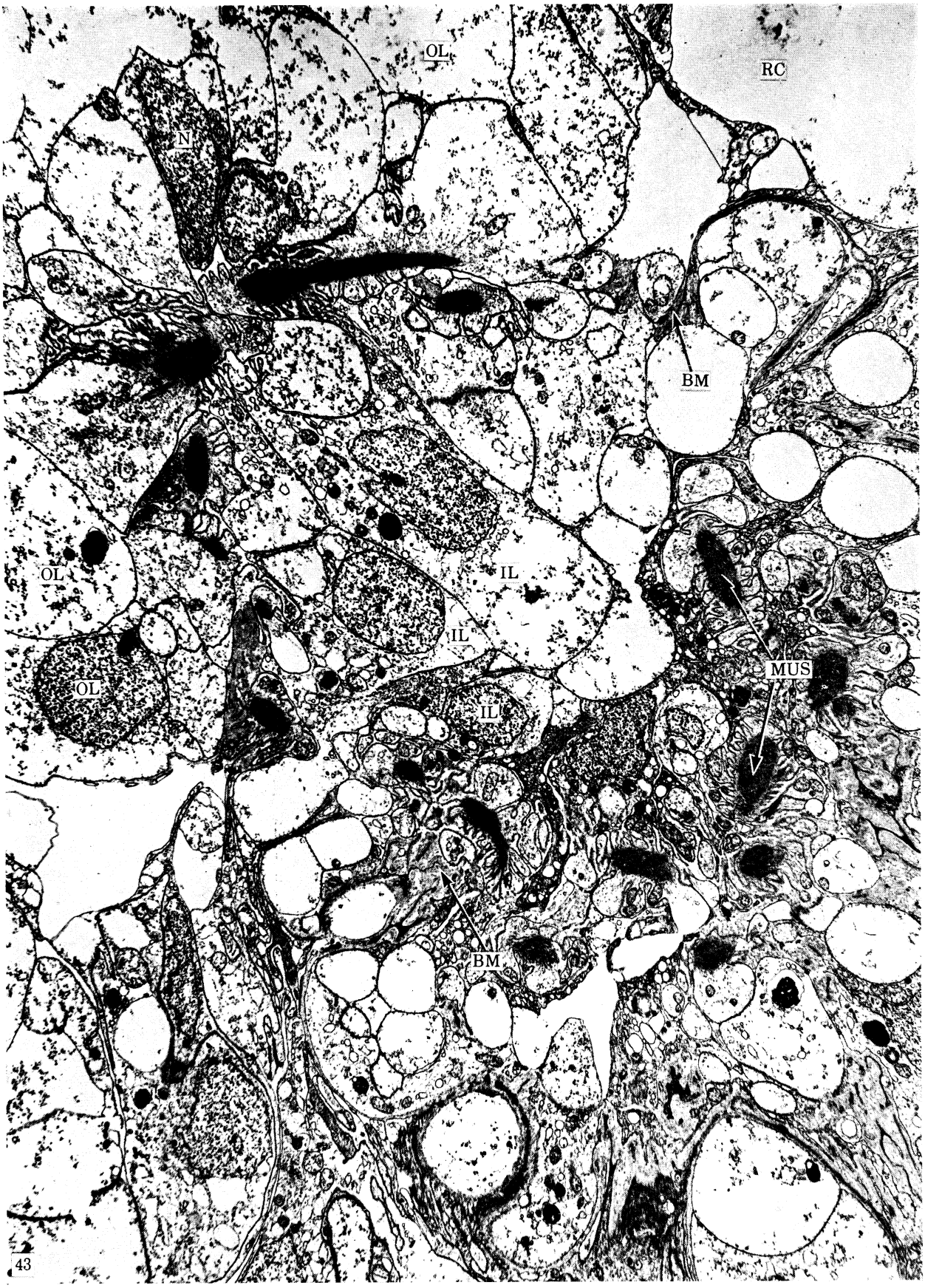
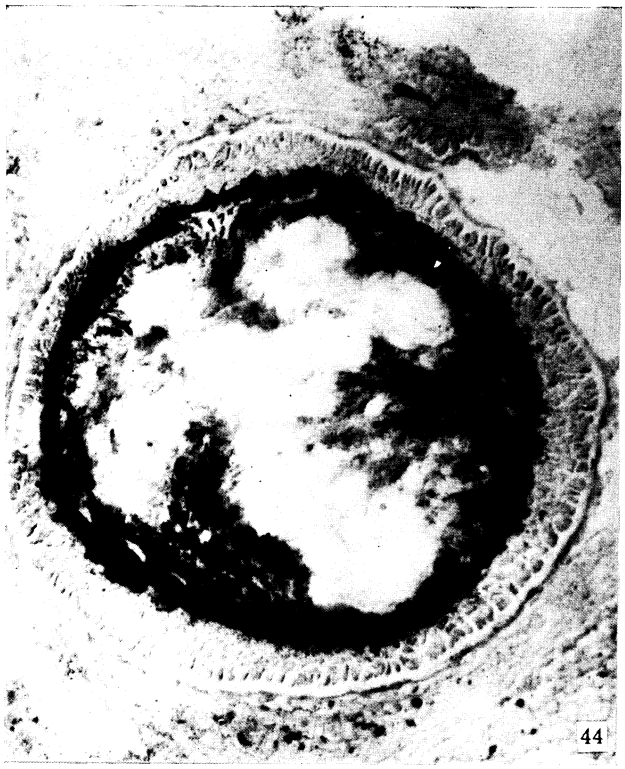
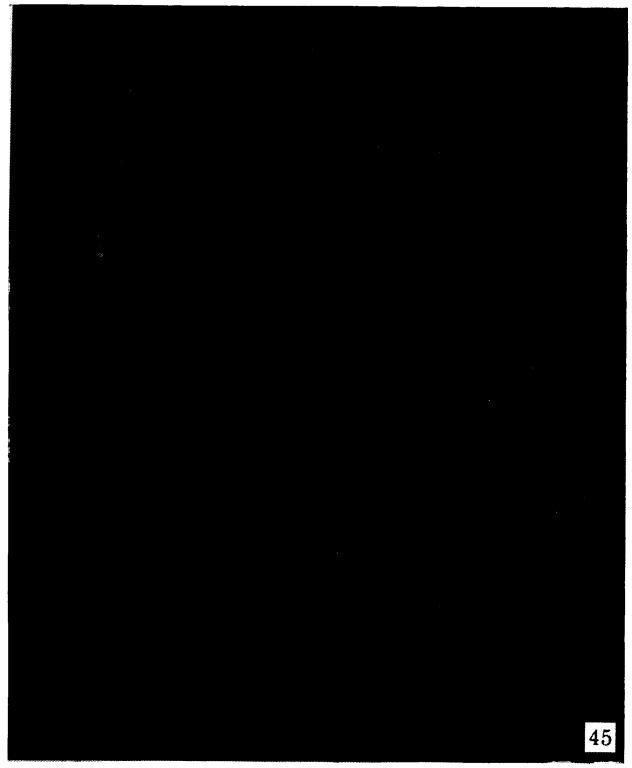
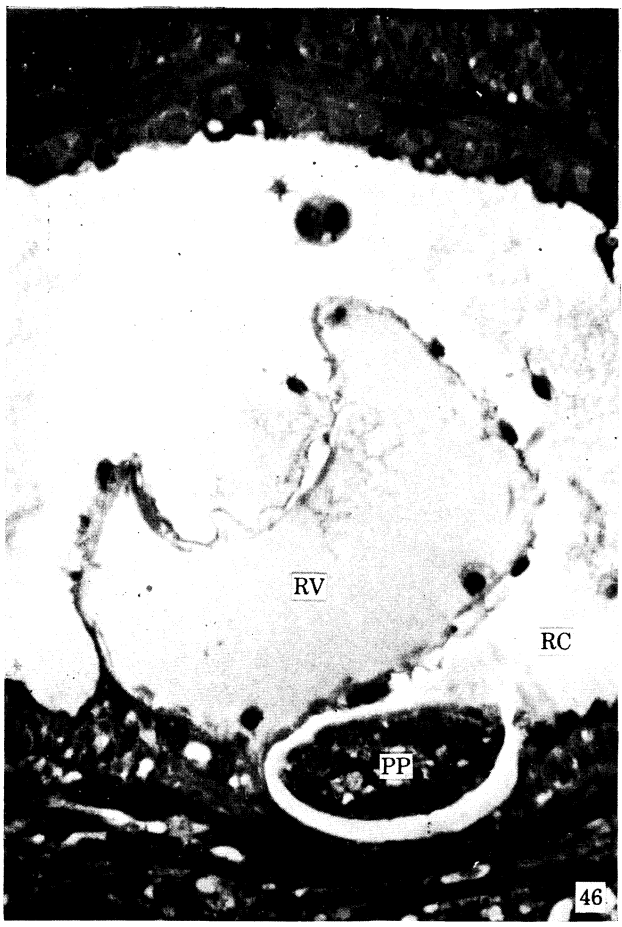


FIGURE 43. Rhynchocoel villus (squared area in figure 6). The lumen of the villus is hardly visible.







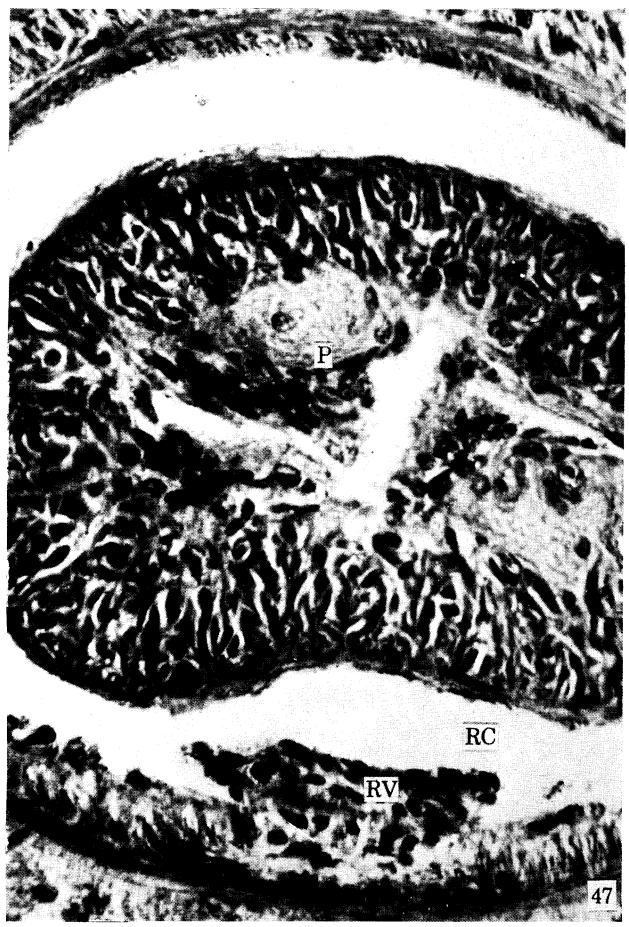
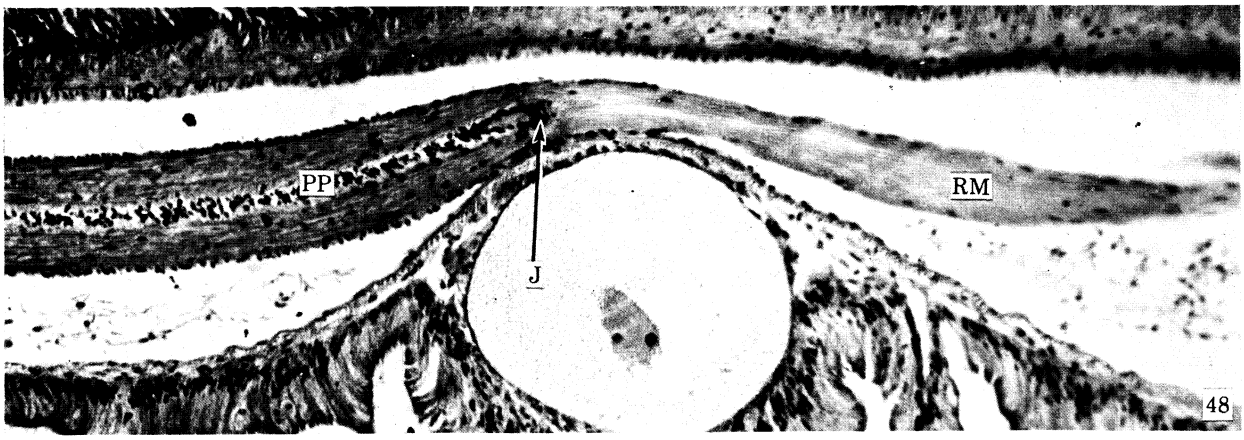


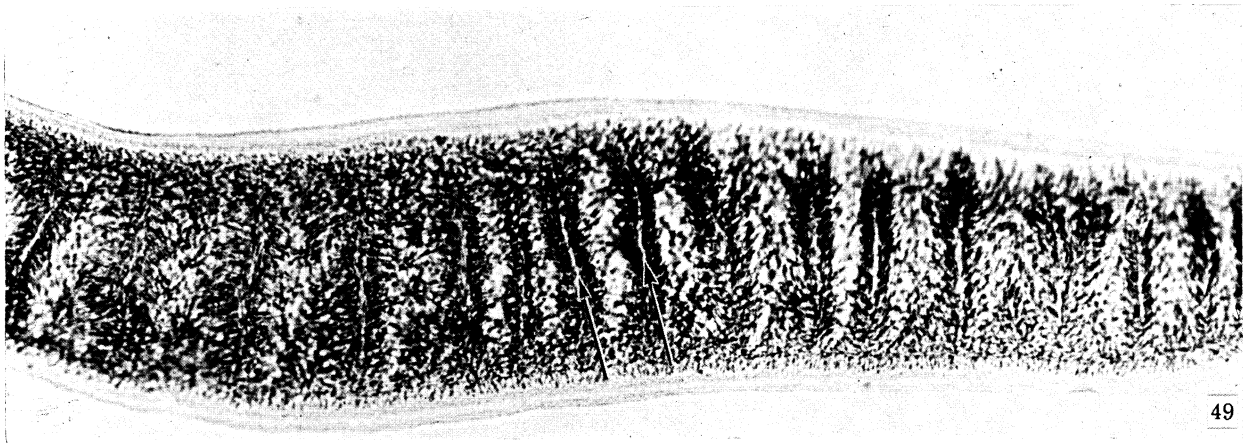
FIGURE 44. Transverse section of the middle proboscis. The layer corresponding to the nerve plexus shows acetylcholinesterase activity.

FIGURE 45. Transverse section of the middle proboscis. The layer corresponding to the nerve plexus gives off a yellowish fluorescence.

FIGURE 46. The rhynchocoel villus becomes dilated when the proboscis is ejected.

FIGURE 47. The rhynchocoel villus is small and flattened when the proboscis is in the rhynchocoel cavity.





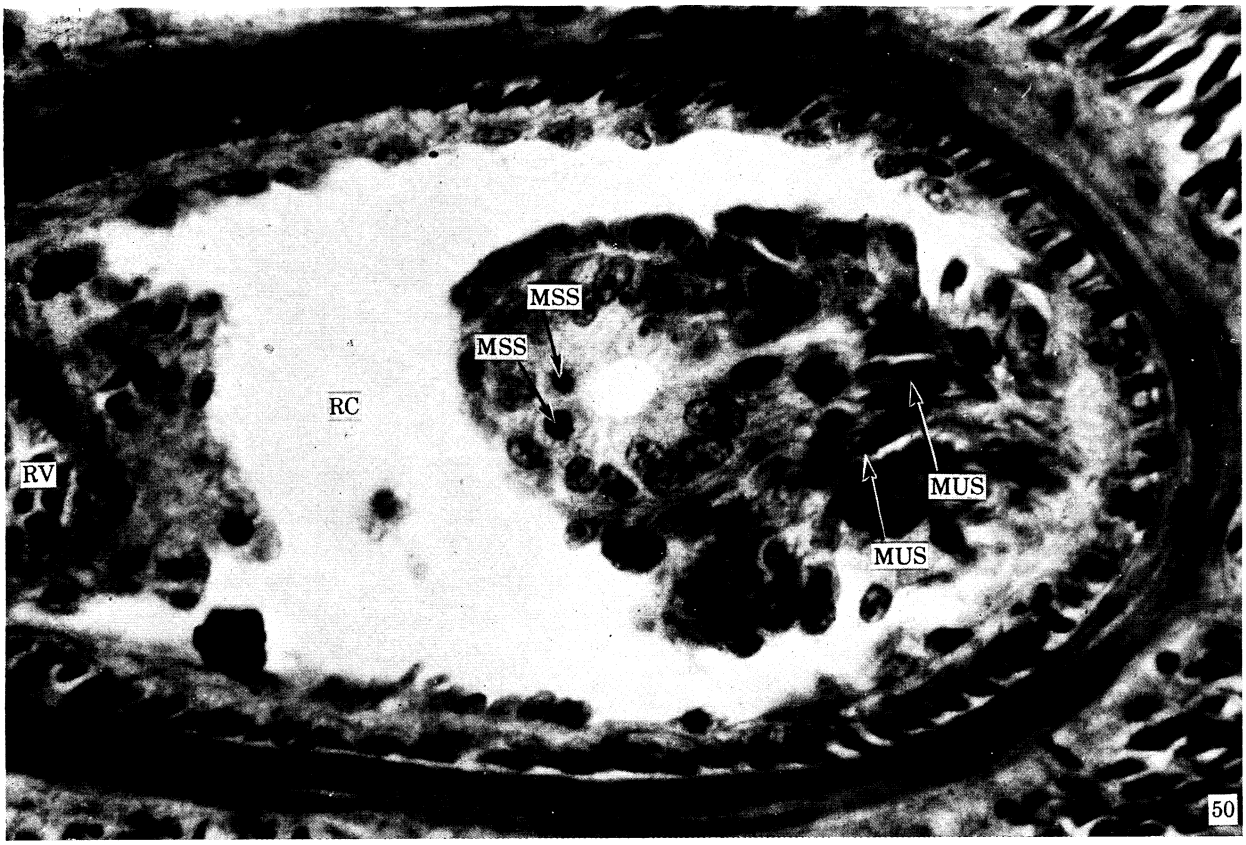


FIGURE 48. Longitudinal section of the worm, showing the junction of the proboscis and the retractor muscle.

FIGURE 49. The circular folds of the middle proboscis (arrows). Whole-mount fresh tissue.

FIGURE 50. Regeneration of a proboscis. Spindle-shaped muscle cells appear to accumulate around a preformed tubular structure. At the same time the lining cells of the tube still proliferate.

Abreviations used in figures

AC	acidophilic cell
AP	anterior proboscis
AR	'accessory rods'
BC	basal cells (zone of differentiation?)
BF	'basal feet'
BM	basement membrane
C	cilium
CF	central fibrils of cilium
Ci	ciliary membrane
CM	circular muscle
CP	'cuticular plate'
CTL	connective tissue layer
Cyt	cytoplasmic interlocking
D	desmosome-like junction
Db	'presynaptic dense body'
DE	distal end of newly formed rhabdite
DR	discharged rhabdites
ER	endoplasmic reticulum
EV	envelope of rhabdite
EV (C)	envelope of rhabdite (outer circular layer)
EV (L)	envelope of rhabdite (inner longitudinal layer)
FS	filamentous structure
I	intestine
IL	inner lining cells
IM	inner basement membrane
IP	inner epithelium
J	junction of proboscis and retractor muscle
LM	longitudinal muscle layer
LU	lumen
M	mitochondria
MC	mucus-secreting cell
MEM	membraneous structure
MM	middle basement membrane
MO	mouth opening
MP	middle proboscis
MSS	mitosis
MUS	muscle cell
MV	microvilli
MVC	cell with well developed microvilli
N	nucleus
Nb	nerve bundle
NE	nerve endings
NP	nerve plexus
NSF	neurosecretory. fibres
O	outer layer of rhabdite
OE	outer endothelium
OL	outer lining cells
OM	outer basement membrane
P	proboscis
PC	pinocytotic channel
PF	peripheral fibrils of cilium
PL	electron-lucent 'pool'
PP	posterior proboscis
PR	partially discharged rhabdites
PS	pinosomes
RC	rhynchocoel
RD	rhynchodaeum
RfC	rhabdite-forming cell
RM	retractor muscle
RV	rhynchocoel villus
S	subendothelial layer of circular muscle fibrils
SC	sensory cell
SOC	electron-dense socket
TC	central tubular core
V	intracellular vacuole

[*Facing plate* 22

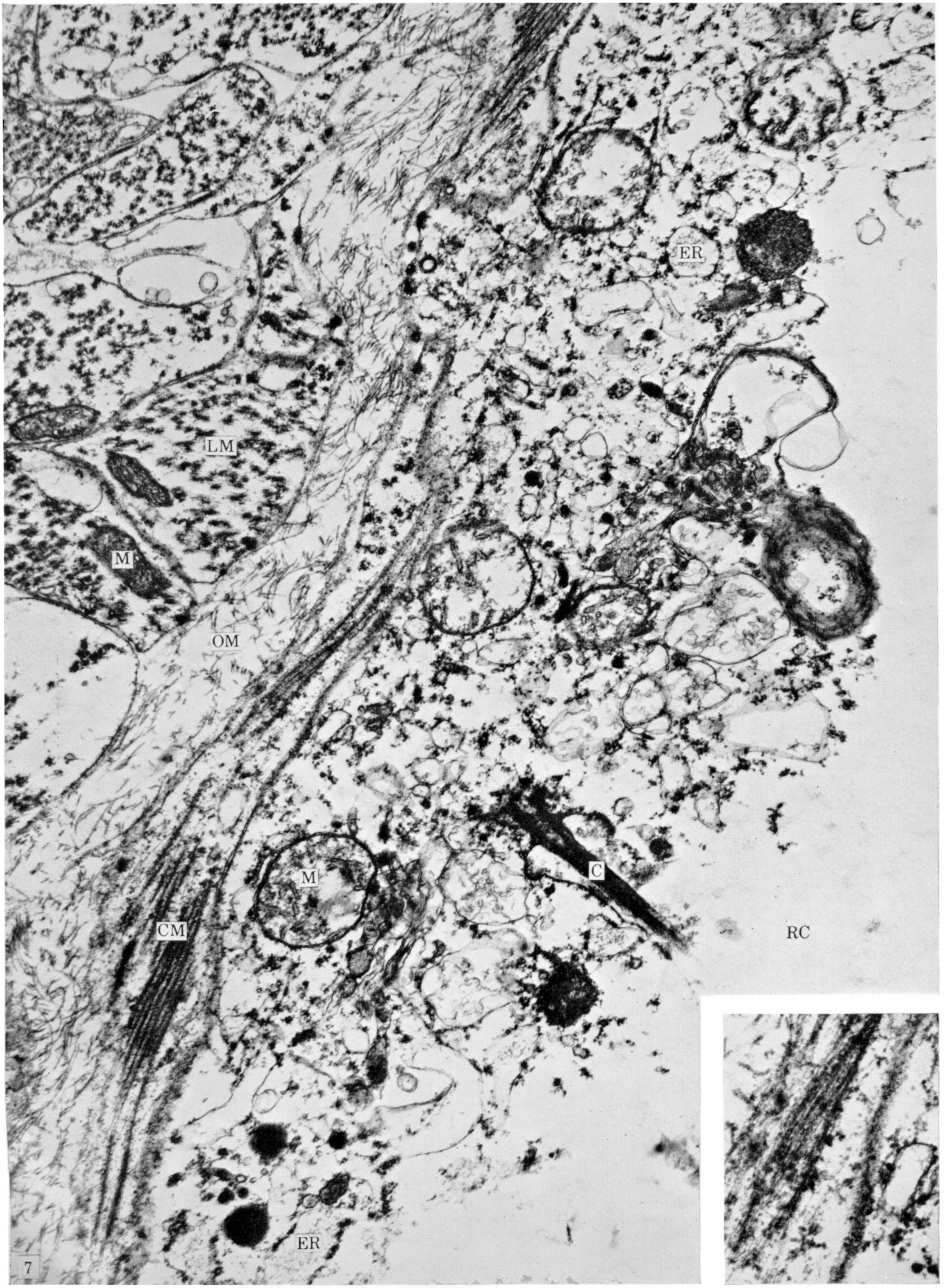
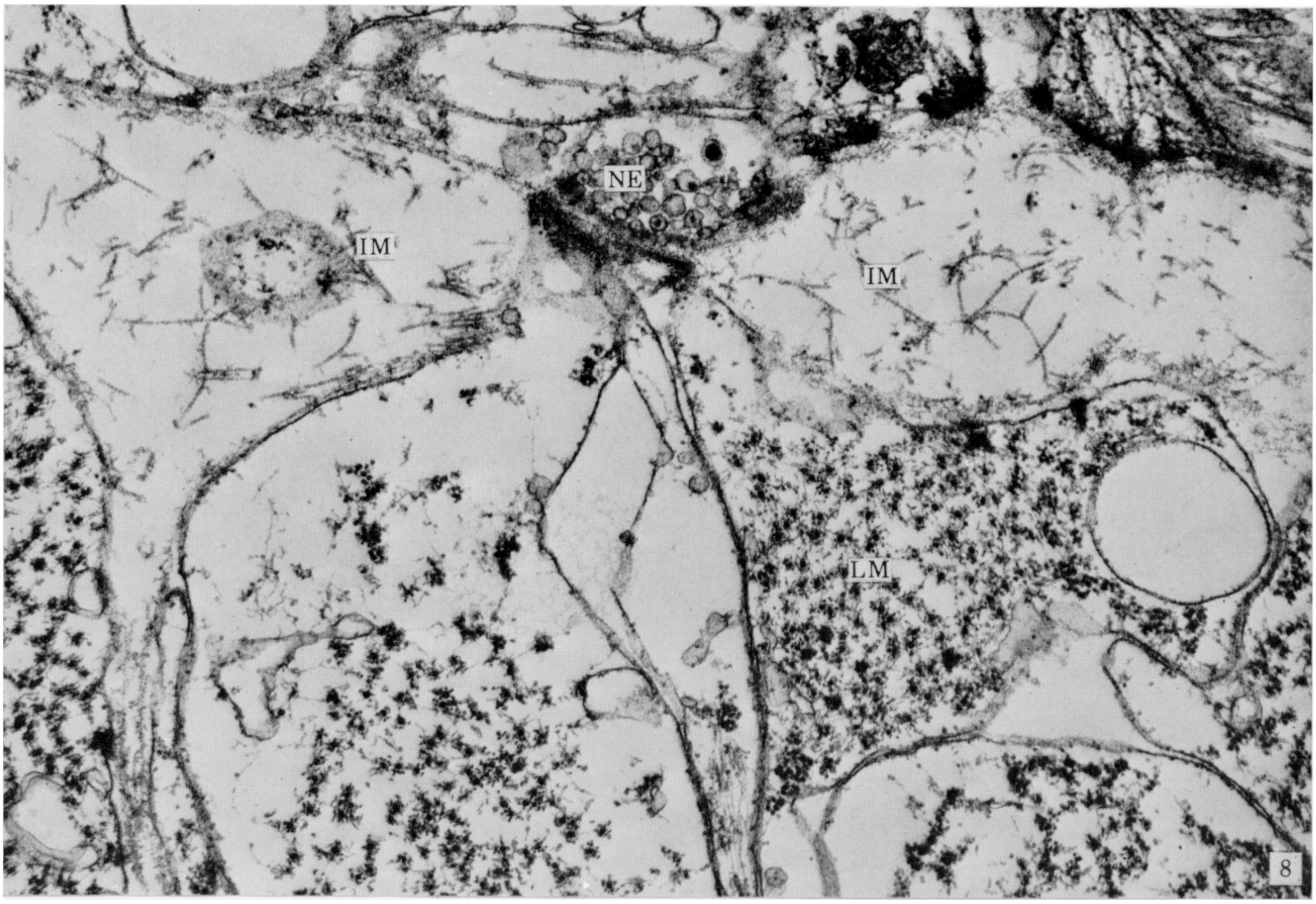
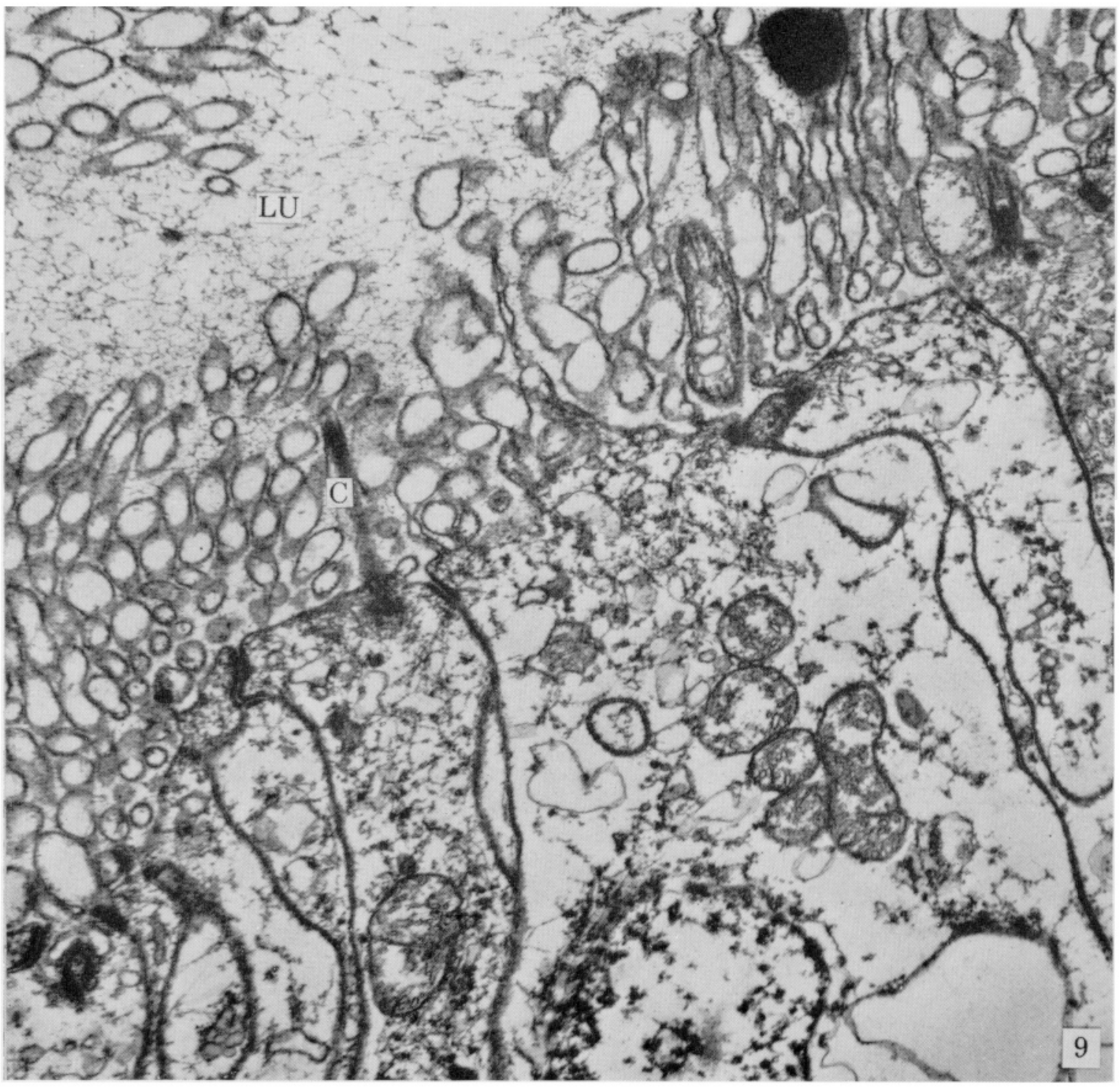


FIGURE 7. Transverse section of the anterior proboscis. An endothelial cell is seen to bear a single cilium projecting into the rhynchocoel. The figure (see inset) also shows that the circular muscle fibres contain two types of myofilaments. (Magn. × 15000.)





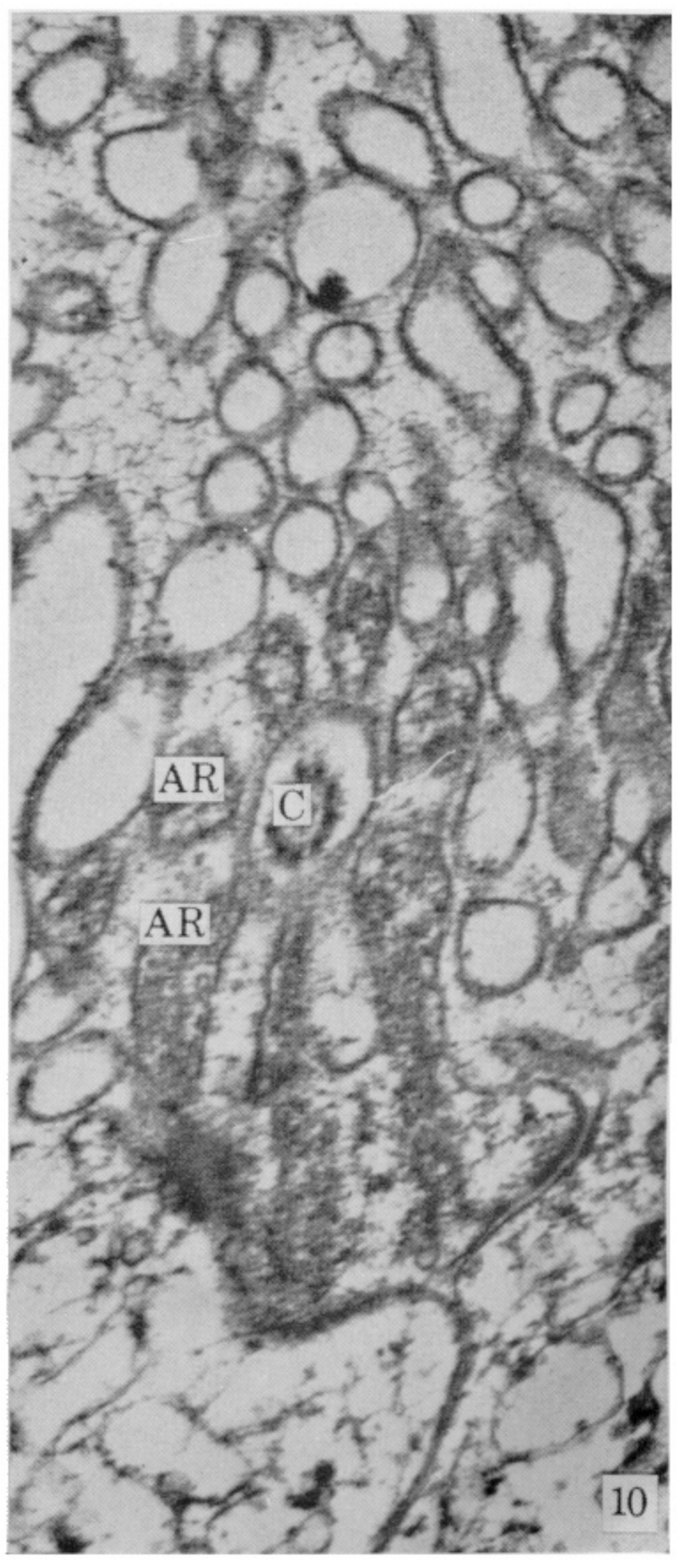
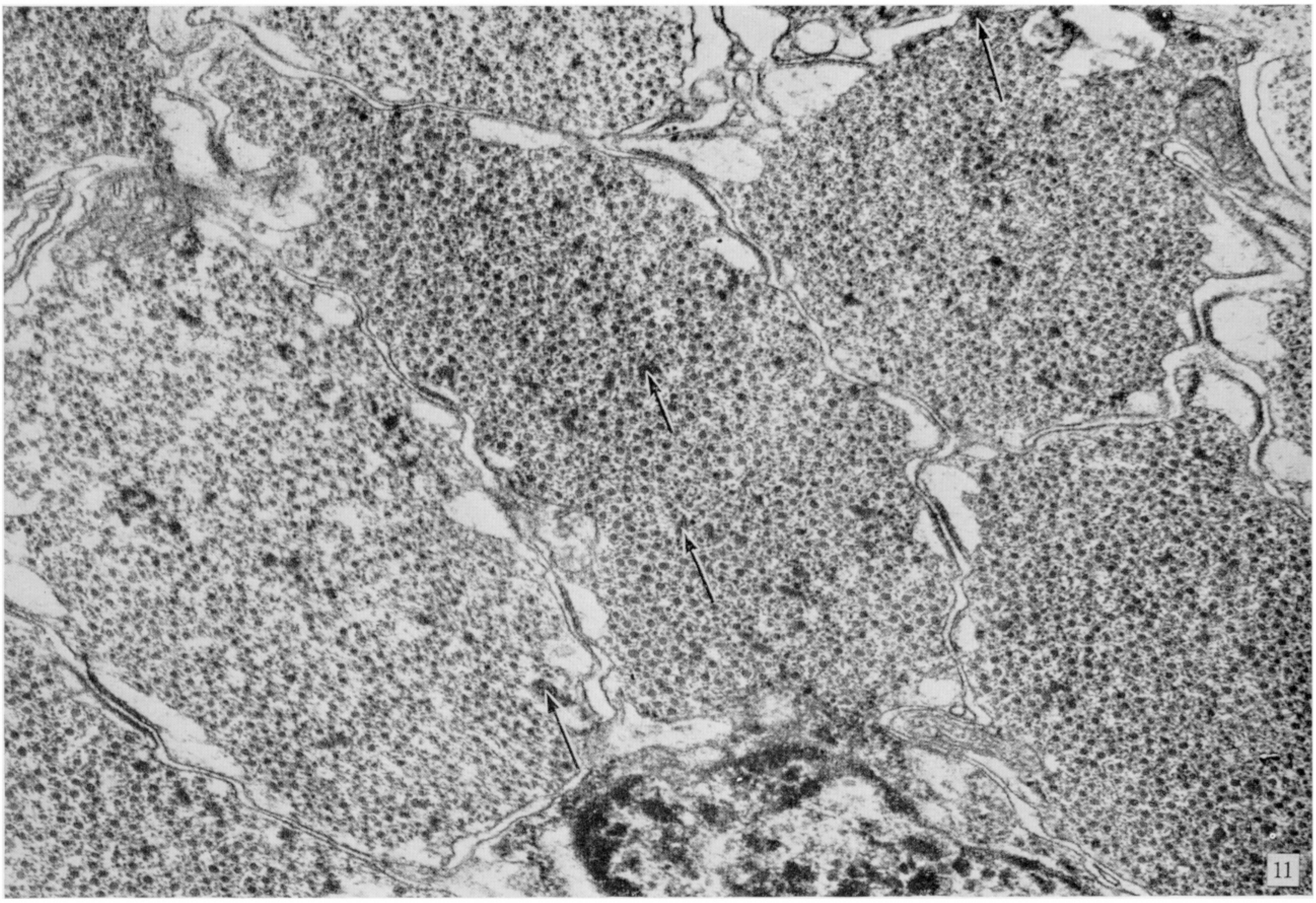
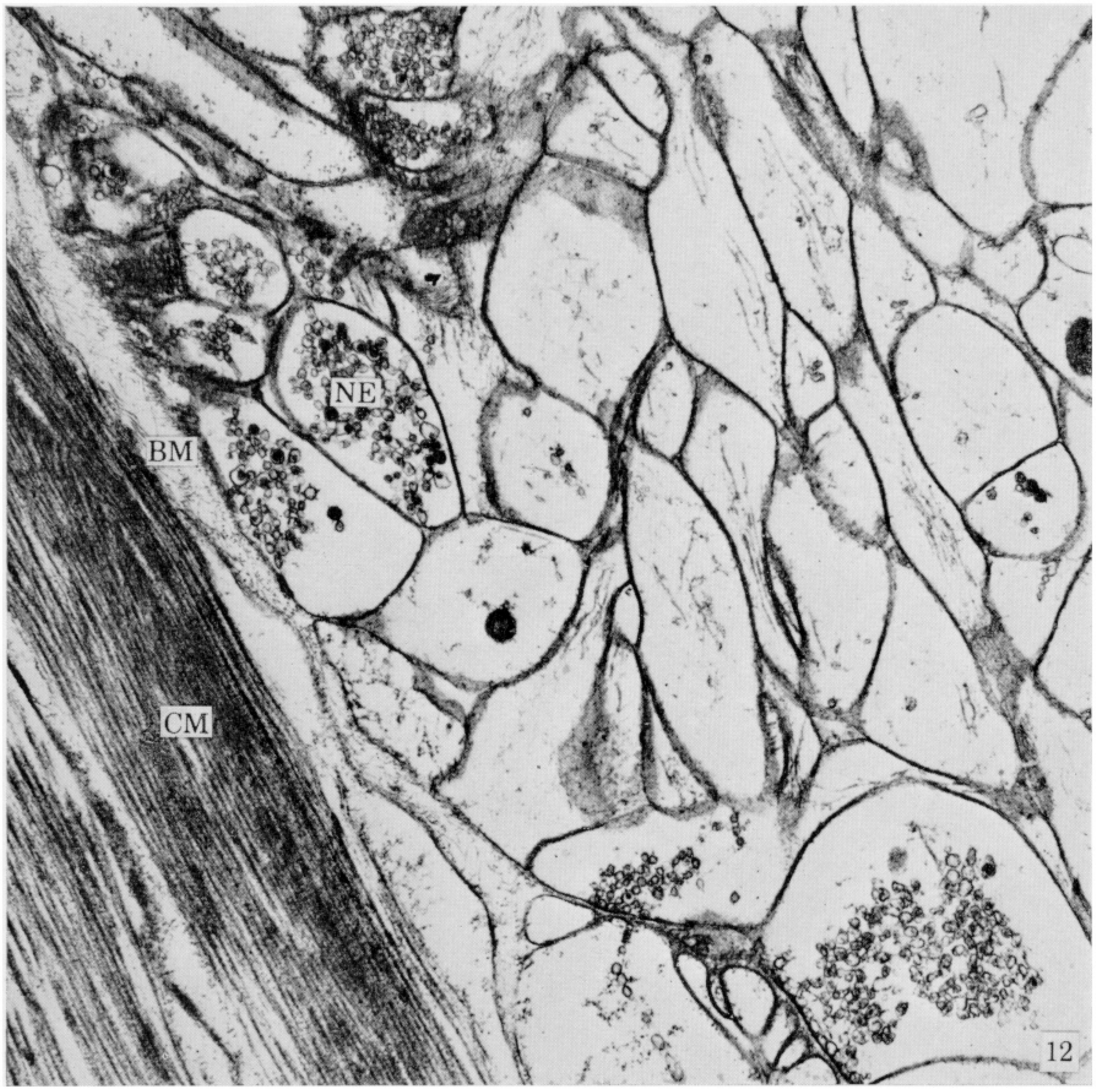


FIGURE 8. The 'innervation' of the longitudinal muscle of the anterior proboscis. The muscle cells contain two types of myofilaments. Note that two types of synaptic vesicles (granular and agranular) are present in the nerve endings. (Magn. × 27 500.)

FIGURE 9. The inner epithelium of the anterior proboscis. Each of the epithelial cells is seen to bear a single cilium. (Magn. × 14 000.)

FIGURE 10. Transverse section of the cilium on the apical surface of an epithelial cell of the anterior proboscis. (Magn. × 18 000.)





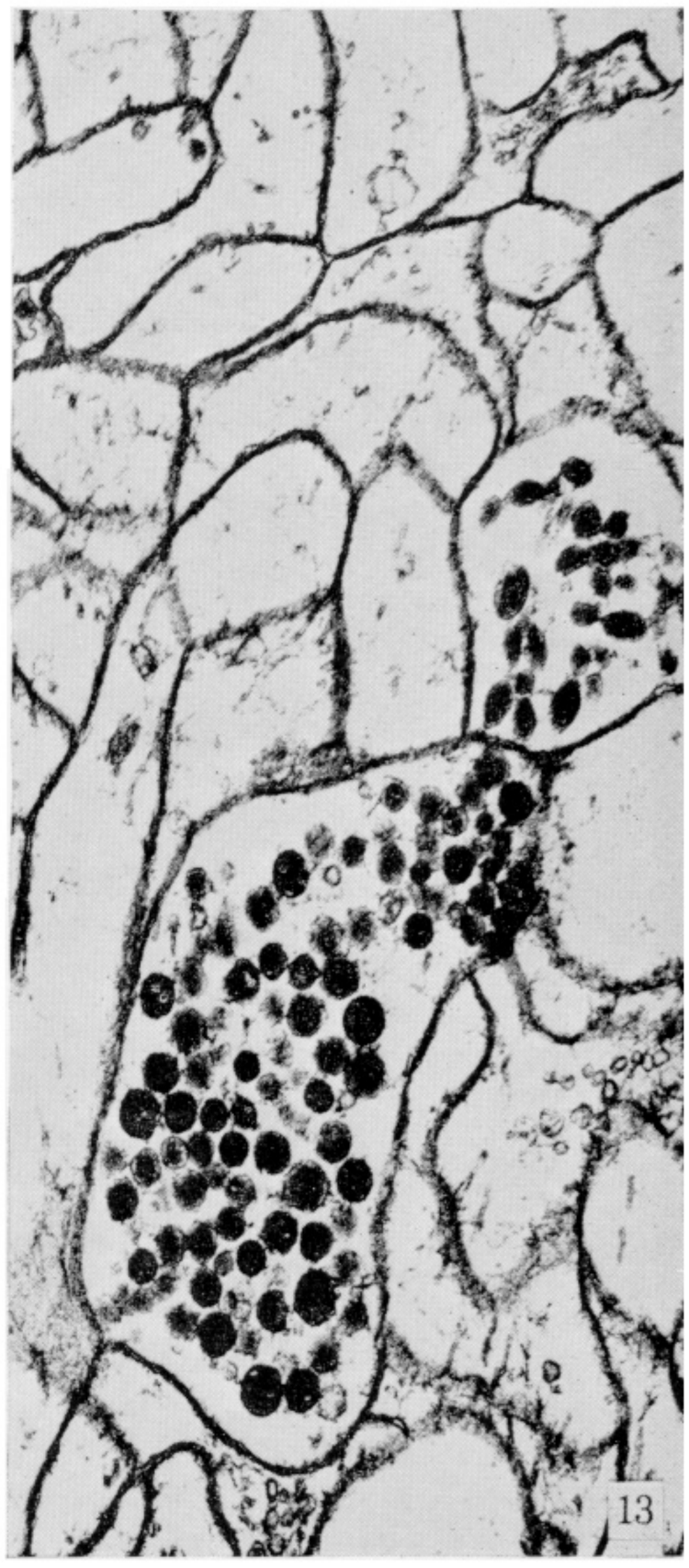


FIGURE 11. Transverse section of the longitudinal muscle of the middle proboscis. The muscle cell contain two types of myofilaments. Arrows indicate 'irregular dense bodies' (see text). (Magn. × 40000.)

FIGURE 12. Transverse section of the middle proboscis. Note many nerve endings are seen closely applied to the surface of the circular muscle cell. (Magn. × 22000.)

FIGURE 13. Two nerve fibres loaded with neurosecretory granules are present in the neuropile of the nerve at the middle proboscis. (Magn. × 22000.)

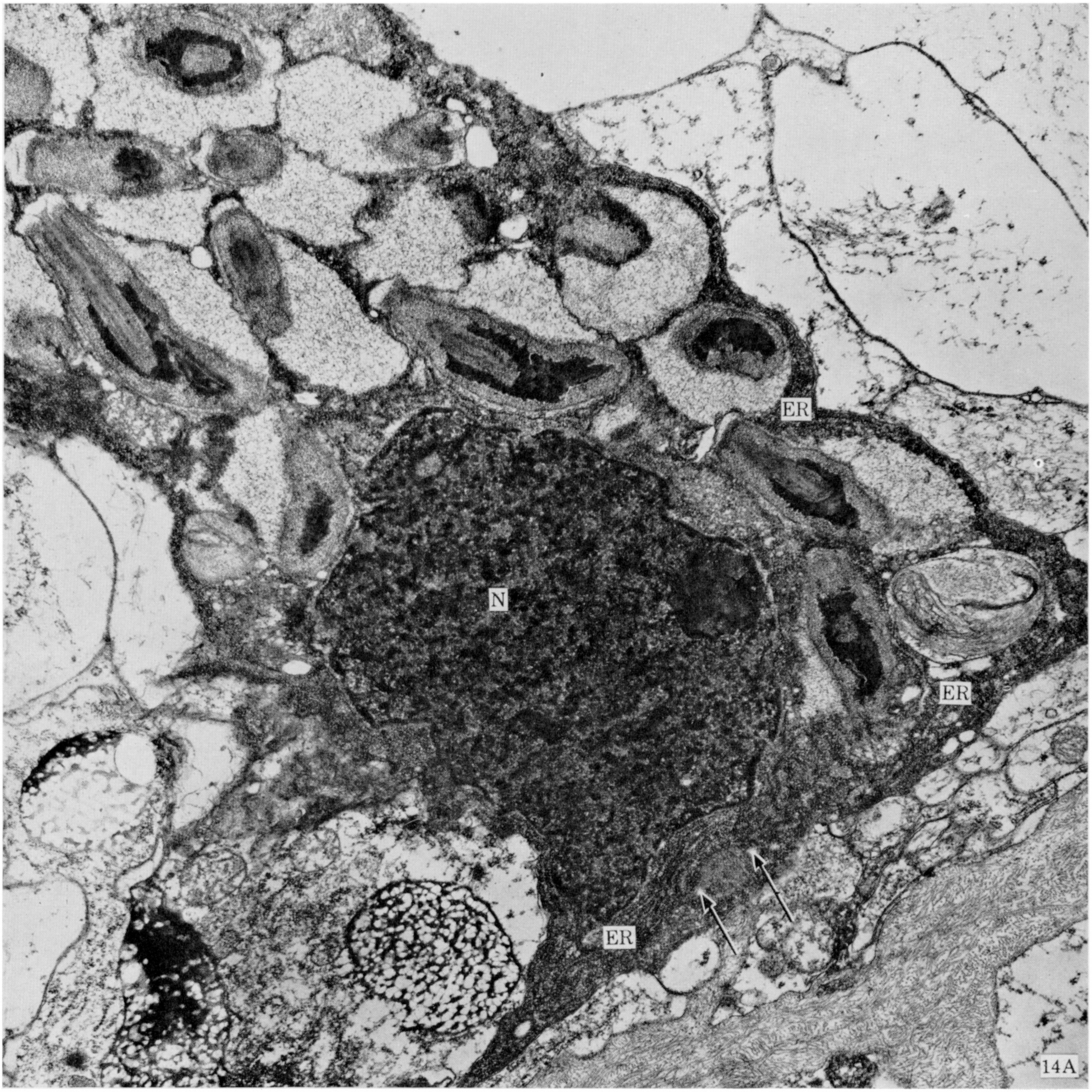


FIGURE 14. A rhabdite-forming cell. Arrows indicate electron-translucent secretion, similar to the material which builds up the electron-translucent 'pool', in the dilated cisternae of endoplasmic reticulum. (Magnifications: *A*, ×15000; *B*, ×26000.)

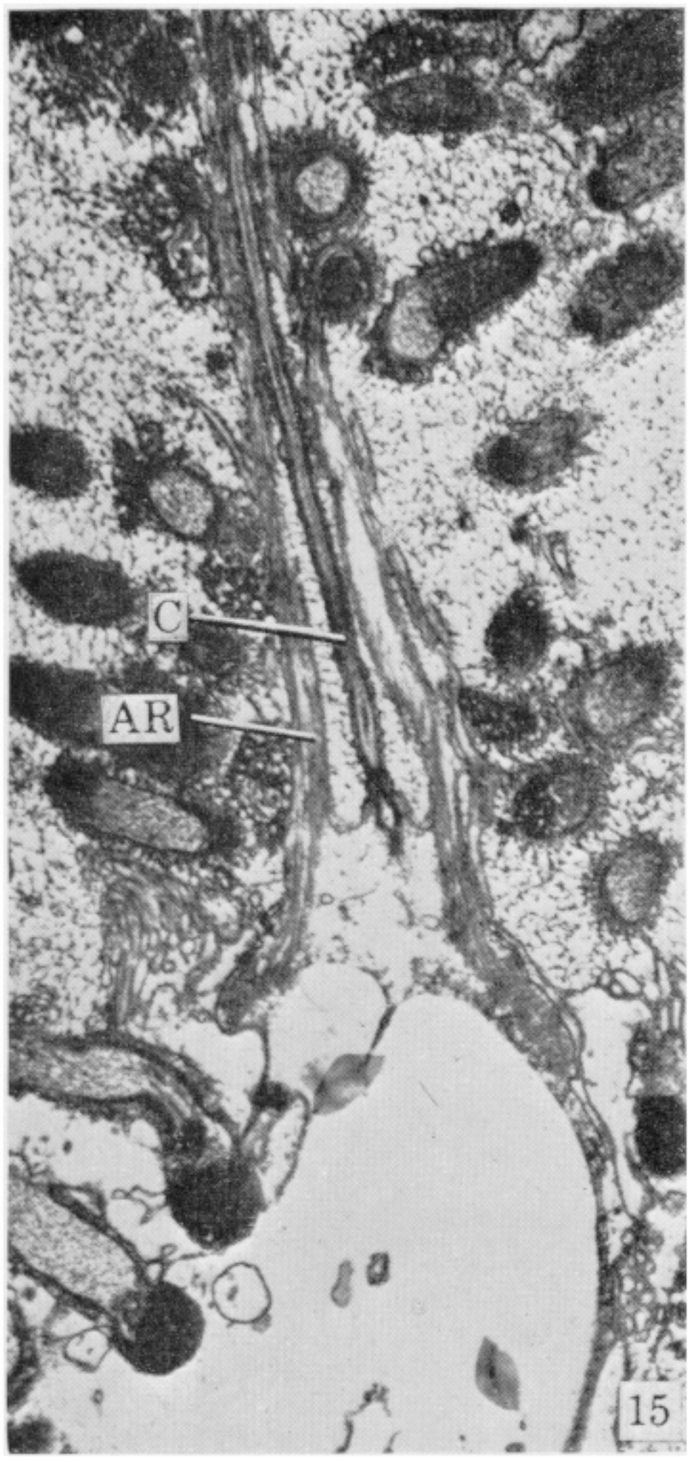
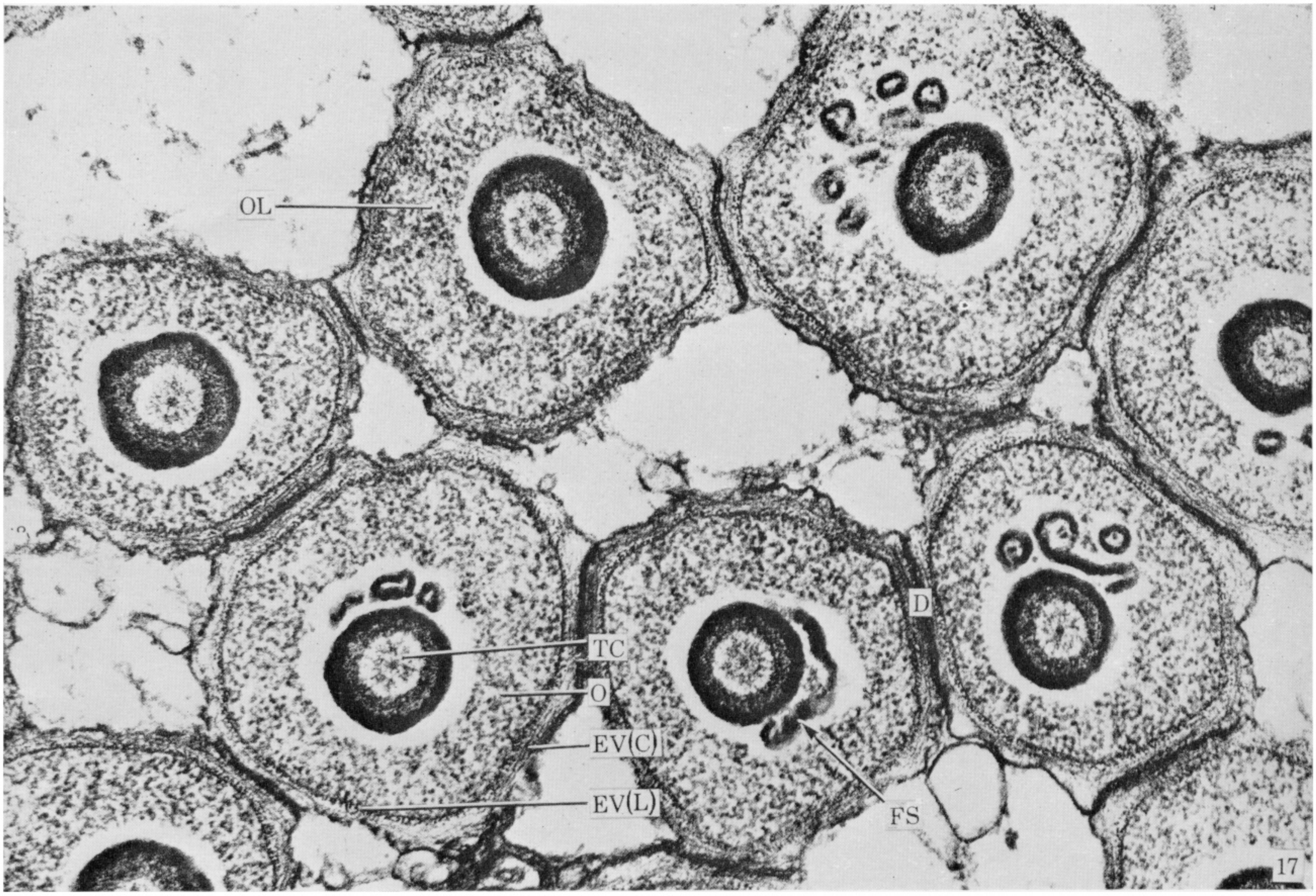


FIGURE 15. The apical surface of a rhabdite cell. A single cilium with its associated structures, is seen to project into the mucus-filled lumen. (Magn. × 6500.)

FIGURE 16. Fully differentiated and partially discharged rhabdites at the apical surface of the rhabdite cells. Note that the central core of the rhabdite is filled with electron-translucent substance (arrows). (Magn. × 24000.)



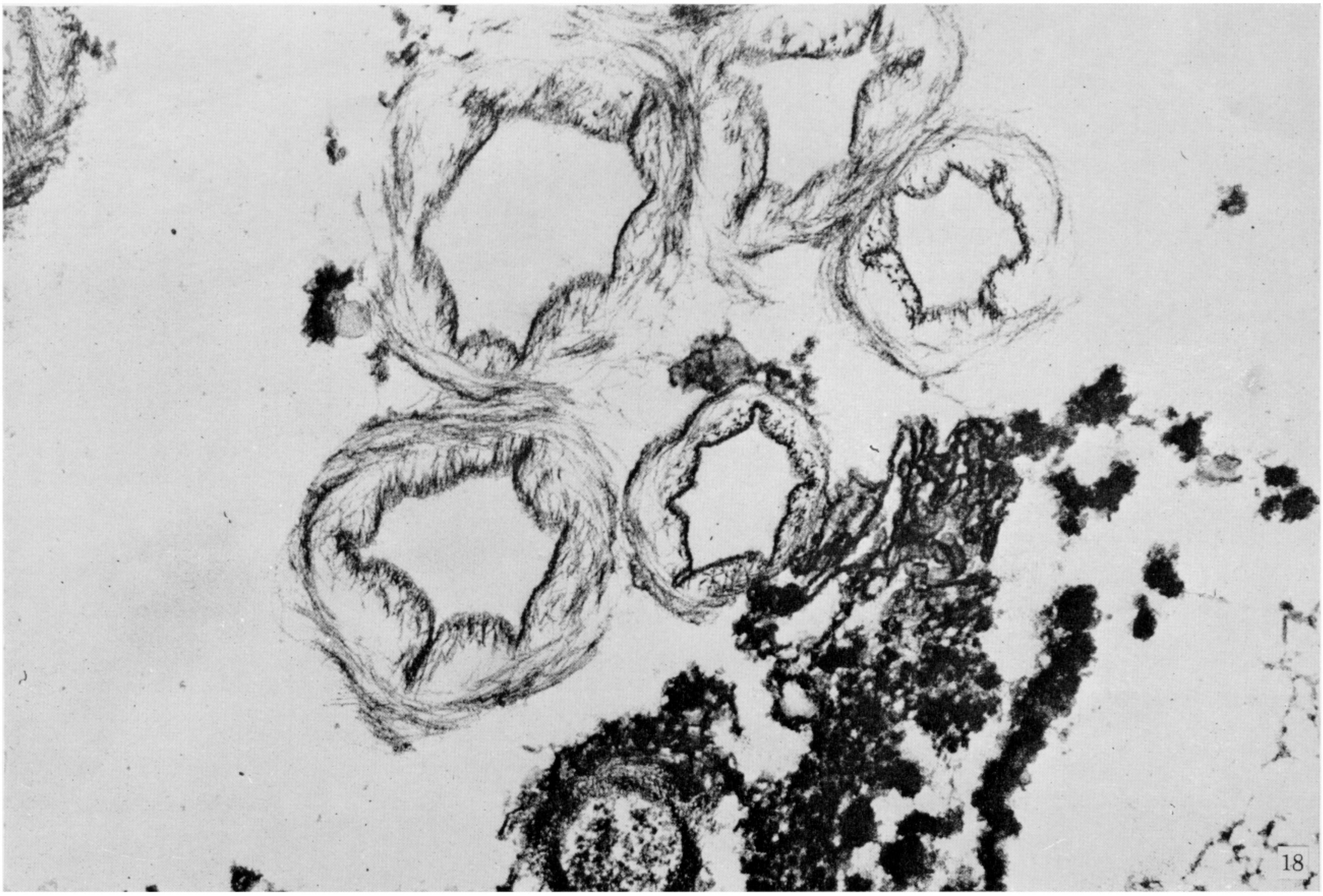
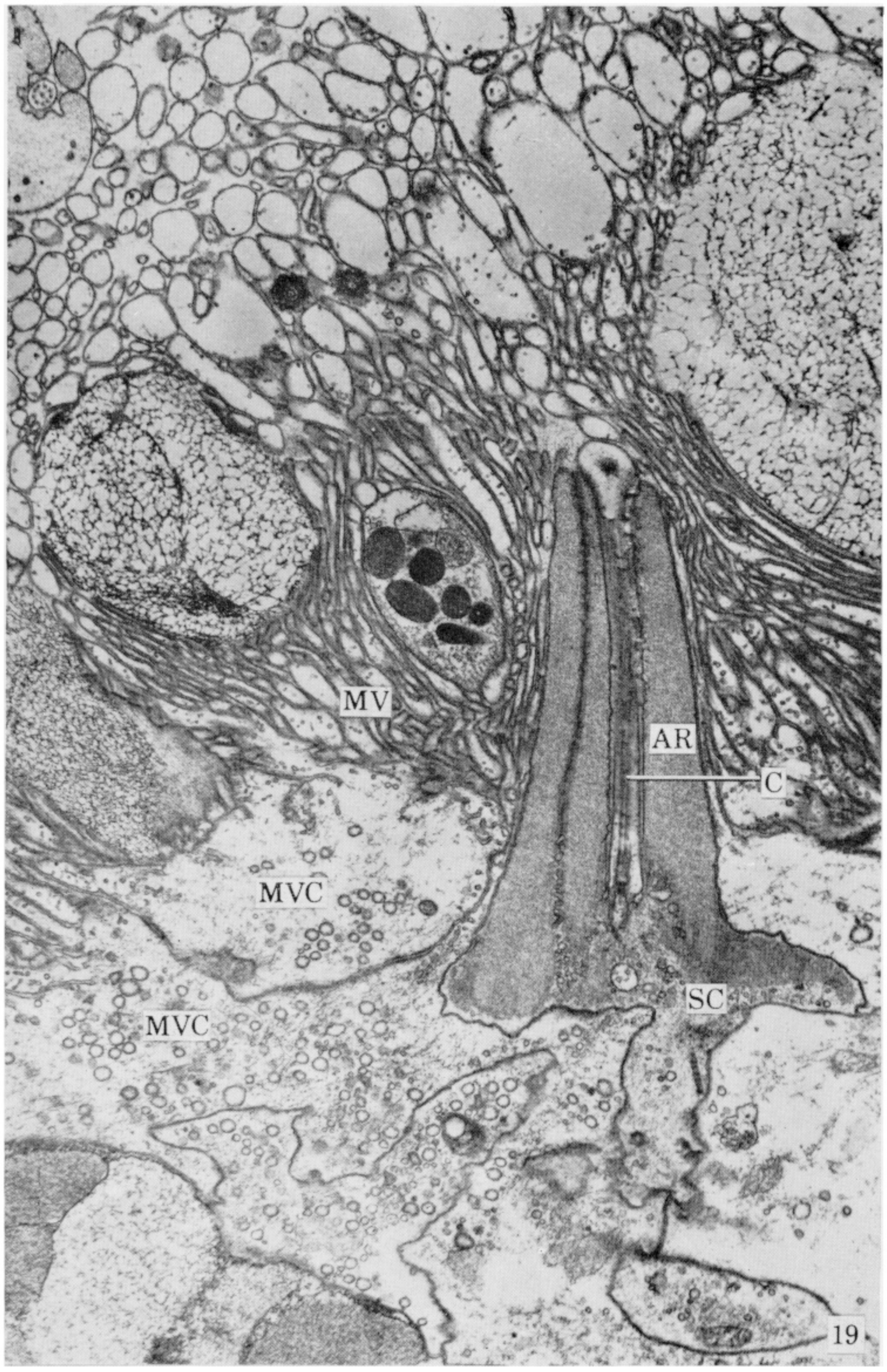
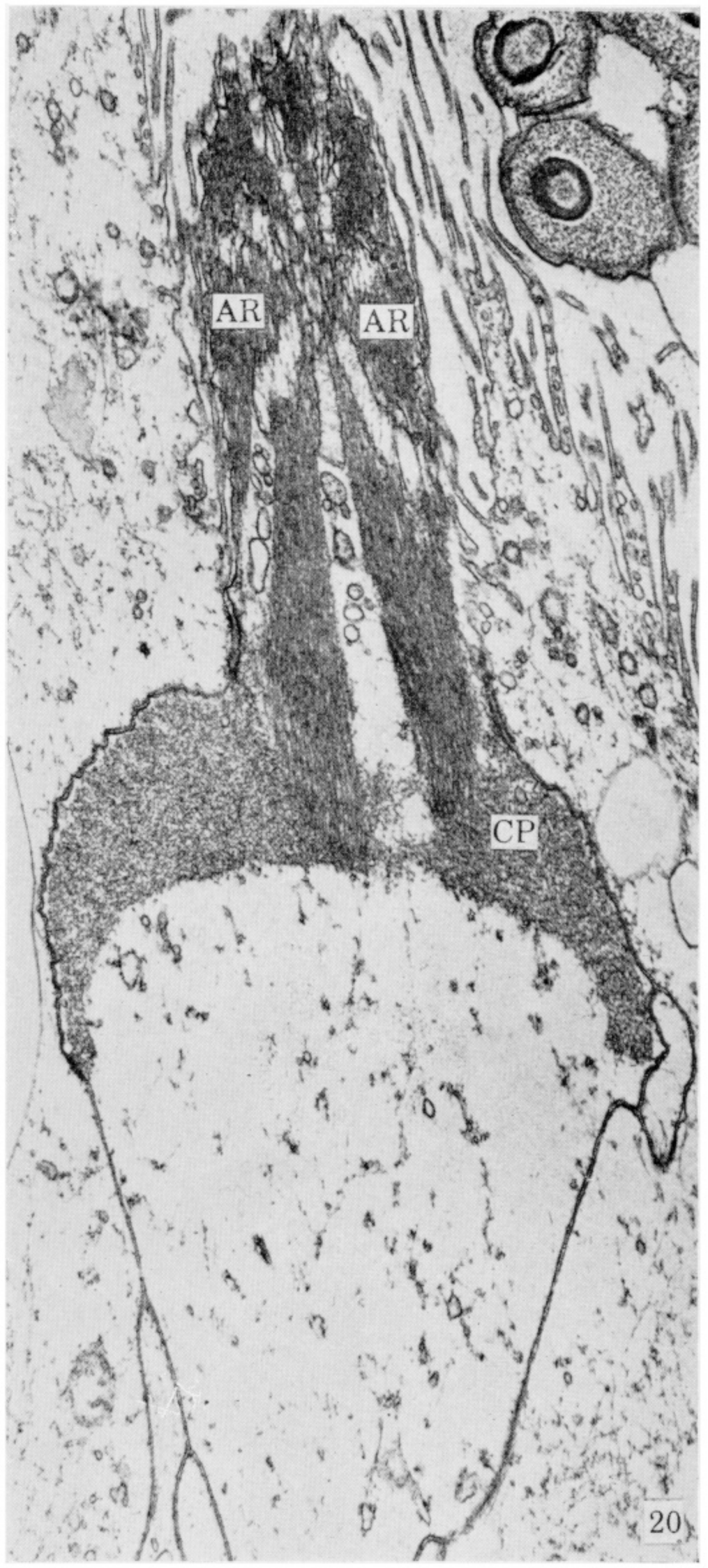
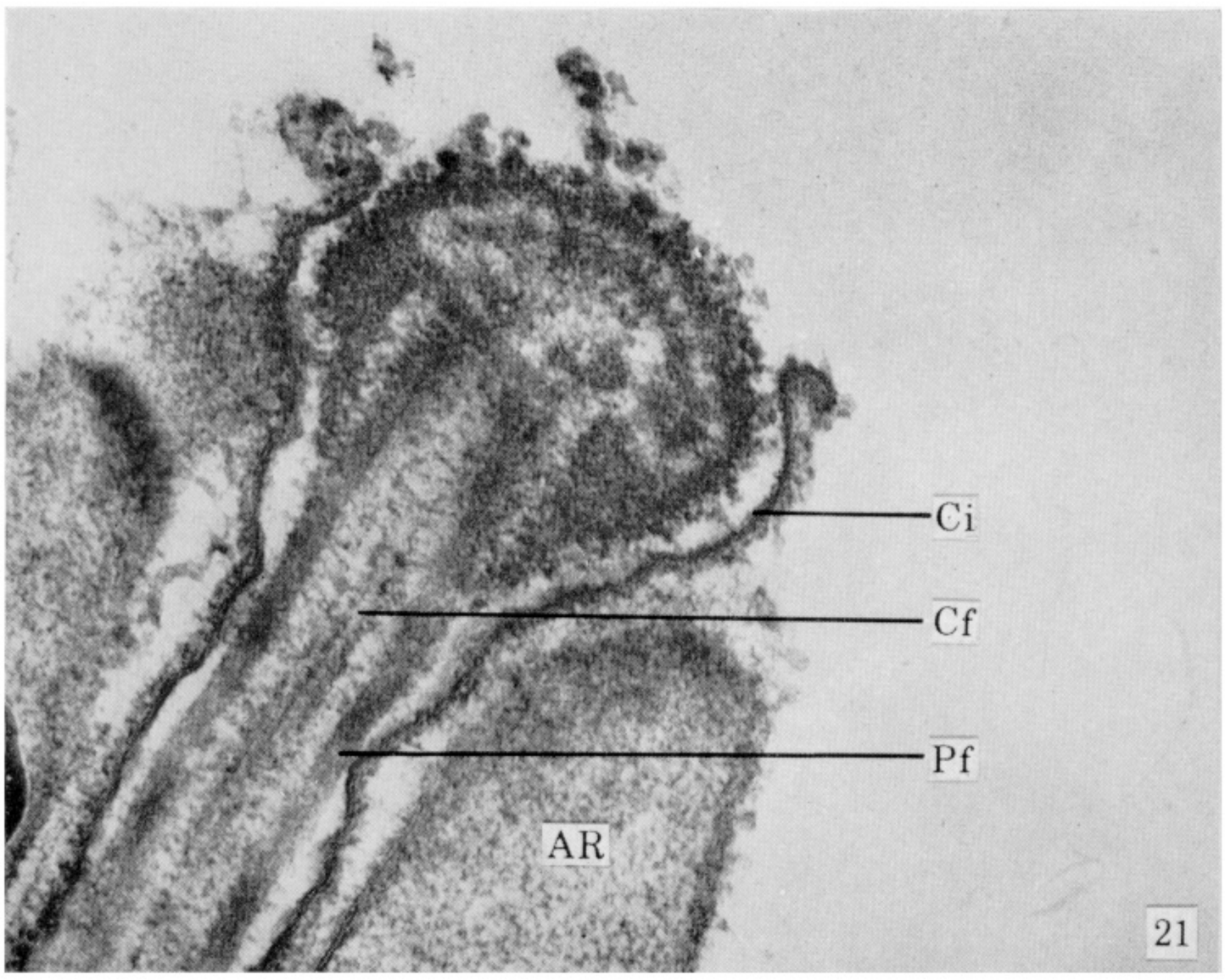


FIGURE 17. Transverse section of discharged rhabdites (see text). (Magn. ×40000.)

FIGURE 18. Transverse section of discharged rhabdites (see text). (Magn. ×40000.)







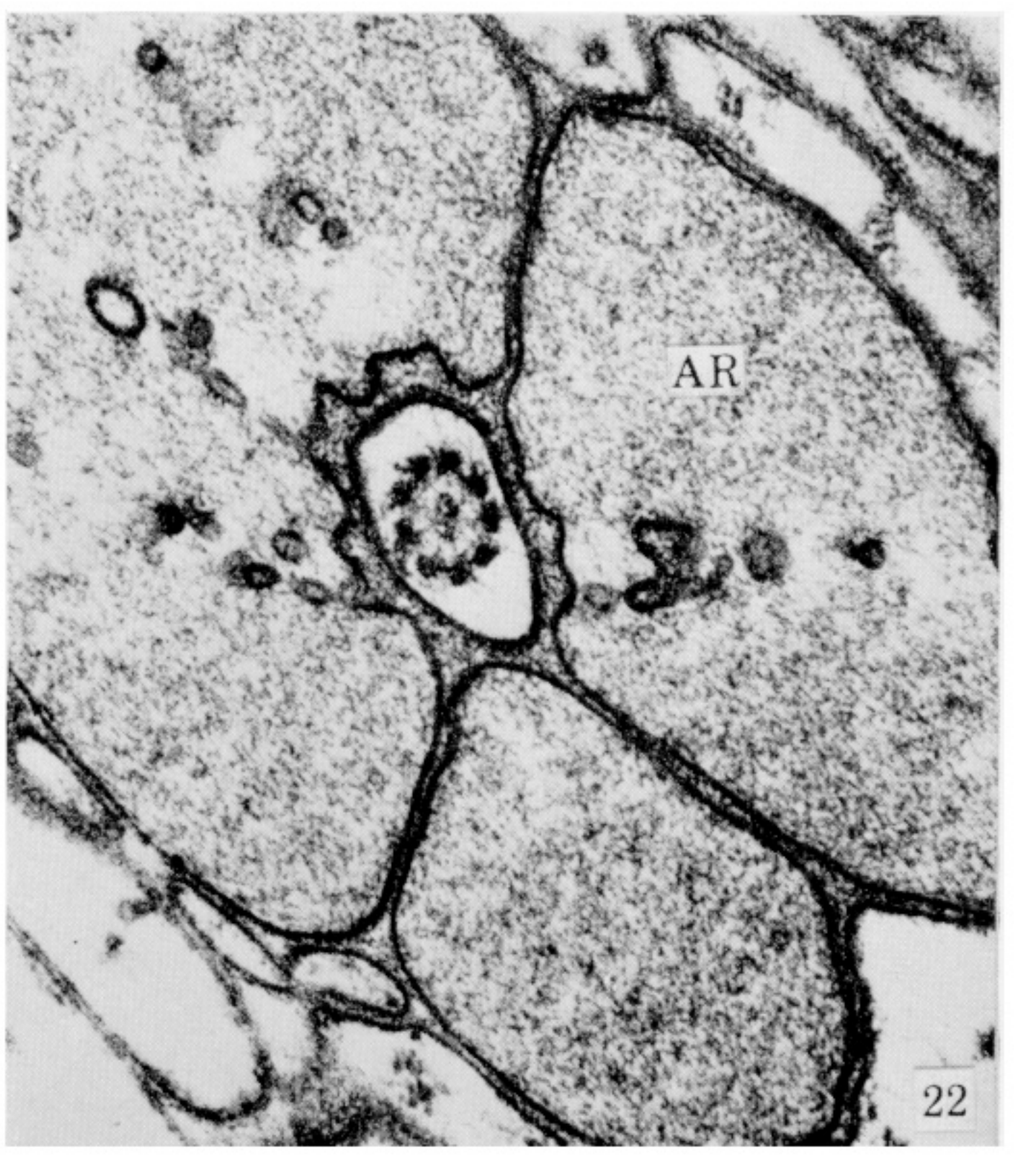


FIGURE 19. 'Sensory' cell and major lining cells (cells with well-developed microvilli). The sensory cell is seen to bear a single cilium. (Magn. × 8000).

FIGURE 20. The cilium of a sensory cell is enclosed by seven 'accessory rods'.

FIGURE 21. The distal tip of a cilium of a 'sensory cell'. (Magn. × 55000.)

FIGURE 22. The 9+2 fibrillar pattern of the cilium of a 'sensory' cell. (Magn. × 40000.)

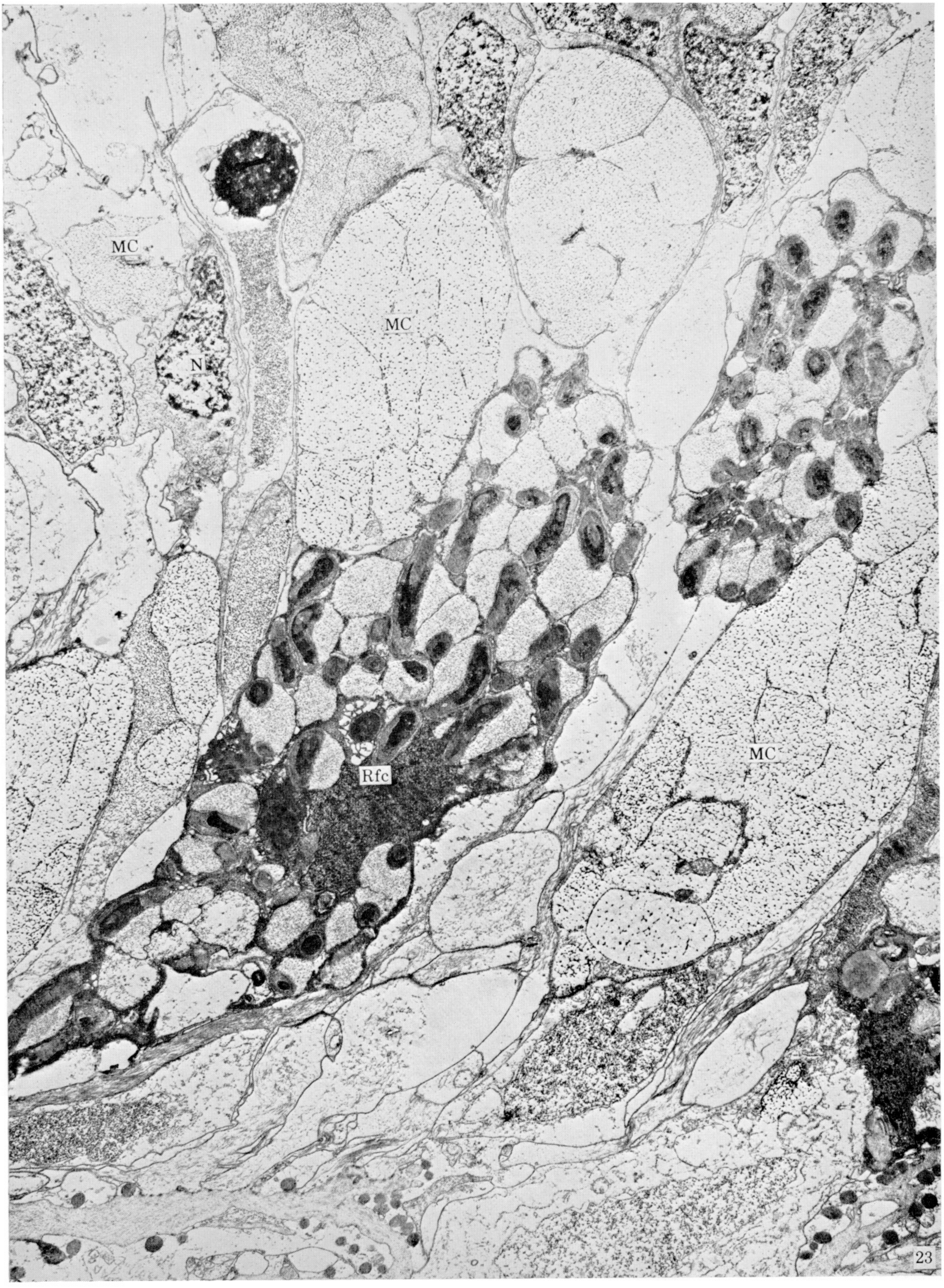
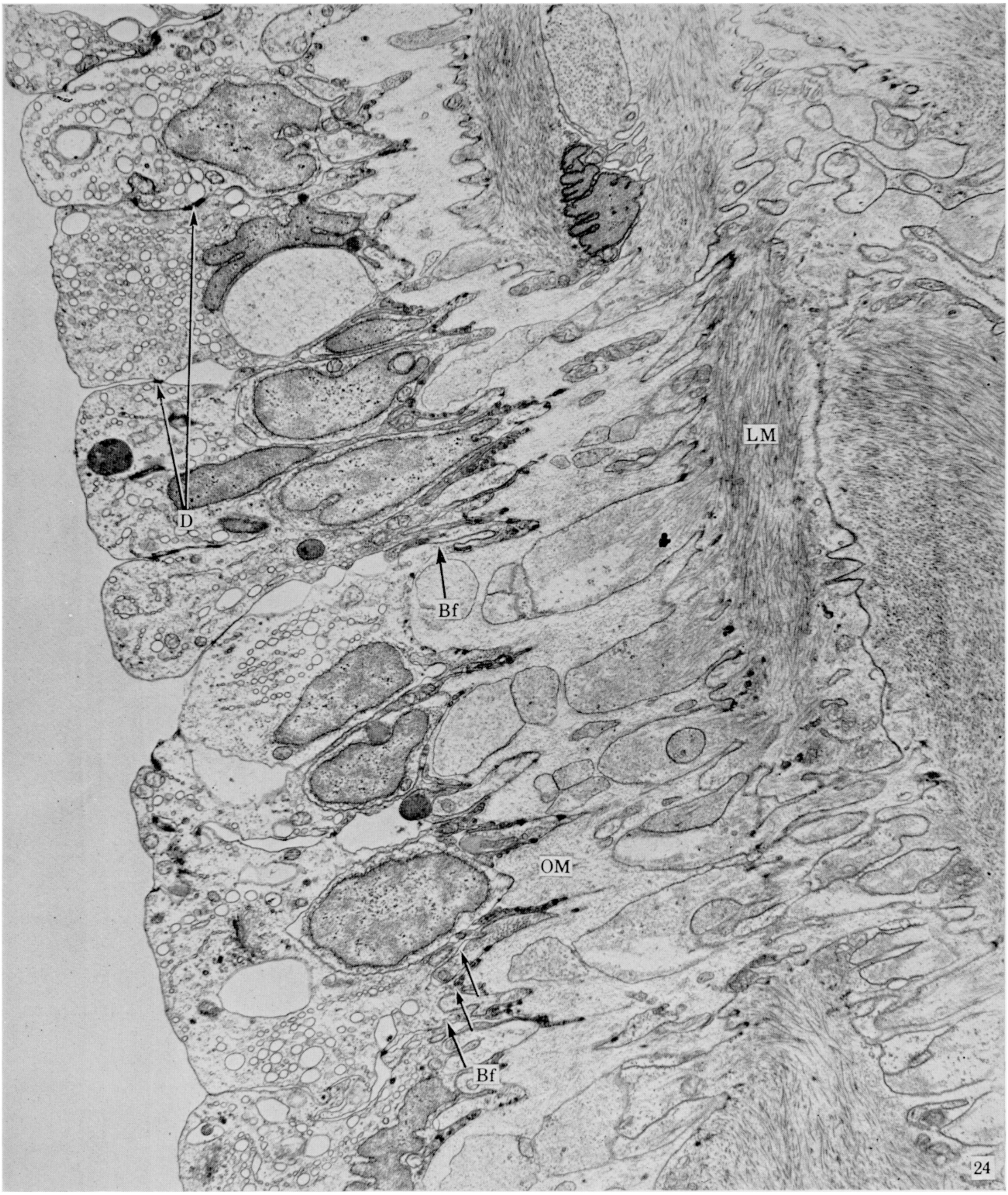


FIGURE 23. Mucus-secreting cells at the middle region of the proboscis. (Magn. × 5400.)



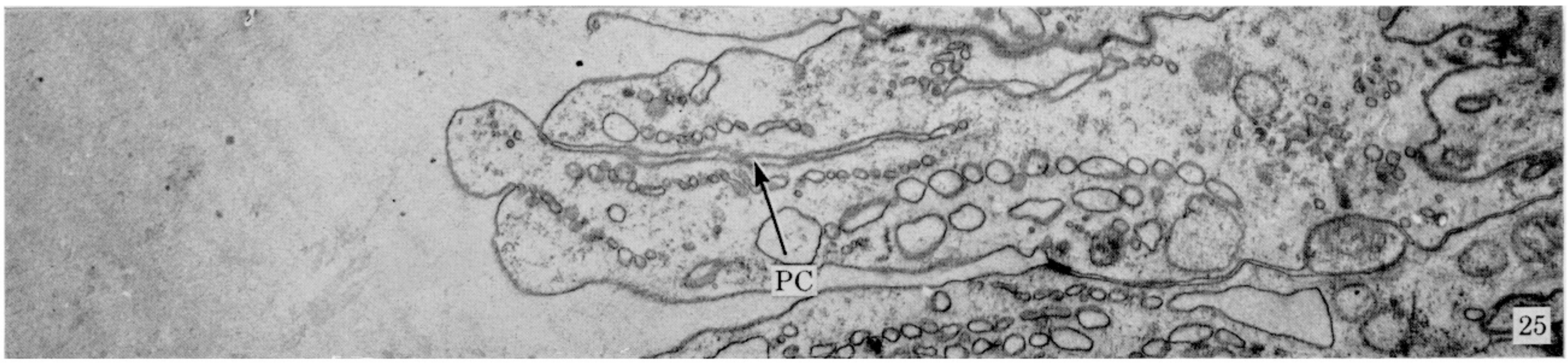


FIGURE 24. The outer endothelial cells of the posterior proboscis. The cells are supported basally by a layer of basement membrane. (Magn. × 6000.)

FIGURE 25. Active pinocytosis in the outer endothelial cells of the posterior proboscis. (Magn. × 10000.)

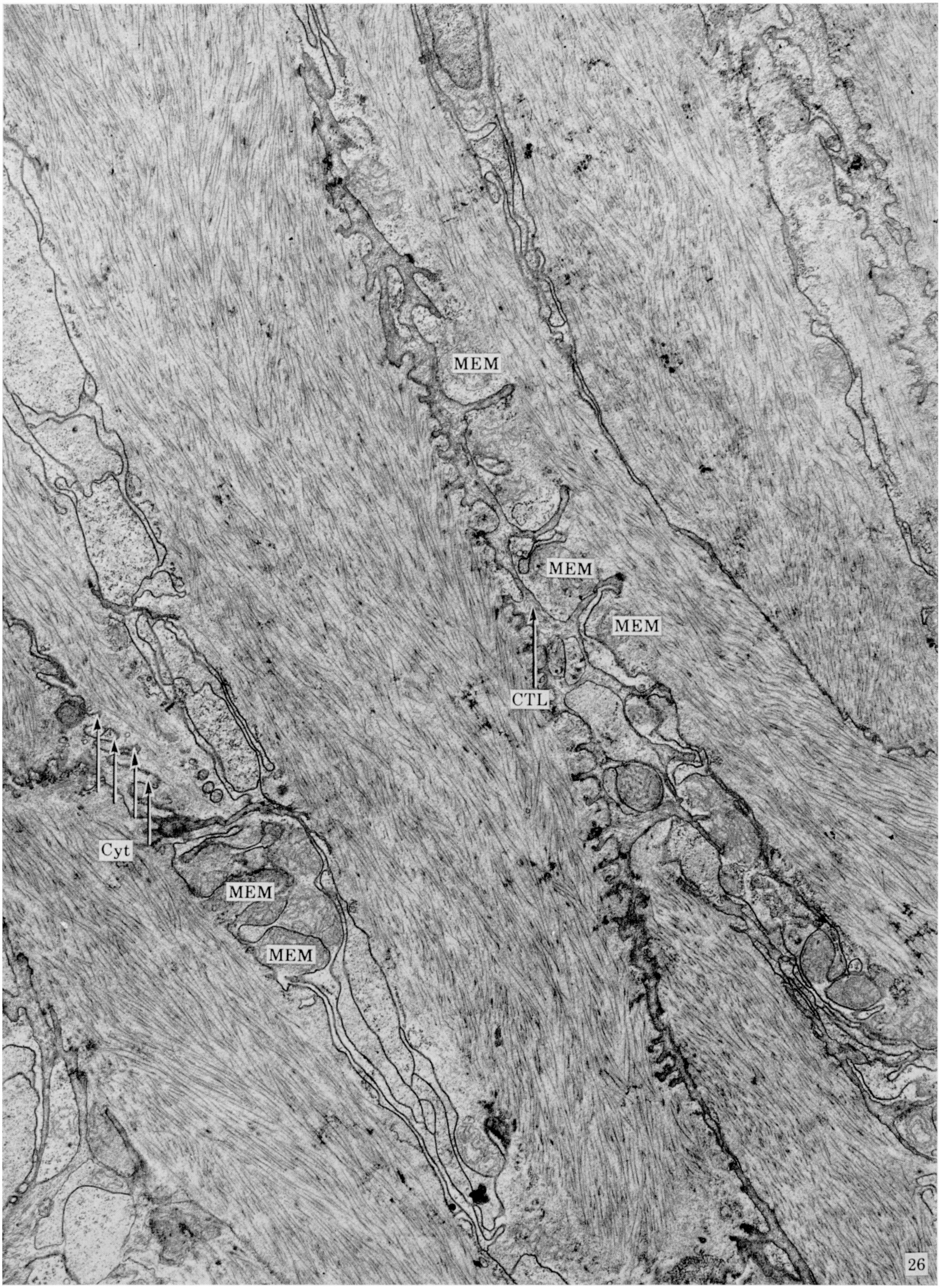
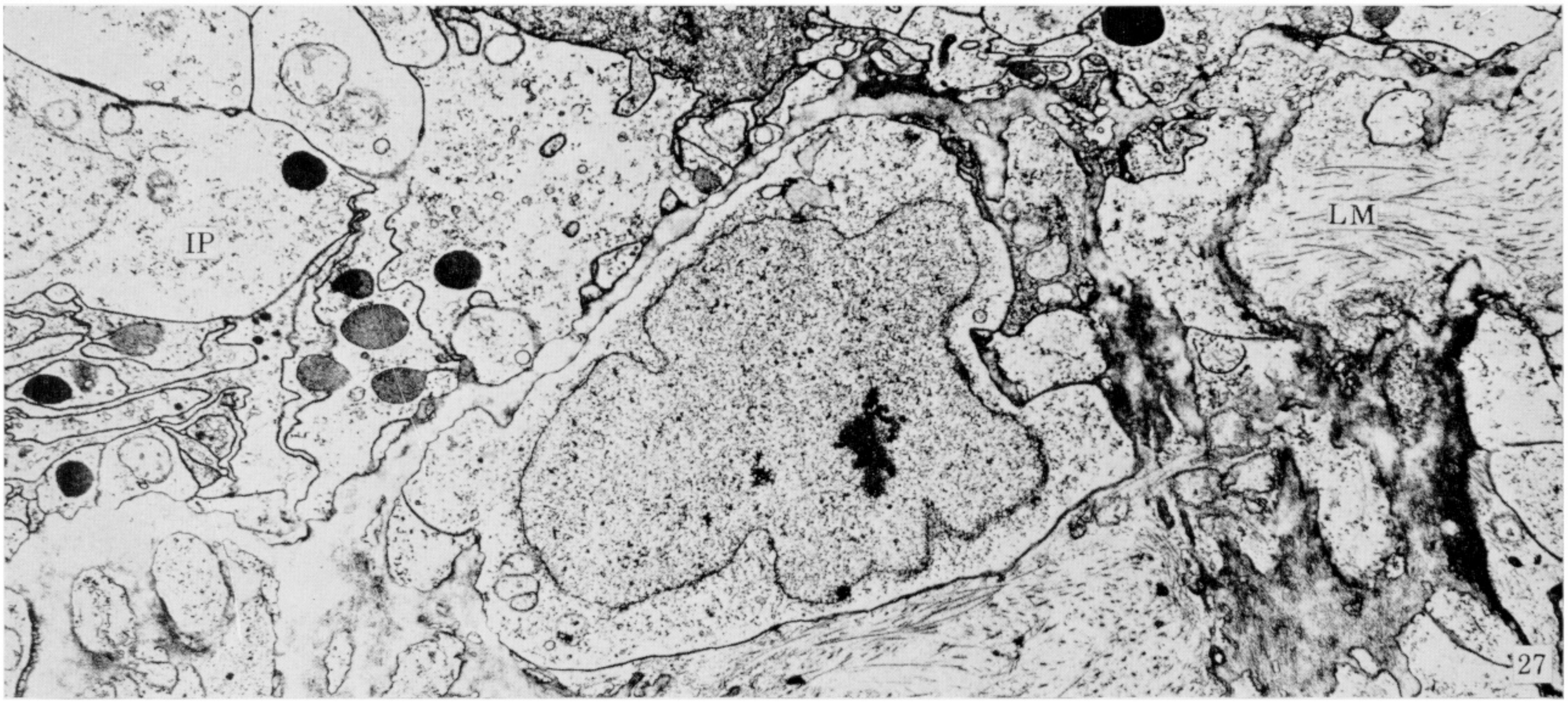
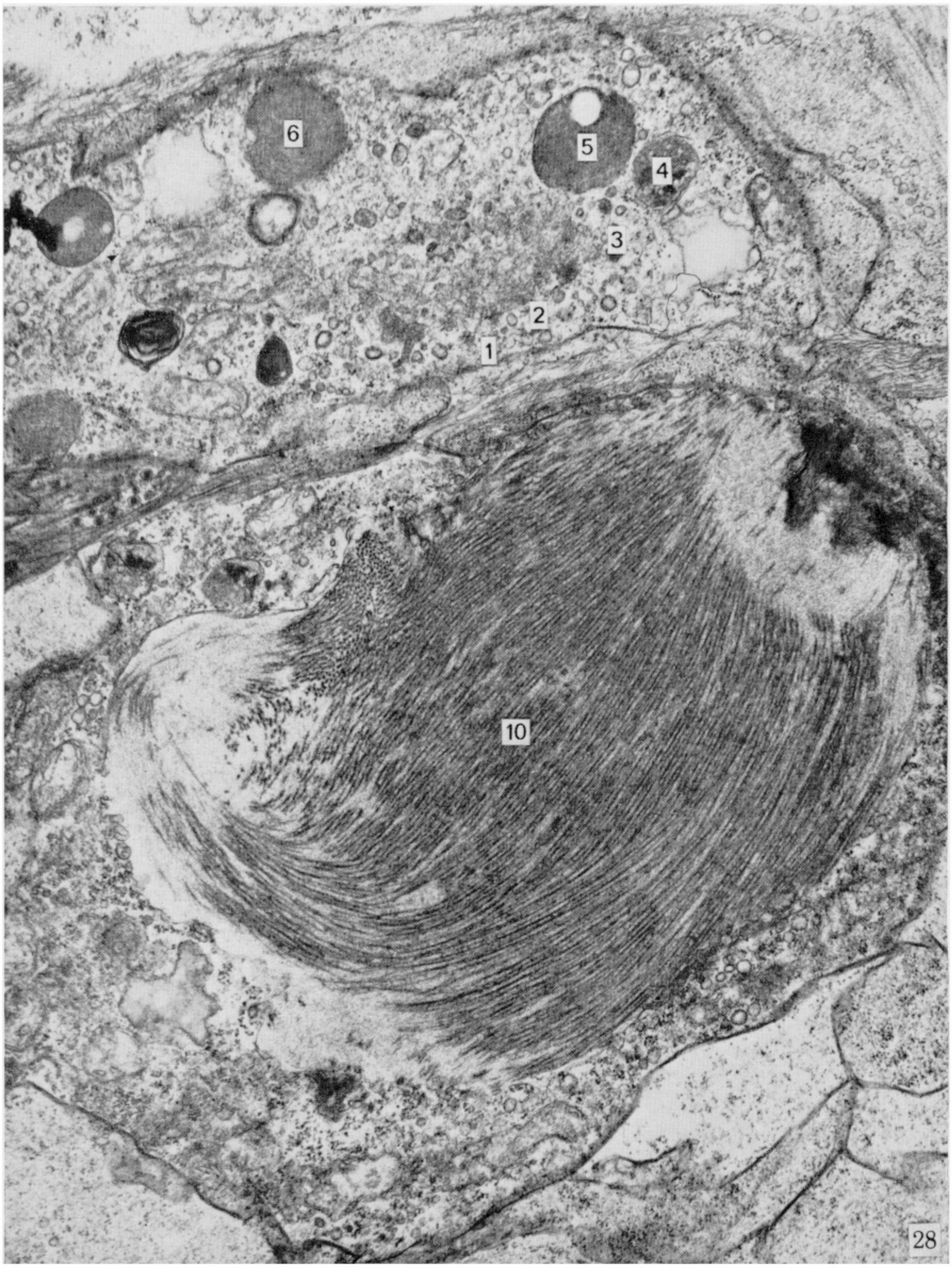
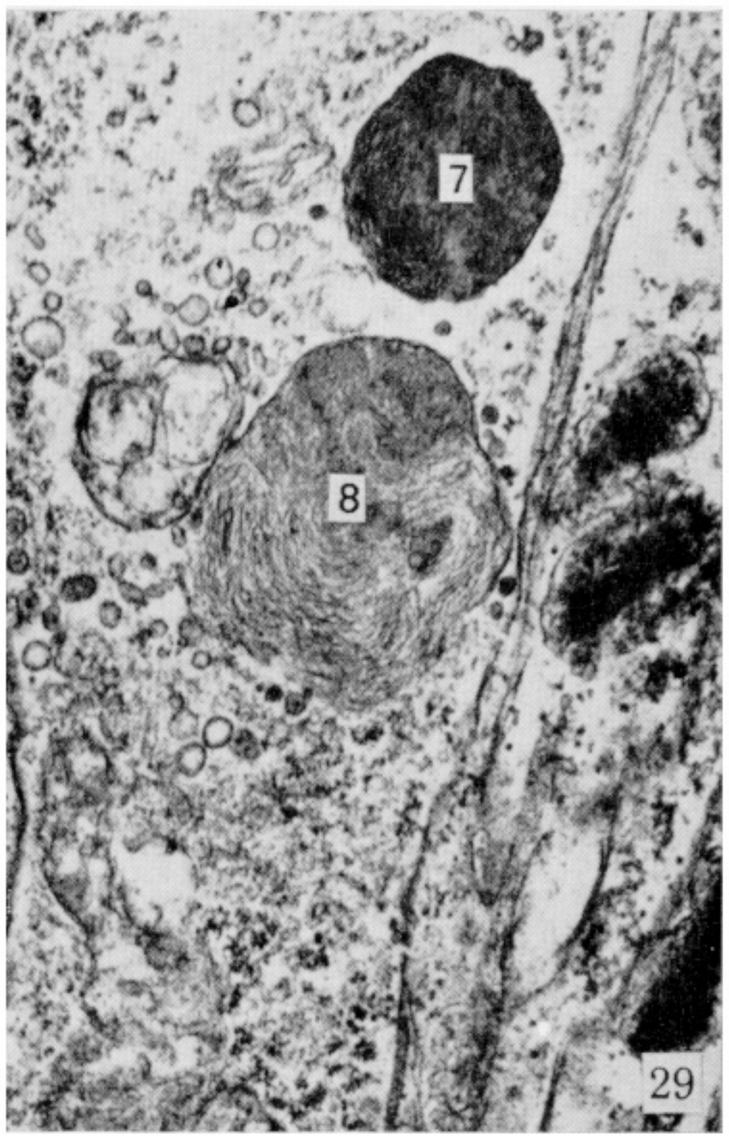


FIGURE 26. Longitudinal section of the longitudinal muscle of the posterior proboscis. (Magn. ×6500.)







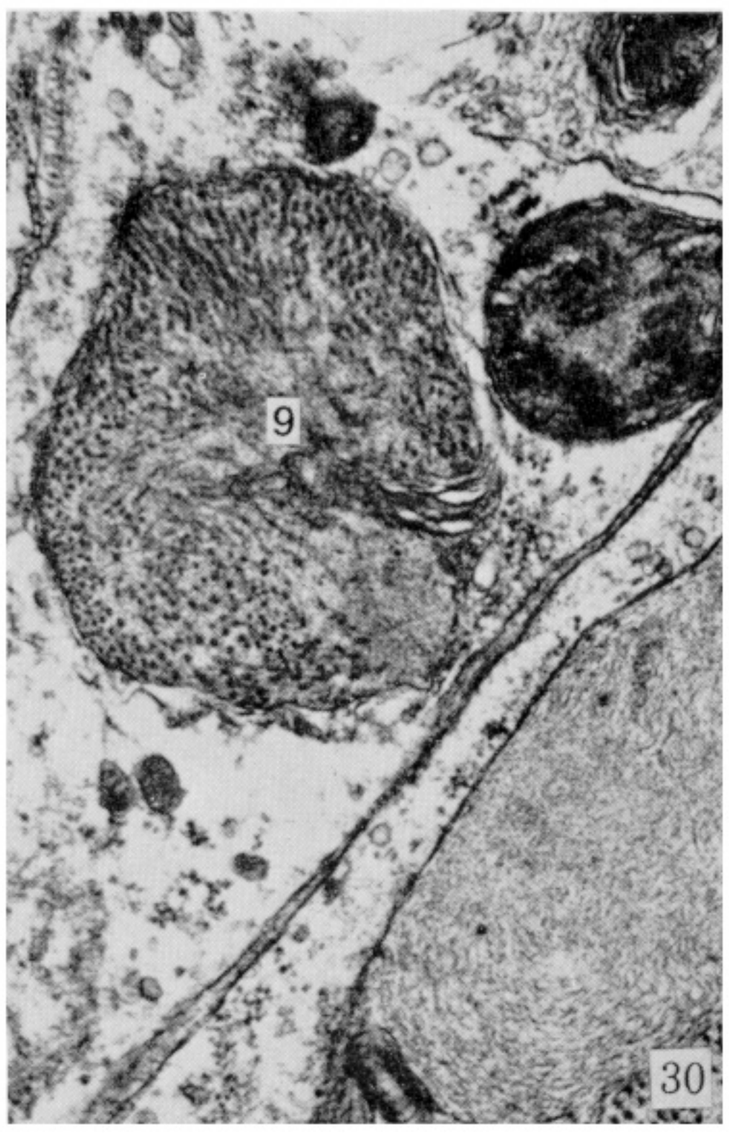
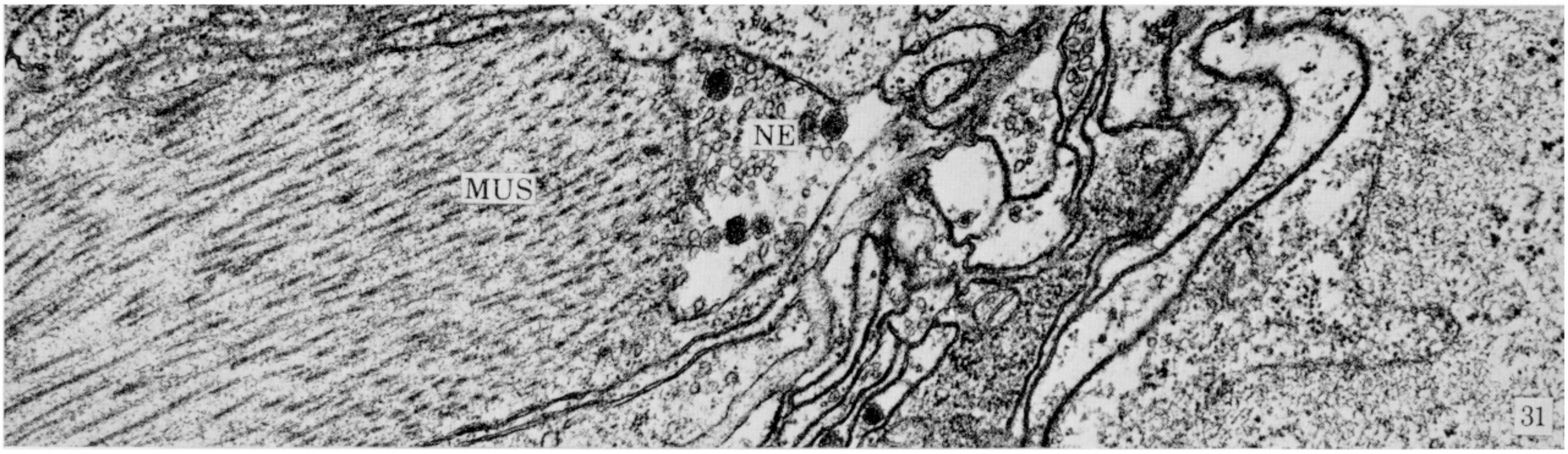
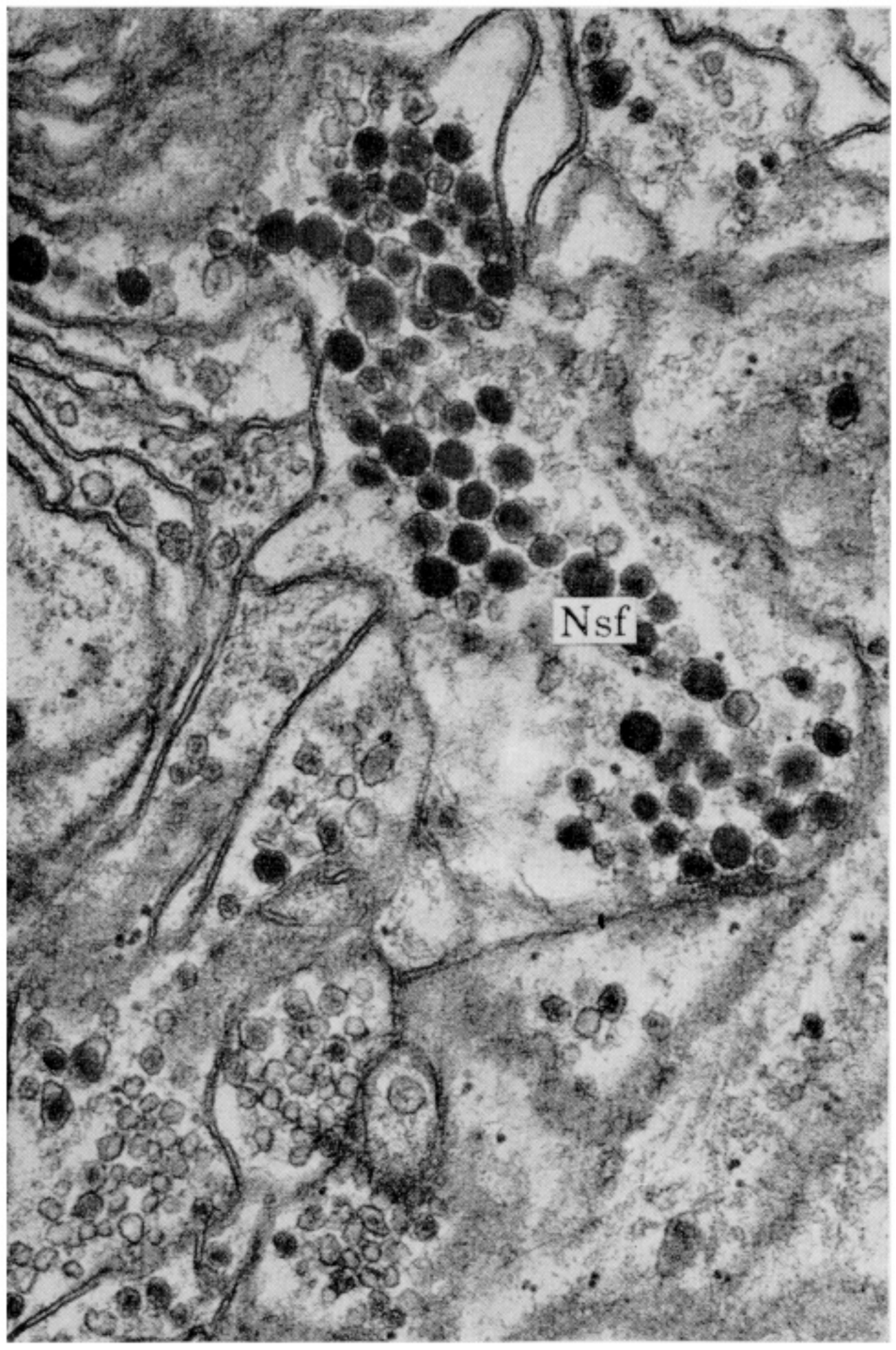


FIGURE 27. An undifferentiated cell found between the inner epithelium and the longitudinal muscle layer. (Magn. × 8000.)

FIGURES 28–30. 'Differentiating cells.' Numbers indicate a possible route by which the myofilaments are formed. Note the cells contain abundant free ribosomes. (Magnifications: figure 28, × 24 000; figure 29, × 16 000; figure 30, × 20 000.)





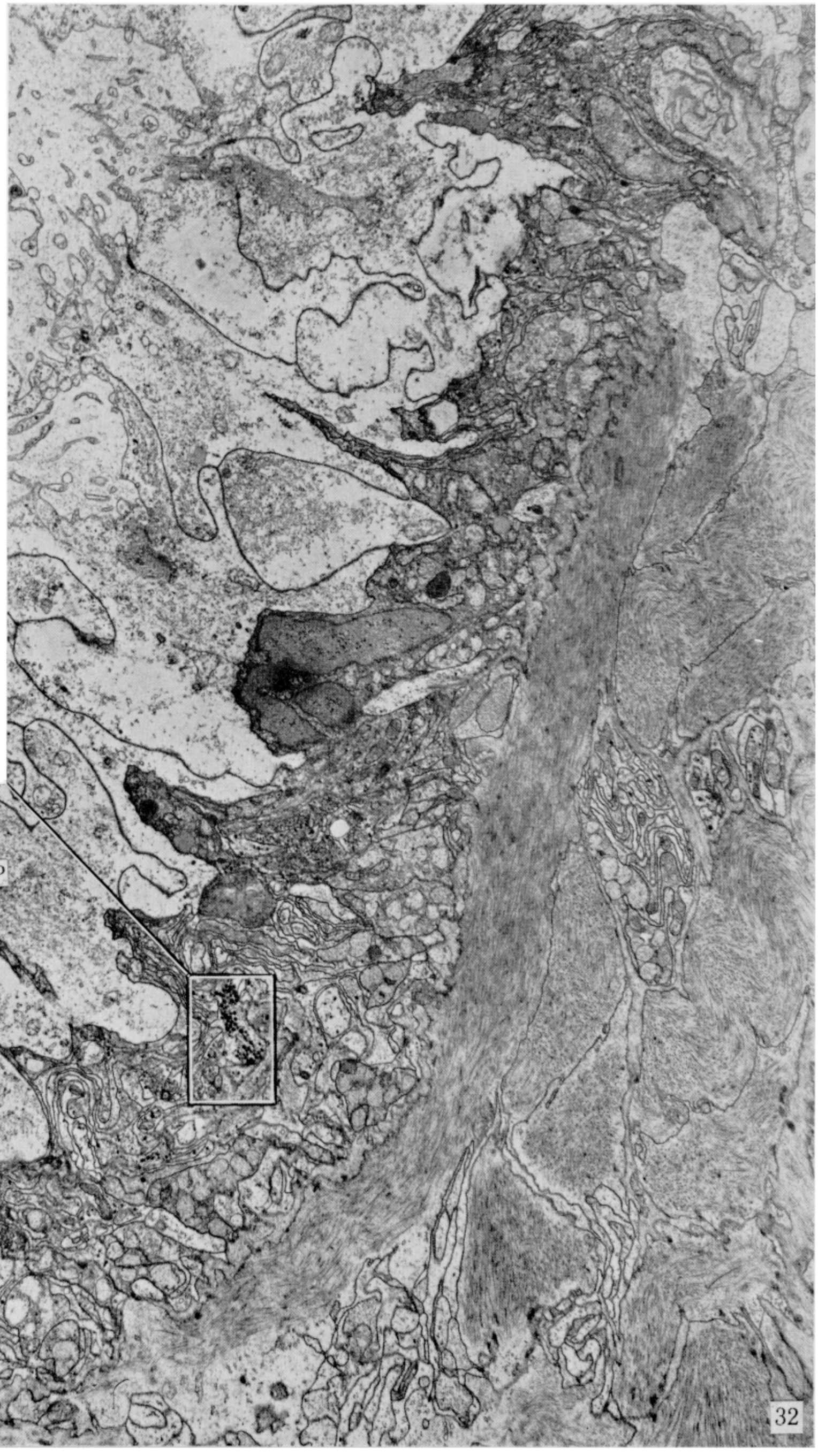


FIGURE 31. The innervation of the longitudinal muscle. (Magn. × 22 500.)

FIGURE 32. Transverse section of the posterior proboscis, showing the epithelial lining cells. Neurosecretory fibres together with the usual terminals are seen beneath the epithelium (see inset; magn. × 30 000). (Magn. × 3300.)

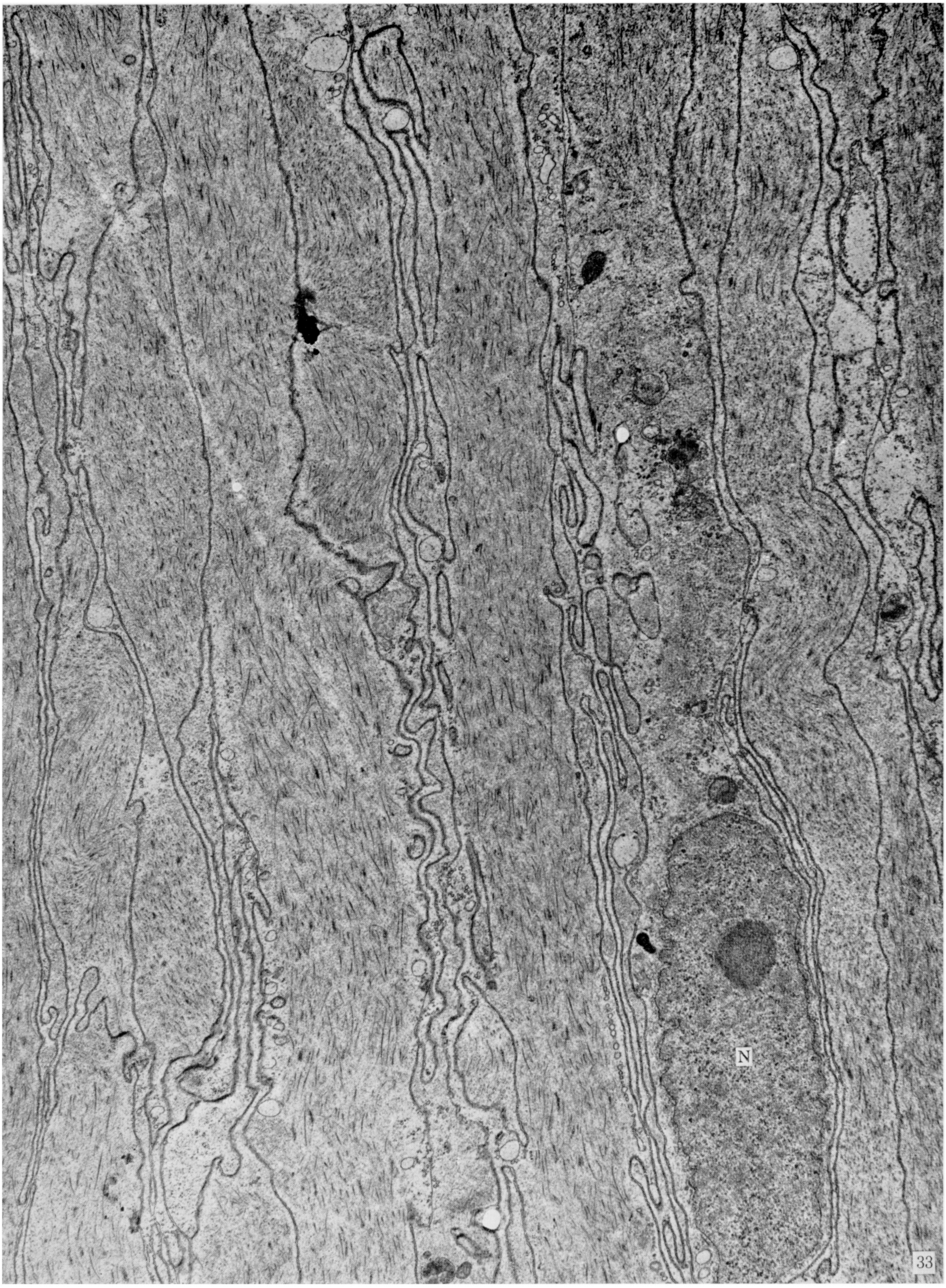
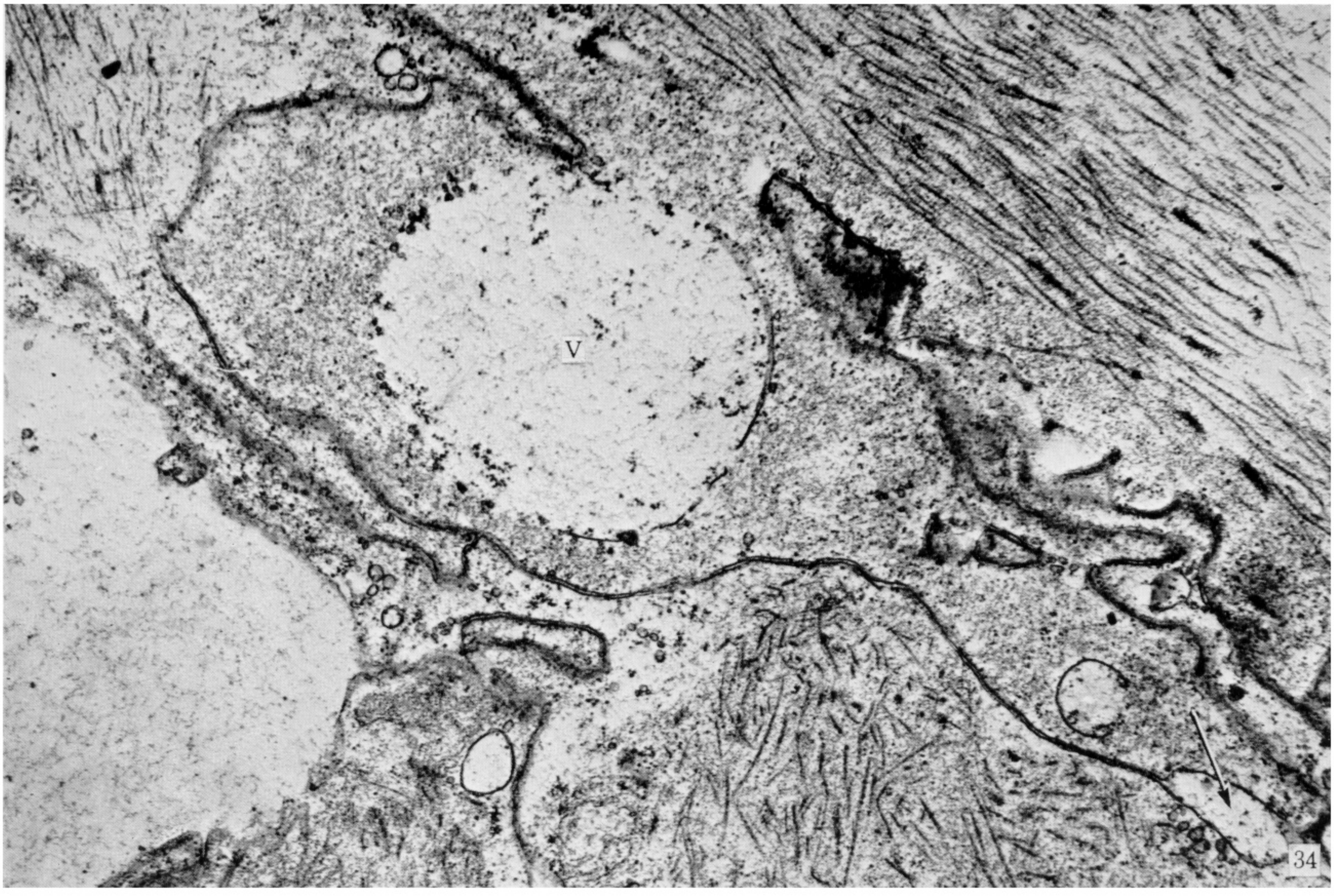


FIGURE 33. Longitudinal section of the retractor muscle. (Magn. × 8000.)



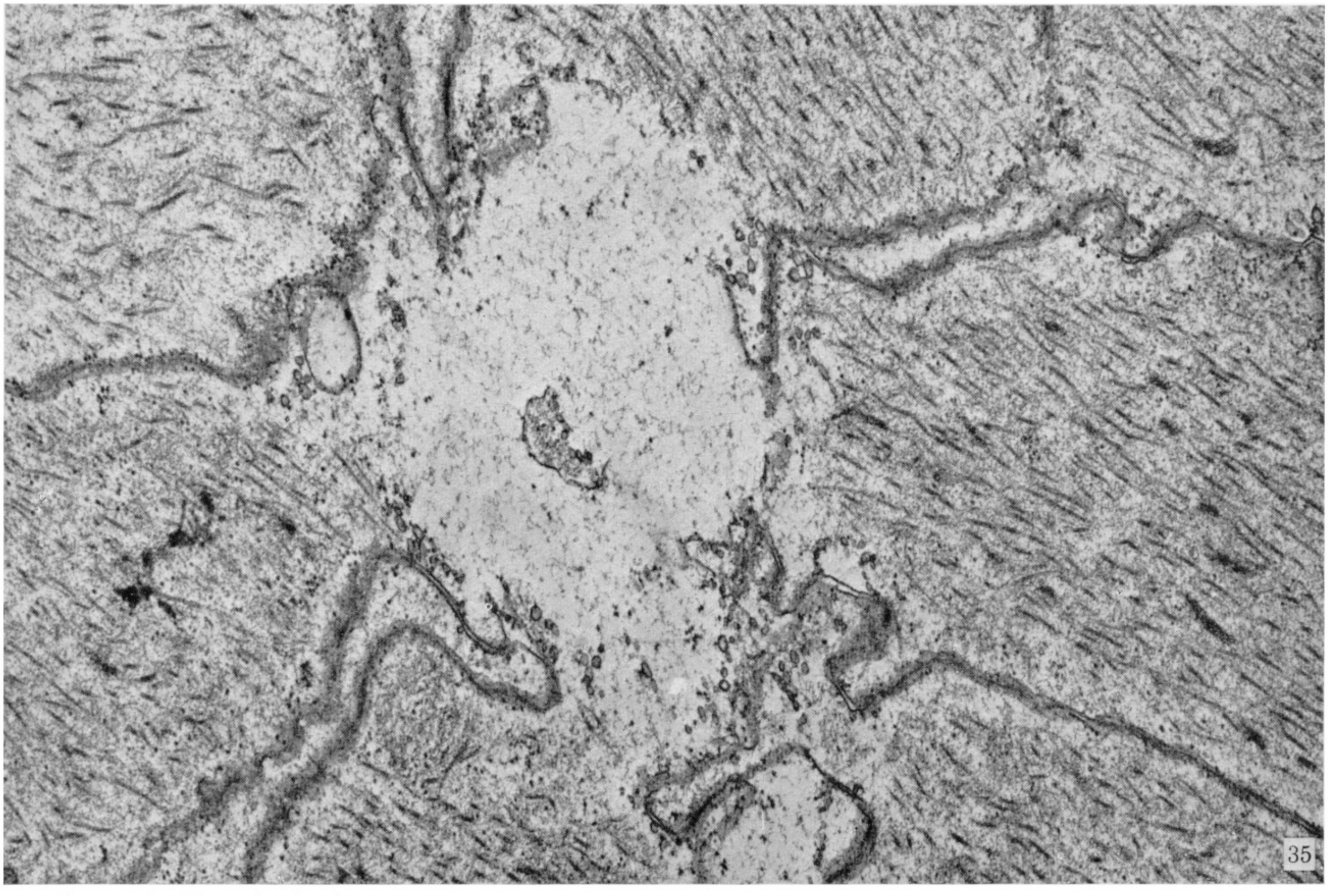


FIGURE 34. Vacuoles in the cytoplasm of the retractor muscle cells. Arrow indicates electron-lucent substance in intercellular space. (Magn. × 18000.)

FIGURE 35. Four muscle cells are seen to 'open' into a common 'secretion pool'. (Magn. × 27500.)

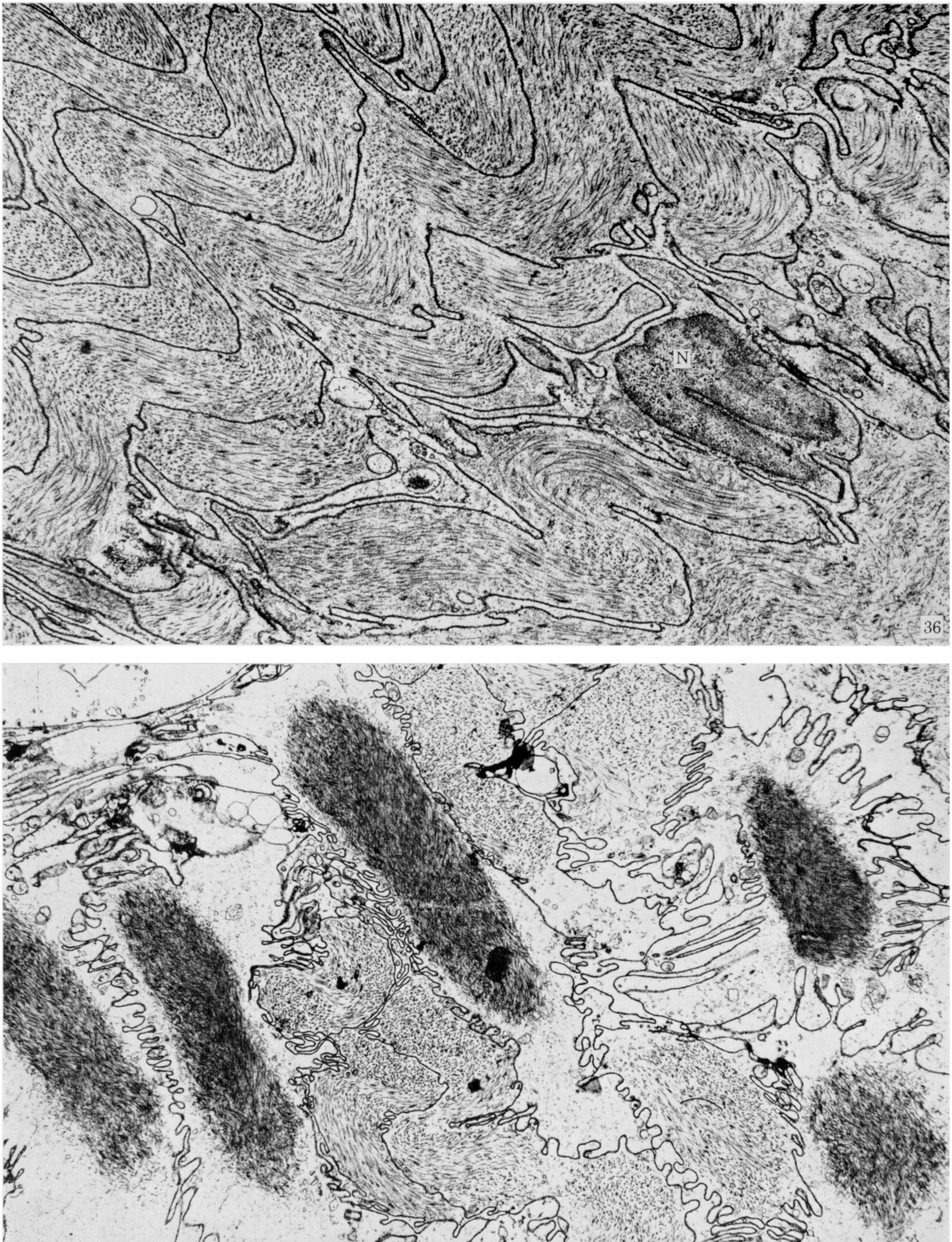


FIGURE 36. Zigzag pattern of serpentine-like muscle cells. Note the deeply infolded nucleus. (Magn. × 7500.)

FIGURE 37. Concertina-like or contracted muscle cells. (Magn. × 4500.)

FIGURE 38. 'Intermediate' muscle cells. (Magn. × 4200.)

FIGURE 39. 'Intermediate' muscle cells. (Magn. × 6000.)

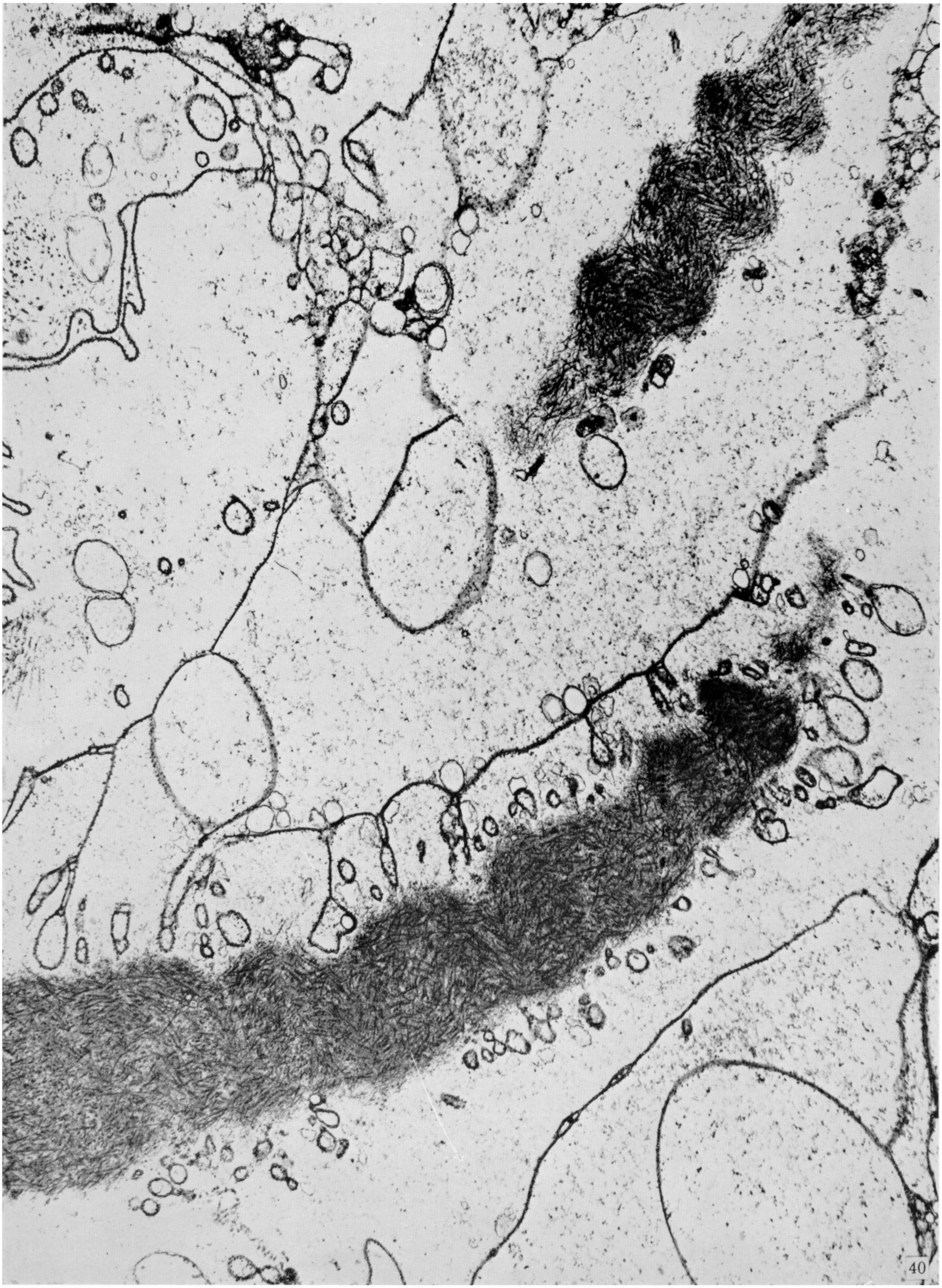


FIGURE 40. Oxytocin-treated. Contracted muscle cells. Note the presence of numerous microvesicles in the cytoplasm. (Magn. × 14000.)

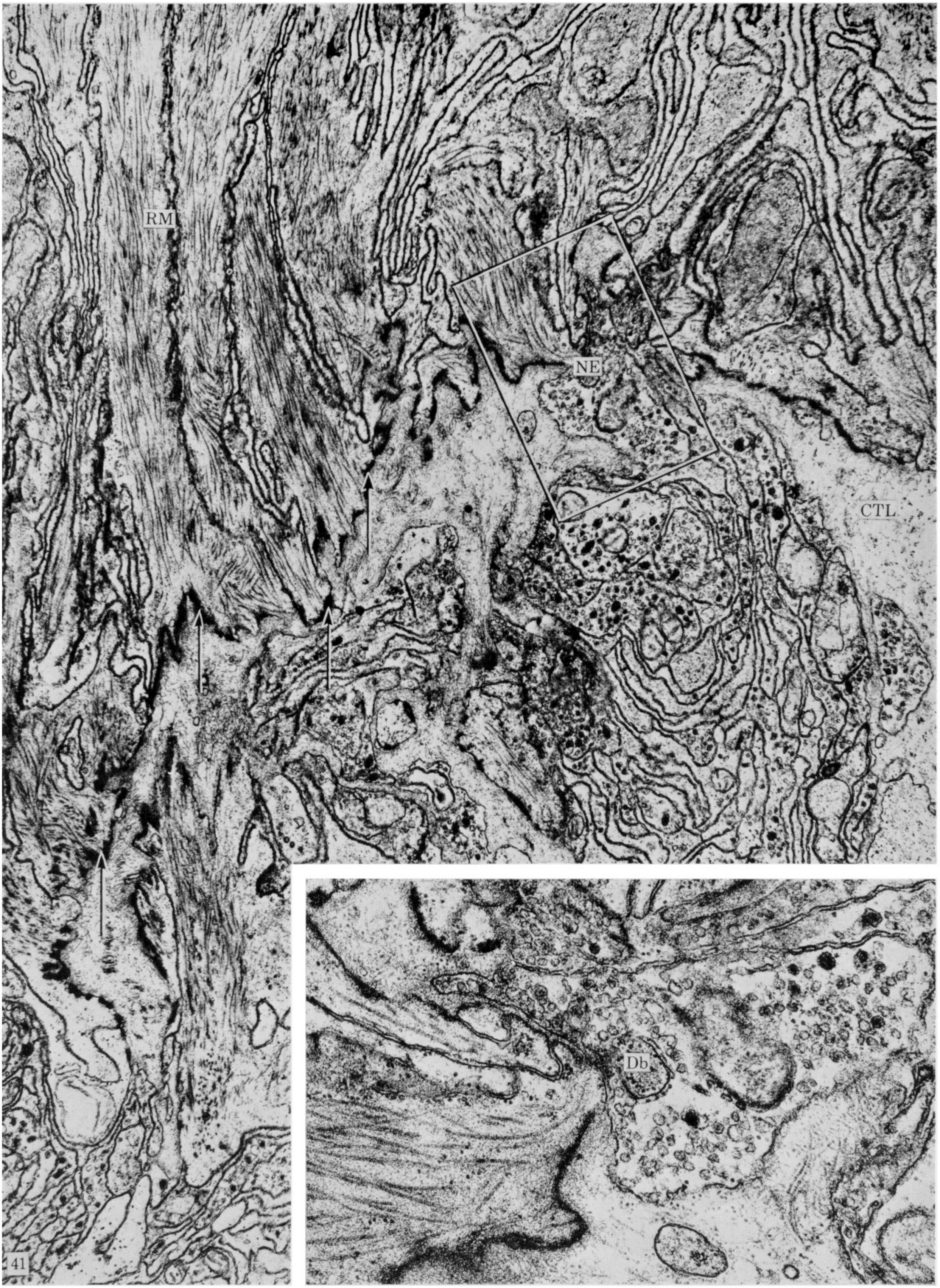


FIGURE 41. Large bundles of non-myelinated nerve endings are seen closely appressed to the connective tissue layer (CTL) separating the retractor muscle from the proboscis. Close contacts between the nerve endings and the retractor muscle cells are not infrequently seen (see inset; magn. ×30000). Arrows indicate 'attachment plaques'—regions where the myofilaments fuse to the cell membranes. See also figure 5. (Magn. ×12000.)

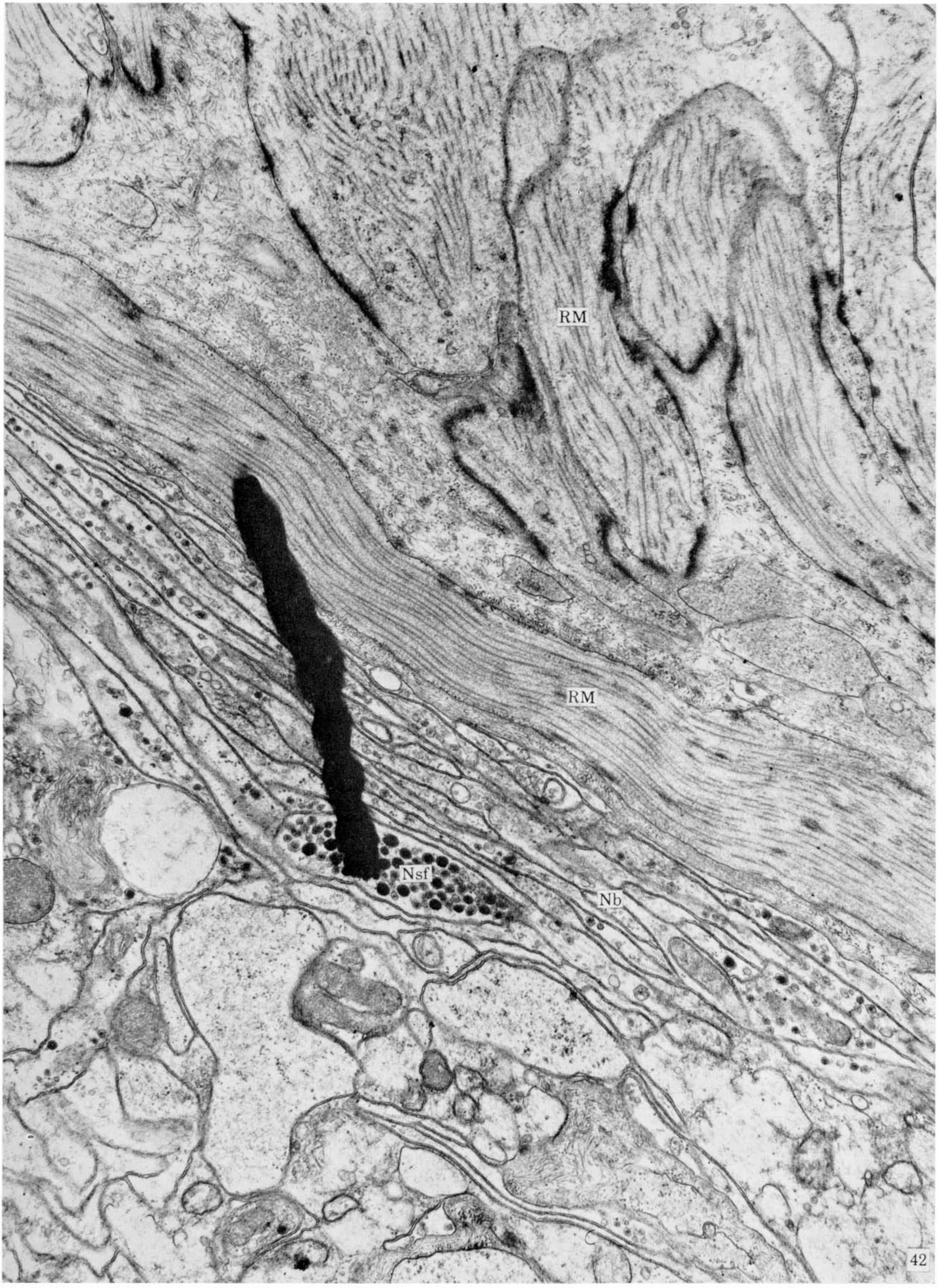


FIGURE 42. Neurosecretory fibres (terminals) are seen in the close vicinity of the retractor muscle. (Magn. × 18000.)

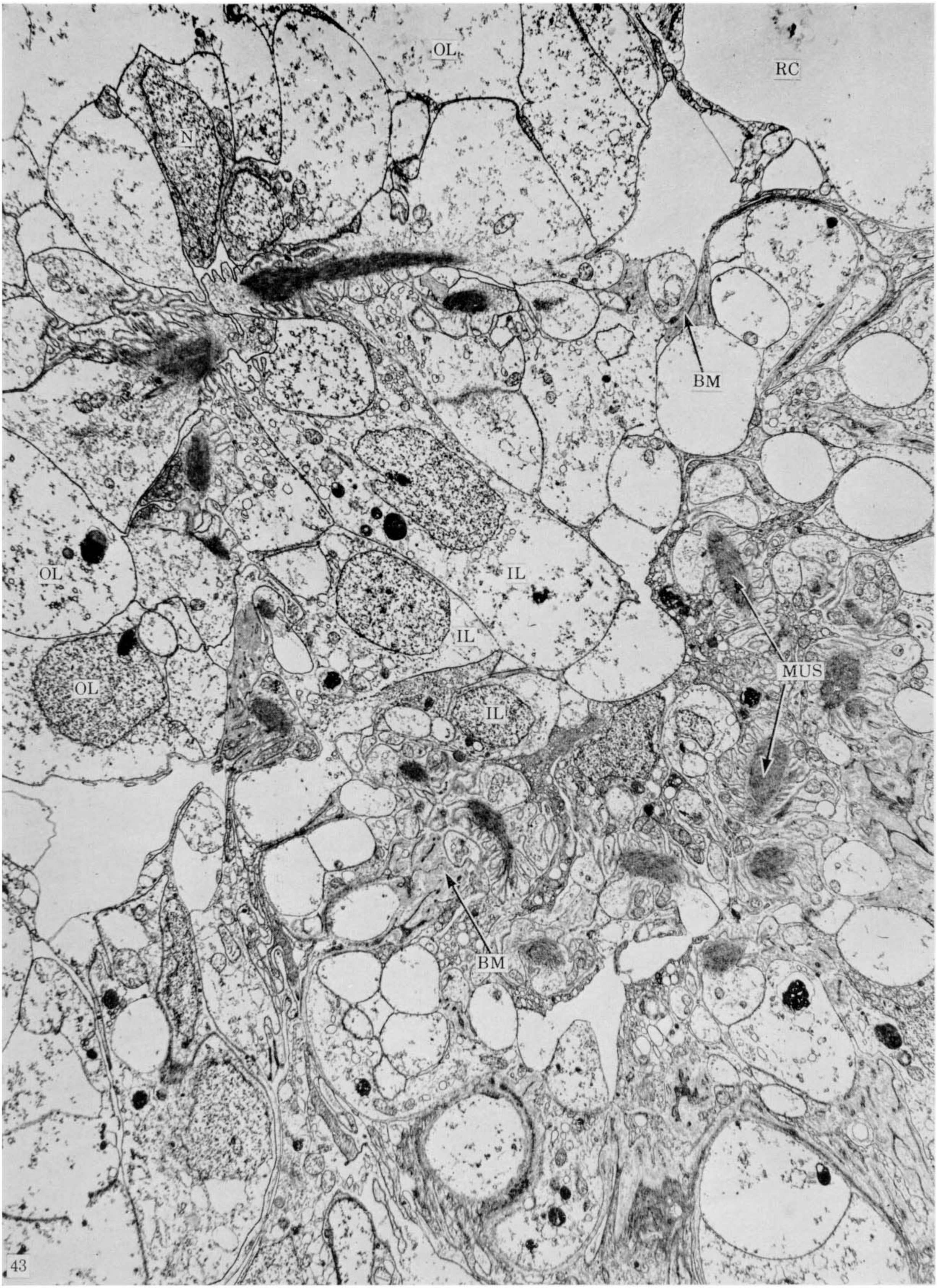
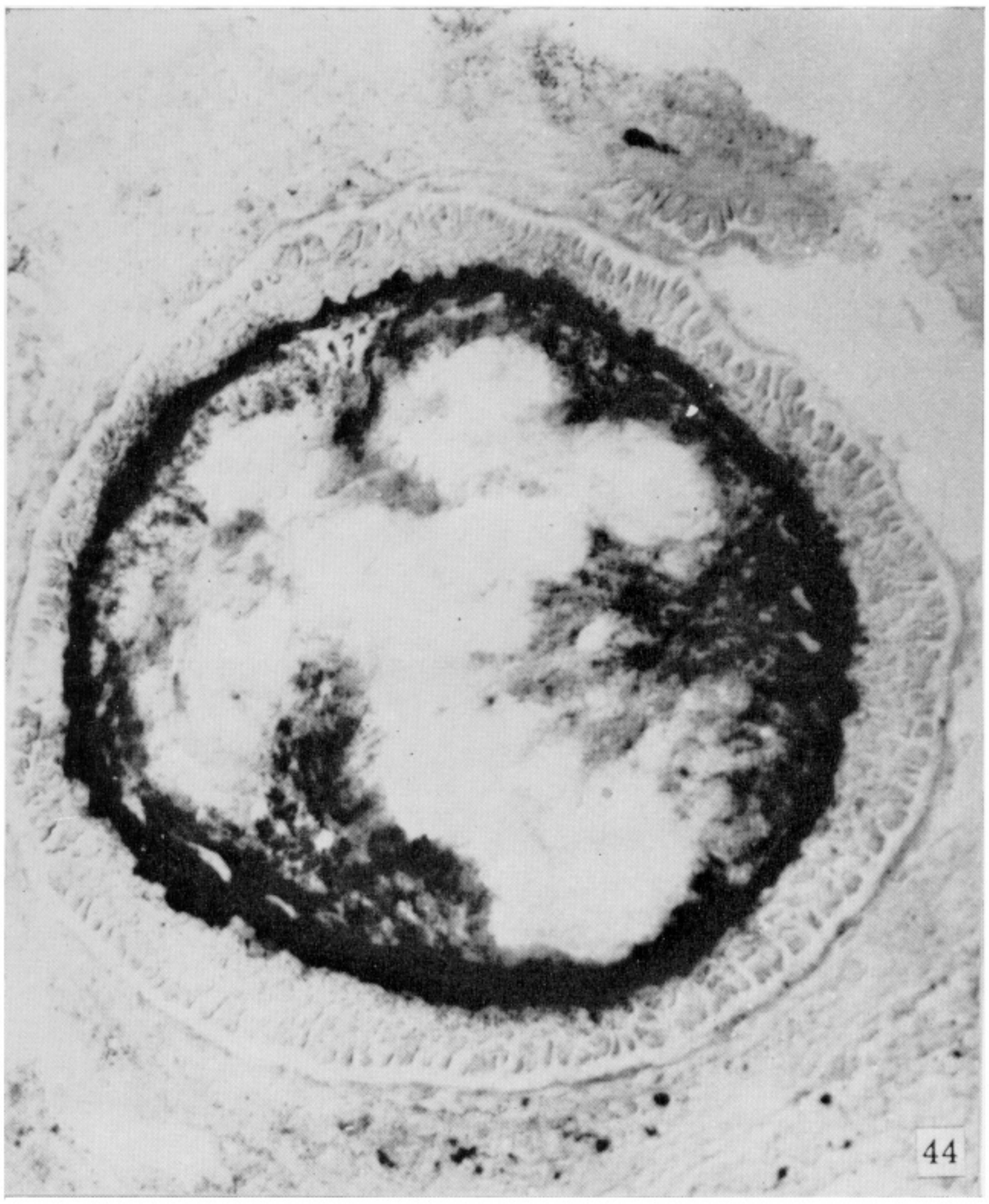
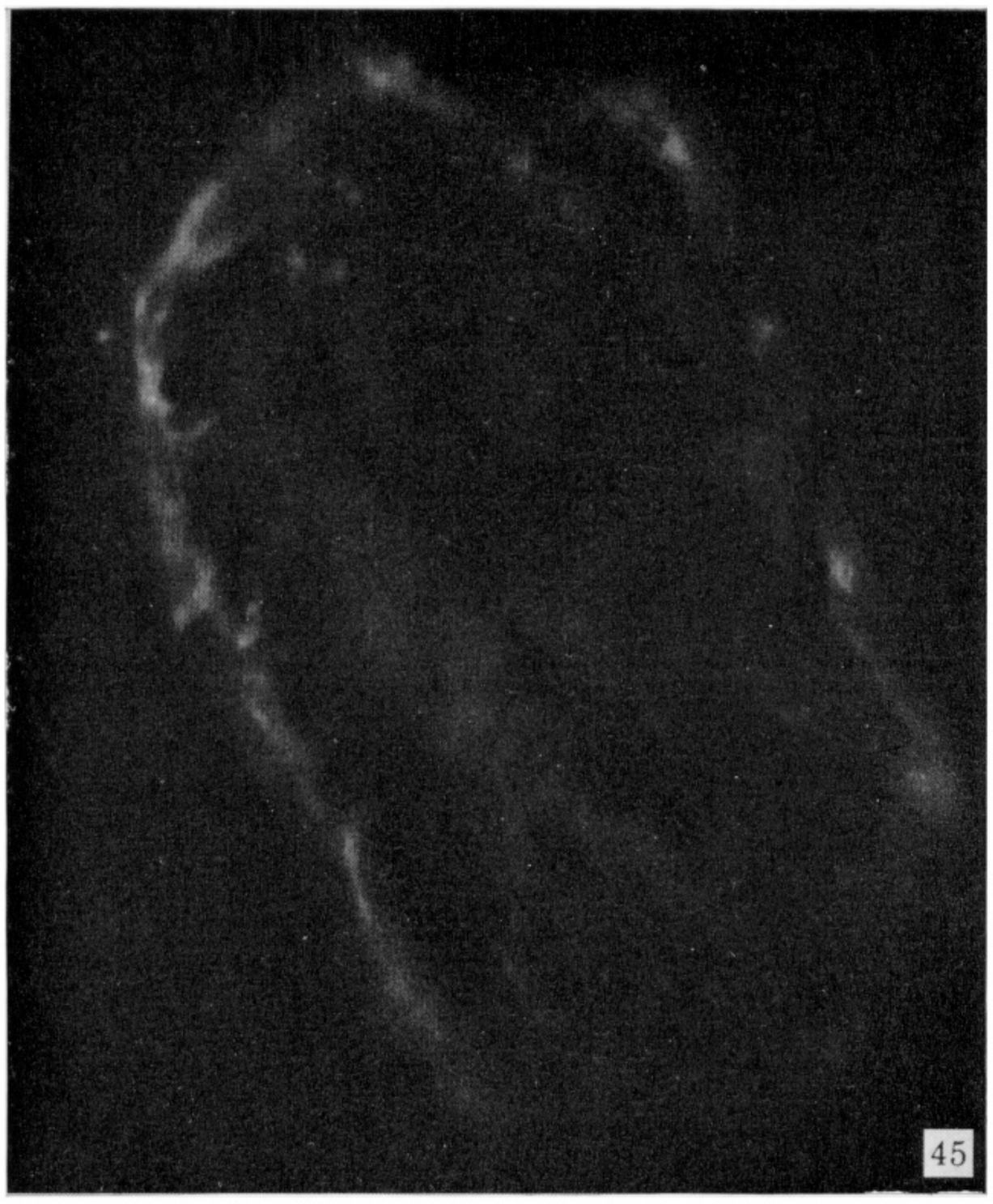
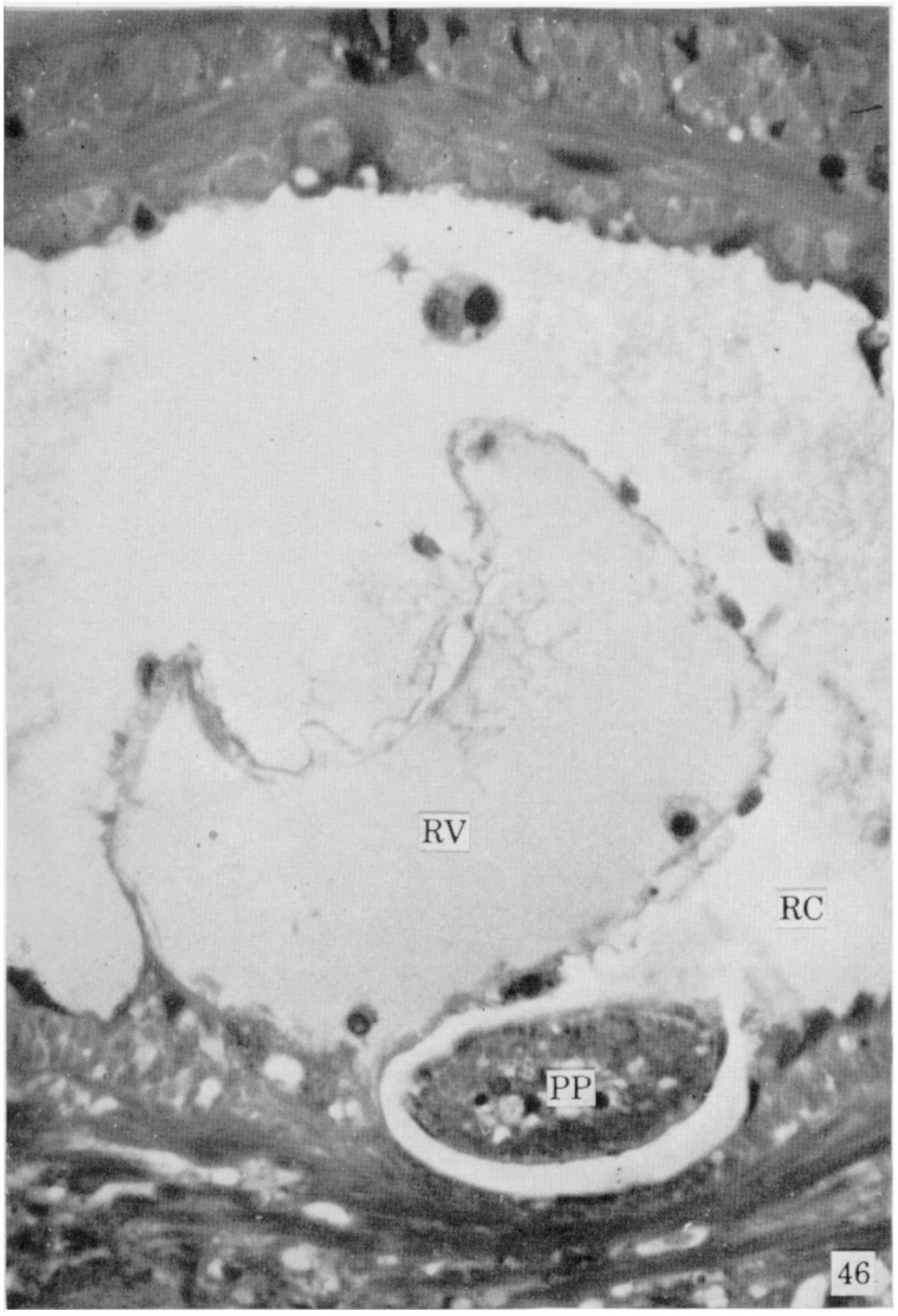


FIGURE 43. Rhynchocoel villus (squared area in figure 6). The lumen of the villus is hardly visible.







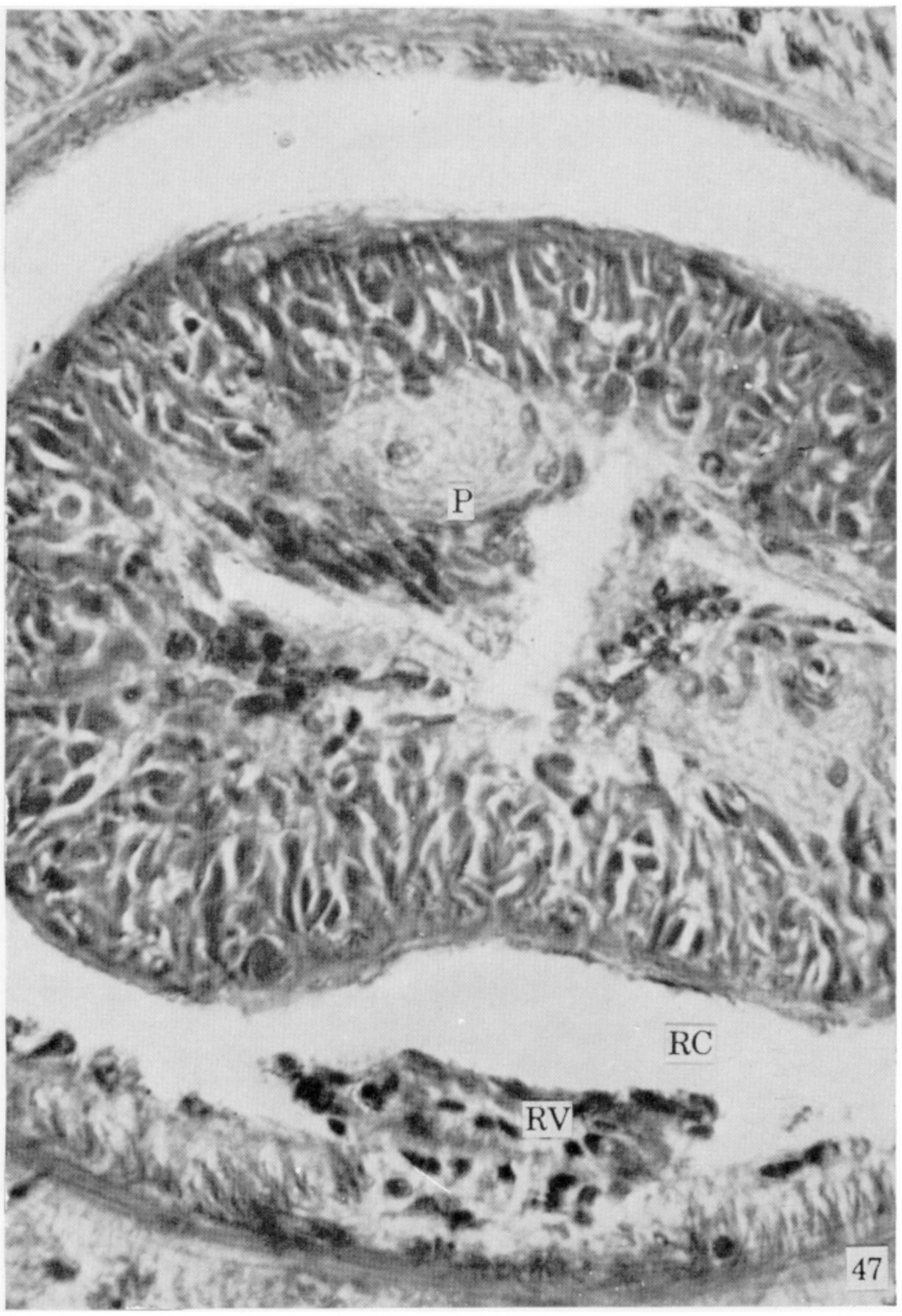
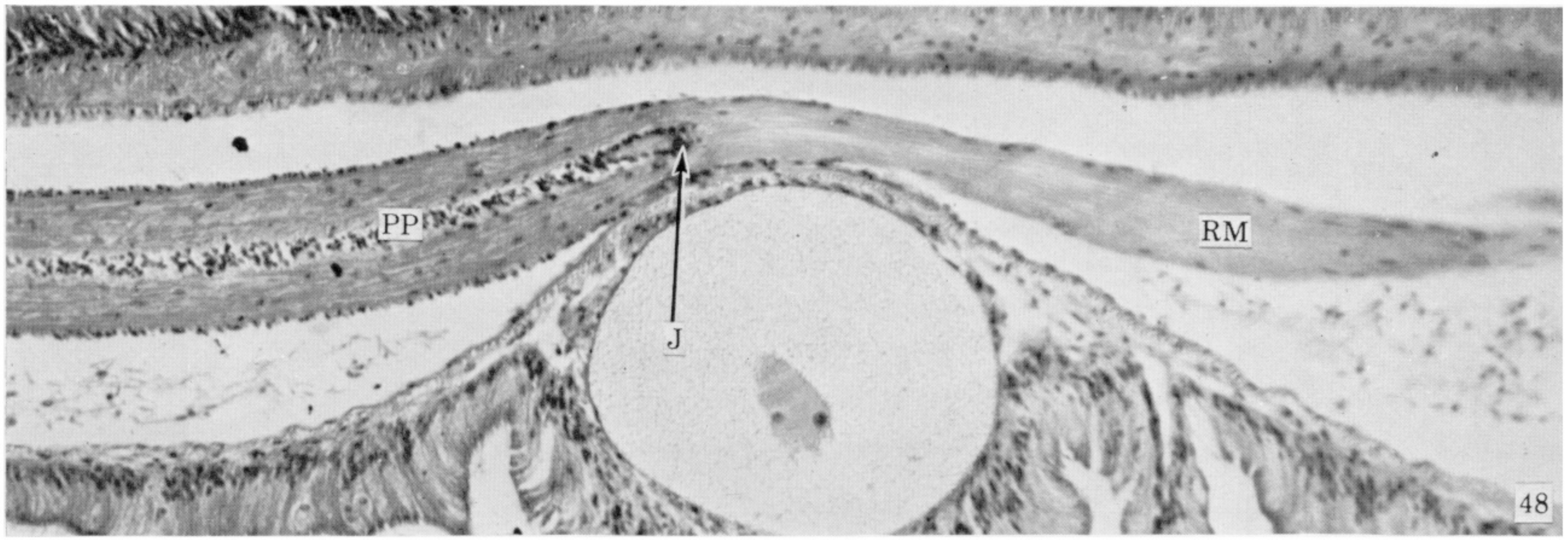


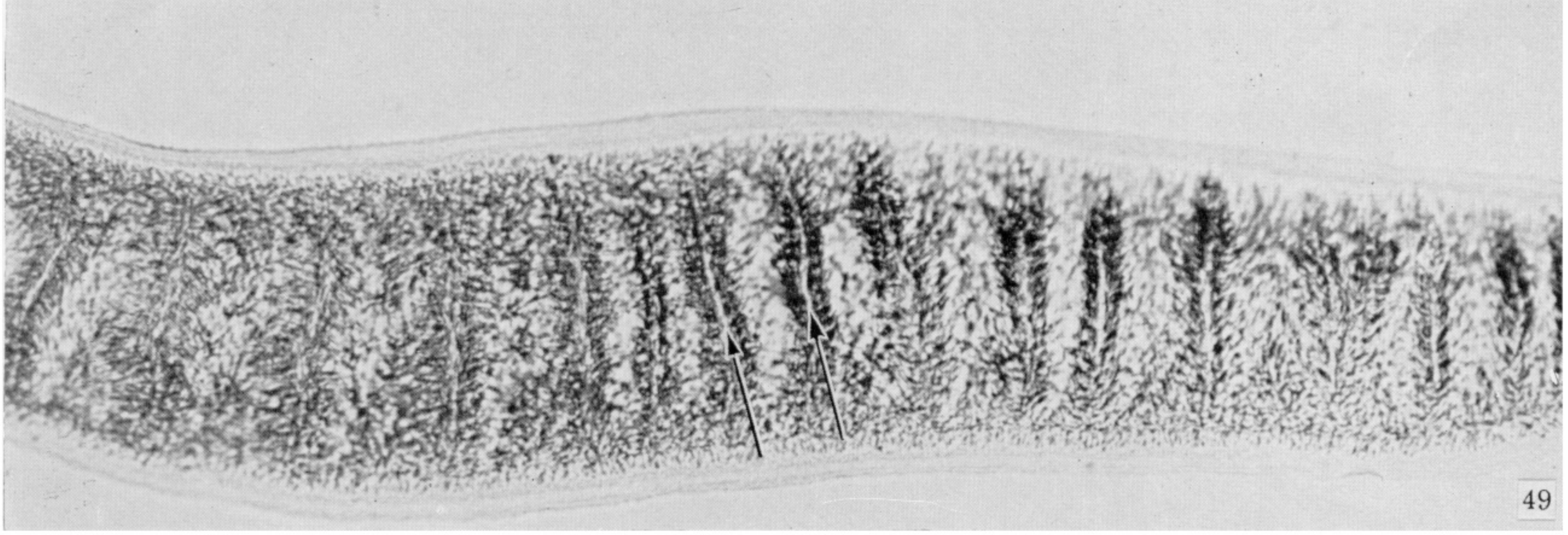
FIGURE 44. Transverse section of the middle proboscis. The layer corresponding to the nerve plexus shows acetylcholinesterase activity.

FIGURE 45. Transverse section of the middle proboscis. The layer corresponding to the nerve plexus gives off a yellowish fluorescence.

FIGURE 46. The rhynchocoel villus becomes dilated when the proboscis is ejected.

FIGURE 47. The rhynchocoel villus is small and flattened when the proboscis is in the rhynchocoel cavity.





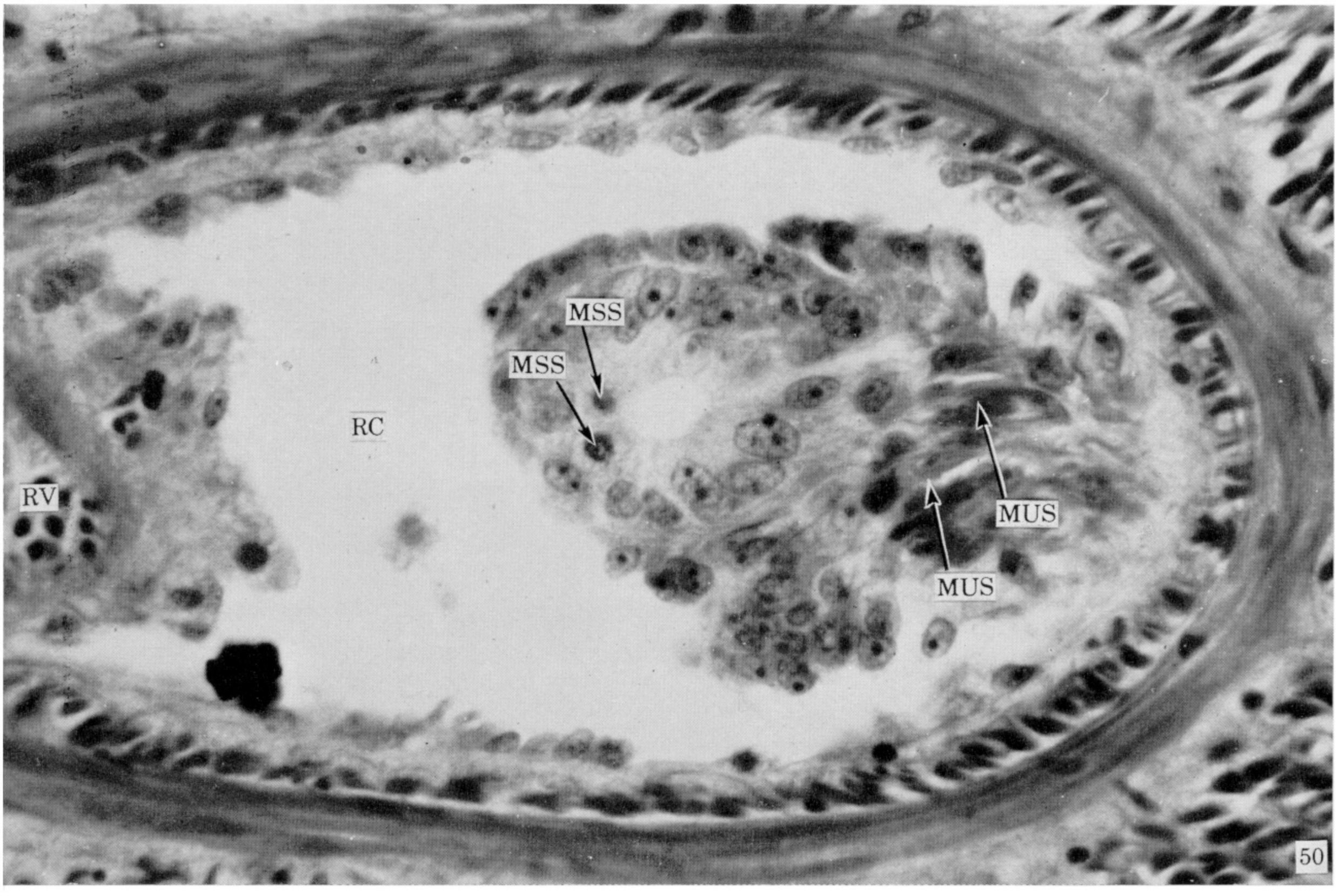


FIGURE 48. Longitudinal section of the worm, showing the junction of the proboscis and the retractor muscle.

FIGURE 49. The circular folds of the middle proboscis (arrows). Whole-mount fresh tissue.

FIGURE 50. Regeneration of a proboscis. Spindle-shaped muscle cells appear to accumulate around a preformed tubular structure. At the same time the lining cells of the tube still proliferate.