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STRUCTURE AND ORGANIZATION OF THE NERVOUS SYSTEM IN THE ACTINOTROCH LARVA OF *PHORONIS VANCOUVERENSIS*

By T. C. LACALLI

Biology Department, University of Saskatchewan, Saskatoon, Saskatchewan, S7N-0W0, Canada

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The nervous system of the earliest functional stage of the actinotroch larva of *Phoronis vancouverensis* is described based on ultrastructural surveys and three-dimensional reconstructions, including serial reconstructions of selected parts of the system.

The central element and main source of fibres in the system is the apical organ. Nerve cell bodies were found here and in the surrounding apical epithelium, but nowhere else in the body. Given the limitations of the methods used, the presence of nerve cell bodies elsewhere in the body cannot be ruled out, but based on this work and a recent study by A. Hay-Schmidt of whole larvae, it seems unlikely they occur in any numbers. The larval nervous system is thus highly centralized, an advanced and rather specialized feature in comparison with some other larval types, specifically those of primitive spiralia and echinoderms, in which nerve cell bodies are more widely distributed in the larval epithelium.

The largest single nerve in the body is the primary hood nerve, which runs around

the pre-oral hood slightly back from its margin. The nerve is a compact, well-defined tract of approximately 40 fibres, with an investment of glial-like accessory cells. A second set of smaller, accessory nerves run parallel to the primary nerve between it and the hood margin. The hood nerves all join at the base of the hood on either side of the mouth to form a pair of adoral nerve centres. A number of small nerves cross the hood from the apical organ to the hood nerves. Three of these are large enough to be considered major nerves: one is medial and connects to the centre of the hood margin, the other two are dorsolateral and connect to the adoral nerve centres. Fibre tracings, which show the distribution of vesicle-filled terminals and varicosities, suggest the hood nerves are mainly involved in neuromuscular control, specifically, in lifting the hood. This involves the stimulation, in sequence, of the radial and circular hood muscles by the primary and accessory hood nerves, respectively. Cells at the hood margin are organized somewhat in the fashion of a conventional ciliary band, but there is no obvious morphological evidence that any of the hood nerves are involved in neurociliary control.

A diffuse plexus of small nerves connects the above apical structures to the nerves supplying the tentacles. There are two main tentacle nerves, the primary tentacle nerve, which runs along the upper, oral margin of the tentacular ciliary band, and a smaller accessory nerve, which arises as a branch from the primary nerve, and runs along the lower, aboral margin of the band. There is also a row of uniciliate sensory receptor cells at the oral margin of the band. Each cell has a basal process ending in a vesicle-filled terminal that abuts fibres in the upper tentacle nerve, and forms junctions with them. The cells themselves produce no other fibres. They appear to be mechanosensory, and are probably involved in initiating the hood lift response, which can be triggered by touching the top surface of the tentacles. Additional large, vesicle-filled terminals branch from the fibres in the primary tentacle nerve. Their positions suggest a neurociliary function. The accessory tentacle nerve is associated mainly with muscle cells. A series of small nerves, which probably arise as branches from the larger tentacle nerves, supply the region below the tentacles, later the site of the telotroch.

The comparative and phylogenetic implications of the above are discussed. Phoronids are generally interpreted as being intermediate between deuterostomes and protostomes, with a curious mixture of characteristics of both groups. Phoronids are probably only distantly related to spiralian protostomes, but they are, strictly speaking, protostomes, and their larvae resemble the trochophore-type larvae of spiralia in many respects. Regarding ciliary band substructure and patterns of innervation, the actinotroch possesses too few features that are clearly primitive to support a detailed comparison with spiralian larvae, but the pre-oral hood band shows a sufficient number of prototroch-like features, to suggest the hood band and prototroch could be homologous. There is evidence for parallel evolution, in the two groups, of an increasingly centralized nervous system that provides improved effector control via nerve cells located in and around the apical organ. No evidence was obtained to support suggested homologies between the post-oral band of the actinotroch and circumoral or post-oral feeding bands in deuterostome larvae. The two appear, in fact, to be quite dissimilar in terms of their innervation. The results thus support conventional interpretations of the relationship between phoronids and other major groups.

1. Introduction

The three lophophorate phyla, Brachiopoda, Bryozoa and Phoronida, are generally accepted as a natural group of related organisms, but the question of the phylogenetic position of the lophophorates, in relation to other metazoa, is a source of continuing controversy. The organisms themselves are oligocoelomate, tentacle-bearing and predominantly sessile filter-

feeders showing clear affinities with deuterostomes (Zimmer 1973; Emig 1982). There are general organizational similarities, for example, in the lack of cephalization and arrangement of the coelomic compartments, as well as anatomical similarities, for example, the simple, plexus-like organization of the nervous system. A strong case can be made, however, based on the embryos and larvae, that lophophorates are more closely allied with spiralian protostomes. Phoronids, which appear to be the closest of the three groups to the most probable ancestral form, are, in fact, protostomes: the blastopore becomes the mouth during development (Emig 1977; Ivanova-Kazas 1986). Furthermore, the type larva of phoronids, the actinotroch, has been interpreted as a modified trochophore, notably by Hyman (1959).

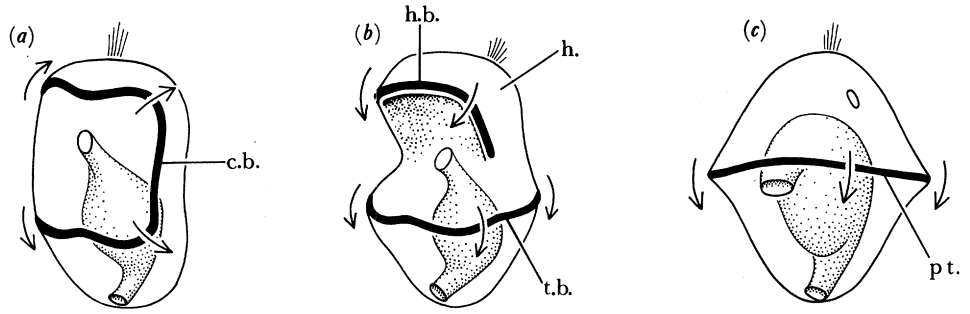


FIGURE 1. Direction of beat (arrows) of the principal ciliary bands in three basic types of marine larvae. (*a*) A deuterostome dipleurula-type larva, e.g. of an echinoderm, which has a single circumoral ciliary band (c.b.) that beats away from the mouth. (*b*) A phoronid actinotroch. The pre-oral hood (h.) has a marginal band (h.b.) that beats towards the mouth, and a post-oral tentacular band (t.b.) that beats away from the mouth. (*c*) A typical spiralian trochophore, e.g. of a polychaete. The pre-oral prototroch (pt.) beats towards the mouth.

While there is some justification for Hyman's view in terms of similarities in overall body form between trochophores and actinotrochs, it does not accord well with current ideas on the functional organization of the major classes of marine larvae. Two general types of organization are now recognized, based on the arrangement of the ciliary bands and their relation to feeding and locomotion: larvae tend to be either 'upstream' or 'downstream' in organization (Strathmann *et al.* 1972; Nielsen 1979, 1987). The two terms refer to the position of the mouth relative to the main ciliary currents. The larvae of primitive deuterostomes are typically organized in an upstream fashion: the principal band is circumoral or post-oral and beats away from the mouth (figure 1*a*). Currents produced by the locomotory cilia cannot then be used, at least directly, to drive food particles towards the mouth. The larvae, in consequence, are usually complex in shape with a large surface area. The band is convoluted, and food capture is indirect via hydrodynamic effects generated at comparatively slow swimming speeds. The dipleurula-type larvae of echinoderms and hemichordates are examples of this type. Most protostome larvae, in contrast, are organized in a downstream fashion. The principal ciliary band is pre-oral and its cilia beat towards the mouth (figure 1*c*). Feeding and locomotory currents can thus coincide. The larvae are generally compact, the bands have dense ciliary arrays, often forming compound cilia, and multiciliated cells predominate. Müller's larvae, the pilidium and the trochophore are examples. Where post-oral circumferential bands do occur, they either beat towards the mouth (for example, the metatroch, an accessory feeding band in some trochophores) or are located well away from the oral region as in the case of various telotrochs, which are mainly locomotory.

The actinotroch seems to fall somewhere between these two types, as it has both upstream

and downstream elements (figure 1*b*). The pre-oral hood has a fringing band of cilia that looks like a proper ciliary band, and it beats posteriorly, towards the mouth. But there is also a post-oral band, running along the margin of the tentacles (the tentacular ridge), that beats away from the mouth, clearly an upstream feature. If the actinotroch is basically a trochophore, the band at the margin of the hood should be prototroch-like in structure and organization. The tentacular band then needs to be accounted for. It occupies approximately the same position as the metatroch in a trochophore, but beats in the opposite direction. In functional terms, actinotrochs clearly feed in an upstream fashion (Gilmour 1978). But if the actinotroch is not a trochophore, it still remains to be shown that there are convincing structural or organizational similarities between the actinotroch ciliary band system and that of conventional deuterostome larvae.

There are various aspects of larval structure and ultrastructure that might be used for establishing homologies between larvae. The nervous system is supposed to be one of the more useful features on the assumption that neural organization is evolutionarily conservative, that is, that patterns of innervation change more slowly than the structures they innervate (Nielsen 1979). There seems to be some justification for this, at least among spiralian larvae, which have a similar intratrochal type of ciliary band innervation and similarly organized oral fields (Lacalli 1984; Lacalli & West 1985).

The purpose of this study is to provide a description of the nervous system of the actinotroch larva for comparison with similar studies of other larvae. A young stage was selected, in part because the principal method used, reconstruction at the electron microscopical level, is best suited to small larvae. In addition, however, the younger the larva, the less its basic organization will be obscured by secondary, late-developing structures. This is a serious limitation of existing descriptions of actinotroch larvae, which are largely based on light microscopy of older, much larger specimens. A special effort has been made in this study to identify structures that might be expected on the basis of the author's previous work on spiralian larvae, for example, sub-oral or subpharyngeal neural centres and rejectory tracts of cilia, but largely without success. The morphological results are reported in §3.1–3.4, and discussed in §4.1. Where possible, morphology has been correlated with behavioural observations (§3.5), which permits at least a rudimentary understanding of how the system functions. An interpretation of the results in comparative, phylogenetic terms is given in §4.2.

2. Methods

Adult *Phoronis vancouverensis* were collected from submerged logs at Jakle's lagoon on San Juan Island, Washington, in May 1978 and maintained at the Friday Harbor Laboratories of the University of Washington. The embryos and larvae are brooded in the adult tentacles to the early four-tentacle stage before release. They will develop for several days without feeding, and during this time they can be staged approximately on the basis of tentacle size.

For transmission electron microscopy, newly released larvae were fixed by the semi-simultaneous method as described previously (Lacalli 1981). This has proven an excellent method for rapid fixation of small larvae, with the added advantage that they can be stored indefinitely in 70 % ethanol for later embedding without deterioration. Specimens were stained in aqueous 2 % uranyl acetate overnight at 60 °C before embedding to avoid the need to stain sections, and were then embedded in Spurr's resin. Five larvae of similar stage, as judged by

tentacle size, were examined, three of these in detail as follows: whole larvae were sectioned in the sagittal plane, and sections were collected on slotted grids provided with a formvar support film, usually eight sections per grid. Roughly 120 grids, covering 80 μm, were required for a typical specimen. Tracings were made of the nervous system in two specimens from sections taken at 2 μm intervals. The resulting reconstruction of one of these is shown in figure 16. Where necessary, for example, for tracing nerves or determining basal body orientation, intervening sections were examined, and more complete series of selected regions, for example, the apical organ and sub-oral region, were also examined. One half of the third specimen was sectioned to obtain serial runs through specific structures for tracing individual fibres and cells: one 14 μm run through the hood nerve (figure 30) and two of about 8 μm through the tentacle nerve (one is shown in figure 45).

For scanning electron microscopy (SEM), fixed larvae were critical-point dried, attached individually to double-sided tape on stubs, and coated with gold.

Behavioural observations were made primarily on older specimens from plankton collected at Friday Harbor and Bamfield, British Columbia, Canada. These are larger and more easily observed, and are identified by their distinct pigmentation (Emig 1982).

3. Results

3.1. *General observations on the four-tentacle stage*

(*a*) *Larval body form and the direction of ciliary beat*

Survey views of the four-tentacle *P. vancouverensis* larva are shown in figure 2 and figures 3–6 (plates 1 and 2). The larva is a typical actinotroch with large pre-oral hood and a post-oral ring of tentacles bearing a dense band of long cilia. New tentacles are added laterally as the larva grows, so the medial pair are the oldest and best developed, while the lateral pair are, in this instance, just forming. Figures 3 and 4 show larvae with the hood raised. The hood is normally lowered during swimming, so the apical organ (figure 5, plate 1), which produces the apical tuft (figure 6, plate 2), faces forward. The inner cavity of the hood is referred to as a vestibule, and leads to the mouth. The digestive tract consists of an oesophagus, stomach, and intestine. The oesophagus is separated from the stomach by a valve (figure 9, plate 2) made of cells with swollen pseudopodial projections. This structure marks the junction between the derivatives of the ectoderm (vestibule and oesophagus) and the endoderm. The former are thus roughly equivalent to the stomodeal derivatives in protostomes. The intestine forms directly from endoderm without a proctodeum according to Emig (1977). The anus is posterior and terminal.

With the exception of scattered cells of particular types, such as mucus cells, the body of the larva is entirely ciliated, but cilium density (the number of cilia per unit area) varies. This account pays special attention to cilium distribution, orientation, and direction of beat as a means of distinguishing the various epithelial domains into which the body surface is divided. Reports exist of biciliate cells in phoronids, but none were observed in this study. Cells with cilia were uniciliate as described by Nielsen (1987). This means cilium density basically reflects cell density, which itself varies mainly with the thickness of the epithelium. Thus the hood, with comparatively sparse ciliation, is thin, while the cells of the apical organ are tall and columnar. Cilium density increases gradually towards the hood margin (figures 7 and 8, plate 2), but changes abruptly to sparse ciliation at the lip of the hood, where the vestibule begins. Nearer

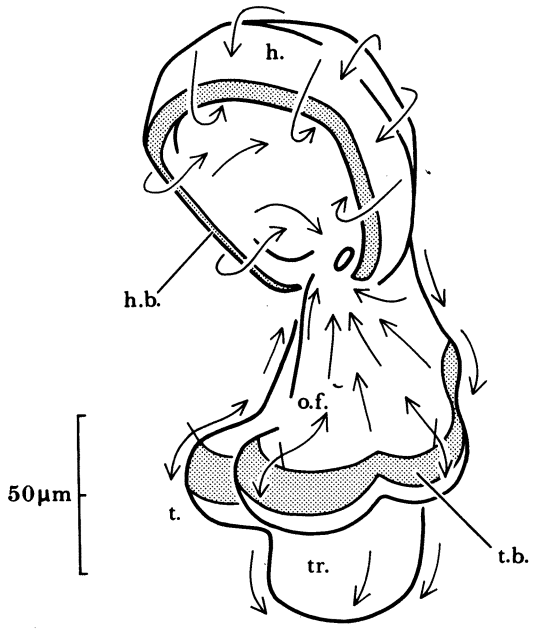


FIGURE 2. Actinotroch larva of *P. vancouverensis* at the four-tentacle stage examined here. Shows the hood (h.), mouth (unlabelled circle), oral field (o.f.), tentacles (t.) and post-tentacular trunk (tr.). The two ciliary bands, at the margin of the hood (the marginal hood band, h.b.) and along the tentacular ridge (the tentacular band, t.b.), are shaded. Arrows show the direction of ciliary beat based on analysis of accessory centriole position (see text).

the mouth cilium density again increases, and below it, extending to the tentacle band, is the heavily ciliated oral field. There is a sharp transition between the oral field and tentacular band, though the obvious change at this point is of cell type and orientation (see below), rather than cell density. Below the band, the body surface is again sparsely ciliated, but cilium density increases gradually towards the posterior end. This is the site of a telotroch in older larvae. The long cilia present at this stage in the posterior region may be an early sign of this structure, but it could not be clearly distinguished on other criteria, for example, in section.

The plane of cilium beat can generally be determined by SEM by using stereopairs. In figure 10, for example, the plane of cilium beat in relation to the mouth is fairly obvious. Inferring direction of beat from scanning micrographs is more difficult. A better method is to examine the position of the accessory centriole in individual cells. Gilmour (1978) and Nielsen (1987), the latter in an exhaustive survey of larval types, have shown that in phoronid larvae, as in deuterostome larvae, the accessory centriole always lies on the downstream side of the basal complex, that is, in the direction of the effective stroke, and is oriented perpendicular to the plane of beat. The basal complex and rootlets of typical epithelial cells in *P. vancouverensis* are shown in figures 13 and 14 (plate 3). Each cilium has two striated rootlets. The principal rootlet is vertical, and runs through the cell adjacent to the nucleus. There is then a second, accessory rootlet running horizontally, parallel to the cell surface, in the plane of beat on the upstream side. These typically attach to the lateral surface of the cell at a junction. The orientation of the rootlet system correlates in the expected fashion with the direction of cilium beat in regions of the body for which the latter is known (for example, in the tentacular band, figure 11, plate 3). There is no reason to suppose it is not a reliable indicator for direction of beat elsewhere in the epithelium. A different type of rootlet system was found in only two places in the larva, in the apical organ (§3.2) and the tentacular sensory cells (§3.4*c*).

Careful examination of positioning of accessory centrioles throughout the body shows the following, summarized in figure 2: cilia on the hood beat away from the centre of the apical organ except for the small cluster of central cells (§3.2), whose accessory centrioles face the centre of the apical organ in the few cases where this could be determined. In the vestibule, cilia beat towards the mouth, as do the cilia of the oral field. The oral field does not appear to extend circumferentially around the whole body. Along the mid-dorsal line, cilia beat posteriorly all the way from the apical organ to the anus, which implies that the oral field has a mid-dorsal gap. The transition between the oral field and tentacular band is sharp, defined by adjacent cells whose centrioles are opposed (figure 12, plate 3). Cilia of the tentacular band and the epithelium posterior to it all beat posteriorly. The tentacle band is continuous around the circumference of the body (figure 6), but is smaller and its cells are less columnar in the dorsal region. Repeated searches for tracts of rejectory cells in the vestibule and oral field, that is, cells with cilia beating away from the mouth, were unsuccessful. A few individual cells were found with accessory centrioles in unexpected orientations, but in all cases these were mucus cells, in which cytoplasmic organization is somewhat disturbed in any case.

(*b*) *The larval nervous system*

The nervous system is intraepithelial and restricted to the ectoderm so far as could be determined. Figures 15 and 16 (plate 4) shows the system as reconstructed from sections for one half of a larva. The other half was essentially the same, that is, the system is bilaterally symmetrical, and reconstructions of a second larva were also similar in all essential features. The results from the reconstructions are summarized in figure 17*a*. Figure 17*b* gives approximate fibre counts at strategic points for one specimen. These are approximate both because of variability between sections, and because processes belonging to glial-like cells (for example, as in figure 45*e*) occur at some points that are easily mistaken for nerve fibres. The main elements of the system are shown in the figures along with smaller nerves of *ca.* 3–8 fibres. The latter vary somewhat from specimen to specimen, which makes a comprehensive description of the system difficult, but they show a similar overall pattern in their organization. Irregular tracts of 2–3 fibres are commonly encountered throughout the epithelium, particularly in the hood and oral field. These were not traced, except in a few specific instances, and are not illustrated or considered further in any detail.

Briefly, the nervous system consists of three main elements, the apical organ, the nerves of the hood margin, and the tentacle nerves. Smaller nerves connect these.

The apical organ (§3.2) is the main neural centre in the larva, with a basal neuropile that is by far the largest collection of nerve fibres in the body at this stage. Cells with nerve-like features occur in various parts of the body in association with nerves, but tracings failed to confirm the presence of nerve cell bodies anywhere except in the apical organ and surrounding epithelium. Three identifiable tracts of nerve fibres leave the apical neuropile. One is mid-ventral; the other two form a pair of dorsolateral nerves. The former breaks up into small fibres that travel to the hood margin, while the two dorsolateral tracts join the nerves of the hood margin at the base of the hood on either side.

The hood (§3.3) is innervated principally by two nerves that run in parallel along its margin. The larger of these, the primary hood nerve, is the largest single nerve in the body and the most regular in organization. It lies about 10 μm back from the margin of the hood, in close association with cells of the radial hood muscle responsible for lifting the hood. The nerve contains *ca.* 35–45 fibres, and is invested in a glial-like sheath of epithelial cells. Most of the

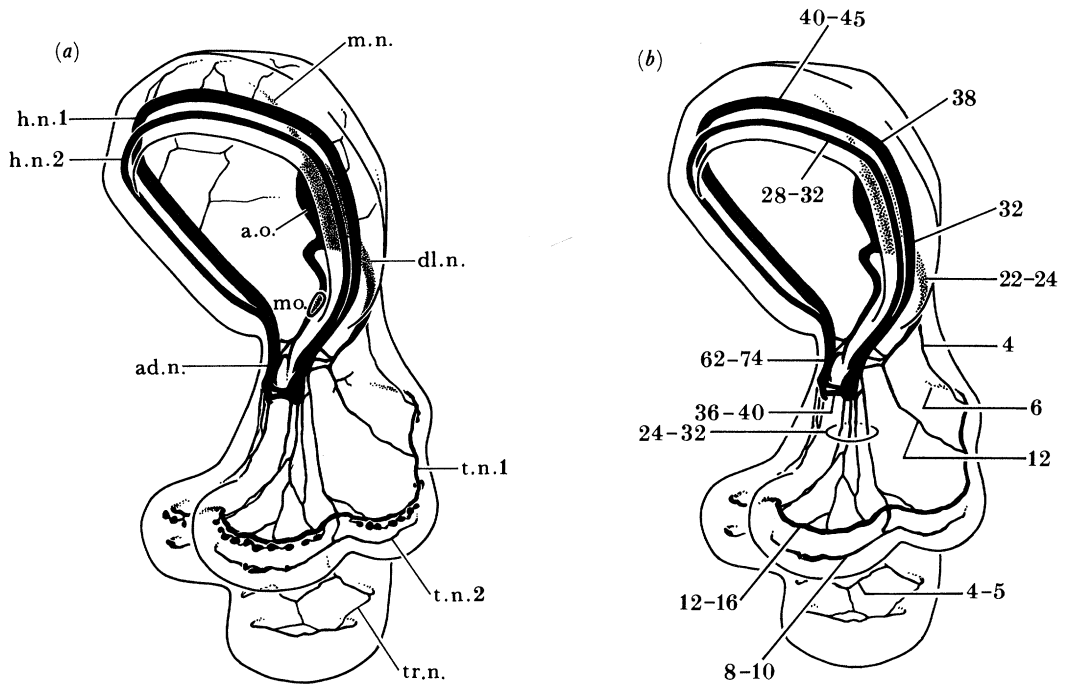


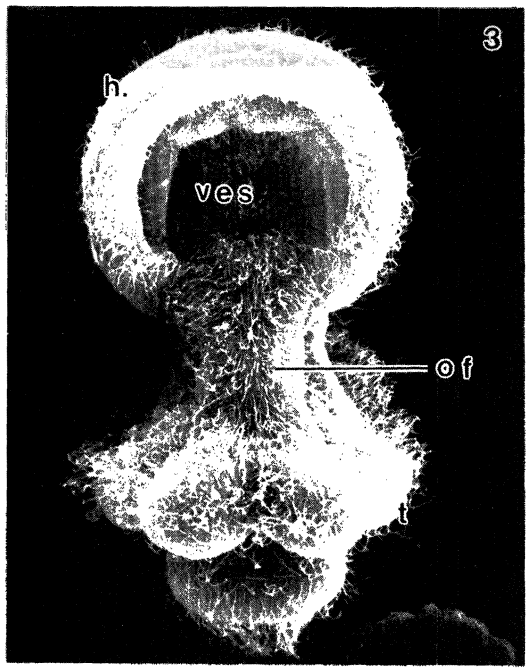
FIGURE 17. (*a*) Survey diagram of the larval nervous system, based on reconstructions (as in figure 16) of two larvae. Shows the apical organ (a.o., shaded where seen through hood), median hood nerve (m.n.), the two dorsolateral hood nerves (dl.n.), primary (h.n.1) and accessory (h.n.2) hood nerves, primary (t.n.1) and accessory (t.n.2) tentacle nerves, and the network of trunk nerves (tr.n.). Small nerves run irregularly through the oral field and hood. Representative examples of these are shown, but no attempt has been made to show nerves with fewer than three fibres. (*b*) Approximate fibre counts at strategic points.

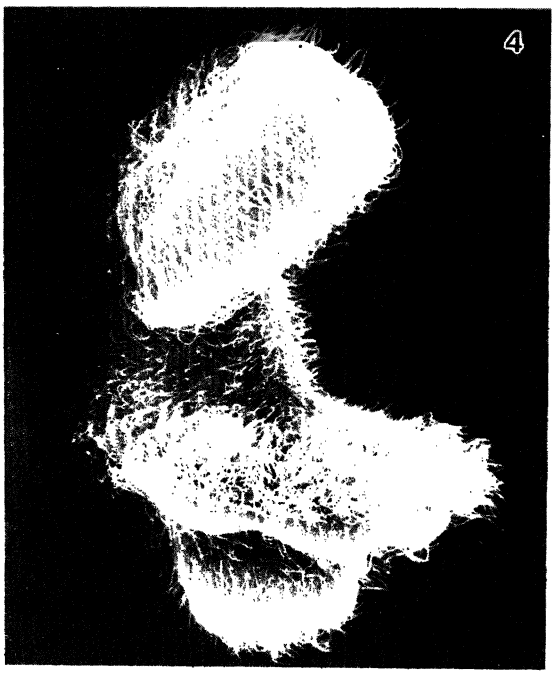
fibres appear to be of a single type, referred to here as type I fibres. They contain predominantly clear vesicles, and comparison with the whole-mount preparations of Hay-Schmidt (1990*b*) suggests they correspond, at least in part, with his glyoxylic acid-stained fibres, which belong to cells whose cell bodies lie in the hood epithelium (§4.1*b*). Single fibres of a second type, referred to here as type II fibres, also occur. These have predominantly dense-core vesicles, and probably correspond with Hay-Schmidt's serotonin-containing fibres, whose cell bodies lie in the apical organ. A second, smaller set of nerves, the accessory hood nerves, run at the hood margin, adjacent to cells of the circular hood muscle. The accessory nerves consist mainly of type I fibres, and there are junctions between these and the muscle cells. On

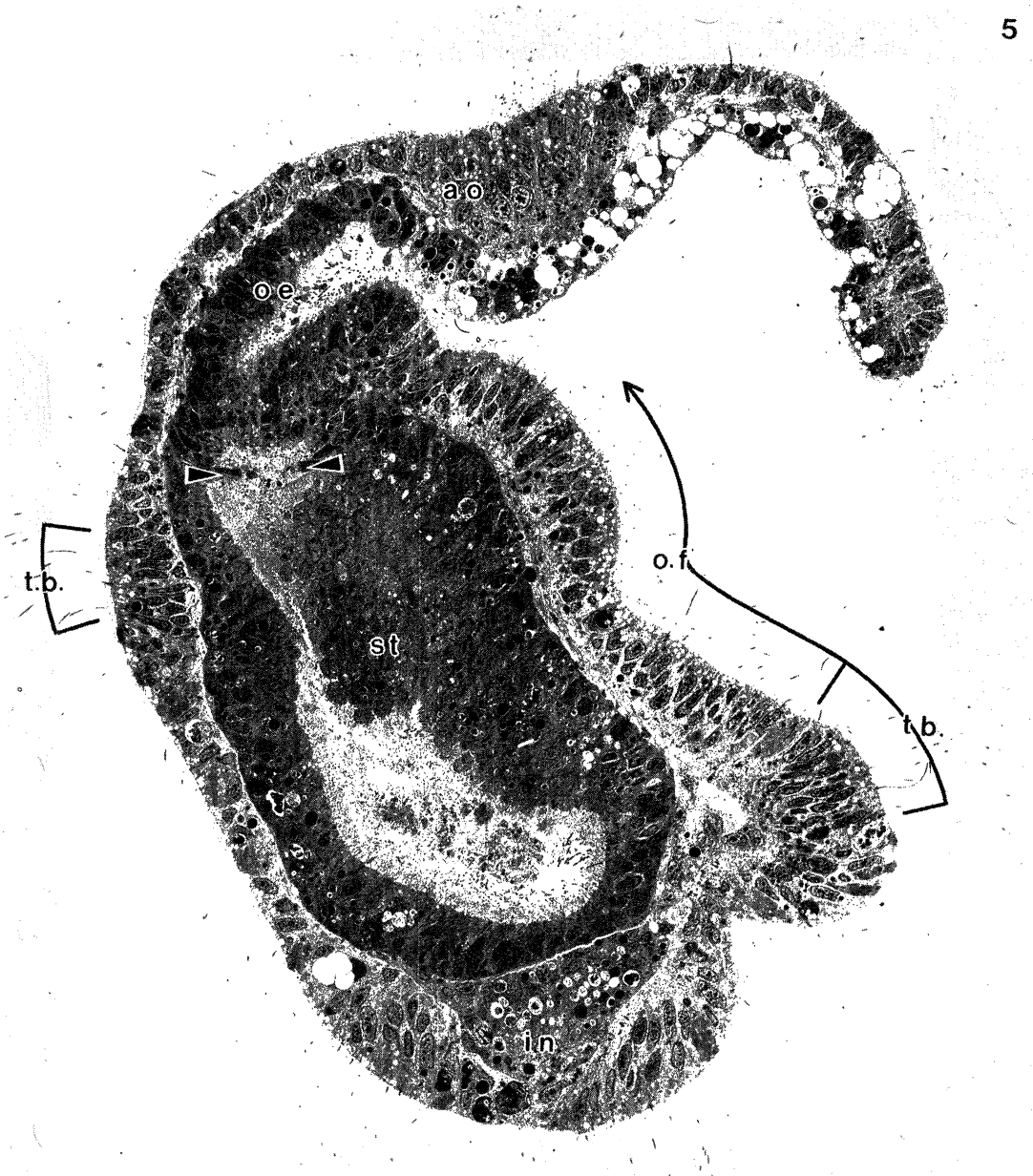
DESCRIPTION OF PLATE 1

FIGURES 3 AND 4. *P. vancouverensis*, survey views of whole larvae at the four-tentacle stage in front and side view. Shows the pre-oral hood (h.), vestibule (ves.), tentacles (t.), and post-tentacular trunk. The zone of dense ciliation includes both the oral field (o.f.) and tentacular band, but stops abruptly at the aboral margin of the latter. (Magn. ×440.)

FIGURE 5. Mid-sagittal section through a four-tentacle larva, oriented as in figure 4. Shows the digestive tract: the mouth, leading to the oesophagus (oe.), oesophageal valve (between arrows), stomach (st.), and intestine (in.). The intestinal lumen and terminal anus are out of the plane of section. Features of interest in the epithelium include the apical organ (a.o.), oral field (o.f.), tentacular band (t.b.), and the vacuolated epithelium forming the inner, vestibular surface of the hood. (Magn. ×1060.)







FIGURES 3–5. For description see opposite.

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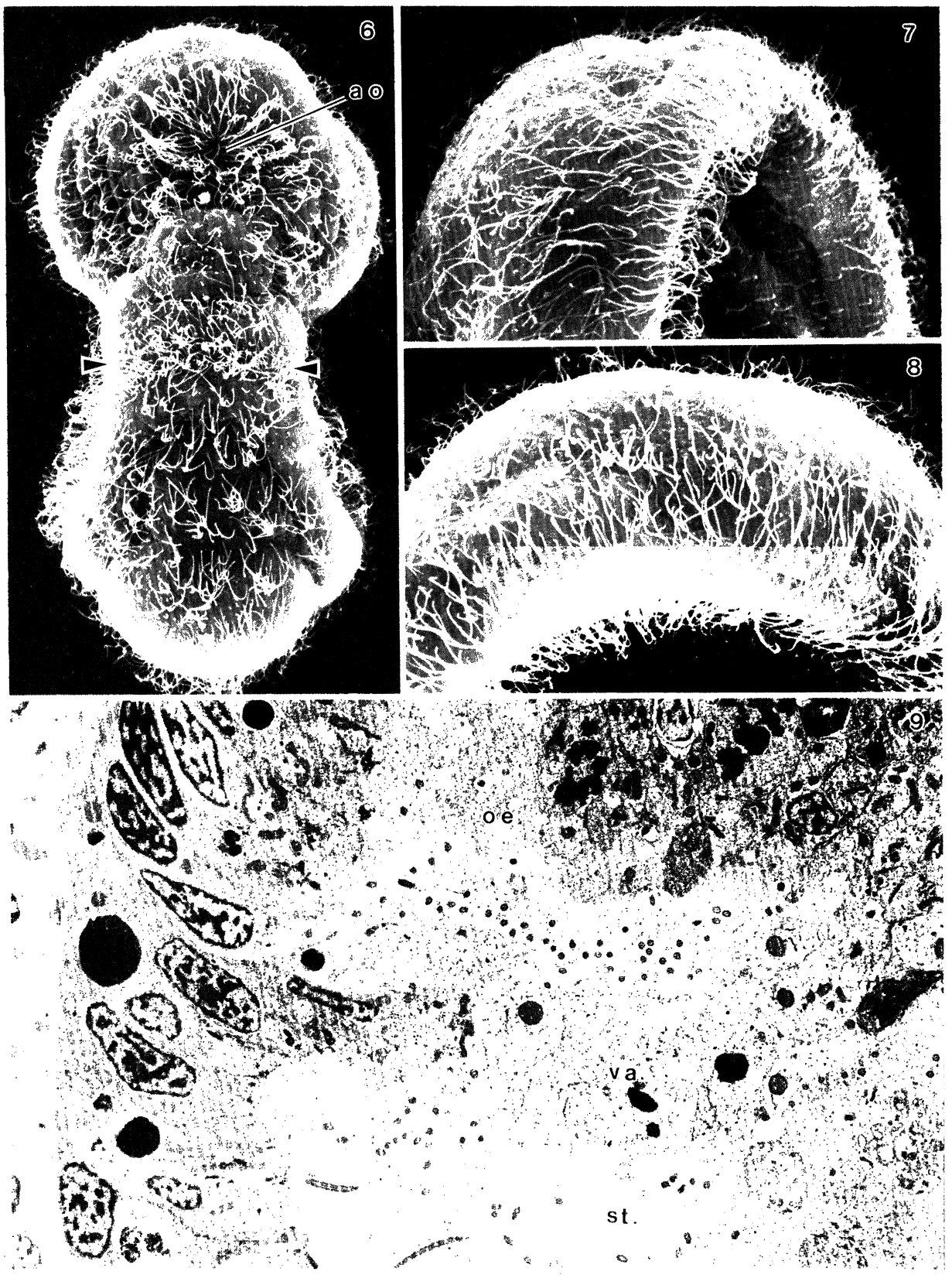


FIGURES 6–9. For description see opposite.

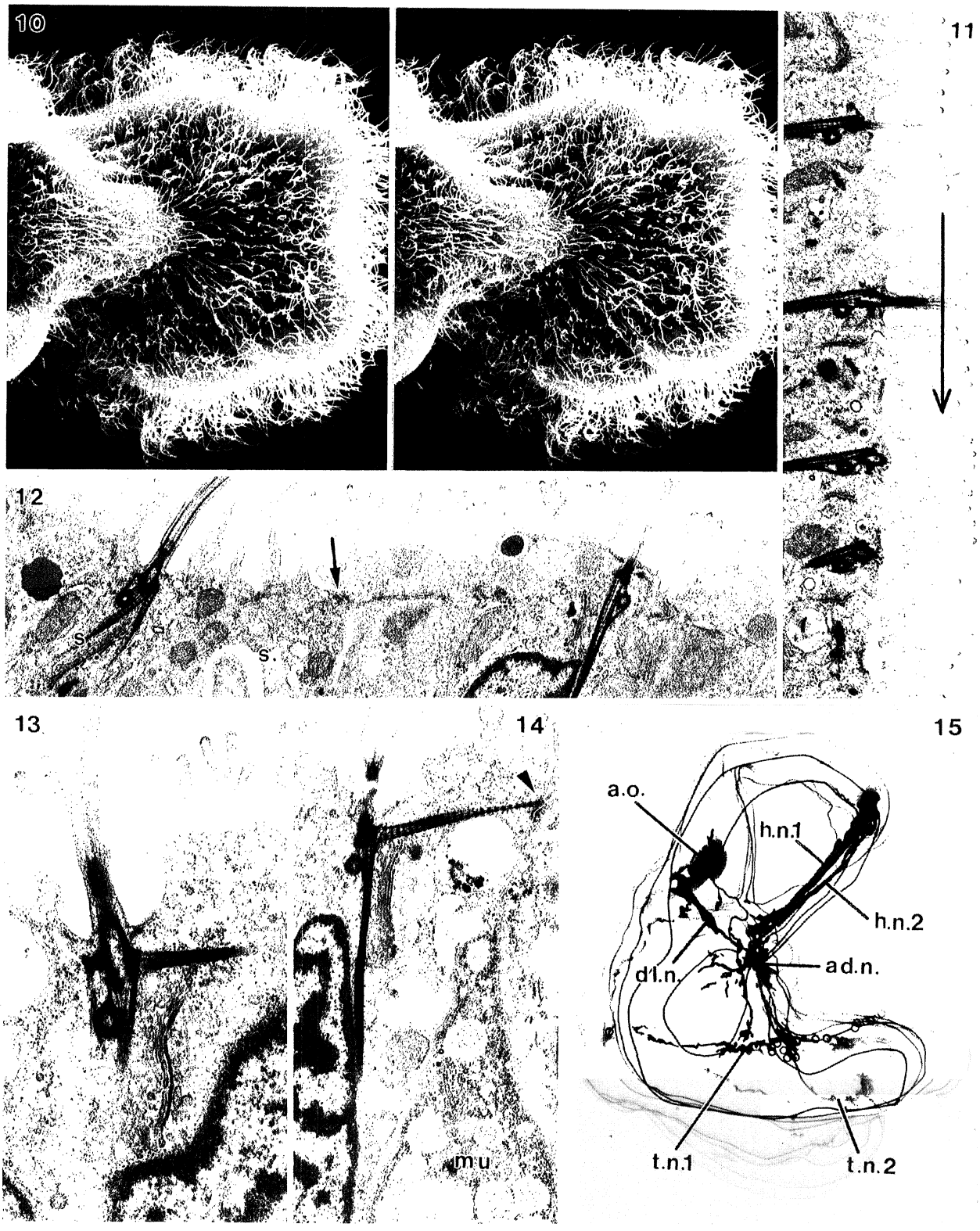
Description of plate 2

Figure 6. Dorsal view of a larva showing the apical organ (a.o.) and the dorsal portion (between arrows) of the tentacular band. The latter is continuous dorsally. (Magn. × 660.)

Figure 7. Side view of hood. The comparatively dense ciliation on the outside of the hood stops abruptly at its edge. The vestibular surface is sparsely ciliated. (Magn. × 1130.)

Figure 8. External ventral view of the hood margin. (Magn. × 1030.)

Figure 9. Sagittal section through the valve (va.) separating oesophagus and stomach. The valve is composed of an annular row of cells with club-like extensions. (Magn. × 7000.)



FIGURES 10–15. For description see opposite.

Description of plate 3

Figure 10. A stereopair showing the oral field seen from above. There is no clear separation between cilia of the oral field and the tentacular band, but metachronal waves are visible in the latter. The plane of ciliary beat, but not direction of beat, can be determined from images of this type. (Magn. ×700.)

Figure 11. A section through the tentacular band to show rootlet orientation. The section cuts through eight cells, and rootlets of four of these are visible. Direction of beat (arrow) is down, and accessory centrioles are positioned on the downstream side of each cilium. (Magn. ×14950.)

Figure 12. Junction (arrow) between the oral field and tentacular band. Cells to the right of this junction belong to the tentacular band. Their accessory centrioles lie on the aboral side of the cilium, and the direction of beat is away from the mouth. Cells to the left of the junction, including sensory cells (s.), belong to the oral field. Their accessory centrioles lie on the oral side of the cilium, implying the effective direction of beat, assuming they do beat, would be towards the mouth. (Magn. ×15800.)

Figure 13. Basal complex, rootlets and associated golgi of a typical epithelial cell. (Magn. ×29200.)

Figure 14. As in figure 13, showing the attachment of the horizontal rootlet at a cell junction. (Magn. ×17830.)

Figure 15. Reconstruction of the nervous system in the right half of a larva, seen from the right side, a guide for interpreting figure 16. Shows the apical organ (a.o.), primary (h.n.1) and accessory (h.n.2) hood nerves, primary (t.n.1) and accessory (t.n.2) tentacle nerves, one of the dorsolateral nerves from the apical organ (dl.n.), one of the adoral nerve centres (ad.n.), and the irregular network of connecting fibres. Open circles show the positions of sensory cell apices at the body surface, but only for about a third to a half of the total number of such cells with cilia. (Magn. ×560.)

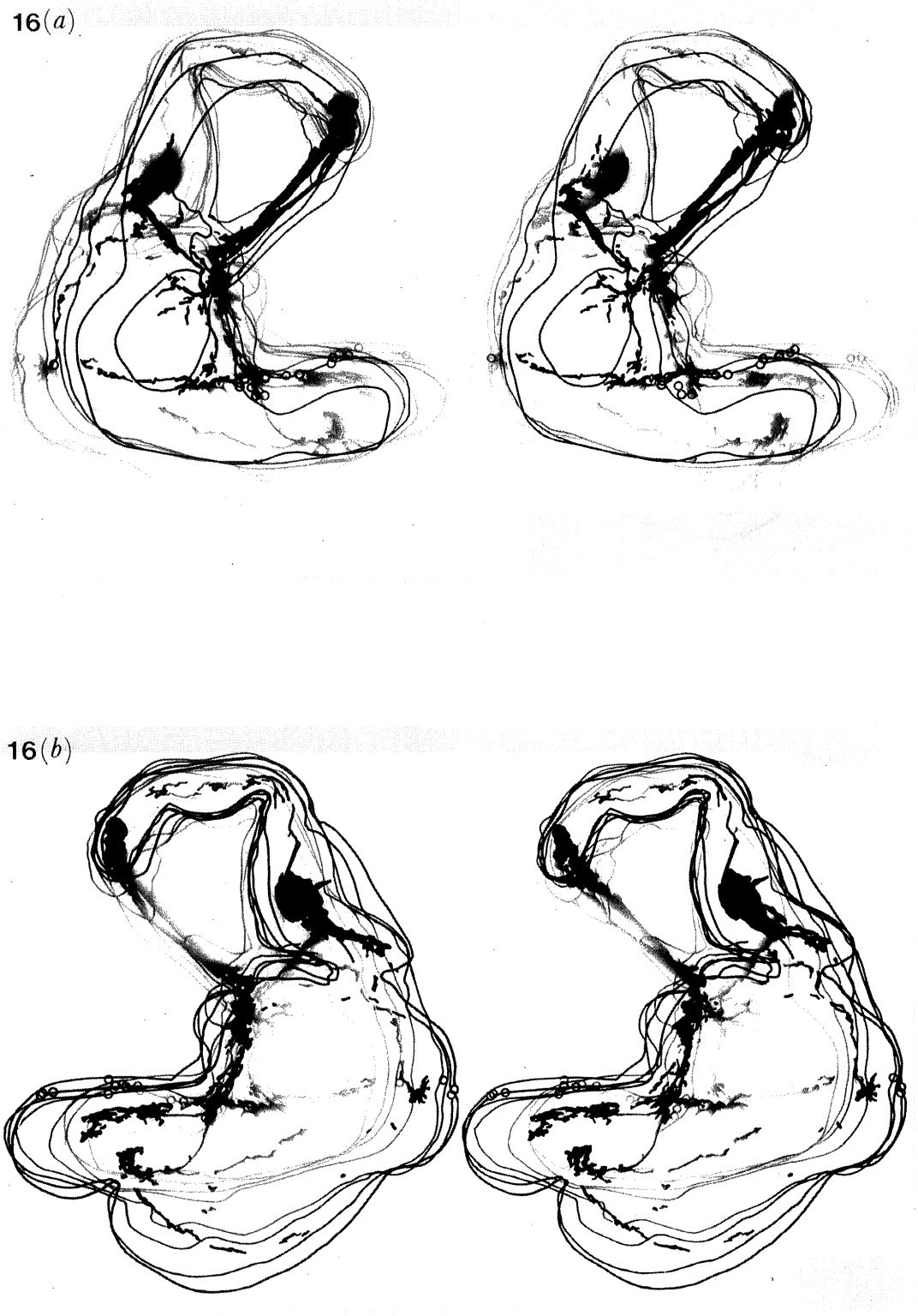
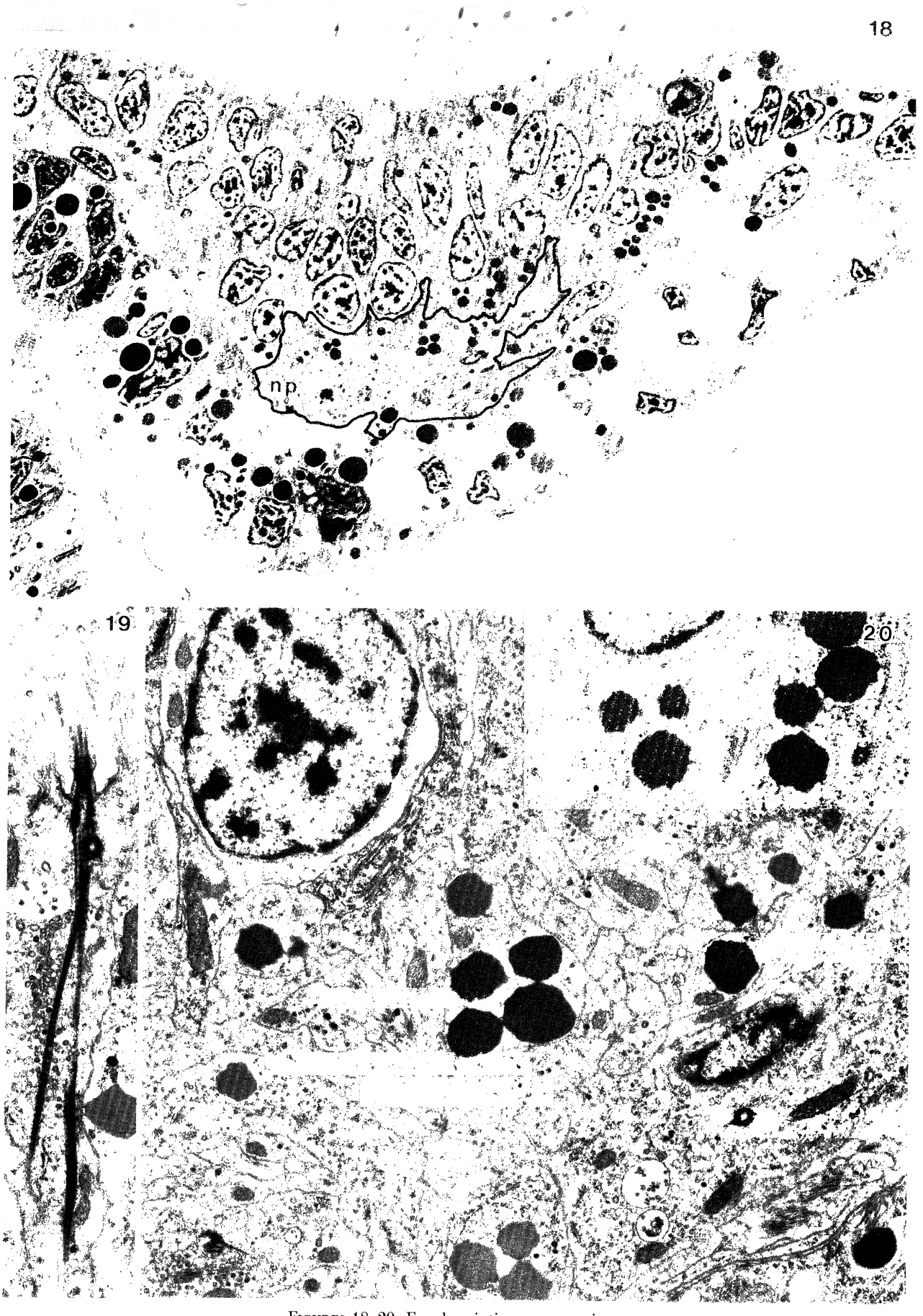


FIGURE 16. For description see opposite.

Description of plate 4

Figure 16. Stereopairs showing the principal nerves and neural centers of a four-tentacle larva. The reconstruction is of the right half of the body, representing about a 45 μm thickness of tissue, from an e.m. survey of the whole larva. Nerve tracings were compiled on 22 sheets with profiles of the body surface on every second sheet, that is, surface profiles are at 4 μm intervals. (*a*) External view from the right side. (*b*) Internal view from the mid-sagittal plane. (Magn. ×570.) See figure 15 for a key.



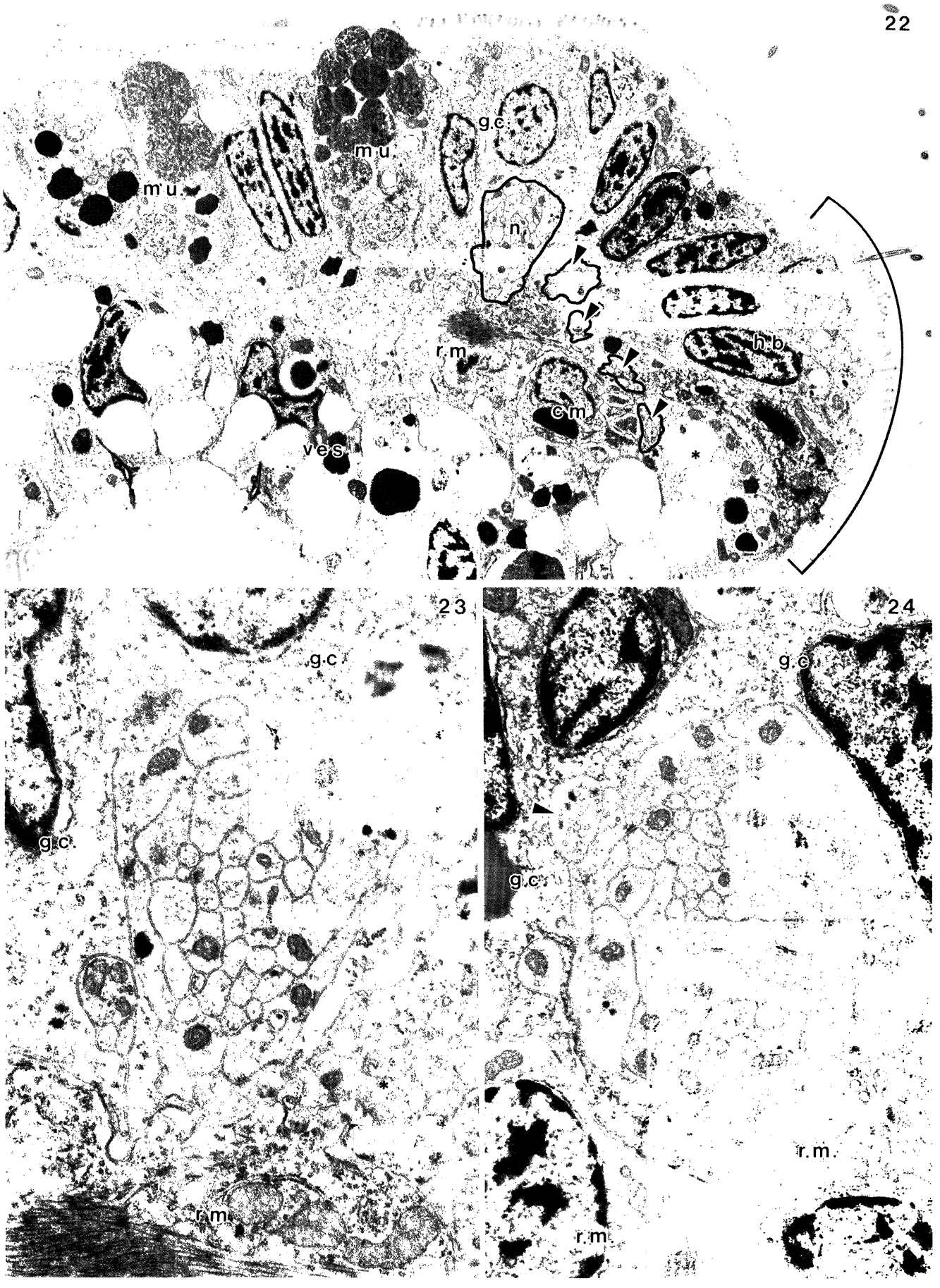
FIGURES 18–20. For description see opposite.

Description of plate 5

Figure 18. Survey view of the apical organ in sagittal section showing the basal neuropile (np., outlined). (Magn. ×3490.)

Figure 19. Apical surface of the uniciliate central cells of the apical organ showing characteristic rootlet structure. (Magn. ×20470.)

Figure 20. Detail of the neuropile, from the centre of the area outlined in figure 18. (Magn. ×16260.)



FIGURES 22–24. For description see opposite.

Description of plates 6 and 7

Figure 22. Survey view of the hood margin, a detail of figure 5 for comparison with figure 21. Shows primary hood nerve (outlined, n.), four accessory hood nerves (outlined, arrows), and cells of the marginal hood band (h.b., approximate extent shown by arc). The vestibular epithelium (ves.) begins at the vacuolated cell marked by an asterisk. (Magn. ×7780.)

Figure 23. Detail of primary hood nerve from figure 22. Shows surrounding glial-like capsular cells (g.c.) and an adjacent radial muscle cell (r.m.). At the lower right are fibres that may be in transit between the primary and accessory hood nerves. There is one type II fibre in this section, one of the small fibres near the centre of the nerve, but it has no vesicles in this section. (Magn. ×26200.)

Figure 24. As in figure 23, a section of the primary hood nerve from another larva, at the same stage, showing its similar size and organization. There is one type II fibre in the nerve in this section, visible at the upper left (arrow). (Magn. ×25520.)

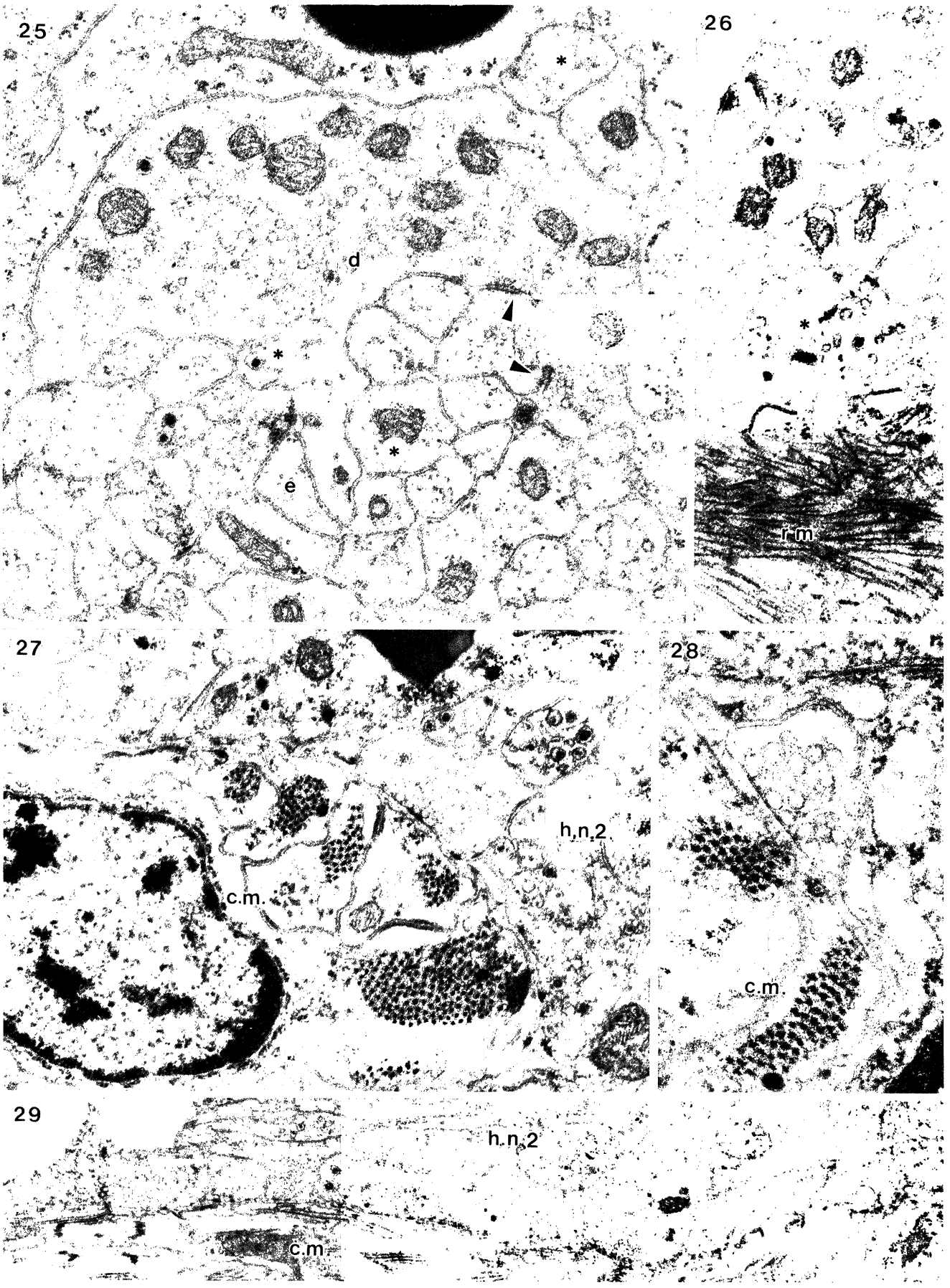
Figure 25. Detail of the primary hood nerve in a section from the serial series used for the reconstruction (figure 30). The large, vesicle-filled varicosity (d) belongs to the fibre shown in figure 30*d*; (e) belongs to the fibre shown in figure 30*e*, which is small at this point. The asterisks mark the three fibres shown in figure 30*c*. Note synapse-like junctions (arrow). (Magn. ×42230.)

Figure 26. As in figure 25, but showing a varicosity (asterisk) belonging to the type II fibre shown in figure 30*f*. No junctional specializations were found between such varicosities and adjacent radial muscle cells. (Magn. ×40490.)

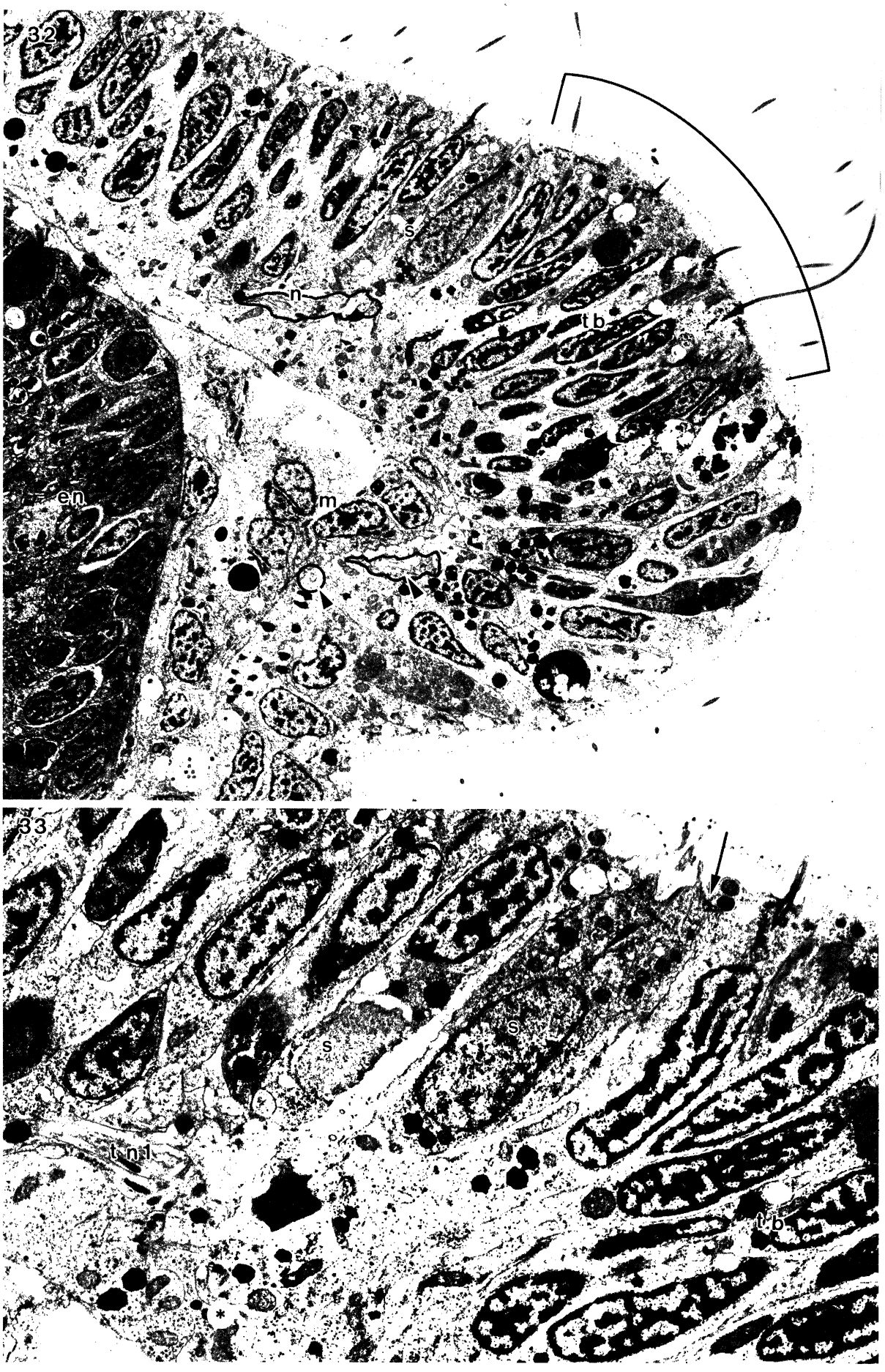
Figure 27. The most distal of the accessory hood nerves in contact with the circular hood muscle (c.m.) at the hood margin. Shows a variety of vesicle types including one terminal containing dense-cored vesicles (upper right), and a neuromuscular junction. (Magn. ×40000.)

Figure 28. As in figure 27, a detail of one of the neuromuscular junctions. (Magn. ×61600.)

Figure 29. A tangential section through the most distal accessory hood nerve showing the extent of its junctional contact with the circular muscle. Only one of the terminals, near the centre, has predominantly dense-core vesicles. (Magn. ×17500.)



FIGURES 25–29. For description see facing plate 6.



FIGURES 32 AND 33. For description see facing plate 9.

Description of plate 8

Figure 32. Survey view of a tentacle, from a section close to that shown in figure 5, for comparison with figure 31. Shows the tentacle band (t.b., extent shown by the arc), sensory cells (s.), primary tentacle nerve (outlined, n.), accessory tentacle nerve (outlined, arrow), and one of the trunk nerves (circled, arrow). (Magn. ×4070.)

Figure 33. A detail of figure 32. Shows two sensory cells (s.), the primary tentacle nerve (t.n.1), several accessory terminals (*), and the junction between the tentacular band and oral field (arrow). (Magn. ×9160.)

Description of plates 9 and 10

Figure 34. Section along the side of one of the medial tentacles, taken tangential to its surface, but at the level of the bases of the sensory cells. Shows the base of one such cell (s.), a basal terminal (arrow), and the primary tentacle nerve, which runs along the top of the figure. A row of accessory terminals (*) runs along the bottom of the figure, and between these and the nerve are the cylindrical bases of 2–3 rows of tentacular band cells. (Magn. ×17970.)

Figure 35. As in figure 34, a detail of a row of accessory terminals and the fibres connecting them. (Magn. ×28460.)

Figure 36. The accessory tentacle nerve. The basement membrane (*, and between arrows) separates it from the trunk musculature (m.). (Magn. ×31870.)

Figure 37. As in figure 36. The muscle cells lie to the right (out of figure) of the diffuse, fibrillar basement membrane (*). (Magn. ×41780.)

Figure 38. Two tentacular sensory cells. Their basal ends are each drawn out (at arrows) to form a slender, microtubule-filled process that connects to a vesicle-filled terminal. The basal processes lie out of the section in this instance, but one terminal from another cell is shown (*) adjacent to the tentacle nerve (n.). (Magn. ×15560.)

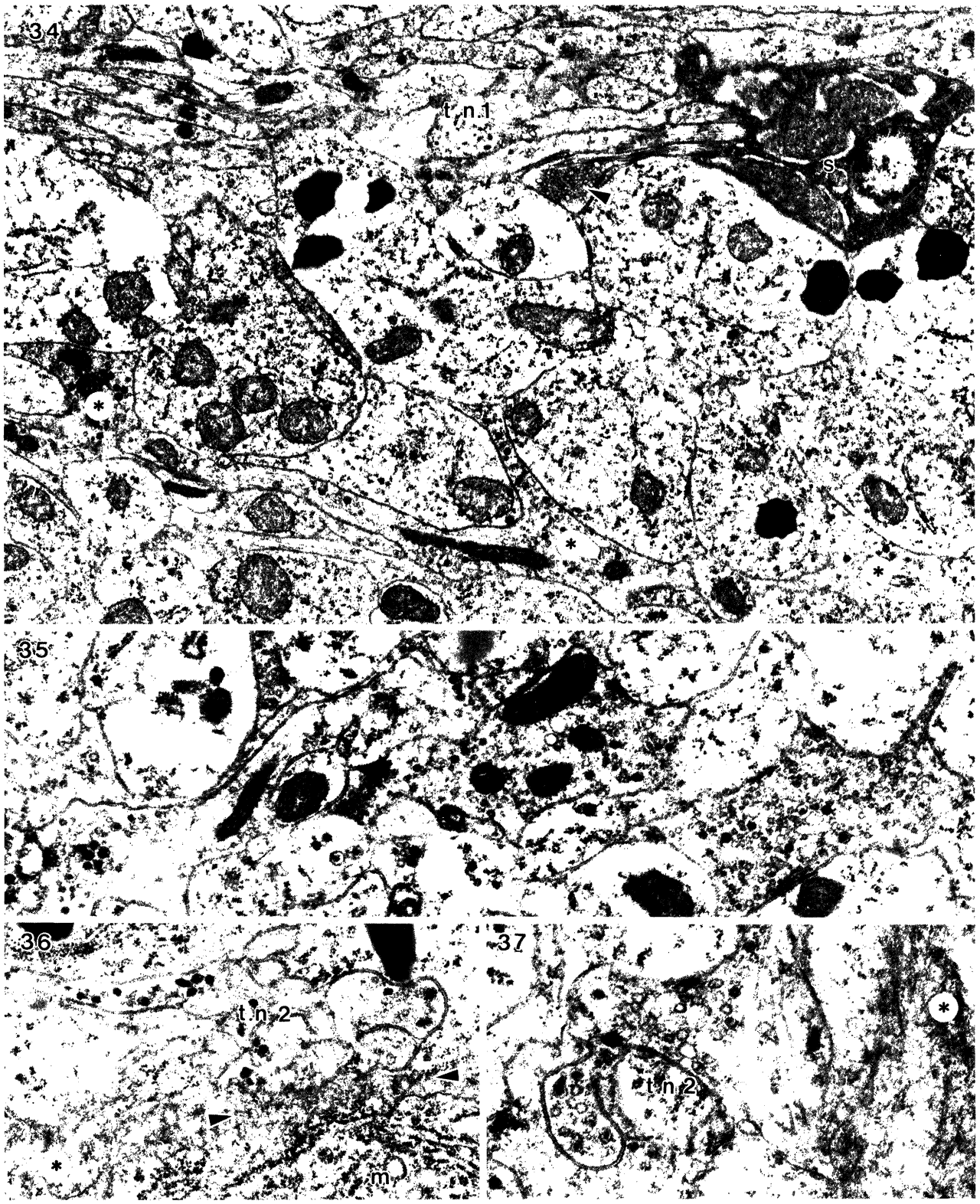
Figure 39. Cilium and rootlet complex of a tentacular sensory cell showing part of the circlet of large microvilli (arrow). (Magn. ×29520.)

Figure 40. As in figure 39, showing the filament bundles (lower arrow) that support each microvillus (upper arrow). (Magn. ×24320.)

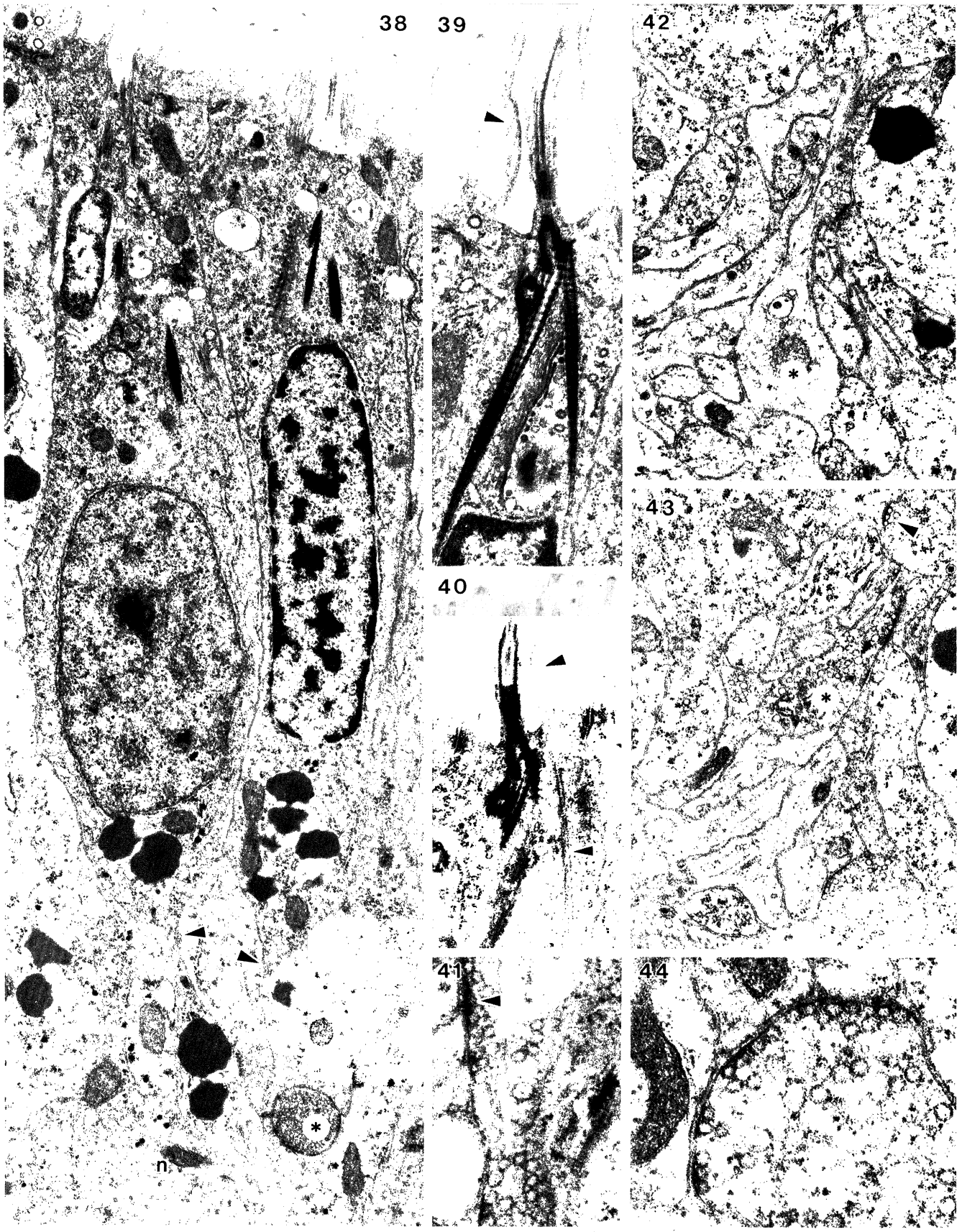
Figure 41. Base of a sensory cell showing the proximal part of the process connecting to its basal terminal. Microtubules fan out at this point, and clusters of vesicles occur (arrow). The adjacent cell is another sensory cell. (Magn. ×50000.)

Figures 42 and 43. Two views of the primary tentacle nerve showing fibres and basal terminals. The terminal in the upper figure connects, in adjacent sections, to the cell whose base appears in the upper right-hand corner of both figures. The same cell is shown in figure 45*f*. Vesicle clusters belonging to this cell can be seen in figure 43 (arrow), along with the basal terminal of the cell shown in figure 45*g*. (Magn. ×22930.)

Figure 44. Detail of the basal terminal in the lower right-hand corner of figure 43. (Magn. ×61180.)



FIGURES 34–37. For description see opposite.



FIGURES 38–44. For description see facing plate 9.

the morphological evidence, the main function of the hood nerves seems to be the coordination of the hood lift response, in which the radial and circular muscles contract in sequence (§3.5).

The two hood nerves join together near the base of the hood on either side to produce large concentrations of fibres, the adoral nerves, that run beside the mouth into the oral field. The two dorsolateral nerves from the apical organ join these, below the mouth. Irregular tracts of small numbers of fibres descend from approximately this point to the tentacles, across the oral field. A cross-connection is formed, below the mouth, forming essentially a sub-oral nerve, but this is not very substantial. The ventral mid-line is otherwise devoid of nerves, consistent with the bilateral symmetry of the system: connections to the posterior structures are basically paired and lateral in position. Despite the large number of fibres in this region, no obvious nerve cell bodies could be identified at the base of the hood or in the sub-oral region.

The tentacle ridge (§3.4) is innervated by two nerves that run in parallel along the upper and lower (oral and aboral) margins of the tentacular ciliary band. The primary tentacle nerve, on the upper surface, is both larger and more complex. Its most obvious function, from behavioural observations, is mechanosensory. Multiple rows of sensory cells are located at the junction of the oral field and tentacle band. They in fact belong to the former, and the primary tentacle nerve runs beneath them. Each sensory cell has a basal process ending in a vesicle-filled terminal that forms synaptic junctions within the nerve, but the cells do not otherwise produce any neurites. They are probably responsible for initiating the hood lift response, triggered in this species by touching the top surface of the tentacles (§3.5). Fibres in the primary tentacle nerve also have branches running into the ciliary band that swell to form chains of accessory terminals. Their function is not clear, but their location suggests a role in the control of ciliary beat. A second nerve, the accessory tentacle nerve, formed by a branch from the primary nerve, runs along the aboral margin of the band. It lies adjacent to the basement membrane, and the presence of muscle cells on the opposite side suggests a neuromuscular function. Both tentacle nerves contain type I and type II fibres, and though it is not clear, the accessory terminals probably belong to the former.

There is a network of small trunk nerves below the tentacles. These were not examined in detail. They presumably provide for innervation of the telotroch as well as other trunk structures, but are irregular in organization and poorly developed at the four-tentacle stage.

In summary, the main nerves in the actinotroch develop in association with the two ciliary bands, but their fibres originate centrally, in or near the apical organ. Except for the sensory cells in the tentacles, there are no peripheral nerve cells bodies in the bands that might provide for local control, for example, of ciliary beat. The available evidence relating to function also suggests that neurociliary control is at most an accessory function of both the tentacle and hood nerves. The primary tentacle nerve clearly has an important sensory function, and the hood nerves appear to be primarily, if not exclusively, involved in neuromuscular control.

3.2. *The apical organ*

The apical organ (figures 18–20, plate 5) has two distinct regions containing cells that differ ultrastructurally in fairly obvious ways. A central pit is visible externally (figure 6), and beneath this is a central zone of about a dozen slender, cylindrical cells with elongate nuclei, pale, extracted cytoplasm, and distinctive ciliary rootlets (figure 19) that split below the accessory centriole. These cells extend the full thickness of the apical organ to the basement

membrane, which leaves no room for a basal neuropile. Instead, small bundles of fibres course between the cells near their bases.

Cells of a second type surround the central zone on front and sides to form an approximately U-shaped domain open dorsally. These cells are flask-shaped: they are narrow apically and expand basally to accommodate an ovoid nucleus. Each has a single rootlet, vertically oriented, and an extensive apical web of filaments. The latter probably account for the constriction of the cells' apical surfaces, which gives curvature to the sides of the apical pit. The cells' cytoplasm is granular with well-developed golgi, membrane systems, and cytoplasmic vesicles, including some dense-core vesicles. They have basal neurites that enter the neuropile, which is also U-shaped, that is, it wraps around the central zone. Figure 20 shows examples of both cytoplasm and neurites from these cells in a section taken tangentially through the apical organ, that is, through one arm of the U. Most of the cells in both figures 18 and 20 are of the flask-shaped type.

The neuropile itself shows no obvious organization, specialized zones or fibre tracts. The fibres are of roughly similar diameter throughout, and many have clusters of vesicles, but no

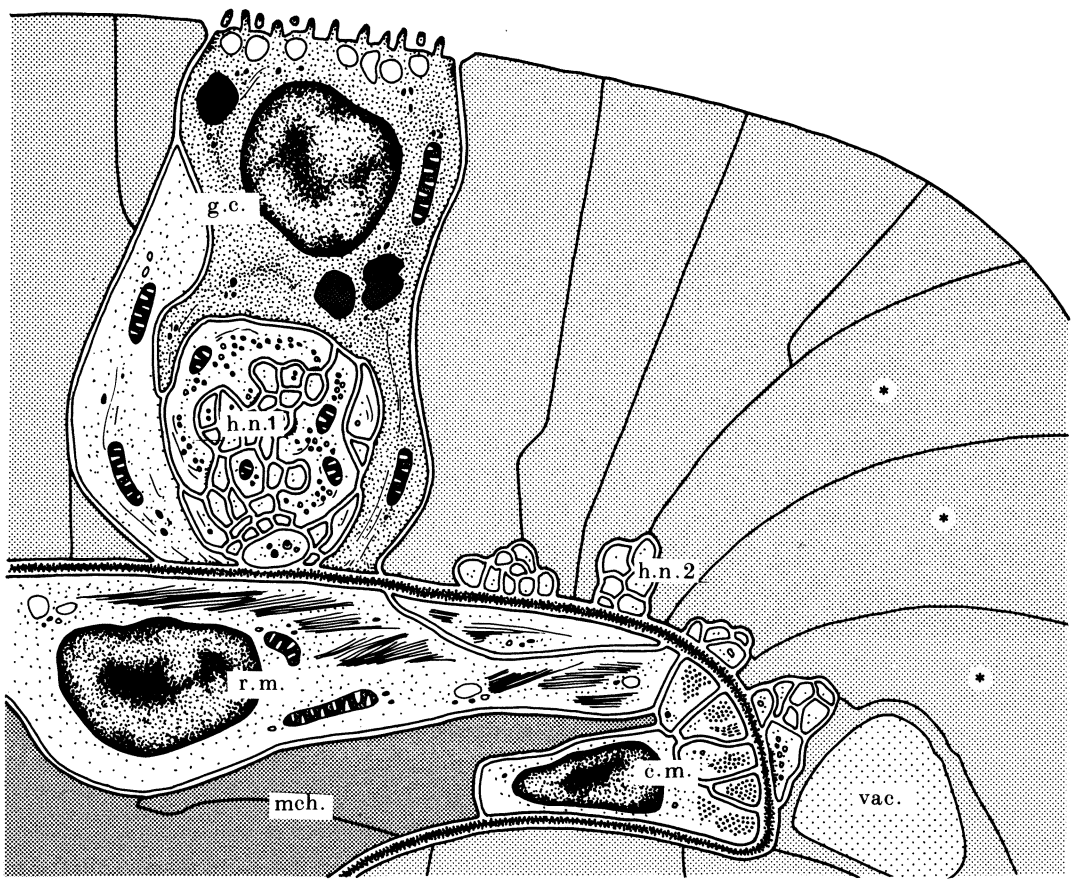


FIGURE 21. Schematic diagram of the hood margin in sagittal section showing its main nerves and muscles. Epithelial cells are lightly shaded, cells of the marginal ciliary band are marked with an asterisk, and mesenchyme (mch.) is darkly shaded. Specific cell types include: glial-like capsular cells (g.c.) surrounding the primary hood nerve (h.n.1), and cells of the radial (r.m.) and circular (c.m.) hood muscle. The accessory hood nerves (h.n.2) comprise four small bundles of fibres at this point, and the most distal of these abuts the first cell of vestibular epithelium, with its characteristic large cytoplasmic vacuoles (vac.).

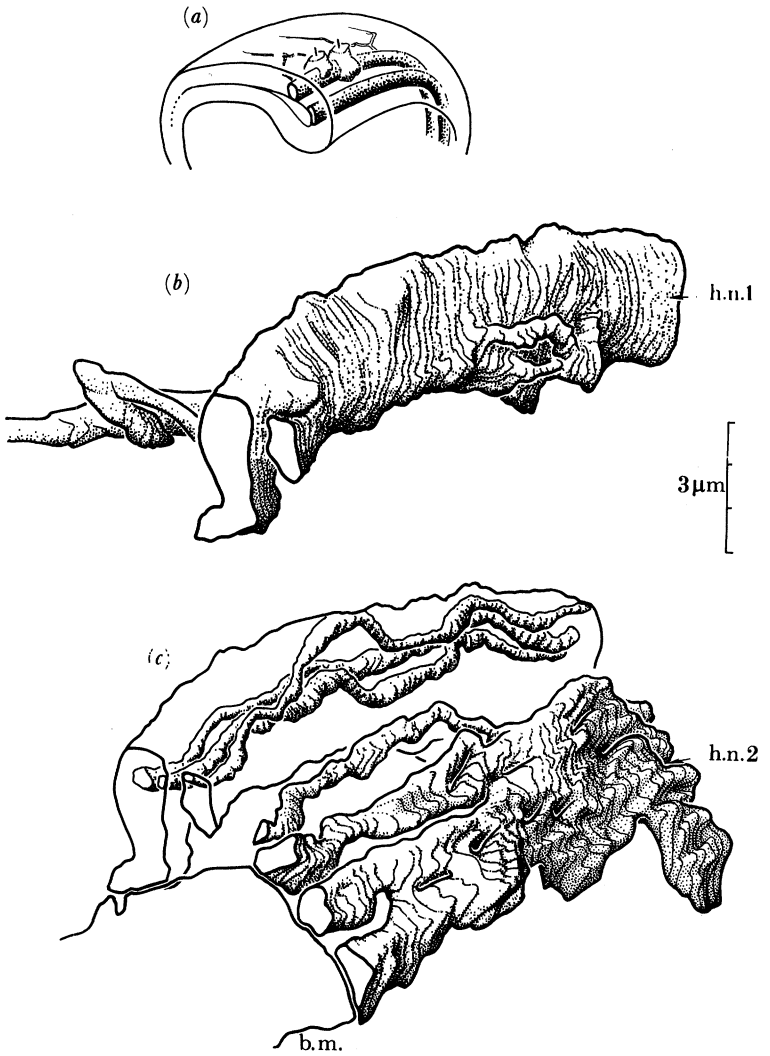


FIGURE 30. For description see p. 667.

specialized terminals, varicosities, or synapse-like junctions between fibres were encountered. The fibres do not fall into clearly defined types. Many contain clusters if dense-core vesicles or mixed dense-core and clear vesicles. Fibres with predominantly clear vesicles also occur, but were common only in the central zone. Further distinctions between cell and fibre types in the apical organ would require a much more extensive study of its structure and organization.

3.3. *Innervation of the pre-oral hood*

The hood epithelium is very diverse in terms of cell types. Various secretory and mucus-containing cells occur, along with cells containing a variety of cytoplasmic vesicles. It was not possible to determine with any confidence which of these, if any, might be nerve cells. Numerous small nerves cross the hood as well. These were not traced in detail, but fibre types and the presence or absence of junctions was noted. The principal nerves, which were examined in detail, run along the hood margin. The hood margin is itself distinctive in several

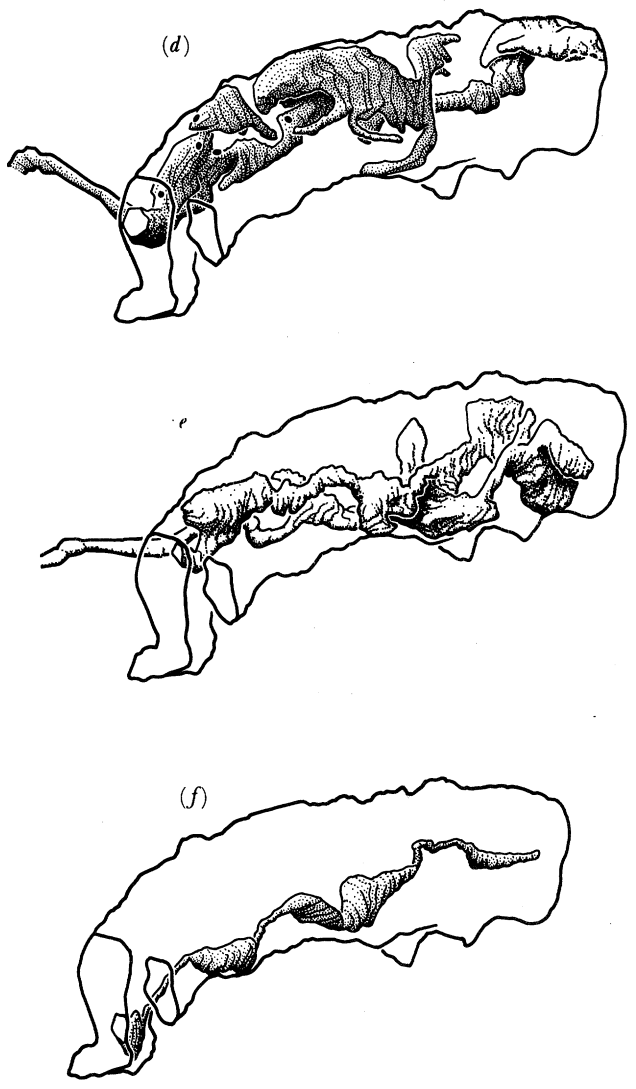


FIGURE 30. For description see p. 667.

respects, with a characteristic arrangement of nerves, epithelial components and muscles (figures 21 and 22). There is a slight depression in the epithelium just back from the edge of the hood (visible in figure 22, plate 6). Between this point and the beginning of the vestibular epithelium lies a zone, 4–5 cells wide, containing cells of a single type, which is the closest thing the hood has to a true ciliary band. Externally, there is no sign of any sudden transition in cilium density except at the junction with the vestibule (figures 7 and 8). The epithelium has considerable curvature at this point, so the cells are wedge-shaped, with tapered bases. This is exaggerated in the case of the cell at the interface with the vestibule, which typically arches over the first of the vacuolate vestibular cells. The largest of the nerves, the primary hood nerve, lies about 10 μm back from the hood margin, that is, well back from the zone interpreted here as ciliary band. The accessory hood nerves run in parallel along the basement membrane between the primary nerve and the edge of the hood. Also at the edge, beneath the ciliary band

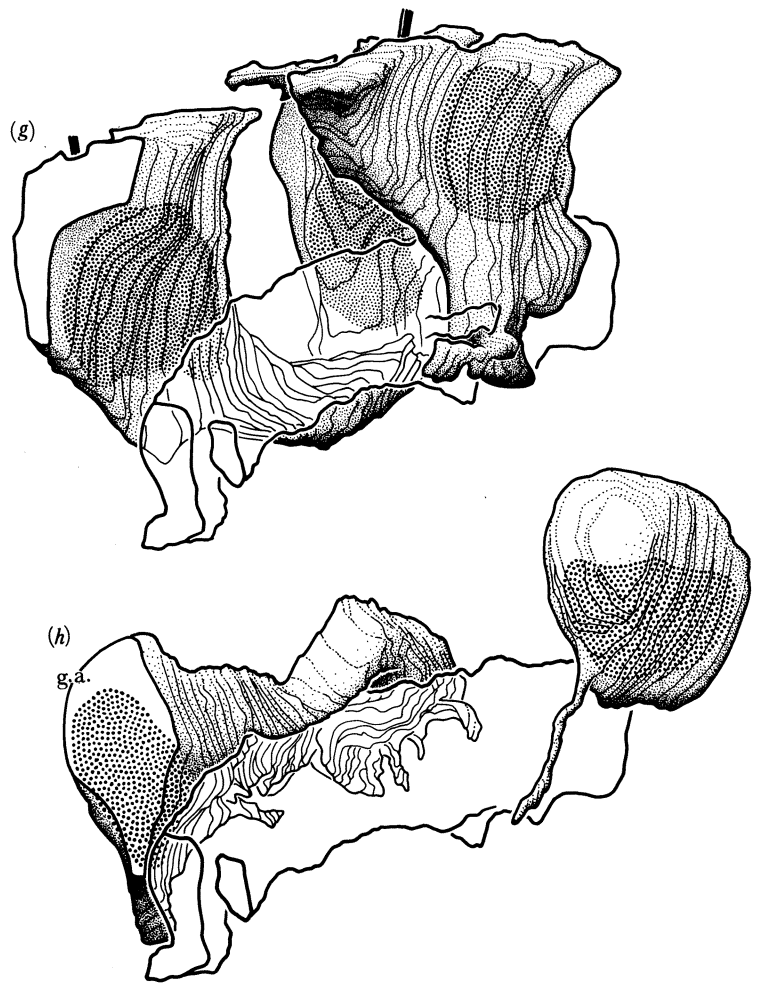


FIGURE 30. Reconstructions from serial sections of a 13 μm segment of the hood nerve showing selected cells and fibres. Profiles were traced from every third section. External surfaces are shaded, nuclei are dotted in. Scale applies to (*b*)–(*h*). (*a*) Overview of the hood showing orientation and plane of section. (*b*) The primary hood nerve. (*c*) The accessory hood nerves (h.n.2), and three typical examples of small type I fibres in transit through the primary hood nerve, which is shown in outline. Both abut the basement membrane (b.m.). (*d*), (*e*) Two large type I fibres, the largest in the nerve. Both expand to form irregular varicosities filled with clear vesicles (cf. cell d in figure 25). Filled circles in (*d*) show sites of synapse-like junctions, but most are hidden from view, on the underside of the varicosities. (*f*) The single type II fibre encountered in the series (* in figure 26). (*g*) Three of the glial-like capsular cells. These form a sheath around the nerve except where it contacts the basal muscle cells. (*h*) Two non-neural cells associated with the nerve. One of these (g.a.), found only rarely, is interpreted as being some type of glial-like accessory cell. The other is one of a number of ovoid cells in the adjacent epithelium with one or more slender, basal processes.

or, more specifically, at the junction between the band and the vestibule, is a large band of circular muscle cells, the largest such muscle in the hood.

(*a*) *Primary hood nerve*

The primary hood nerve is shown in transverse section in figures 23–26 (plates 6 and 7), and in reconstruction in figure 30. It is a cylindrical nerve, enclosed in a glial-like fashion by adjacent capsular cells everywhere except basally, where it makes contact with cells of the

radial hood muscle. The capsular cells are modifed epithelial cells, forming a double row along the nerve, with encapsulating processes that typically lie along one side of the nerve or the other as shown in figure 30*g*. Some capsular cells straddle the nerve, however, and at several points these were observed to send thick processes into it, which has the effect of splitting the nerve into two parts. Small bundles of fibres also leave the nerve and then rejoin it (two examples of this are shown in figure 30*b*), which seems to be a situation where fibres have been diverted around the back of processes from one or more capsular cells. Both situations may be significant in relation to what is observed in later development in *P. muelleri* (Hay-Schmidt 1989), in which two or more primary-type nerves run in parallel. The nerve may be progressively split during development by the intrusion of glial cell processes.

All of the fibres in the nerve, with the exception of one, appeared to be of a similar type, referred to here as type I fibres. Though possibly an oversimplification, differences between these can be interpreted as differences in size or degree of development. Type I fibres, as ultrastructurally defined, have axial microtubules, and contain predominantly clear vesicles, *ca.* 45–50 nm in diameter (figure 25). Most sections through the primary hood nerve are dominated by two or three large varicosities belonging to fibres of this type (for example, figures 23 and 24), which make synapse-like junctions with adjacent smaller fibres. The reconstructions are of the specimen shown in figure 23, and in this case, the large varicosities arise from only two fibres, shown in figures 30*d* and 30*e*. The remaining fibres include medium-sized ones with smaller varicosities and some vesicles, and smaller, simple fibres, like those in figure 30*c*, with very little in the way of distinguishing features beyond a few scattered vesicles. In the segment of nerve shown in figure 30, as an example, there were 36 identifiable fibres passing fully or at least part-way through the section series. Of these, two were very large, as mentioned, ten were medium-sized, and the rest were small. Of 43 synapse-like junctions identified, large or medium-sized fibres were presynaptic in all cases, and small fibres were postsynaptic in most cases. The small fibres may in fact represent a distinct and separate fibre type for reasons discussed in §4.1*b*.

Regarding junctions in the reconstruction, a small proportion of the fibres had most of the junctions: the large fibre in figure 30*d* was presynaptic in 20 of 43 cases, and one small fibre was postsynaptic in 13 of 43 cases. A single, glial-like accessory cell (g.a. in figure 30*h*), which appears not to be a neuron, was postsynaptic in nine cases. This raises the question of whether the junctions are really synapses, or only adhesion plaques of some type. Clusters of vesicles are associated with them in most cases (for example, as in figure 25), but this is only circumstantial evidence for synaptic function.

One type of fibre was encountered in the primary hood nerve clearly of a different type from the type I fibres just described, but usually only one such type II fibre was found in any given segment of the nerve. The type II fibre from the reconstructed segment is shown in figure 30*f* and in section in figure 26. Typically, type II fibres have periodic swellings containing mixed vesicles including dense-core vesicles with irregular, often oval, profiles. The dense-core vesicles are typically larger (*ca.* 65–80 nm in diameter) and more numerous than the clear vesicles, which are similar in size and appearance to those in type I fibres. Between swellings, the type II fibres become very slender, with diameters down to 0.1 μm at many points, and are uniformly dense without visible internal structures. In contrast, type I fibres are seldom smaller in diameter than *ca.* 0.2 μm, which is large enough that their axial microtubules are always visible in section. The paths of type II fibres through the nerve is often irregular. They are

frequently found at the bottom of the nerve, adjacent to the underlying radial muscle, as in figure 26. Junctional specializations were not observed at such points.

Small nerves from the hood enter the primary hood nerve all along its length. Some of the fibres then branch and travel in both directions along or within the hood nerve, as is the case for the two largest type I fibres (figures 30*d*, *e*). Examined in transit, beneath the hood epithelium, the nerves typically consist of 3–4 type I fibres and an occasional type II fibre. Synapse-like junctions between type I fibres are not common, but do occur. There are also junctions between the fibres and adjacent mucus cells. Type I fibres were traced into cells in only two instances, in both cases to cells located well back from the hood margin. The cells were ovoid and rather nondescript, but with scattered clear vesicles in their basal cytoplasm. Along with these, a number of cell types were encountered in the hood epithelium that looked promising from the point of view of being nerve cells, but too few tracings were done to confirm any of these as a major source of fibres. Among the cells examined were a number of dense, ovoid cells with slender basal processes that lie near the primary hood nerve. An example is shown in figure 30*h*. Cells of this type lie on both sides of the primary nerve and occur in some numbers, but their processes terminate without becoming neurites, as in the example shown. In fact, no evidence was obtained to show that any of the cells located adjacent to or even near the primary hood nerve or the accessory hood nerves (see below) produced neurites of any identifiable type, that is, none of them are nerve cells.

(*b*) *Accessory hood nerves*

This is a collection of small nerves running in parallel along the basement membrane between the primary hood nerve and the hood margin. There were usually four of them in the specimens examined, as in figures 22 and the reconstruction (figure 30*c*), each consisting of 4–8 type I fibres. All but the most distal of the nerves, that is, the nerve closest to the hood margin, are poorly supplied with vesicles. The latter is distinctive in several respects. It passes along the interface between the distal-most cells of the ciliary band and the vestibular epithelium, while the other nerves travel beneath or between the cells of the band itself. Furthermore, vesicle-filled varicosities are common, and these form frequent junctions with the adjacent cells of the circular hood muscle, which runs just beneath the nerve (figures 27–29, plate 7). Occasional fibres with dense-core vesicles are seen, for example, as in the upper right of figure 27, but most of the varicosities that make junctional contact contain only clear vesicles.

The origin of the fibres in the accessory nerves is not clear. They do not come from cells in the band. Basal processes from band cells mingle with the nerve fibres, and the two look very similar in section, for example, in terms of cytoplasmic density and contents. However, repeated tracings showed that the basal processes from band cells are, without exception, attachment structures. They contain tonofilament bundles, and pass between the nerves, often through quite narrow gaps between them, to the basement membrane. Also, though many individual micrographs show neurites that seem to cross between the primary and accessory hood nerves (for example, as shown in figure 23), it was not possible to confirm this in tracings. The primary and accessory hood nerves thus seem to be separate except at the base of the hood, where they fuse, and fibres may move from one to the other at this point. There is an additional route for incoming fibres, at least for those in the most distal of the accessory nerves, through the vestibular epithelium. This occurs as follows and was observed at several points: small nerves of 3–4 fibres, approaching the primary hood nerve, were diverted through the basement

membrane to the vestibular epithelium. These travelled along beneath or between vestibular cells to the margin of the hood, and then entered the accessory nerve from the vestibular side. There are so few fibres in the most distal of the four accessory nerves that most if not all of them could arrive by this route. Though less substantial than the outermost circular muscle, at the hood margin, there are other circular muscles in the hood, many of them near the apical organ. Vesicle-filled terminals like those in the accessory nerve also occur adjacent to these muscles, on the vestibular side, and make junctions with them. As in the case of the circular muscles at the hood margin, innervation here seems to be via fibres that pass through the vestibular epithelium on route to their targets.

3.4. *Innervation of the tentacles*

Sections taken near the midline of one of the medial pair of tentacles are shown in figures 32 and 33 (plate 8), and in diagrammatic form in figure 31. The tentacle band is a clearly defined zone delimited on the oral side by the oral field, whose cells have oppositely oriented rootlets (§3.1 *a*), and on the aboral side by the resumption of normal epithelium with diverse cell types and sparse ciliation. The band cells seem to be all of one type. They are slender,

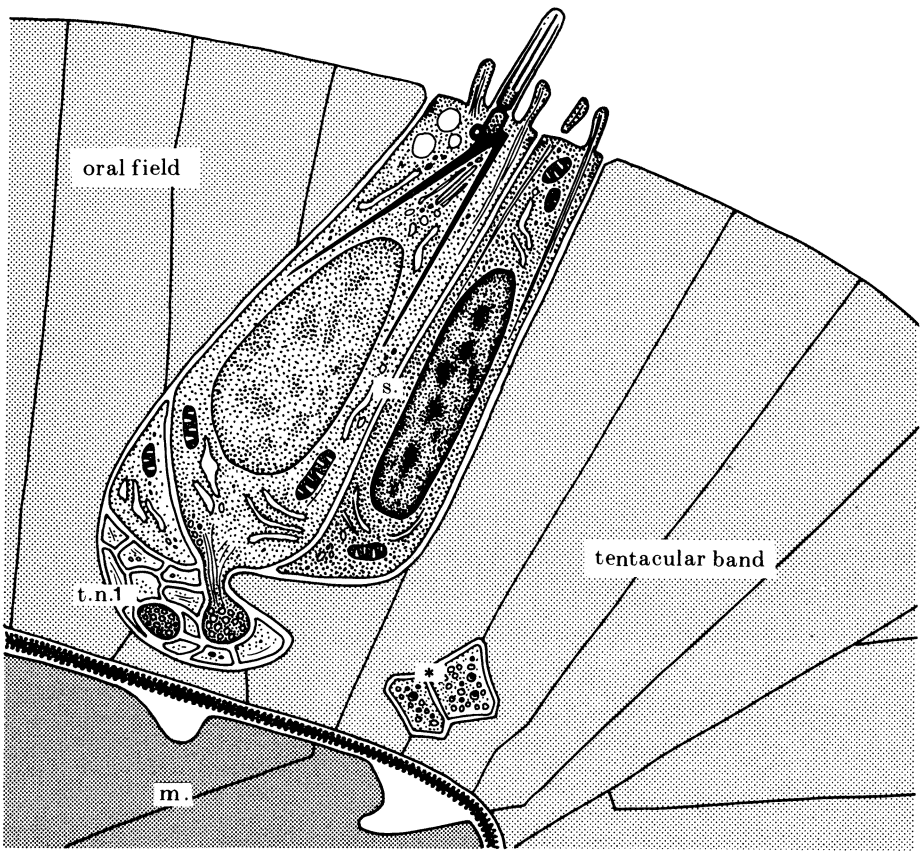


FIGURE 31. Schematic diagram through the top surface of a tentacle, in sagittal view, to show the relation between the tentacular sensory cells (s.) and the primary tentacle nerve (t.n.1). Conventions as in figure 21. The sensory cells lie between the oral field and tentacular band, but belong to the former, judging from the orientation of their cilia. They have basal terminals that enter the nerve, but produce no neurites. Larger vesicle-filled terminals (the accessory terminals, asterisk), arising from the primary tentacle nerve, form a chain that runs between the bases of the tentacular band cells several cell diameters from the oral margin of the band. Their morphology is more clearly shown in the reconstruction (figure 45*c*).

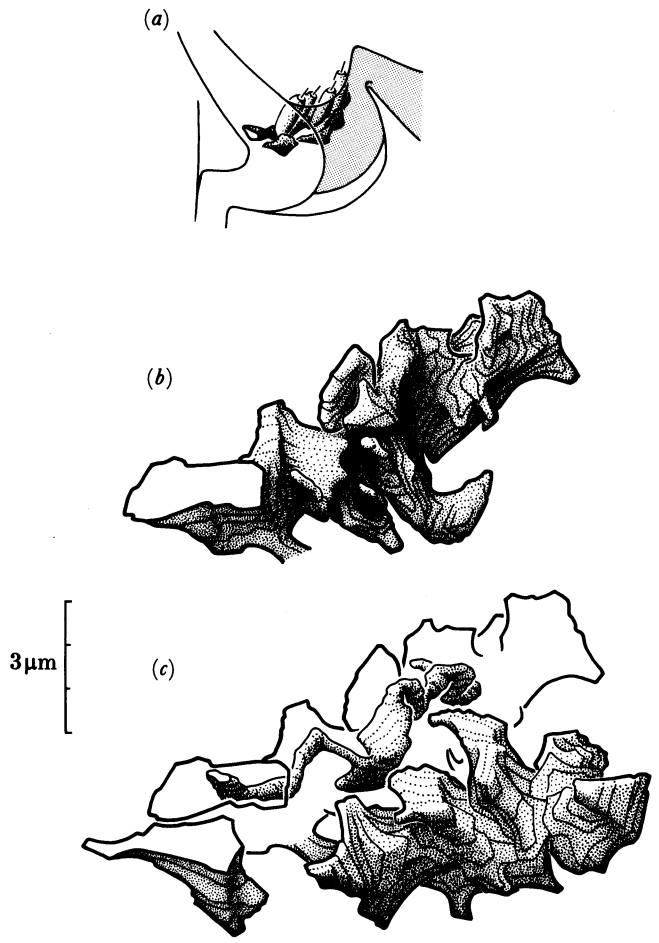


FIGURE 45. For description see p. 672.

cylindrical cells with elongate nuclei, comparatively clear cytoplasm, and long cilia. Two major nerves run along the tentacle band, the primary tentacle nerve on the oral side, and the accessory nerve on the aboral side. Neither actually runs under the band, through branches from the former along with vesicle-filled terminals push into the band, between its cells, along the oral margin. Various small trunk nerves are found in the post-tentacular part of the body. In section, they appear as distinct small bundles of fibres, and in the ventral region (figure 32), they come quite close to the accessory tentacle nerve, from which they may originate.

(*a*) *Primary tentacle nerve*

The primary tentacle nerve is shown in section in figures 33, 34 (plate 9), 42 and 43 (plate 10), and in reconstruction in figure 45. It is the larger of the two tentacle nerves, but is small and irregular in shape in comparison with the primary hood nerve (figure 45*b*). This is presumably because it is a simple bundle of fibres, lacking any glial investment, that must squeeze between the bases of the epithelial cells. The nerve travels completely around the body, that is, it is continuous dorsally, but is smaller on the dorsal side. Major branches from the primary nerve pass beneath the band at the junctions between the medial and lateral tentacles on each side and connect to the accessory tentacle nerve. Otherwise, the only branches from

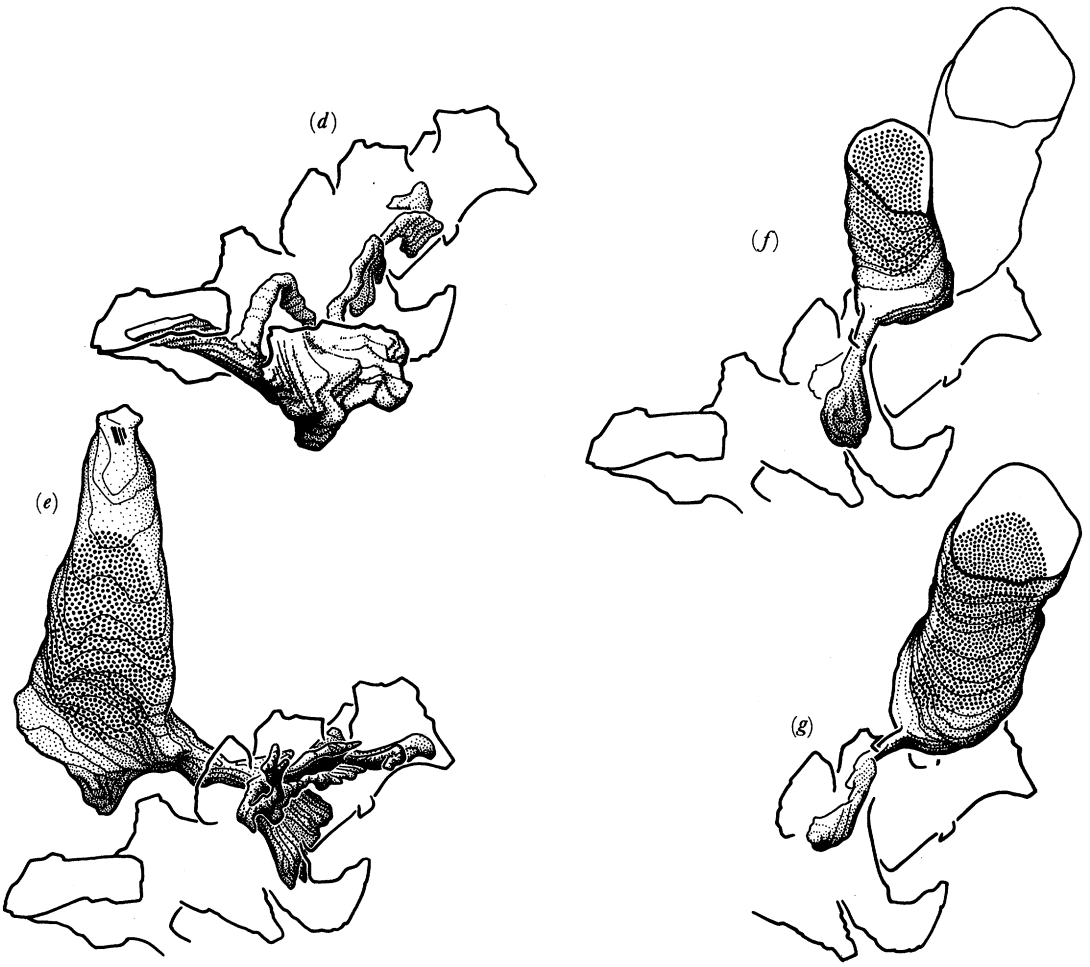


FIGURE 45. Reconstructions from serial sections of a 7.5 μm segment of the primary tentacle nerve showing selected fibres, accessory terminals, and sensory cells; conventions as in figure 30. (*a*) Overview of a tentacle showing orientation and plane of section. The tentacular band is shaded. (*b*) The primary tentacle nerve with its characteristic irregular shape. (*c*) The nerve (in outline), with one constituent fibre and a cluster of accessory terminals. (*d*) A fibre responsible for one of the terminals of the cluster shown in (*c*). (*e*) A glial-like accessory cell with branched processes and cilium (basal body shown at top). (*f*), (*g*) Basal halves of two sensory cells, each with a basal terminal. The cell in (*g*) is shown in outline in (*f*); it lies behind the other cell.

the nerve actually entering the band are short ones that expand into chains of large, vesicle-filled accessory terminals that run along the band 2–3 cell diameters inside its oral margin (figures 34, 35 (plate 9) and 45*c*). These have the largest concentration of vesicles found anywhere in the body. They lie close to the basement membrane, but do not make contact with it, and do not form junctions with any of the surrounding cells.

Both type I and type II fibres occur in the primary tentacle nerve, but there are few vesicles or junctional specializations, so it is difficult to distinguish fibre types with any degree of confidence. The accessory terminals seem to be all of one type, with some mixed vesicles, but a predominance of clear ones. Junctions were found in some instances between fibres leading to the terminals, which further suggests they are type I fibres, and in two cases traced in detail (figure 45*d* shows one), this was confirmed.

At least some of the processes in the primary tentacle nerve are non-neural. These arise from

nearby cells in the oral field that send branching processes into the nerve. These contain some microtubules, but no vesicles, and those traced do not travel very far. An example, interpreted here as a glial-like cell, is shown in figure 45*e*. Such cells are not very numerous.

(*b*) *Accessory tentacle nerve*

This is a small nerve that originates as a branch from the primary nerve on either side at the junction between the medial and lateral tentacles. It lies in direct contact with the basement membrane along much of its length (figures 36 and 37, plate 9), and the presence of muscle cells on the opposite side of the basement membrane near the nerve suggests a neuromuscular function. Both type I and II are present with small to moderate accumulations of vesicles, but no large terminals. The nerve is incomplete dorsally at the four-tentacle stage.

(*c*) *Sensory cells*

The sensory cells (figure 38, plate 10) form a continuous zone 2–3 cells wide at the edge of the oral field. Each cell produces, at its base, a single process containing microtubules that projects into the primary tentacle nerve, where it swells to form a vesicle-filled terminal (figures 42, 43, 45*f* and 45*g*). There may be some diversity of cell type among sensory cells, as there is considerable variation in shape and ultrastructural appearance. All the cells have basal bodies, but not all have cilia, and fat, ovoid cells are frequently paired with elongate ones, as in figure 38. The former typically have uniform, finely granular cytoplasm, while the latter tend to have extracted cytoplasm and clumped chromatin. Figure 33 shows two cells of the first type, and between these is a thin strip of cytoplasm belonging to one of the more elongate cells. It is possible that the sensory cell population is actively proliferative, for example, in preparation for a period of rapid tentacle growth, in which case the differences between cells could be related to the cells' progress through the cell cycle. Mitotic figures or other signs of proliferative activity were not, however, encountered.

It is very likely that the sensory cells, at least those with cilia, are mechanosensory. Each cilium is surrounded by a circlet of large microvilli (figure 39, plate 10) supported by bundles of fibrils that extend well into the cell along its lateral surfaces (figure 40, plate 10). There are four rootlets that together form a cage around the nucleus. The principal and accessory rootlets are vertical, and extend in the median plane (that is, the plane of cilium beat) along the oral and aboral surfaces of the nucleus, respectively. Smaller lateral rootlets extend from the two ends of the accessory centriole along the sides of the nucleus.

The basal terminals of sensory cells contain only clear vesicles, 50–75 nm in diameter. Dense material along the inner face of the terminal membrane suggests some type of junctional specialization (figure 44, plate 10). Small junction-like regions and clusters of vesicles are also found in the cell body near the point of origin of the terminal process (figures 41, 43, plate 10). In all cases examined, the adjacent cell, on the post-junctional side, was also a sensory cell. The basal terminals vary in terms of number of vesicles. Some are packed with them (for example, as in figure 38, plate 10), while others have relatively few (figures 42, 43). In two instances, a tiny fibre (*ca.* 0.1 μm diameter) was traced from one terminal to another, so they can occur in chains, but most appear to be single.

The sensory cells themselves are unusual in relation to those found elsewhere among invertebrates and invertebrate larvae. They lack obvious neurites, and in this respect resemble the secondary sensory cells of some vertebrate sense organs (for example, acoustical hair cells).

It is, nevertheless, relatively easy to envisage the origin of the actinotroch-type cell from a primary sensory cell more like what is found in other larval ciliary bands (§4.2), by reduction of the long neurites of a primitively bipolar or multipolar cell to a single, short process with a terminal.

3.5. *Larval behaviour*

Older eight- or ten-tentacle stages, taken from the plankton, were more suitable subjects for behavioural observations than the newly released four-tentacle stage. The latter are small and swim continuously, at moderate speed compared with trochophores of similar size, but rapidly enough so that observing unrestrained, swimming larvae is difficult. The young larvae normally swim with the hood lowered against the apron-like expanse of the oral field. A few instances of hood lifting were observed, and fixation causes the hood to be partially or fully raised, as shown in figures 3 and 4.

By the eight-tentacle stage, the prominent perianal telotroch has developed, and takes over from the tentacular band as the main locomotory organ. Larval behaviour by this stage is much more complex. Eight- and ten-tentacle larvae swim slowly, executing prolonged pauses that seem to be due to changes in the pattern of beat of the telotroch. Continual minor adjustments to body posture are made during such pauses, mainly involving the trunk musculature. The hood is held close against the apron of the oral field. There is a narrow gap between the two, presumably to allow for feeding currents. Mechanical stimulus to the apical organ or hood, that is, touching it with a fine glass needle, elicits no obvious response, even during swimming. Touching the front surface of the tentacles causes an immediate lifting of the hood, which is held up for 1–2 s, and then closed. The most sensitive spot seems to be at the base of the tentacles where they connect with the oral field. Repeated touches cause either a series of lifts or a sustained lift with periodic contractions of the hood margin that partially close the hood. The response can be reversibly abolished by placing larvae in a 50% mixture of isotonic magnesium chloride and sea water. This causes some distortion of body shape as well, but does not affect swimming.

Large *P. vancouverensis* larvae are, in general, rather sluggish compared with other large planktotrophic actinotrochs. Behaviour seems to vary between species; it is not clear that the feeding mechanism is necessarily the same, and some of the other actinotrochs the author has tested do not respond as described above for *P. vancouverensis*.

4. Discussion

4.1. *Organization of the larval nervous system*

(*a*) *General features*

This study is the first to attempt a comprehensive description of the nervous system of an early stage actinotroch at the ultrastructural level. The main nerves are traced, and characteristic organizational features can be identified in most instances. Because the system as a whole is rather diffuse, with many small nerves, it is difficult to identify nerve cell bodies with certainty, but the information obtained, together with what is known from work on stained whole mounts (Hay-Schmidt 1990*b*, see below), provides a relatively complete picture of the structure and organization of the nervous system at the four-tentacle stage.

A young stage was chosen for this study to minimize the complications that arise in late stages with the development of additional structures and the nerves associated with them. The

existing literature deals mainly with advanced larvae, which are more complex, and most of the older studies, based on light microscopy, are incomplete. Usually only the largest nerves are identified (see Zimmer 1964 for a review). Recent studies by Hay-Schmidt (1989, 1990*a*) on *Phoronis muelleri* are far more complete. Based on his account, most of the structures reported here for *P. vancouverensis* have counterparts in advanced larvae. Some of these are modified during development, but most remain small and comparatively unmodified, so in advanced larvae they appear as minor components beside larger, late-developing nerves. The main nerves in the four-tentacle stage are those supplying the hood margin and the tentacle band, and three small apical nerves (one medial, two dorsolateral) that leave the apical organ. Of the latter, the median nerve, which connects to the hood nerve by diffuse processes in the young larva, becomes a well-defined tract of three parallel nerves in advanced larvae. A large sensory patch of ciliated cells develops at its junction with the hood nerve in some species. The hood nerves increase in size with the larva, and so are major nerves at all stages. The nerves supplying the tentacles remain small, however, as do the connections between the tentacles, and between the tentacle nerves and the hood. The difficulty of tracing these in older larvae has led to some confusion about how the hood nerves and tentacles nerves are linked together, and there may be variation between species. The arrangement reported here for the four-tentacle stage matches Hay-Schmidt's results fairly closely. In whole mount preparations of *P. muelleri*, fibres from the hood nerve pass beside the mouth on either side and fan out across the oral field on their way to the tentacle nerves. There is no evidence for sub-oral nerves crossing between the two sides, but there are tracts of small nerves, referred to as lateral epistome processes, that correspond with the dorsolateral nerves described here. In both cases, these form connections between the apical organ and the adoral nerve centres. The older literature does not mention such nerves, but they are so small that they could easily have been overlooked.

The main nerves found in advanced larvae, but not in the four-tentacle stage, are the large, paired trunk nerves. These leave dorsally from the apical organ and loop below the tentacle ring to form the post-tentacular ring nerve. The latter is the main nerve supply to the tentacles in the adult (Silén 1954). The tentacles are basically adult structures precociously differentiated to serve a larval function. Their initial innervation in the larva, from the oral side, appears to be provisional, and a matter of necessity. This would explain why the plexus of oral field and tentacular nerves is a comparatively minor component of the nervous system in older larvae.

As in previous studies, the apical organ is recognized here as a major neural centre and source of fibres. In fact the apical organ, together with the surrounding epithelium, may be the only source of fibres. With the exception of the tentacular sensory cells, which do not produce fibres, careful search has failed to turn up peripheral nerve cells of any kind. Glial-like cells were found, some with elongate processes that entered nearby nerves, but no identifiable peripheral nerve cells. The larval nervous system would appear, on this basis, to be highly centralized. Hay-Schmidt (1990*b*) has reached the same conclusion from his study of stained whole mounts of young *P. vancouverensis* larvae. The results are summarized in figure 46. He identified two types of cells, one stained by the glyoxylic acid method for catecholamines (CA cells), the other with antibodies to serotonin (S cells). CA cell bodies lie in a ring around the apical organ. They are either bipolar or multipolar. Each sends a fibre into the apical organ, where an irregular plexus is formed, and one or more fibres outward to the hood nerve. From the hood nerve, the CA fibres fan out across the oral field in transit to the tentacles. The cell bodies of the S cells lie in the apical organ, in a U-shaped domain. Their fibres leave dorsolaterally as single fibres

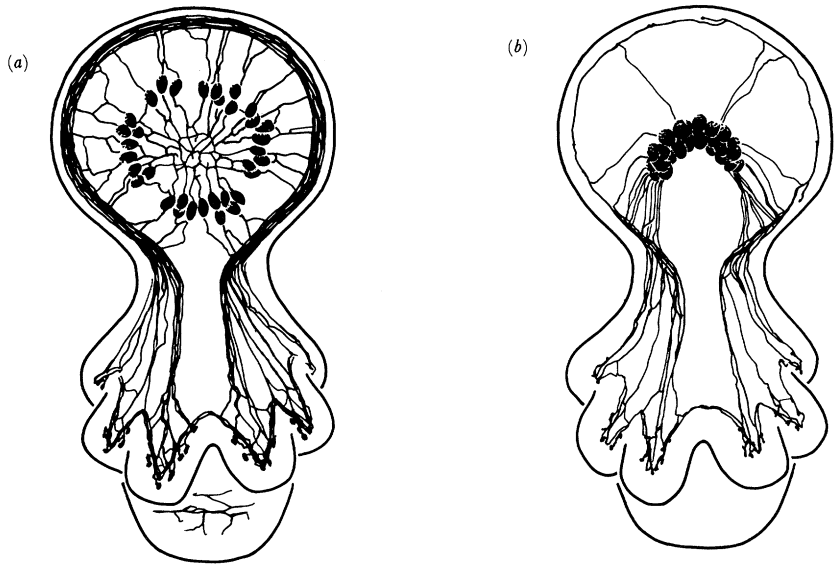


FIGURE 46. Drawings of stained whole mounts of *P. vancouverensis* larvae showing two fibre types and the cell bodies responsible for them, after Hay-Schmidt (1990*b*; personal communication). Shows the results obtained with (*a*) the glyoxylic acid method for catecholamines, and (*b*) antibodies to serotonin (see text for discussion).

or in small bundles, and again, fan out across the oral field in transit to the tentacles. Hay-Schmidt found no cell bodies outside the apical region, although in *P. muelleri*, glyoxylic acid stains clusters of nerve-like cells at the junction of the hood and oral field (Hay-Schmidt 1990*a*, his mesosomal cells), approximately at the site of the adoral nerve centres described here.

The main nerves in the *P. vancouverensis* larva are those associated with the two ciliary bands, at the hood margin and along the tentacle ridge. In neither case, however, is the nerve really as integral a part of the band as it seems to be in most other marine larvae with ciliary bands (§4.2). Usually, in these, at least some of the nerve cell bodies lie in or near the band, and the nerve fibres at least run under the band, in contact with the band cells. In the actinotroch, nerve fibres run parallel to the ciliary bands but not under them in the case of both the primary hood nerve and the two tentacle nerves. Only the accessory hood nerve has fibres that run directly under the band, and only the primary tentacle nerve produces vesicle-filled terminals that are associated with band cells. Neither of the bands contains identifiable nerve cells, that is, there is no evidence for intratrochal innervation, and even the tentacular sensory cells belong to the oral field rather than the tentacle band. The various nerves also have little in common in terms of their internal organization. Each is different: the primary hood nerve is highly organized, its accessory nerves are not, but have well-developed terminals. The primary tentacle nerve has sensory input and neurociliary terminals whereas the accessory nerve has neither. The evidence is thus for diversity in terms of nerve organization reflecting, presumably, the functions each nerve performs. Most of the system, in fact, seems to be concerned with neuromuscular reflexes (§4.1*c*), despite its morphological association with ciliary bands.

In summary, the larval nervous system is highly centralized with regard to the locations of nerve cell bodies, but diverse in terms of the organization and patterns of innervation of the peripheral nerves. To the extent that a diffuse and unspecialized plexus can be considered primitive, both features argue that the actinotroch is an advanced larva whose nervous system

has shown considerable flexibility in evolving to meet the functional needs of the larva. Neuromuscular control, rather than neurociliary control, seems to have been most important, and any form of intratrochal innervation that might once have existed has been replaced by direct innervation by cells located in and around the apical organ.

(*b*) *Fibre types*

The two main fibre types reported by Hay-Schmidt (1990*b*) correspond reasonably well, based on their distribution, with the type I and II fibres identified on ultrastructural criteria. The primary hood nerve consists mainly of type I fibres, so some or all of these must be catecholamine-containing (CA) fibres (figure 46*a*), despite the fact that they contain mainly clear vesicles. Catecholamines are usually associated with dense-core vesicles, but similar glyoxylic acid-staining nerves are seen in echinoderm larvae (see, for example, Burke 1983; Nakajima 1987), and these fibres also contain mixed populations of vesicles in which clear vesicles often predominate. Type II fibres probably correspond with Hay-Schmidt's serotonin-containing (S) fibres. There appears to be only one such fibre at the hood margin, approximately where the primary hood nerve should be, and small branches from this nerve run out to the very edge of the hood, that is, to the site of the accessory hood nerves, at several points. The tentacle nerves stain strongly for both fibre types, and roughly equal numbers seem to be present if one traces fibres in the whole mounts. Both are irregular with side branches and swellings, but the CA fibres have more terminal-like projections suggesting, as in the ultrastructural results, that they are responsible for the accessory terminals.

The above suggests there is a dual innervation of most larval structures by the two main fibre types, but it is probably an oversimplification to think in terms of just two types of cells. Hay-Schmidt's *P. vancouverensis* preparations show a population of fibres that contain FMRFamide, though these are poorly defined and no cell bodies are visible. He has also found FMRFamide-containing cells in *P. muelleri* in the same location as the sensory cells described here for *P. vancouverensis*. They may, in *P. vancouverensis*, contain some other peptide transmitter. It is also possible the type I category of fibres needs to be further subdivided, for example, that the large, medium and small fibres represent distinct types. The large ones, with abundant clear vesicles, could be cholinergic or peptide-containing, for example, which would still leave enough fibres to account for the intensity of CA staining observed in the hood nerve in Hay-Schmidt's preparations. Another alternative is that the small, post-junctional fibres are non-neural. They could be slender glial processes like those in the tentacle nerve. Neuro-glial junctions are known from other invertebrates (see, for example, Cobb & Pentreath 1977; Roubos & Moorer-van Delft 1979). They could have a structural role, which would account for the orderly appearance of the primary hood nerve in comparison with other larval nerves. A third fibre type, whether neuronal or glial, might also explain anomalies in the fibre counts (figure 17*b*). The problem with these is that far more fibres enter the adoral centres from the primary and accessory hood nerves than leave in transit to the tentacles. The difference can be approximately accounted for if about half the fibres on each side cross to the other side via the sub-oral nerve, but no such fibres are seen in Hay-Schmidt's preparations. This suggests the need for an additional cell type whose fibres run mainly in the hood nerves and which cross sub-orally, but it is not clear where the cell bodies should be expected to lie. The adoral region is a possibility, for example, they could be Hay-Schmidt's mesomal cells or be associated with them, or they could lie somewhere in the hood. Further work is clearly needed, but the total number of cell and fibre types is likely to be manageably small.

(*c*) *Functional aspects*

Marine larvae with ciliary nerves typically show some capacity to control ciliary beat, for example, to arrest or reverse ciliary beat as a means of controlling locomotory or feeding currents. Actinotrochs are ciliary feeders, but there is disagreement as to the precise mechanism involved. According to Gilmour (1978), complex water currents within and around the hood generated by the normal pattern of ciliary beat are largely responsible for driving particles to the mouth. Strathmann (1973, 1982) considers ciliary reversals to be an essential part of the mechanism. Their studies are of advanced larvae much larger than the ones examined here, and of species other than *P. vancouverensis*. Young *P. vancouverensis* are not particularly suitable for behavioural observation. In the present instance, obvious examples of controlled alteration in beat of the tentacle cilia were not observed, but their occurrence cannot be ruled out.

Control of hood posture is clearly important to the larva. Lifting the hood is an obvious behavioural response that develops early, and hood posture seems to be precisely controlled during normal feeding: the hood is normally held against the apron of the oral field with only a narrow slit between the two. Lifting the hood would then be useful as a means of clearing the vestibule of debris, and this seems to be what happens in some species (T. H. J. Gilmour, personal communication). There is also evidence that the hood can be used to capture large particles (R. R. Strathmann, personal communication). Lifting the hood requires a rapid, coordinated contraction of the radial hood muscles followed, after some delay, by contraction of the circular ones. Normal posture, with the hood lowered, probably depends to some degree on the circular muscles: in fatigued larvae, the surface of the hood loses its smooth, symmetrical shape and becomes wrinkled and irregular. Based on this study, the hood-lift response depends on sensory input from the primary tentacle nerve and neuromuscular control via the primary and accessory hood nerves, but there could also be continuous sensory feedback from the tentacles that would maintain the hood in a correct posture when in the lowered position.

It is difficult to be more specific about the role of the various cell types identified here in larval behaviour. One could envisage the type I and II fibres being sensory and motor, respectively, for example. This means there might be two classes of the latter, operating the two antagonistic sets of muscles, as well as some integrative centre, perhaps the apical organ. As an alternative, one or both cell types could be sensorimotor, with receptive processes in the tentacles and terminals in the hood nerves, and act antagonistically. The main problem with the morphological evidence is that it so far provides no clear means of deciding whether the cells have separate dendritic and axonal processes or of distinguishing between these. With the identification of some of the transmitters involved in the hood lift response, simple behavioral tests with specific blocking agents may make it possible to choose between alternative interpretations.

4.2. *Comparative and phylogenetic considerations*

The central issue here is whether a sufficient number of structural or organizational similarities can be shown between the ciliary bands of the actinotroch and either pre-oral bands in protostomes, or post-oral bands in deuterostomes, to show a degree of homology.

(*a*) *Trends in the spiralia*

In the primitive spiralian larvae so far examined, that is, Müller's larva (Lacalli 1982) and the pilidium (Lacalli & West 1985), the ciliary bands have a characteristic intratrochal type of innervation. Tracts of fibres run beneath the bands, and these arise predominantly from

uniciliate sensory cells located in the bands themselves. The trend in more advanced larvae seems to be away from a local, intratrochal type of innervation to more direct control via nerves from the apical region, either from specific structural entities like the apical organ and cerebral ganglia, or from cells scattered in the apical epithelium. The trochophore larvae of serpulid polychaetes shows one step in this direction (Lacalli 1984). Here the post-oral ciliary bands directly associated with feeding, the metatroch and neurotroch, are innervated by cells in the bands themselves, but nerves to the pre-oral prototroch come from the pretrochal apical epithelium and apical organ. The same seems to be true of *Polygordius*, whose trochophore Woltereck (1902) describes as having a pretrochal network of multipolar nerve-like cells. This observation needs to be confirmed, but the implication is that nerve cell bodies occur rather generally in the apical epithelium in trochophores, and that these are involved in innervating the pre-oral band. The trend away from intratrochal innervation seems to have gone a step further in specialized trochophores like that of *Phyllodoce* (Lacalli 1986, 1988). Here the rudiments of the cerebral ganglion develop precociously, and comparatively large, paired dorsolateral connectives are formed. These appear to supply both the oral region and the prototroch nerve. There are some peripheral cell bodies, but the cerebral centres are clearly the dominant ones. There are other similarities between the prototroch nerve in *Phyllodoce* and the primary hood nerve in *P. vancouverensis*, though these are probably due to convergence. Both are large nerves, with more fibres than would seem to be necessary. Both have a glial-like sheath, and in both the largest terminals and junctions seem to be neuromuscular, despite the fact that both nerves are generally thought of as being ciliary nerves.

Centralization seems to be carried furthest in veligers, in which fibres from the cerebral ganglia cross the velar epithelium and branch extensively to innervate the trochal cells (Mackie *et al.* 1976). In none of these larvae are the fibre types well defined or the transmitters known with any certainty. Only for the veliger, where the nerve is involved in ciliary arrest, is there clear neurophysiological evidence regarding its function.

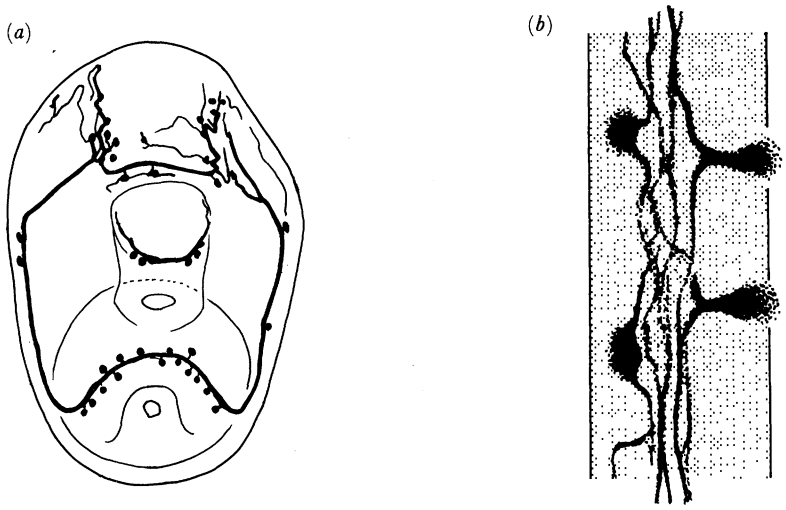


FIGURE 47. Band innervation in starfish bipinnaria larvae, two examples of the type of results obtained from glyloxylic acid-stained preparations. (*a*) Whole mount of an *Asterias* larva, from Nakajima (1987, figure 2*a*). The nerve follows the band exactly, and cell bodies lie on either side. (*b*) Detail of the ciliary nerve in a *Pisaster* larva, after Burke (1983, figures 32, 33). The extent of the band is shown by shading, and the stained fibres form an irregular plexus beneath it. Cell bodies lie either along the nerve or beside the band, on the aboral side in the latter case according to Burke's interpretation.

(*b*) *Band innervation in deuterostome larvae*

There is only limited information available on ciliary nerves in tornaria larvae (Nezlin 1988), but the three main types of planktotrophic echinoderm larvae, the pluteus, bipinnaria and auricularia, are relatively well studied (Burke 1983; Burke *et al.* 1986; Bisgrove & Burke 1987; Nakajima 1987, 1988). The latter all have similarly organized ciliary nerves and a similar complement of fibre types. Serotonin-containing fibres have been identified, coming from cells in either the apical part of the band (in the pluteus, Bisgrove & Burke (1987)) or the dorsal epithelium, just behind the apical region (bipinnaria, Nakajima (1988)). Innervation of the ciliary band is diffuse, however, and involves cells mainly located within or beside the band. The nerves are best demonstrated with the glyoxylic acid method, which stains fibres that probably contain dopamine (Bisgrove & Burke 1987). The nerves run along the whole length of the band, forming an irregular tract of fibres beneath it (figure 47). The cell bodies appear to lie either along the nerve beneath the band (bipolar type I cells, Burke (1983), immediately adjacent to the band (multipolar type II cells, Burke (1983)), or in the adjacent epithelium (bipolar cells, Bisgrove & Burke (1987)). The larvae also have circumoral or adoral bands immediately beside or around the mouth, whose cilia beat towards the mouth, and both cell bodies and fibre bundles are found in association with these.

Functional evidence suggests the nerves trigger ciliary reversals involved in locomotory control (Mackie *et al.* 1969).

(*c*) *Assessing the actinotroch*

The larva most closely related to the actinotroch is probably that of bryozoans, the cyphonautes. Jägersten (1972) compares the two, and suggests that the cyphonautes mantle, which encloses the large vestibule, might be the precursor of the actinotroch hood. His scheme is summarized in figure 48, but with the direction of this transition reversed. If the actinotroch's tentacles are accepted as a secondary addition, there is no reason to suppose that something like an actinotroch, but not necessarily with tentacles, could have given rise to the cyphonautes. There are a number of differences between the two larval types, for example, the cyphonautes is far more specialized cytologically, with a coronal band containing complex multiciliated cells (Nielsen 1987; Stricker *et al.* 1988), but sufficient similarity to suggest that an actinotroch-like larva, with a pre-oral hood, could be a basic larval type at least as old as the common ancestor of phoronids and bryozoans. There are some unresolved problems. For example, the hood margin in the actinotroch could be homologous with the whole of the corona or only the anterior part. If the latter, then the posterior corona and lateral ridges need to be accounted for, and the fact that together they are circumferential and post-oral suggests they could be derived from one or both of the post-oral bands in the actinotroch.

Comparing the actinotroch with the larval types, the most meaningful similarities seem to be with the larvae of protostomes rather than deuterostomes. Similarities with the latter, taking echinoderm larvae as the only examples sufficiently well studied, appear to be superficial on closer inspection. The principal ciliary band in echinoderm larvae, as in the actinotroch, is post-oral and beats away from the mouth. Echinoderm larvae also have circumoral and adoral bands within the oral field, with cilia that beat towards the mouth and basal tracts of nerves, comparable in this respect to the pre-oral hood in actinotrochs. Both also have catecholamine- and serotonin-containing nerves, the former associated with the ciliary bands, and the latter

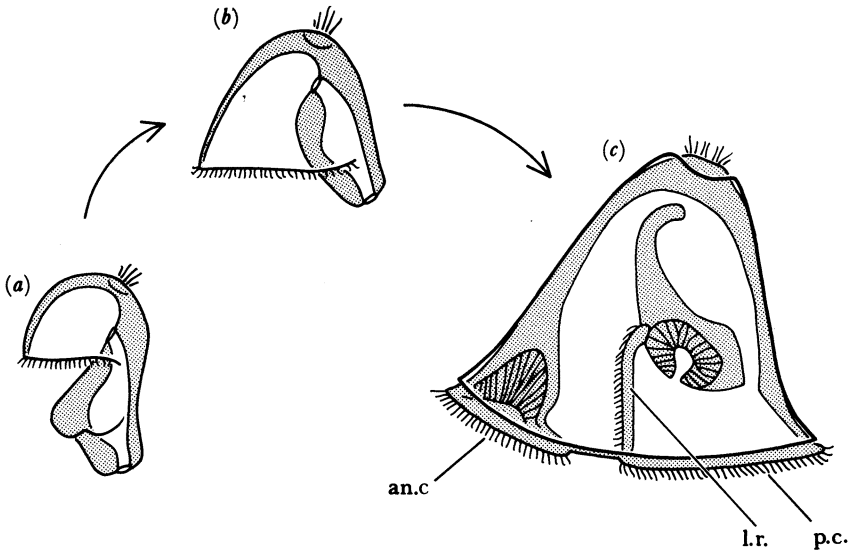


FIGURE 48. An interpretation of the relation between the actinotroch (*a*) and the bryozoan cyphonautes larva (*c*), after Jägersten (1972, figure 12). The principal ciliary band of the latter is the corona, which has separate anterior (an.c.) and posterior (p.c.) parts. There is also a lateral ciliary ridge (l.r.) on each side on the inside surface of the mantle. The sequence (*a*)–(*c*) shows how expansion of the actinotroch pre-oral hood could produce the cyphonautes mantle. This sequence is the reverse of Jägersten's, who takes the cyphonautes to be the ancestral form.

with the apical region of the larva. Otherwise, however, patterns of innervation appear to be quite dissimilar. In the actinotroch, the ciliary nerves run largely beside rather than under the bands, and the bands themselves contain neither nerve cells nor sensory cells. Innervation of the post-oral band is concentrated on the oral side, and fibres cross the oral field to get there. In contrast, in echinoderm larvae, the ciliary nerves run under the bands, and the nerve cells themselves lie in or adjacent to the band. When cells outside the band are involved, their fibres appear to enter from the aboral side in at least some instances. On this basis, the two types of larvae appear to be at most, only distantly related.

Comparisons with other protostome larvae, that is, of spiralia, are more satisfactory, but not conclusive. Primitive spiralian larvae and actinotrochs both have heavily ciliated oral fields with an important role in feeding. The actinotroch has no metatroch-like band at the posterior margin of its oral field, but the sensory cells located here could be remnants of a band innervated along standard spiralian lines that has since been lost. There is, however, no sign of median sub-oral structures in the actinotroch like those found in spiralian larvae, for example, rejectory tracts of cilia or sub-oral nerve cells. The sub-oral nerves that were found in the actinotroch appear to be no more than part of a larger set of nerve tracts rather than being neural centres of any importance in themselves. When pre-oral bands are compared, the actinotroch seems to have most in common with advanced spiralian larvae like the trochophore. In both, the band is associated with large nerves, but the fibres come from the apical region, that is, the apical organ and surrounding epithelium. Ivanova-Kazas (1987) has argued that pre-oral bands in the different protostome groups evolved independently, that is, their common ancestor had an atrochal larva. The suggestion here is that pre-oral bands in the actinotroch and advanced spiralia may be homologous, but if so, both are secondarily and

similarly modified with respect to their innervation. In both, external innervation replaces the intratrochal pattern seen in primitive spiralian forms which, presumably, are closer to the common ancestral pattern. Converting a primitive form, for example, something organizationally equivalent to Müller's larva, to either a trochophore or an actinotroch is relatively easy. Several possibilities are shown in figure 49.

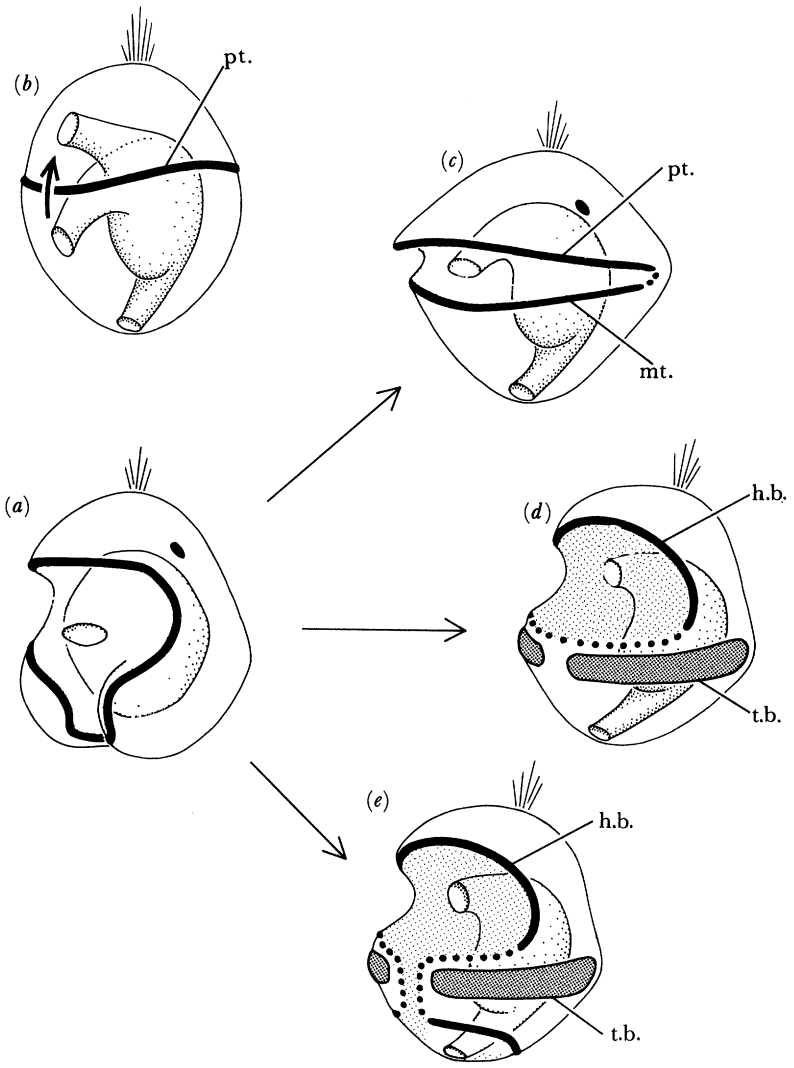


FIGURE 49. Hypothetical scheme for producing diverse protostome larvae by rearranging the pre-oral ciliary band. The starting point, (*a*), is taken to be something simple like Müller's larva, with a pre-oral band (heavy line) that surrounds the ciliated oral field (light shading). (*b*) Assuming the band is first converted to a circumferential prototroch-like band, one way to make it post-oral in the actinotroch is to shift the position of the mouth (arrow). Pre-oral structures like the hood would then have to arise *de novo*, which does not seem very likely. (*c*)–(*e*) Require less invention. (*c*) Shows a trochophore in which both prototroch and metatroch are derived from the primitive band. They lie at the top and bottom margins, respectively, of the oral field, which expands laterally to form a ciliated food groove running around the whole body. (*d*) and (*e*) Show two ways of making an actinotroch. In both cases the pre-oral band is retained in a pre-oral position. In both, the post-oral band (dark shading) is a secondary, lateral addition, and the part of the band lying along the lower margin of the oral field is lost (dotted line). In (*d*), the anus originates outside (that is, porterior to) the oral field; in (*e*) it originates in the oral field and the perianal part of the band becomes a telotroch. A similar scheme could apply to the trochophore as discussed by Nielsen (1979), to account for the telotroch in that larva as well.

The above analysis suggests that phoronid larvae have diverged about as much from the ancestral type shared with spiralians as have the more advanced trochophores. There is another way of looking at the process of divergence that reinforces the idea of parallel trends in these two groups. First, consider the body surface as divided into epithelial domains distinguished on the basis of whether or not each is involved in neurogenesis. In primitive spiralia, nerve cells seem to be restricted to the ciliary bands and the apical epithelium. In both the actinotroch and advanced spiralia, the former has become more important at the expense of the latter. And in both, with progressive anterior movement of the mouth and elaboration of trunk structures, the pretrochal apical region has become progressively smaller. The neural centres are thus more concentrated. This contrasts with the situation in deuterostome larvae. They not only have intratrochal innervation, which could be a retained primitive condition, but nerve cells are found variously throughout the epithelium, near bands, as well as in them. It is difficult to define an apical region as such in these larvae, at least in terms of its being a restricted or preferred site for neurogenesis, because so large a proportion of the larval surface seems to participate in this process. This is not surprising if one accepts that the mouth is a truly secondary structure, appearing *de novo* well forward of the primitive mouth. The entire oral field is then really an apical structure, and the surrounding epithelium is pre-oral, apical ectoderm capable of behaving like typical apical ectoderm in terms of producing nerve cells.

The above serves a reminder of the very substantial gulf that may separate lophophorate protostomes and deuterostomes. One way to deal with the origin of a digestive system with two openings is to have both of them develop from the primitive blastopore. Fusing the two sides of the blastopore leaves openings at both ends; one could be the mouth and the other the anus. This may have happened in the spiralia (Nielsen 1985): in some groups mouth and anus originate so close together that they may both derive from the blastopore. Changes in the timing of developmental events then make it relatively easy, in principle, to adjust whether the mouth or anus forms first. It is possible, on this basis, to envisage a gradual transition from protostomy to deuterostomy occurring with progressive anterior movement of the primitive mouth. Phoronids could be seen as representing an intermediate stage in this process, that is, protostomes that have not quite become deuterostomes. The alternative is to equate the blastopore and the anus in deuterostomes, but exclude the mouth entirely. If the deuterostome mouth is truly secondary, then deuterostomy represents a major departure from the protostome condition in both developmental and organizational terms, and there is no way phoronids can be seen as developmental intermediates. They are simply one of several branches of protostomes, independent of the spiralia, with larvae that are trochophore-like in terms of general organization, notwithstanding that the adults retain many features also seen in primitive deuterostomes.

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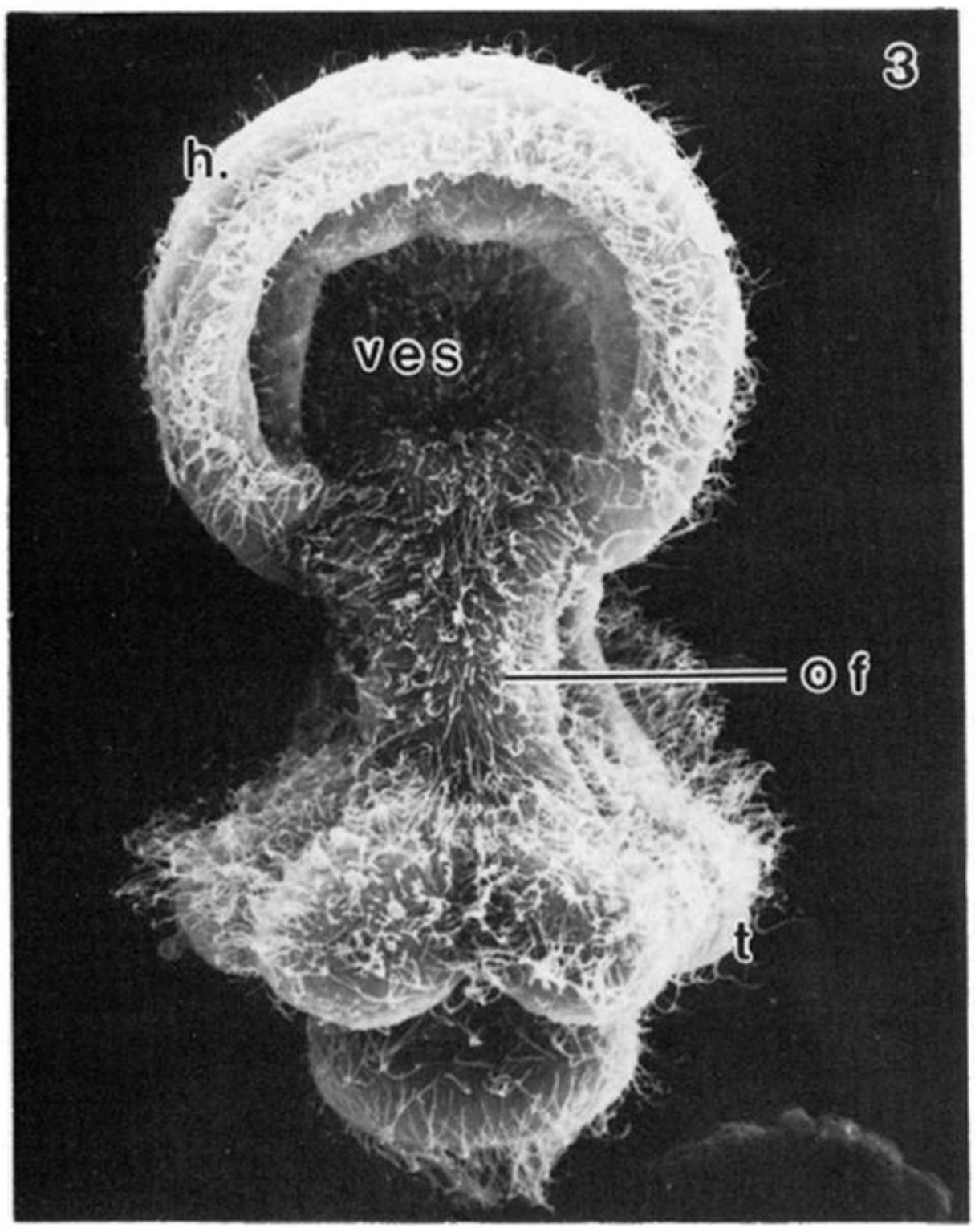
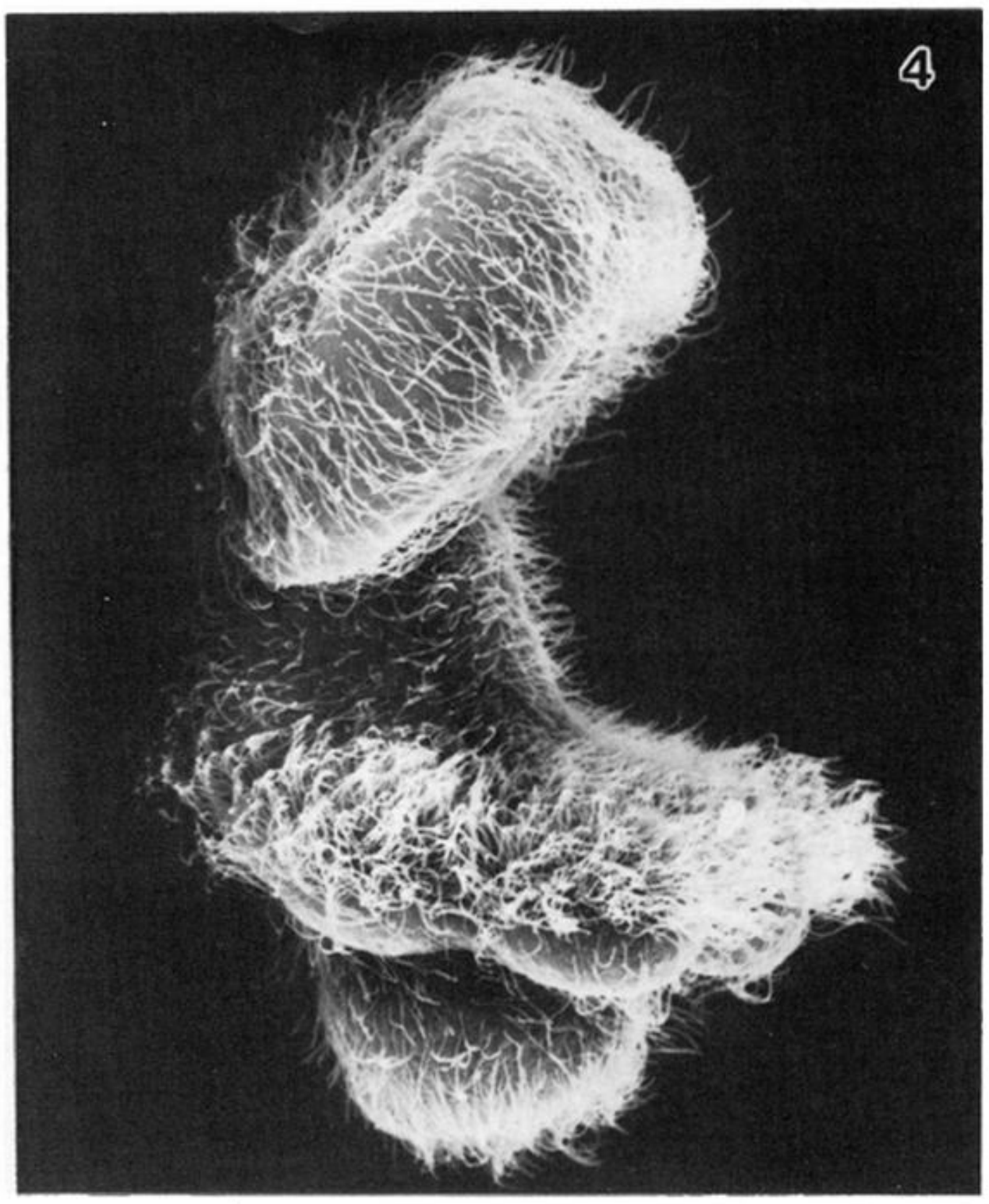
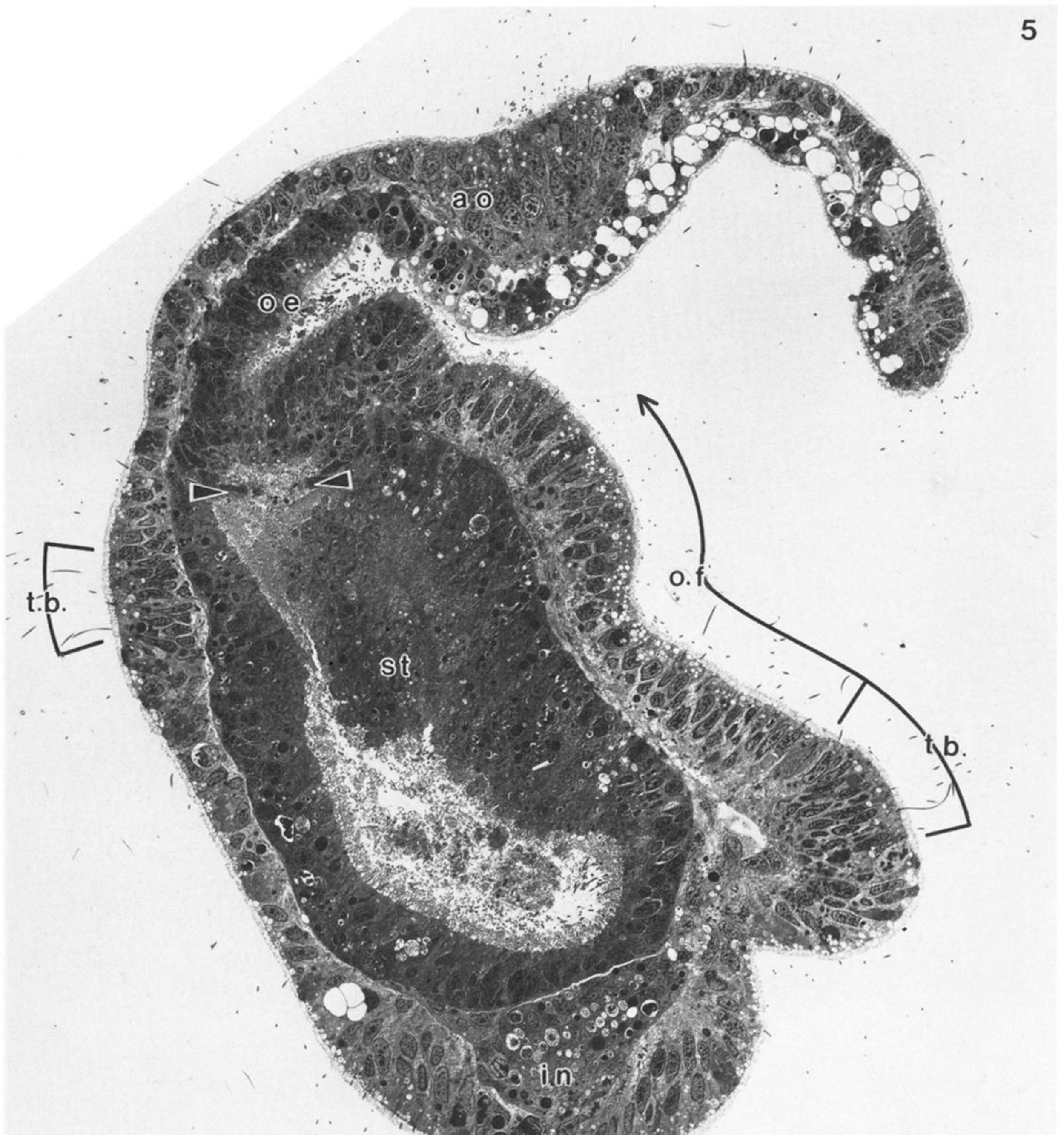
KEY TO ABBREVIATIONS USED IN FIGURES

Abbreviations for terms used in relation to larvae other than the actinotroch are shown by (+).

a.o.	apical organ
ad.n.	aboral nerves
an.c.	anterior corona (+)
c.b.	ciliary band (+)
c.m.	circular hood muscles
en.	endodermal cells or tissue
g.a.	glial-like accessory cells
g.c.	glial-like capsular cells
h.	pre-oral hood
h.b.	marginal ciliary band of hood
h.n.1	primary hood nerve
h.n.2	accessory hood nerve
in.	intestine
l.r.	lateral ridges (+)
m.	mesodermal tissues or muscle cells
m.n.	median hood nerve
mch.	mesenchyme
mo.	mouth
np.	neuropile
o.f.	oral field
oe.	oesophagus
p.c.	posterior corona (+)
pt.	prototroch (+)
r.m.	radial muscles of hood
s.	sensory cells
st.	stomach
t.	tentacles
t.b.	tentacular ciliary band
t.n.1	primary tentacle nerve
t.n.2	accessory tentacle nerve
tr.n.	trunk nerves
va.	oesophageal valve
vac.	vacuoles or vacuolate cells
ves.	vestibule or vestibular epithelium
*	as shown in figure description







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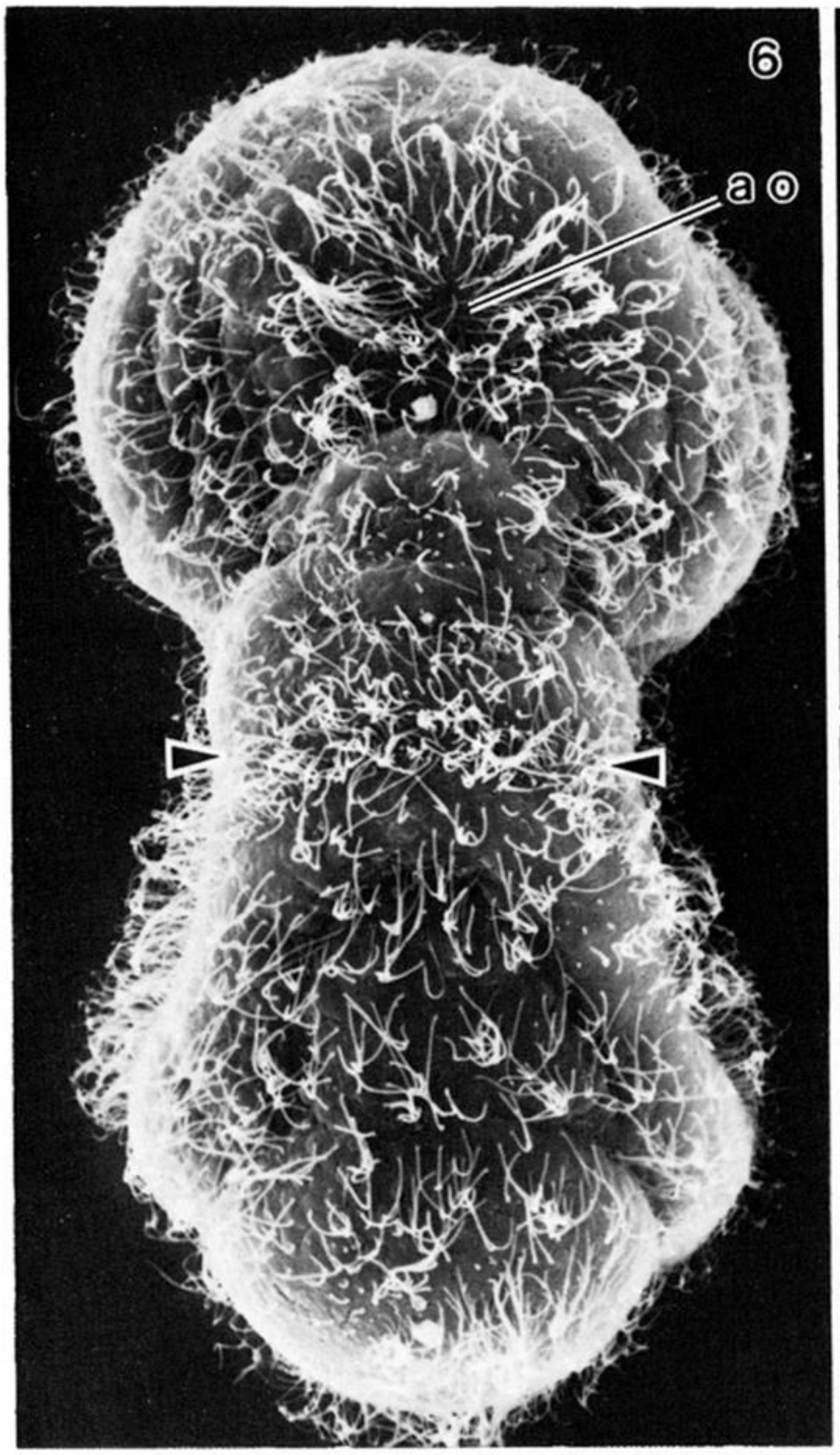

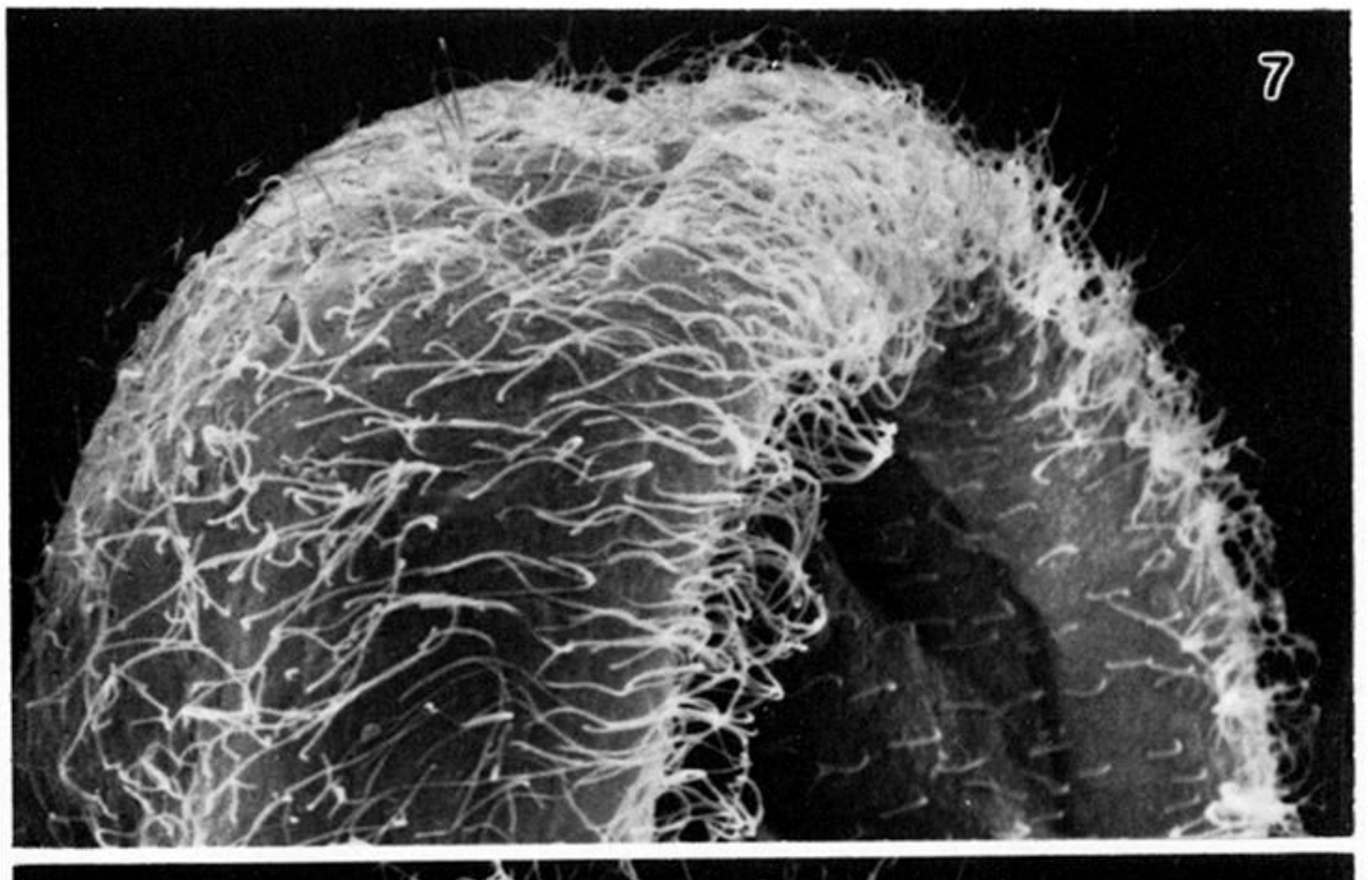

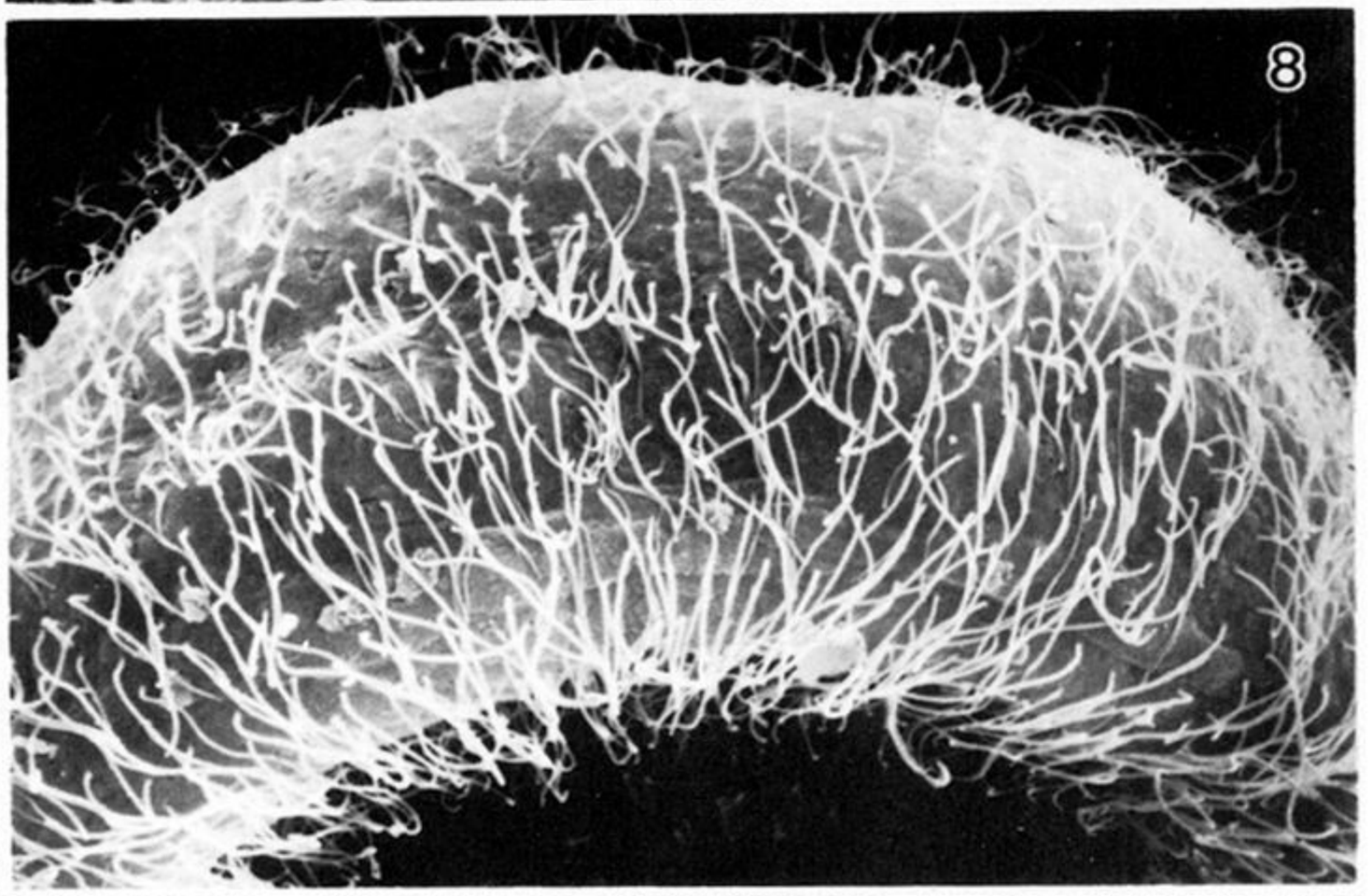

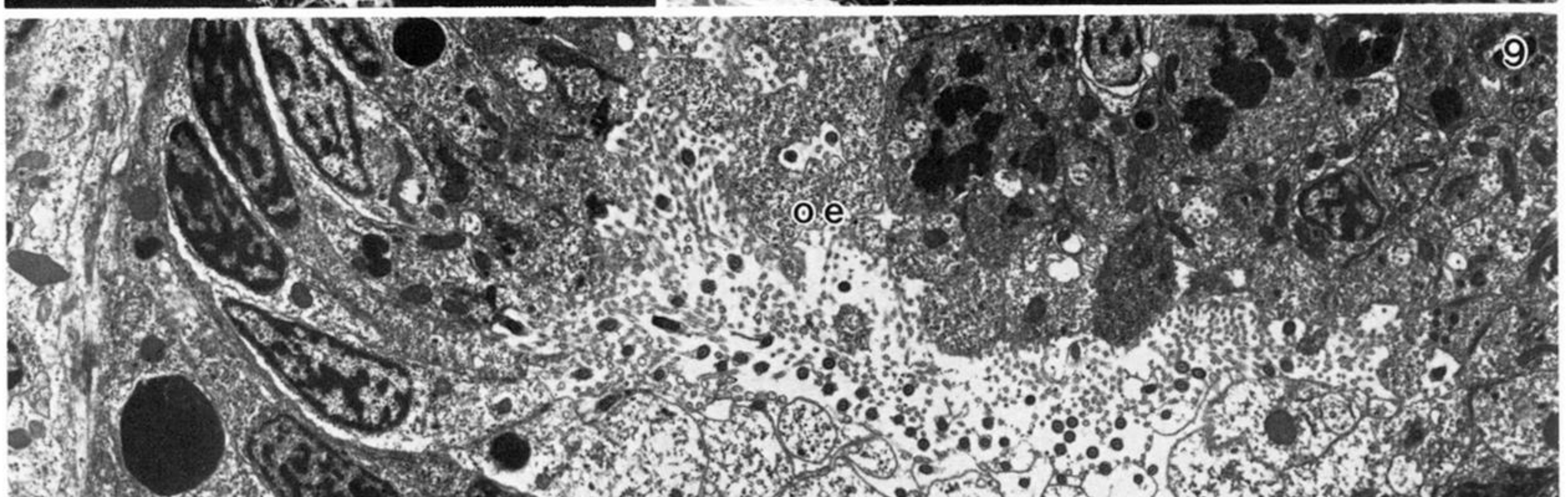

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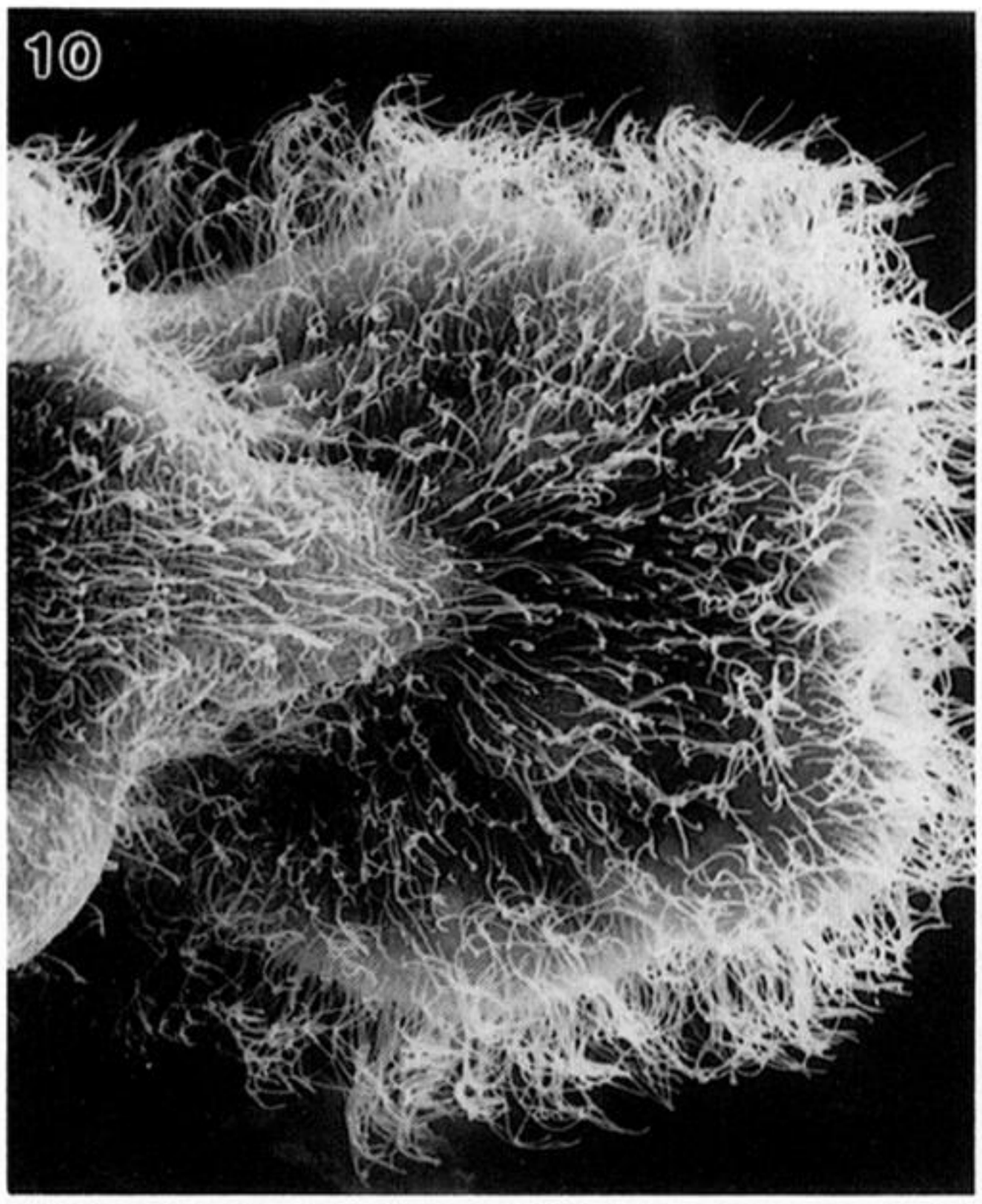

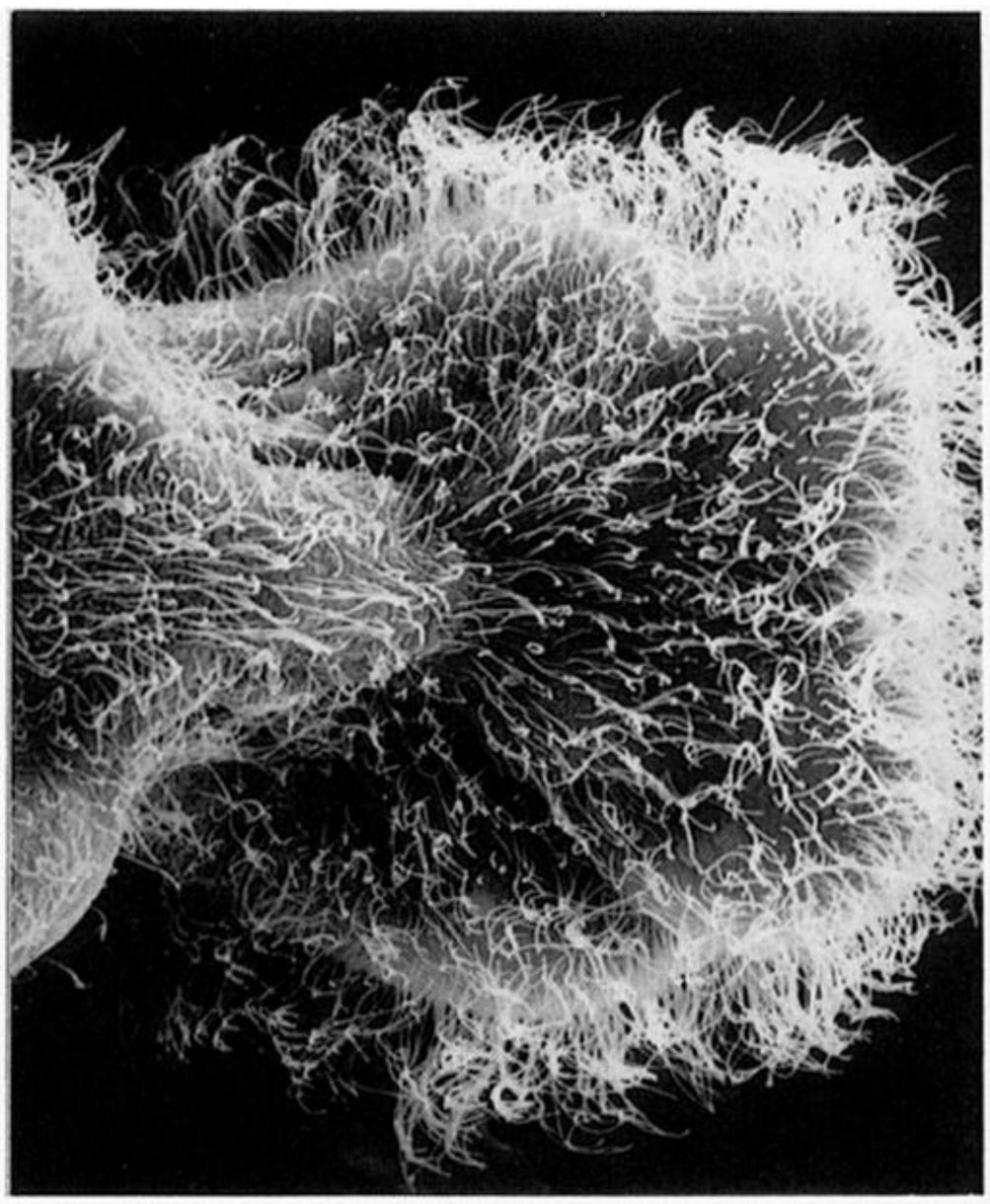

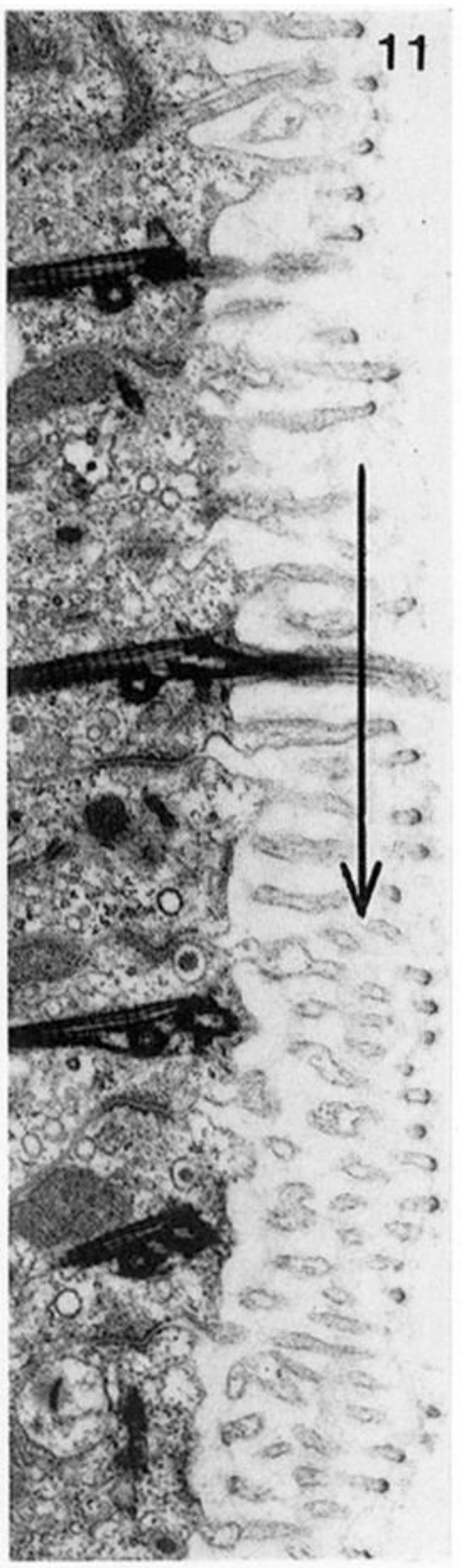

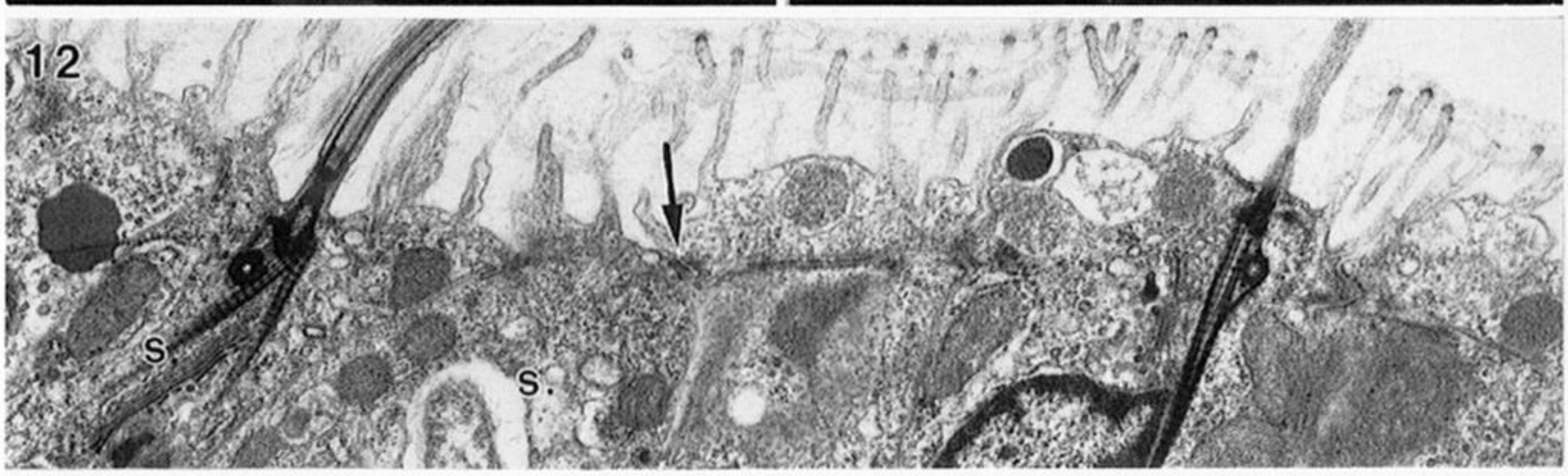

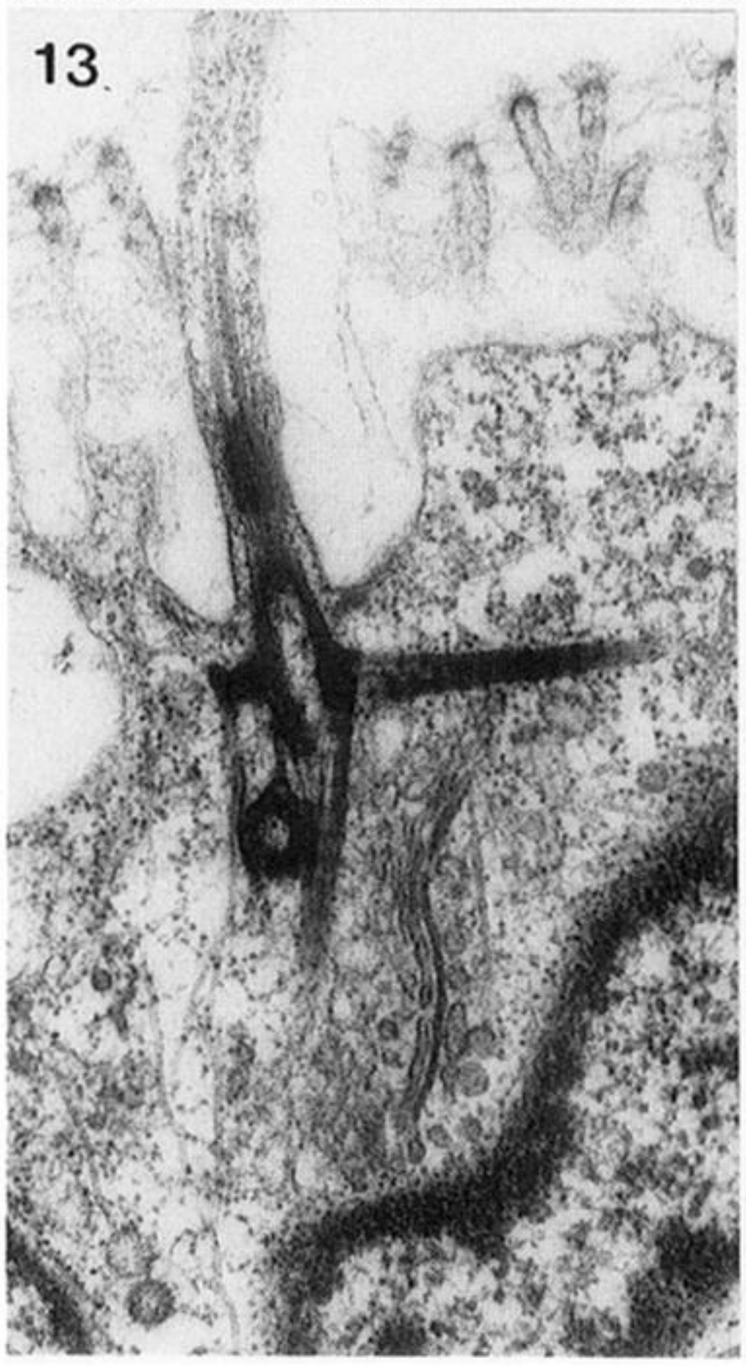

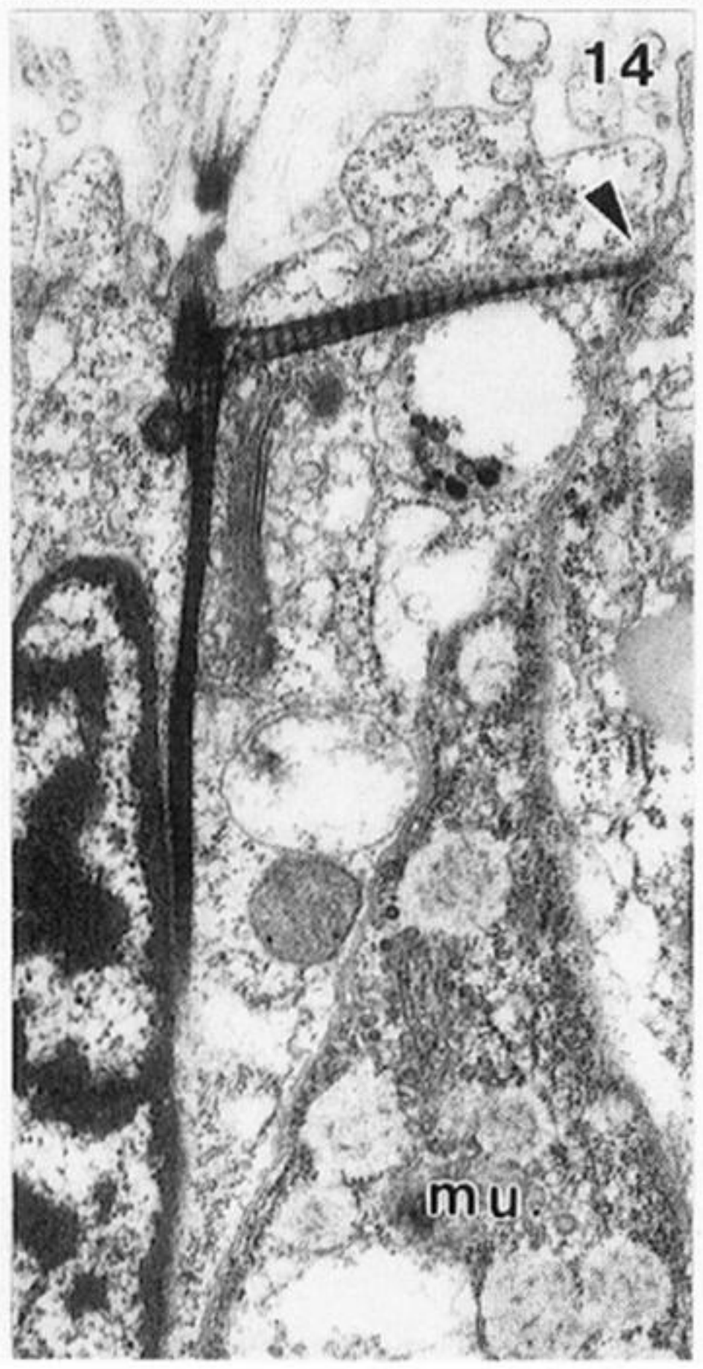

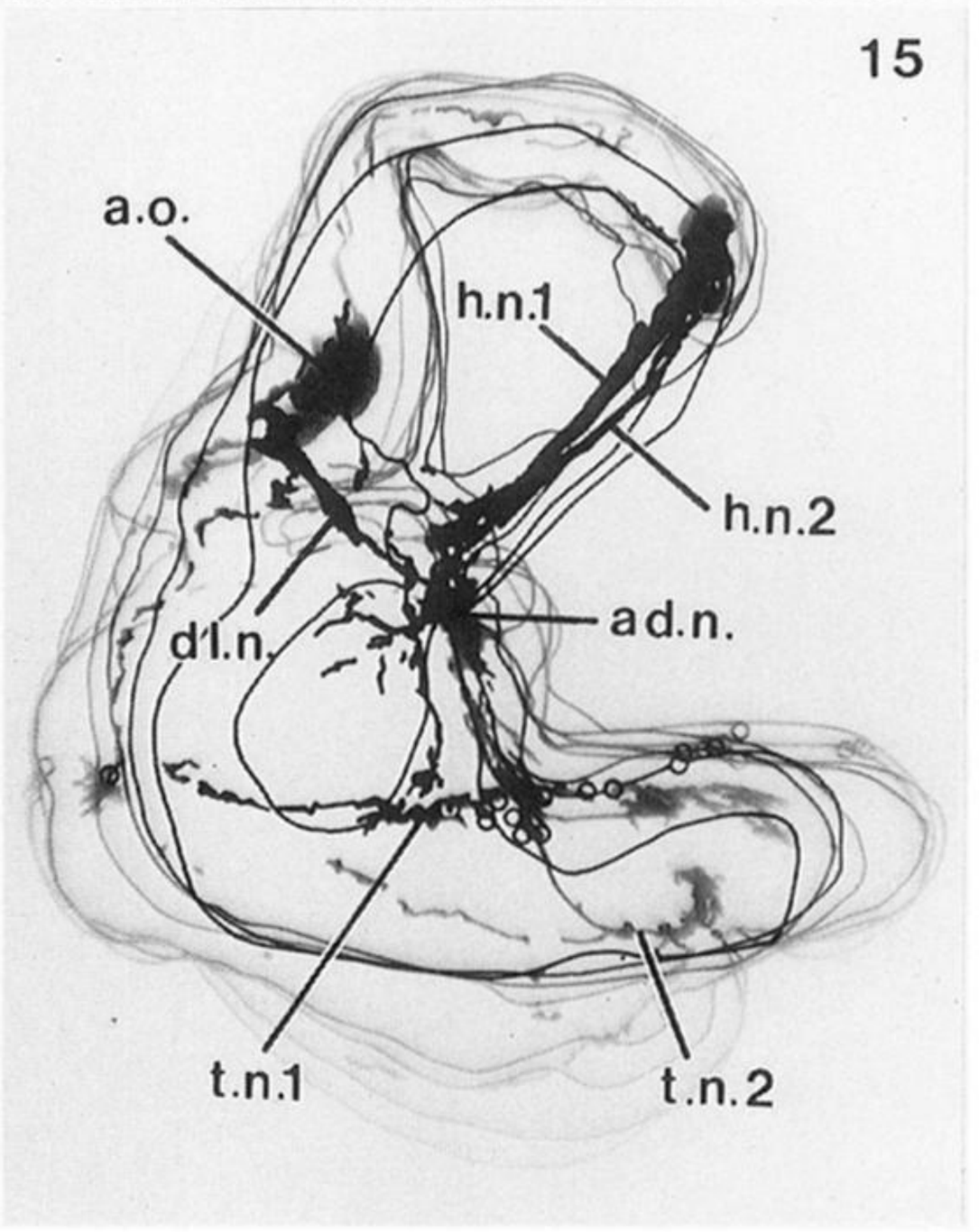

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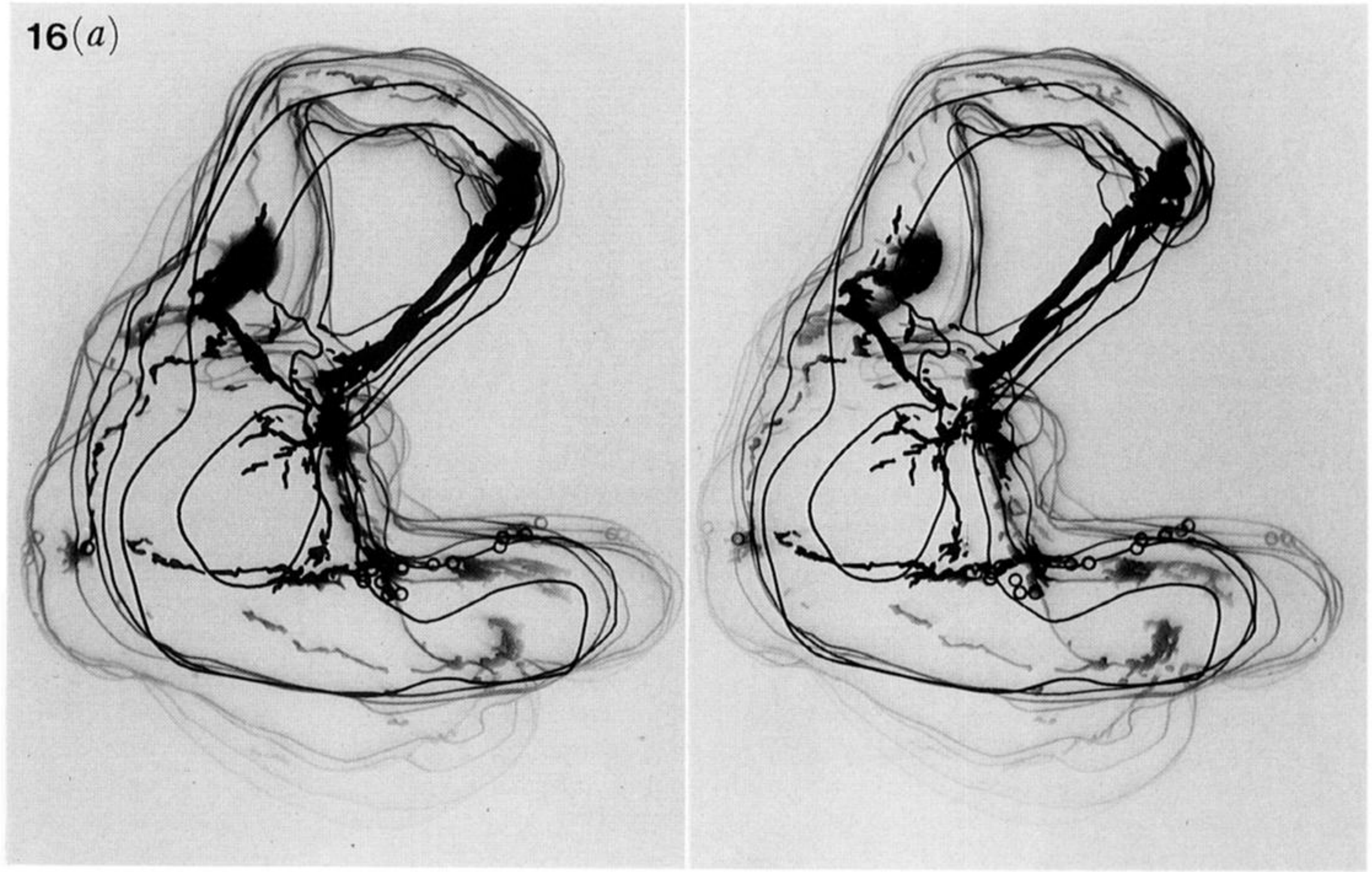

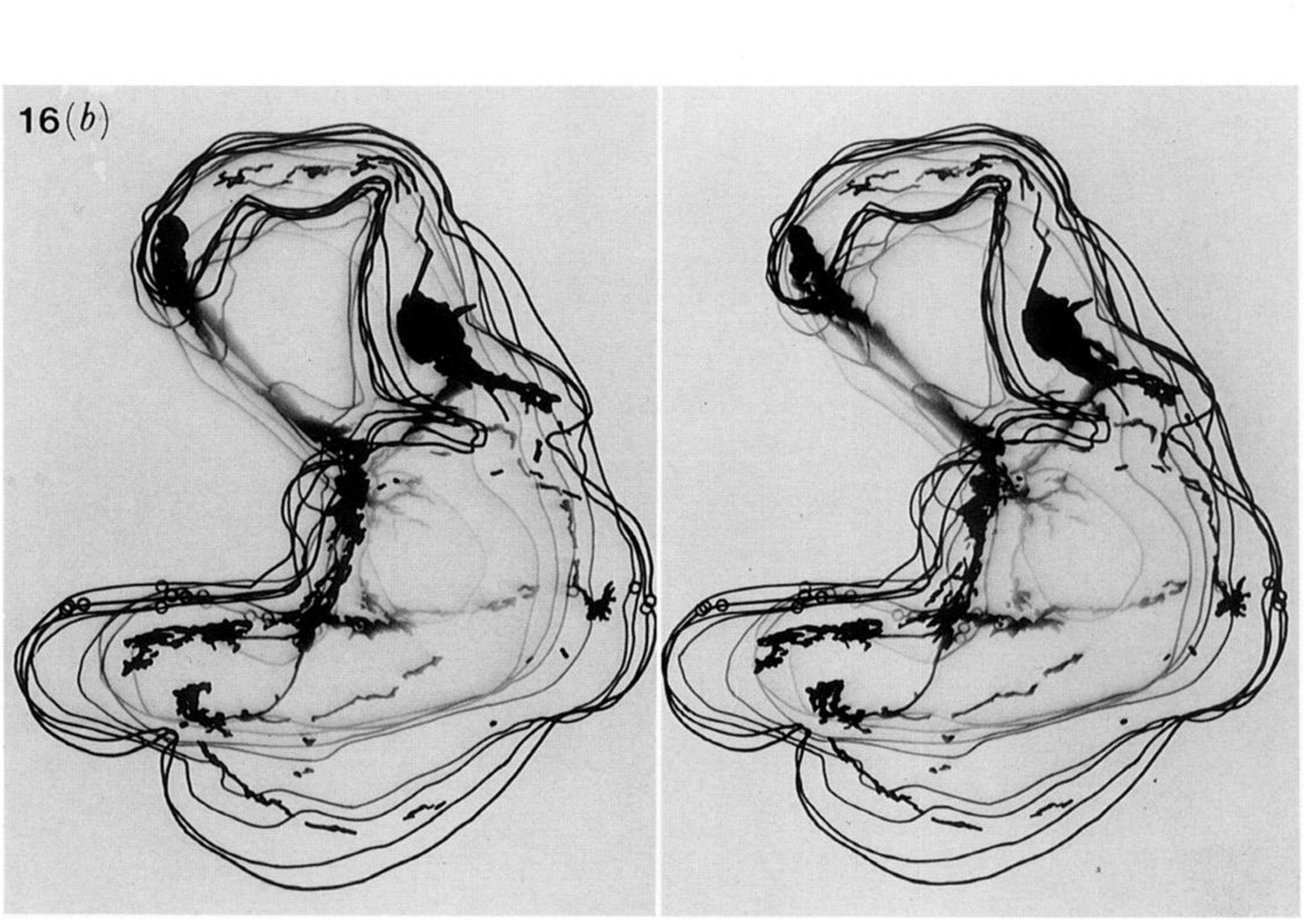

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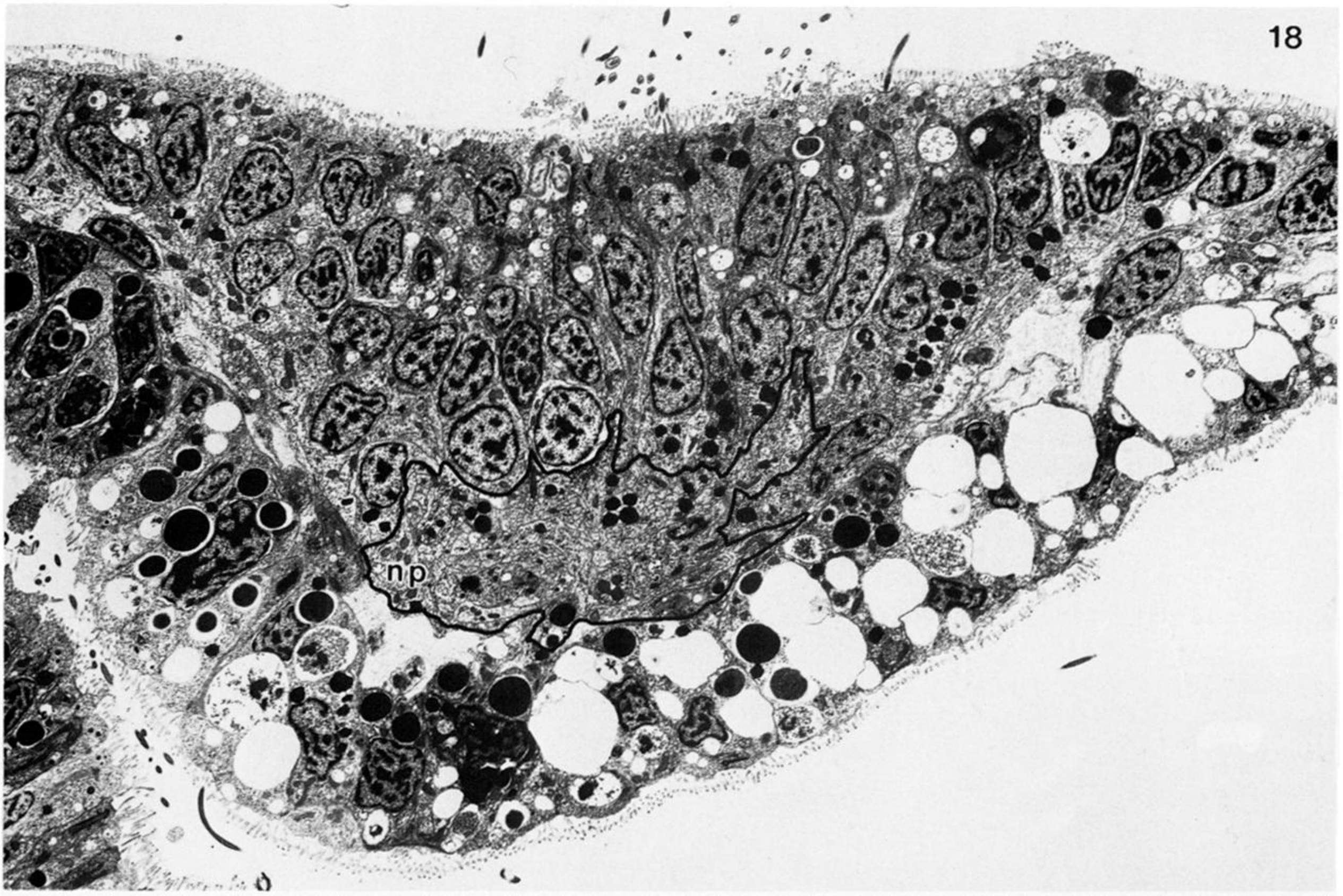

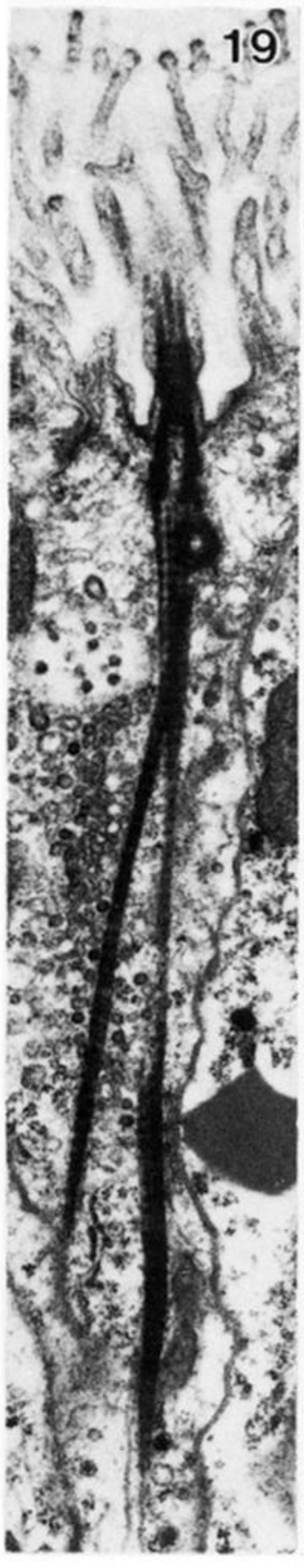

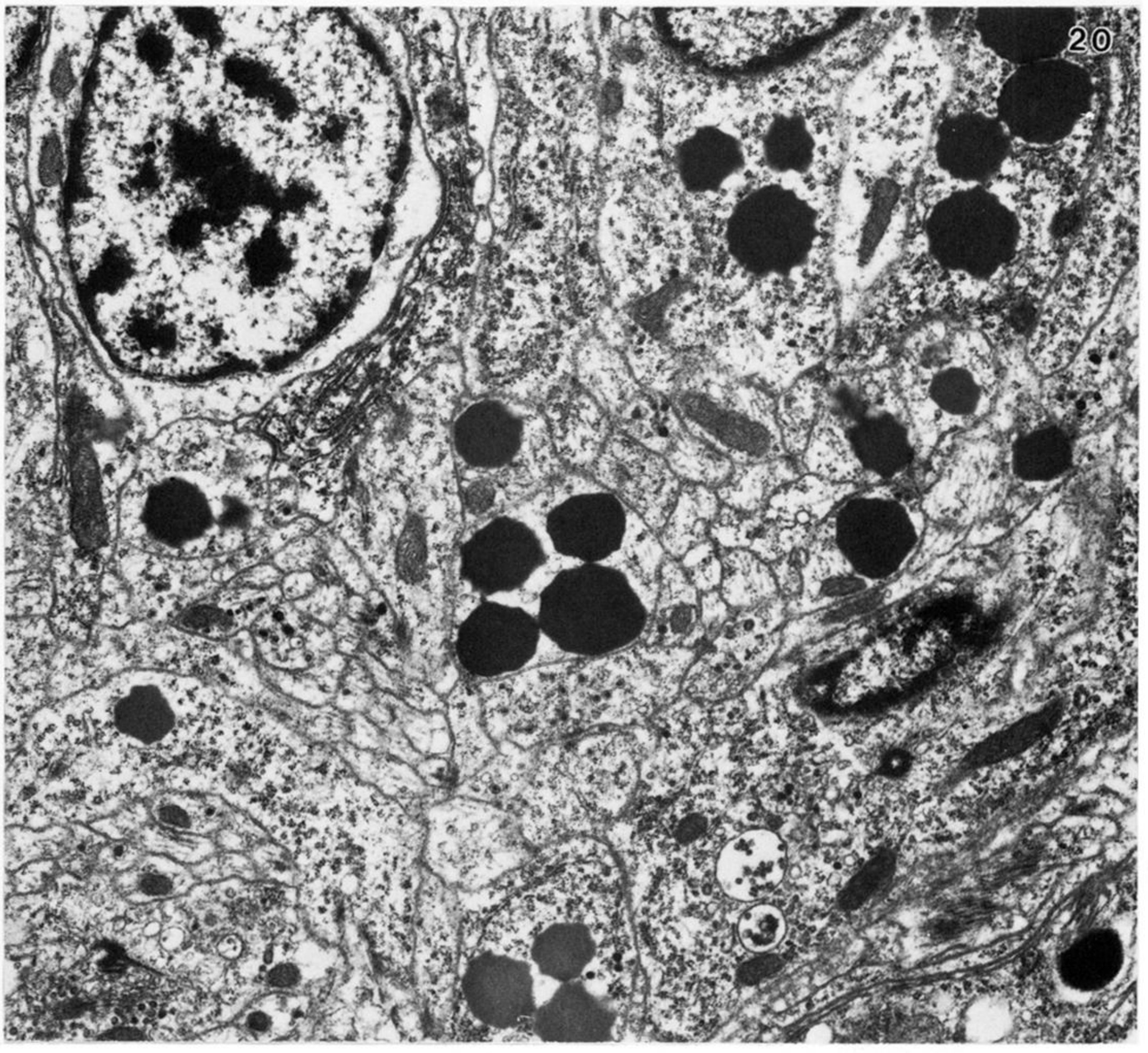

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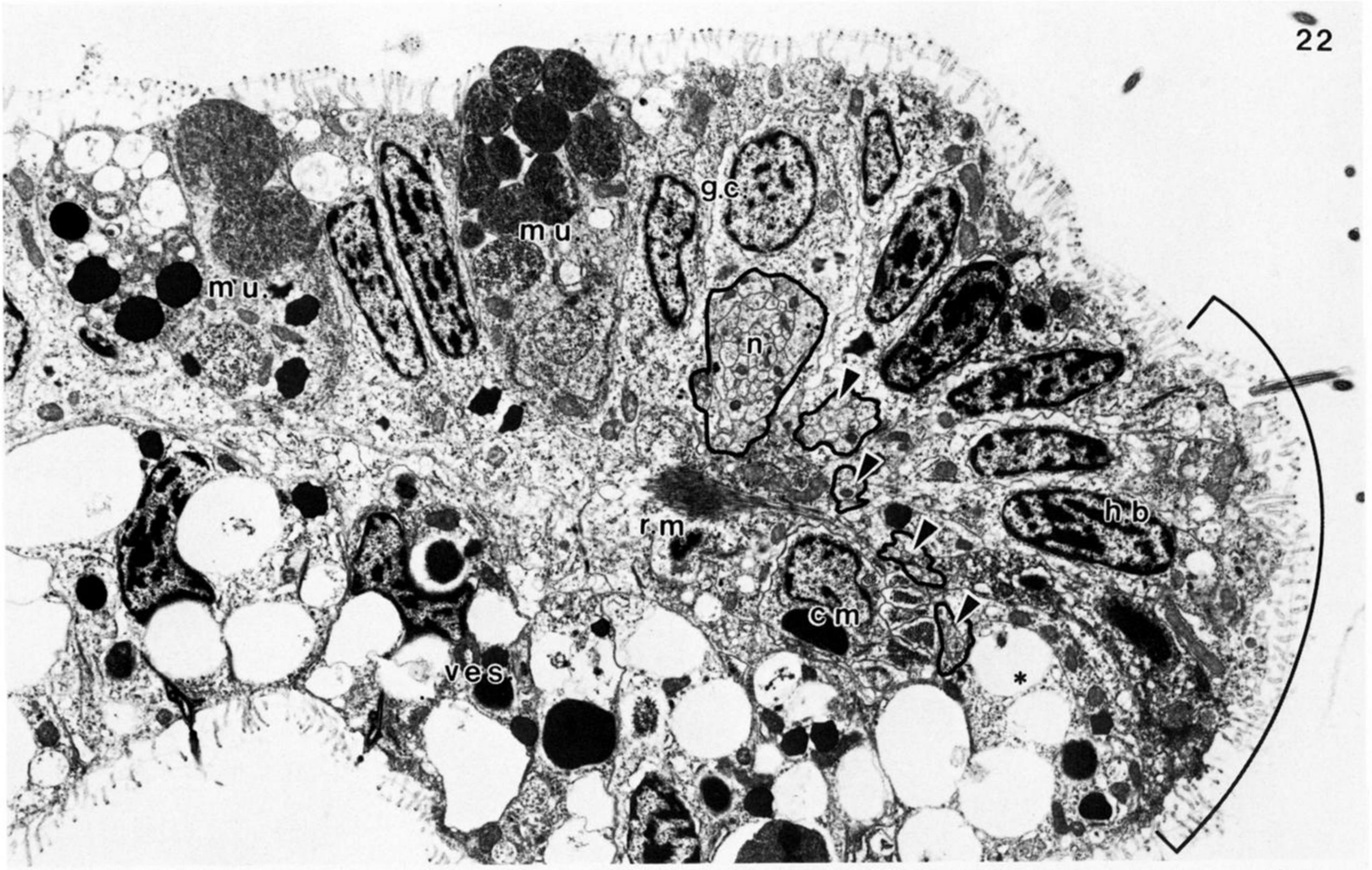

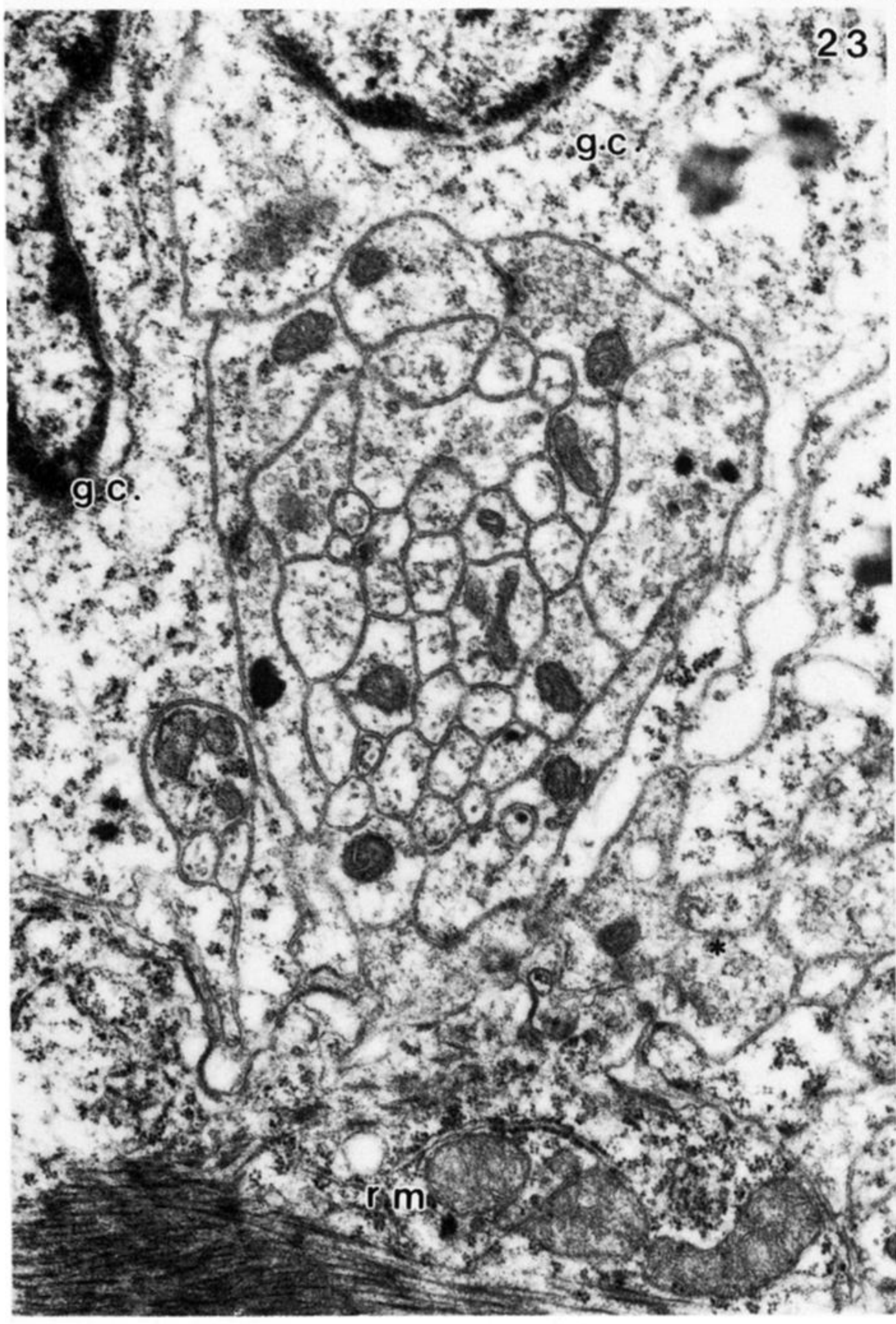

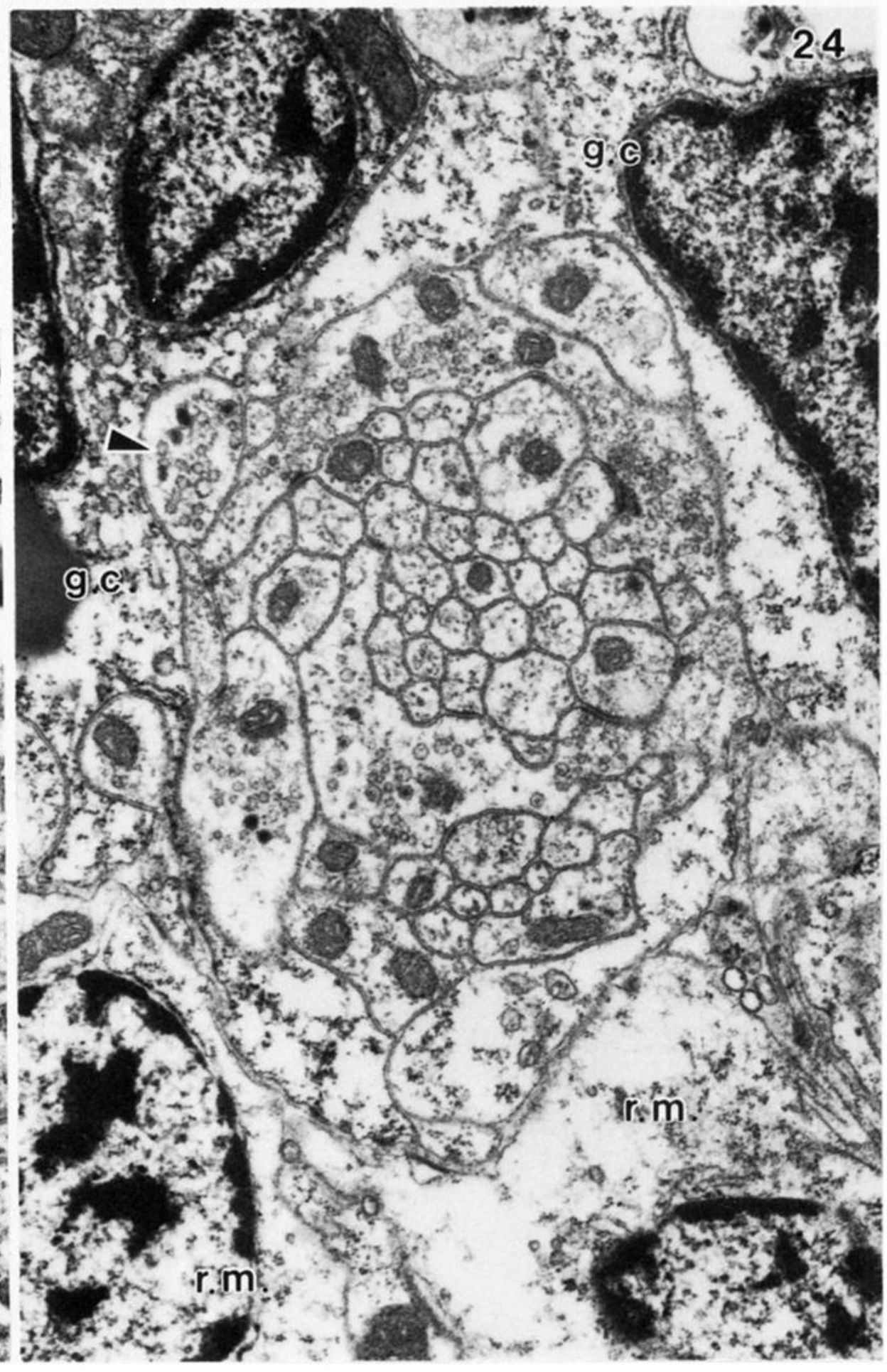

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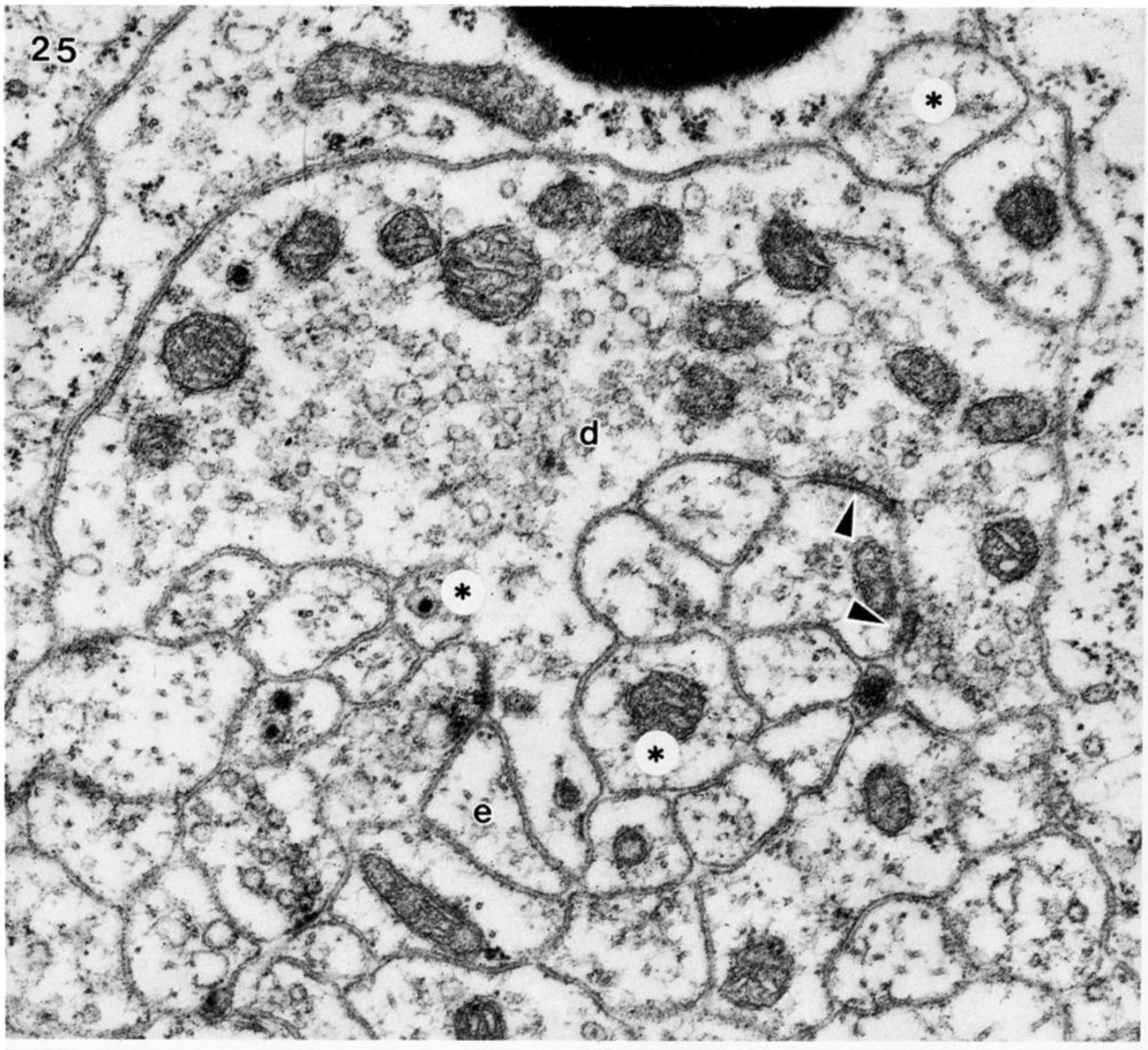
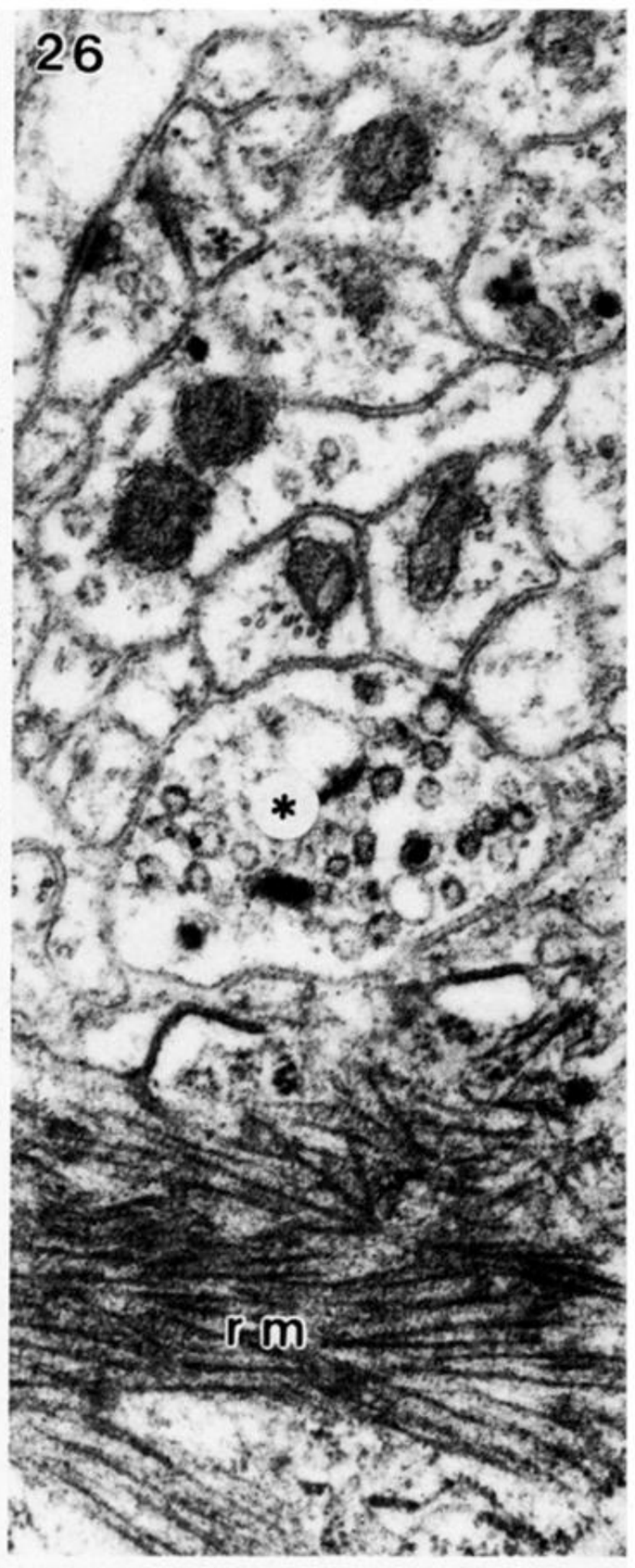
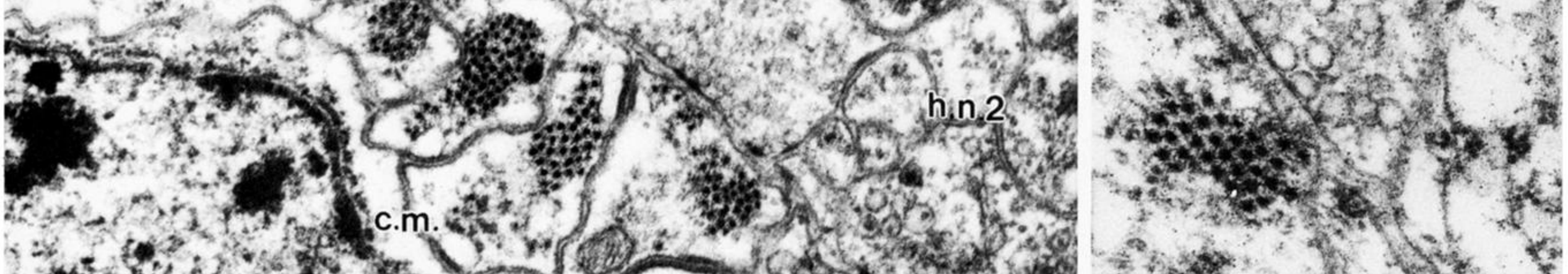
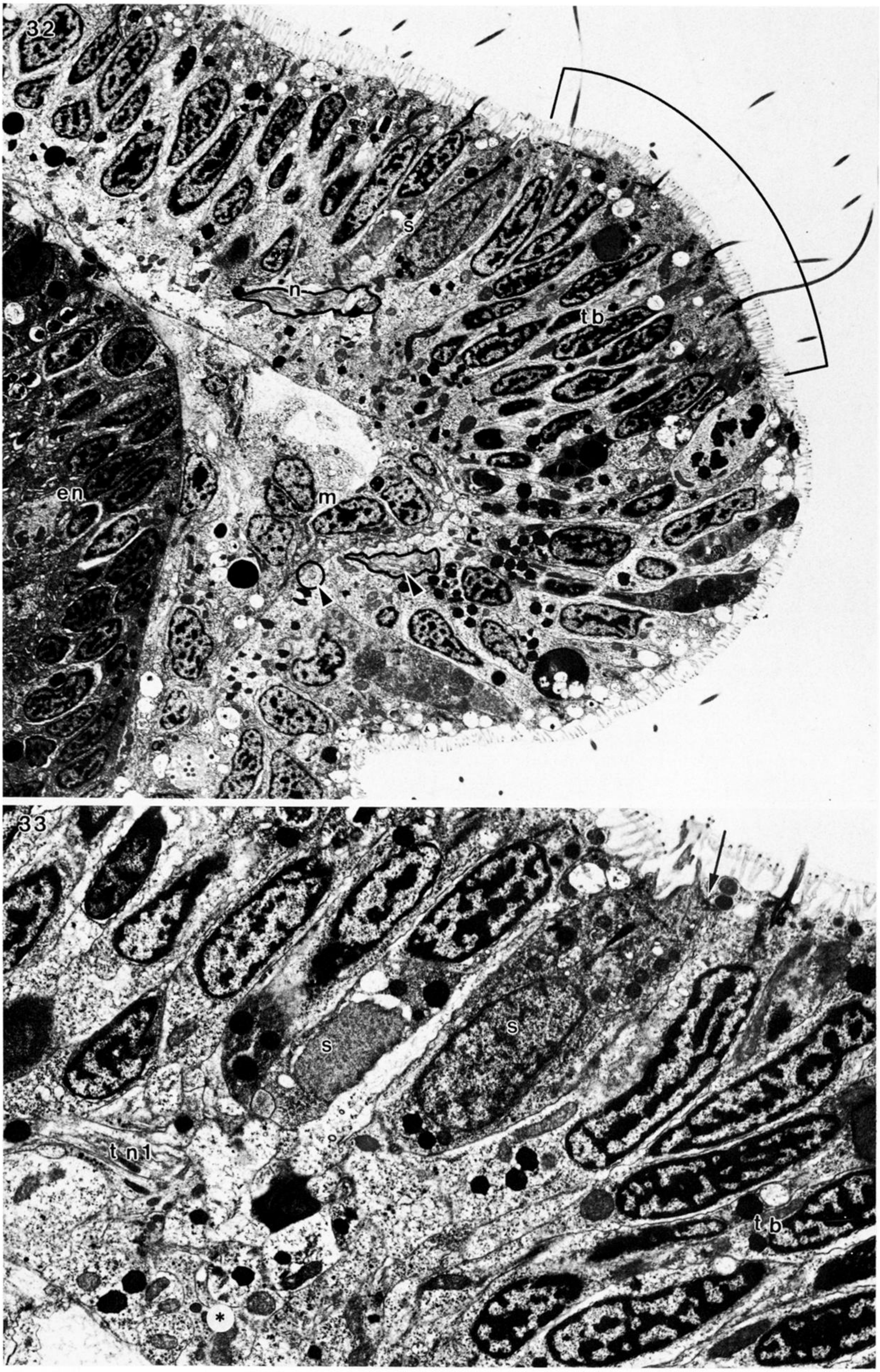


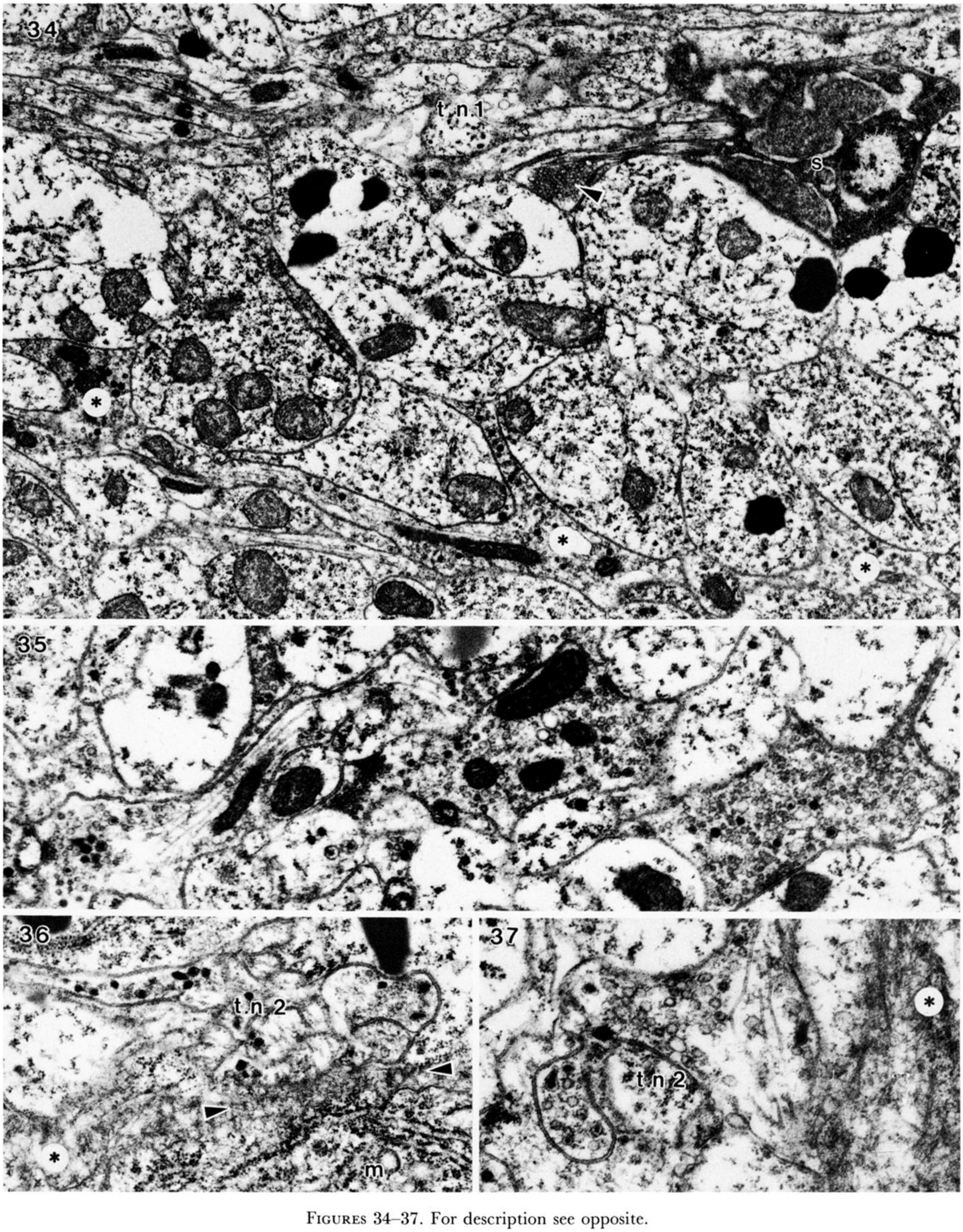




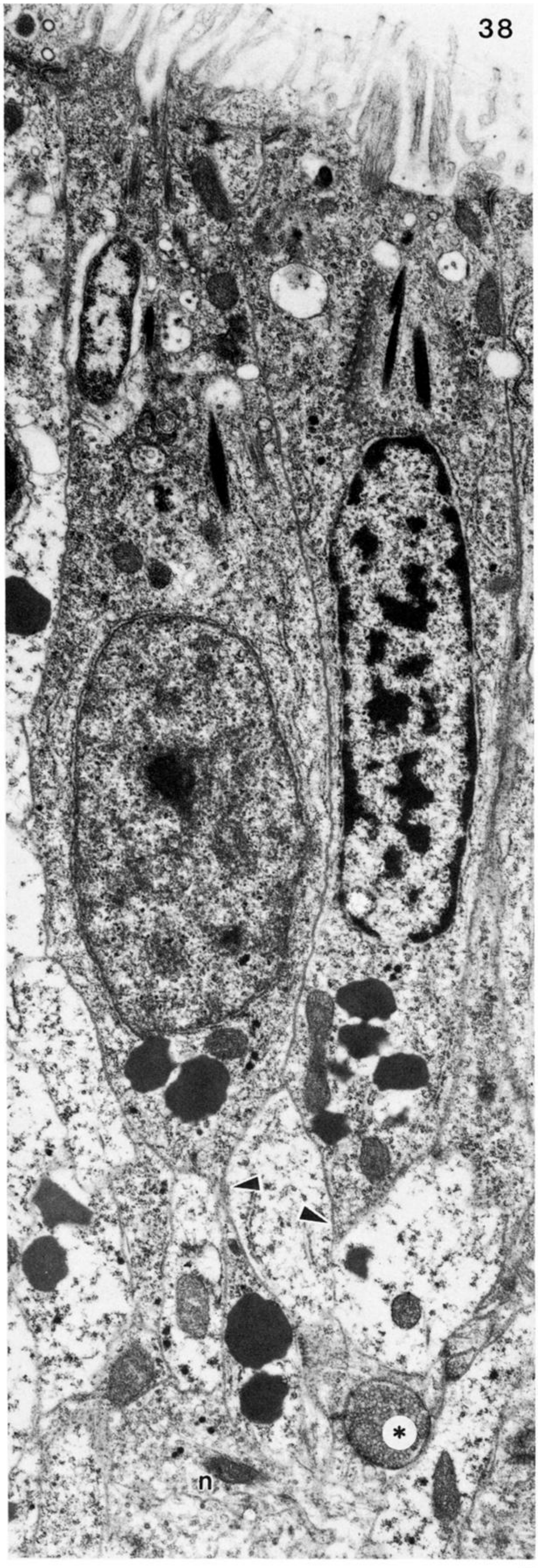
FIGURES 25–29. For description see facing plate 6.

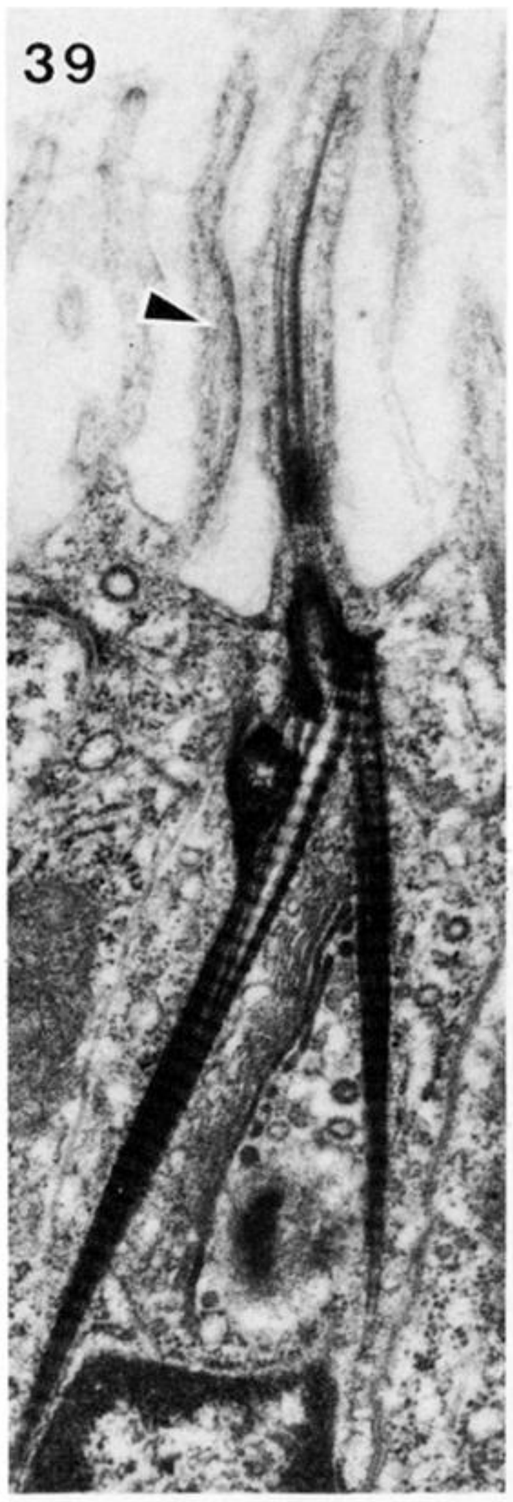


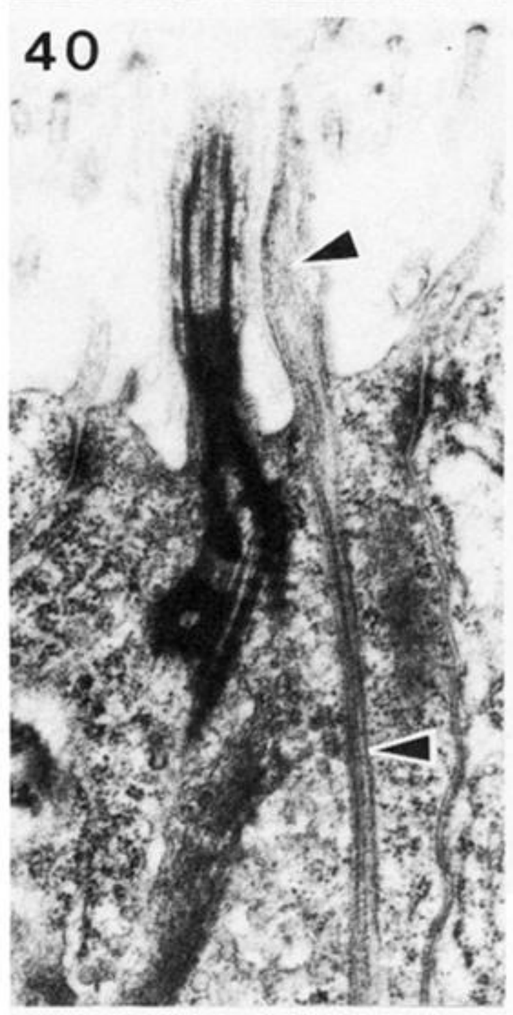
FIGURES 32 AND 33. For description see facing plate 9.

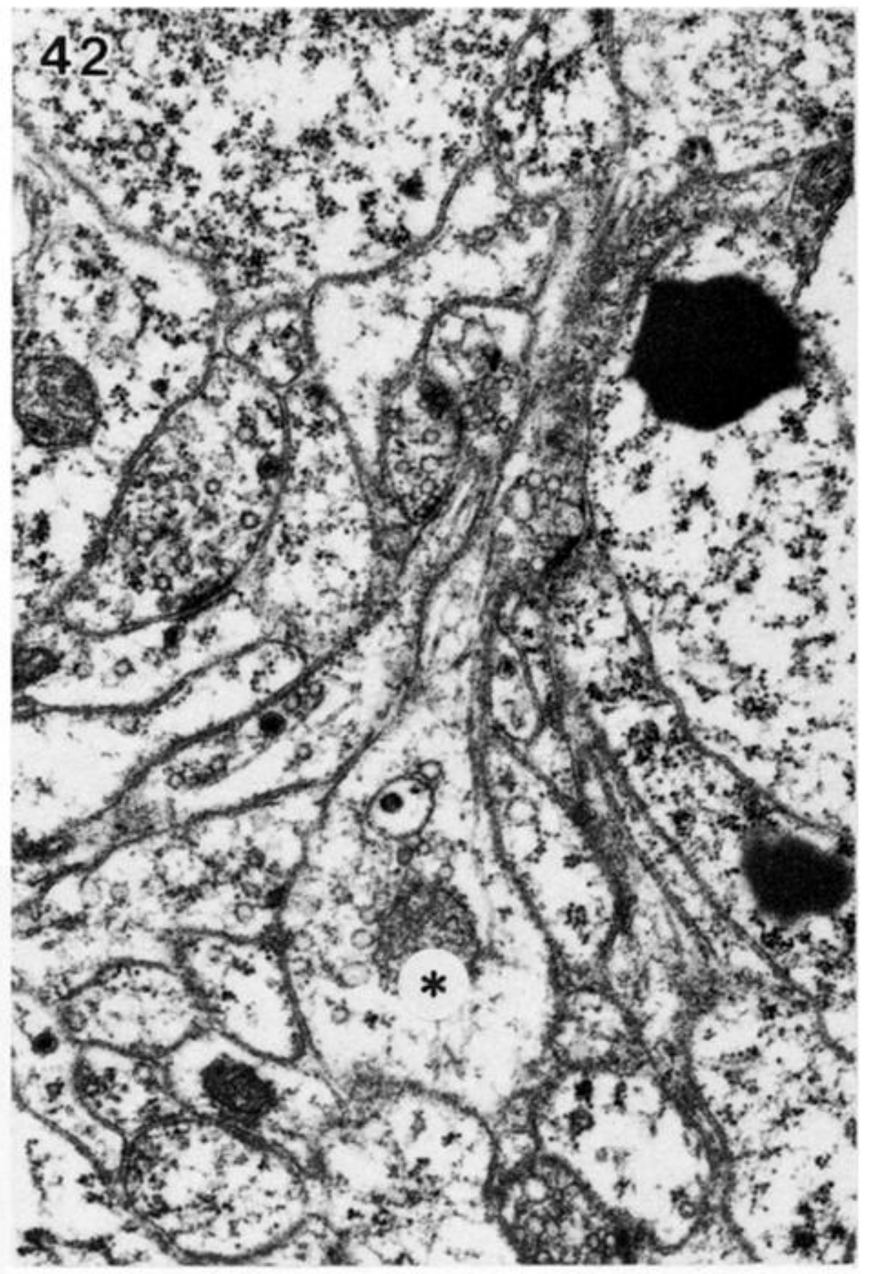


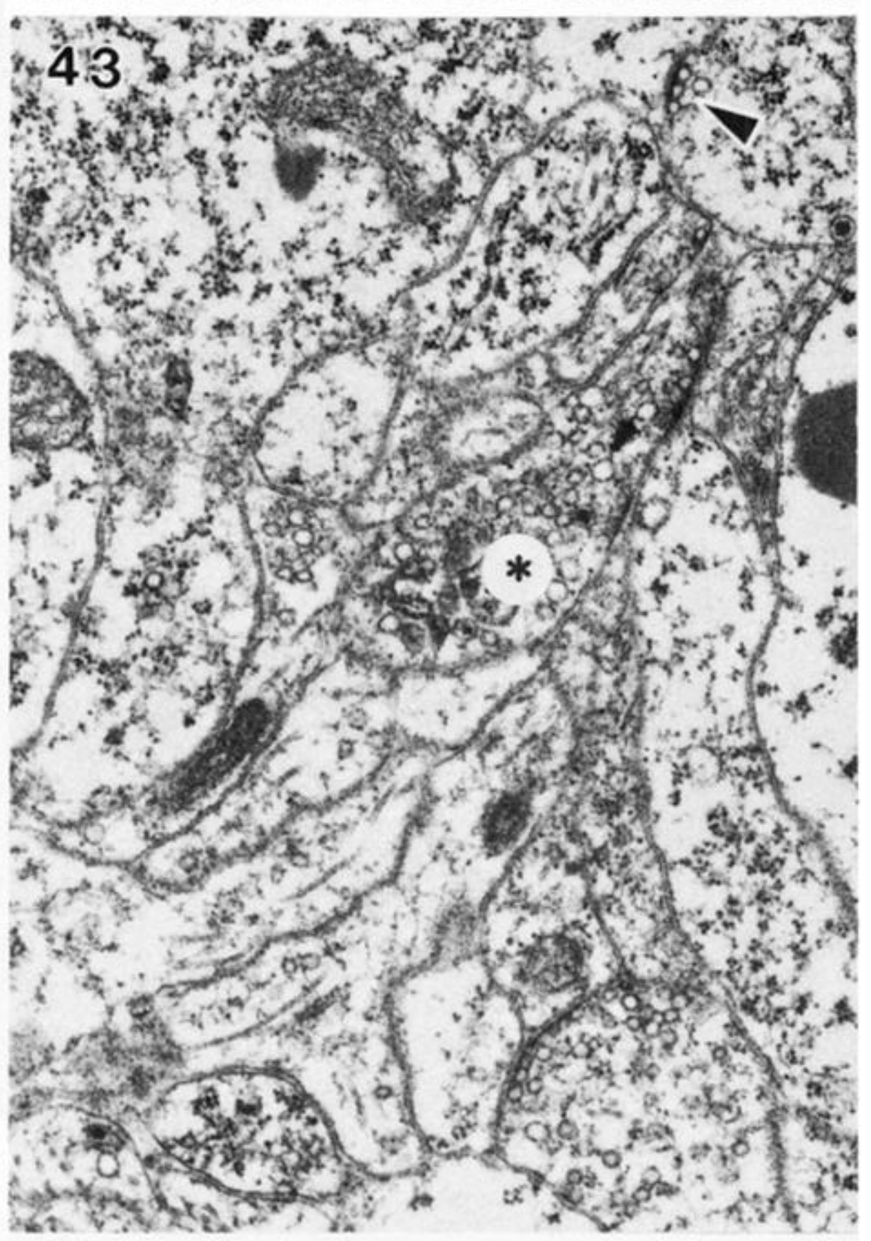
FIGURES 34–37. For description see opposite.











FIGURES 38–44. For description see facing plate 9.