STRUCTURE AND ORGANIZATION OF THE NERVOUS SYSTEM IN THE TROCHOPHORE LARVA OF *SPIROBRANCHUS*

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[Plates 1–19]

CONTENTS

The trochophore larva of the polychaete *Spirobranchus polycerus* is described, based on ultrastructural surveys and three dimensional reconstructions, with emphasis on the structure and organization of the nervous system. A complete and detailed description is provided of the larval parts of the nervous system at the cellular level for the 48 h stage, by which time the larval system is fully developed in most respects. The adult nervous system, whose rudiments form a largely separate system of nerves and nerve cells, appears progressively during later development. Its principal structures, the brain, commissures and ventral cords, are briefly described based on an examination of the metatrochophore.

The larval nervous system is entirely presegmental and is divisible into two parts: (1) a system of pretrochal cells and nerves arising from them that innervates the prototroch, linking it to the apical organ and the single larval eye, and (2) a system of intratrochal and intraepithelial nerves supplying the feeding apparatus of the larva. The latter consists of two nerves that encircle the pharynx and join basally beneath the cluster of cells that make up the basal pharyngeal complex. The pharyngeal nerves are then linked by means of a suboral complex of four sensory cells and their nerves to the nerves supplying the metatroch and neurotroch. The two parts of the larval system are anatomically separate and develop separately, each in association with its own organizational centres. These are: the apical organ and its central plexus in the case of the pretrochal system, and the suboral and pharyngeal complexes in the case of the oral and pharyngeal nerves. Like the larva itself, the larval nervous system is specialized and highly reduced. There are comparatively few cells, but a number of distinctive cell types. At 48 h, the larval system comprises 36 cells, including among these between 16 and 18 recognizably different types of sensory and non-sensory nerve cells and non-neural accessory cells. The majority of the cells are individually identifiable by morphology, ultrastructure and location, and are invariant or nearly so from larva to larva. The development of the system as a whole involves production of fibres by certain of these followed by fibre growth either along preestablished pathways, for example along the trochal bands or cells derived from these, or towards identifiable targets, for example, the apical plexus or pharyngeal complex. The resulting system varies little from larva to larva, and neurogenesis appears therefore to be a very precisely controlled developmental process. However, the individual cellular events that occur as parts of this process, do exhibit considerable diversity,

both in terms of the cell types involved and of the types of interactions that occur between them, which raises the question of how the degree of developmental precision required by *Spirobranchus* is achieved. Cell lineage and lineage-dependent phenomena are clearly important, but it is not clear how concepts arising from linage studies in other organisms, e.g. in nematodes or other spiralia, should be applied in dealing with this particular case.

Besides being anatomically separate, the two main parts of the larval nervous system evidently also have different evolutionary origins. Comparison of the *Spirobranchus* trochophore with the closely related Müller's larva of polyclads supports the idea that the pretrochal system of the former is derived secondarily from the adult nervous system of some ancestral form despite the fact that it innervates a strictly larval organ, the prototroch. Conversely, the nerves supplying the trochophore oral apparatus, which includes secondarily-derived adult structures like the pharynx, are of larval origin, probably derived by rearrangement from the nerves of a series of primitive trochal bands. The basic features of the oral apparatus in both Müller's larva and the trochophore can be accounted for by assuming the existence of an ancestral larva with three circumferential trochal bands. Two of these would then be incorporated into the stomodeum as it evolved, with their nerves being retained as stomodeal structures in modern forms. This interpretation emphasizes (1) the evolutionary conservatism of the larval nervous system, i.e. larval nerves change less in organization and arrangement than the structures they innervate, which makes them important phylogenetic indicators, and (2) the importance of the evolutionary continuity of the mouth in protosomes as a justification for comparative studies of the oral apparatus in spiralian larvae that seek to establish homologies between them. In the case at hand, it is concluded that the oral apparatus of Müller's larva and the trochopore, excluding the anus of the latter, are homologous.

The functional operation of the larval nervous system in *Spirobranchus* is discussed briefly and in general terms. The larval nerve cells show a low degree of morphological differentiation, and specialized cell junctions (e.g., synapses) are largely absent, so only a rudimentary understanding of the circuitry of the larval system is possible. Further, it is not clear to what extent the morphological and ultrastructural differences between the various larval cell types and between larval and adult nerve cells reflect significant functional and physiological differences. It would be most interesting if such differences did exist: the trochophore would then have to be accorded independent status as an organism physiologically quite different from the adult polychaete with, in particular, a far more primitive nervous system.

1. INTRODUCTION

The polychaete trochophore is generally accepted as the type larva of the phylum Annelida, so a detailed knowledge of trochophore structure is essential for understanding the ontogeny and basic organization of the annelid body. Similar trochophore and trochophore-like larvae occur among the marine representatives of other spiralian phyla. To the extent that such larvae share primitive structural and organizational features, the trochophore is also central to an understanding of spiralian phylogeny and interrelationships, and to speculations concerning the early evolution of invertebrate larval forms.

General features of trochophore structure and development are described in numerous classical studies at the light microscopical level (reviewed by Anderson 1966). While complete in some respects, these seldom deal very thoroughly with the nervous system, which is unfortunate, since considerable importance is often attached to the larval nervous system in comparative surveys and phylogenetic speculations (Nielsen 1979). In light microscope sections, only the larger elements of the nervous system are usually visible in larvae, that is, the

ganglia and cords of the developing adult system in most cases and the largest of the larval nerves (e.g. the prototroch nerve, Korn 1960) in some large trochophores. Most larval nerves are too small to be distinguished from surrounding tissue, and past attempts at a comprehensive description of the larval nervous system are unreliable for this reason. Larvae of two genera, *Polygordius* and *Lopadorhynchus*, figure especially prominently in past studies, and serve to illustrate the shortcomings of the existing literature. The *Polygordius* trochophore has a thin, transparent episphere crossed by eight radially directed nerves that link the apical organ with the prototroch according to Woltereck (1904). The approximate eightfold symmetry of these nerves has been used repeatedly in support of hypotheses linking bilateral phyla, via their larvae, to radially symmetric cnidarian or ctenophore-like ancestors (in, for example, Beklemishev 1969), but recent studies (Åkesson 1967) confirm the existence of only two of the nerves, the two circumoesophageal connectives. The remaining six have yet to be convincingly demonstrated. From his studies of the large planktotrophic trochophore of *Lopadorhynchus*, Meyer (1901) described a complex orthogonal system of longitudinal cords and circumferential ring nerves. This larval orthogon has been used as evidence for a direct relation between the trochophore and adult Turbellaria, which also have orthogonally arranged nerves. Re-examination of the *Lopadorhynchus* larvae (Åkesson 1967) shows Meyer to have been mistaken on a number of points, confirming in general only those elements of the orthogon (e.g. the prototroch nerve) known from other trochophore larvae. Phyllodocid trochophores, of which the *Lopadorhynchus* larva is one, also possess an unusual reticulum of large multipolar cells that are apparently neurosecretory (Lacalli & Marsden 1977 and unpublished), and this complicates any attempt to interpret the underlying organization of their nervous systems.

There is, in summary, considerable confusion regarding the organization of the larval nervous system in trochophores based on past reports. The intent of this study is to provide a complete and comprehensive description, at the electron microscopical (e.m.) level, of the nervous system of a representative trochophore, in this case, of a serpulid trochophore. Serpulid trochophores have been the subject of numerous previous studies (e.g., Hatschek 1885; Shearer 1911; Segrove 1941), and their large blastocoel and transparency make them favoured microscopical subjects. It is probably a mistake to consider them 'typical' trochophores, but they share with *Polygordius* larvae an almost diagrammatic simplicity, and a complement of ciliary bands including an accessory feeding band, the metatroch, that appears to represent a basic type from which the arrangement seen in other trochophores is probably derived. Serpulids have the additional advantage, for e.m., that their eggs are among the smallest of any polychaete. The larvae are then also small, being specialized in this regard in comparison with other trochophores. They have a minimal number of cells, sufficiently few in number in both neural and non-neural tissues that most of them can be identified and dealt with on an individual basis. *Spirobranchus* was chosen by the author from among other sepulids examined because so many of its larval cells have distinctive ultrastructural features, and this further facilitates cell and cell type identification.

Both anatomical description and functional interpretation of the larval nervous system in *Spirobranchus* is then possible, at least in principle, at the cellular level. The descriptive account that follows (§§3.1–3.6) is comparatively complete in this regard; the various parts of the nervous system are dealt with in turn and at the cellular level. With regard to the functional operation of the system (§3.7) the results are less satisfactory. The larval nervous system is small and simple in organization, but its cells' low level of morphological differentiation and

the absence of synapses in most parts of the system preclude any meaningful interpretation of its circuitry on morphological and ultrastructural grounds alone. Instead, the author's interpretation of the nervous system as a whole, and its organization (§§4 and 5), is mainly in comparative anatomical terms. The larval system is shown to be surprisingly similar in general organization, and in some respects in fine detail, to the nervous system of the type larva of polyclad flatworms, Müller's larva. This permits first, some specific suggestions about the origin and evolution of the main elements of the larval system in the trochophore and, second, confirmation of the evolutionary conservatism of the larval nervous system in general and its consequent phylogenetic significance. This study is not intended primarily as a developmental one, but enough different stages were examined to permit some inferences regarding the developmental phenomena responsible for producing a complete and correctly constructed nervous system. The cellular activities and interactions involved are shown to be roughly comparable to what is seen in the developing nervous systems of other invertebrates. The more general question of how the results reported here on the nervous system fit with what is known about the mechanism and strategy of developmental control in spiralian invertebrates is also discussed.

2. METHODS

The anatomical descriptions and e.m. results reported here, and all the figures in §3 except figures 4–9 and 89, are of larvae of *Spirobranchus polycerus* reared by the author during two visits to Barbados, in 1975 and 1980. The adult worms were collected from intertidal limestone shelves at Round Rock, Silver Sands, a collecting site described by Lewis (1960), and maintained in the laboratory at the Bellairs Research Institute, St James. *S. polycerus* is a small worm that forms large, encrusting colonies at this site. It is often confused with the larger, but otherwise very similar reef-dwelling species, *S. giganteus* (see ten Hove 1970). When removed from their tubes, ripe worms release gametes that can then be combined and will develop in fingerbowls. Successful fertilizations can be performed at most times of year since a small percentage of ripe individuals is almost always present. Unripe gametes will in some instances fertilize, and these produce a high proportion of abnormal larvae. It was therefore necessary to carefully screen the cultures and discard those containing any abnormal larvae. Rearing was done at 27 °C.

This study focuses on one particular stage, the 48 h trochophore, which is the terminal stage of development for the larvae if they are not fed and appears, in feeding larvae, to be a stage of at least partial developmental arrest. To obtain more advanced stages, the larvae were fed wild algae collected locally by net and cultured *Dunaliella salina*. With both diets, the larvae become progressively less synchronous as development proceeds, and subsequent staging is better done in terms of significant developmental events rather than by age. A diet of cultured algae gave less variability through the metatrochophore stage, but poor success at settlement, and so was judged less reliable than a diet of wild algae, which gave greater variability among larvae in a given culture, but more robust larvae. Fixations of metatrochophores were therefore of larvae from these latter cultures though, in fact, the study is restricted to stages that developed in a comparable and apparently normal way under both types of culture conditions.

Larvae were fixed for e.m. by the semisimultaneous method described previously (Lacalli 1981), and were stained in uranyl acetate overnight before embedding to avoid the need to stain the sections later. Spurr resin was used for embedding. Individual specimens were then

sectioned in their entirety and all sections were collected on slotted grids supplied with formvar support films. Generally a 1–1.5 μm thickness of tissue could be collected on each grid in this fashion, and complete or nearly complete serial series were obtained through selected parts of the larvae. The results rely principally, however, on reconstructions done at intervals of about 1 μm (i.e. one or two sections per grid on average), rather than serial series, since this is adequate for the purpose of tracing nerves and documenting the position and overall morphology of individual cells in most instances. Three specimens were reconstructed in detail in this fashion: one each of the 24 h and 48 h trochophore stages, both unfed, and one 9 d metatrochophore at the three-eye stage. All of the reconstructions in §3 except figure 21*b*, and all of the micrographs except figures 4–14, are of these three specimens, and most are either of the 48 h larva or the metatrochophore. The 24 and 48 h stages were both sectioned longitudinally in a plane slightly oblique to the sagittal plane, as is evident in the reconstructions (e.g. as in figures 49 and 50 for the 48 h larva). The metatrochophore was sectioned transversely in a plane with a slight lateral tilt from the horizontal, as is evident in figures 79 and 80. Six additional specimens were sectioned for comparison and reconstruction of selected parts: two at 16 h, one at 24 h, two at 48 h and one metatrochophore. While two to three larvae of a given stage represents a rather limited sample on which to base conclusions, particularly when variability at the level of individual cells is being dealt with, the considerable similarity evident between most larval structures, regardless of stage, permits all the specimens to serve to some extent as checks on each other. A number of specimens were sectioned at 1 μm for light microscopy, and osmium-stained mounts of whole larvae and larval fragments were prepared, but these types of preparations were of limited use as a means of demonstrating the nervous system.

Because of its availability from suppliers, *S. spinosus* is more easily obtained for experiment in the author's laboratory in Saskatoon than *S. polycerus*, and this has provided more opportunity to observe and photograph live larvae of this species. Observations on *S. spinosus* larvae are included where appropriate. The adult worms were obtained from Pacific Biomarine Co. of Venice, California, U.S.A., and the larvae were reared in artificial seawater at 14 °C and fed on cultured *Dunaliella salina* and *Isochrysis* sp. To better observe particle capture by the pharynx, larvae were fed suspensions of Aquadag colloidal graphite (Acheson Colloids Ltd, London) having a particle diameter of the order of 1 μm. It has proved much more difficult to observe routinely capture of the larger *Dunaliella* cells, or to discover an artificial particle in this size range that the larvae will consume.

3. RESULTS

3.1. *General observations on larval body form and structure*

Spirobranchus polycerus possesses a typical serpulid trochophore (figure 1) that is essentially fully developed by 48 h at 27 °C. Further cell differentiation occurs in some larval organs after this stage (e.g., in the pharynx, see §3.4), and structures that normally appear toward the end of the first 48 h are developmentally retarded in larvae that have not been fed (e.g., the longitudinal muscles, see below). With regard to most larval structures, however, the 48 h stage represents a stage of developmental arrest from which the larvae are released only after a period of several days' feeding. It is thus a convenient developmental landmark, and serves as a suitable focal point for this account.

The schedule of development in *S. polycerus* at 27 °C is, briefly, as follows. Cleavage and

gastrulation are discussed by Marsden (1960, as *S. giganteus*). Apical and trochal cilia appear, and the late gastrulae begin to swim, at 10–11 h. By 14 h the blastocoel and cavities of the stomach and stomoedeum are visible, and larval muscle cells associated with the latter can be distinguished from surrounding cells. At 16 h, the larvae are 80 μm in diameter, the apical organ is visible as a well defined thickening in the apical ectoderm, and contractions begin in the front part of the pharynx. The pigment granules of the single larval eye first appear at this stage, and in some larvae it is possible to see the tapered outline of the receptor cell and the thread-like pretrochal nerve arising from it. In section, each of the four larval muscle cells associated with the gut occupies its characteristic, final location (figure 3), and all have begun their differentiation. Small neurite profiles can be identified among the cells of the prototroch and pharynx, but no attempt was made to reconstruct the nervous system in detail at this stage.
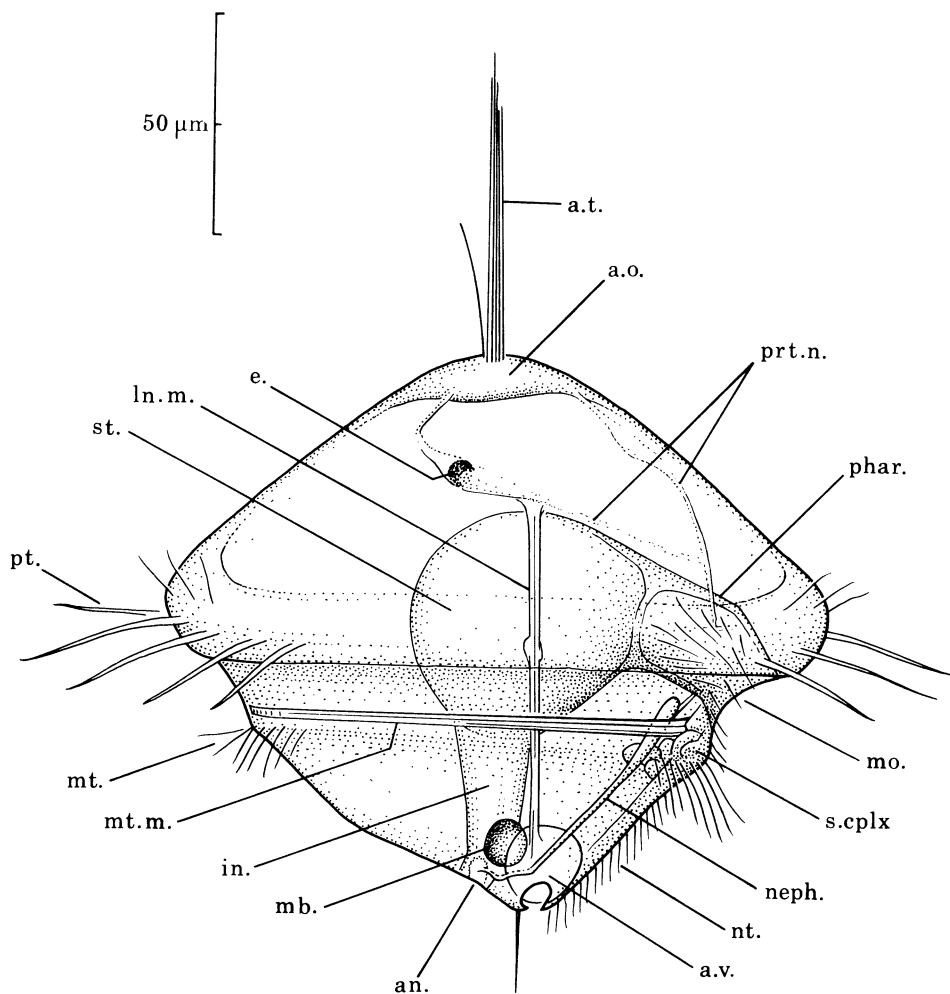
FIGURE 1. Fully developed *S. polycerus* trochophore seen from the right side. In feeding larvae this would correspond to the 48 h stage. Unfed larvae are similar in size and appearance at 48 h, but lack the paired longitudinal muscles (ln.m.). Shows the apical tuft (a.t.), apical organ (a.o.), prototroch (pt.), metatroch (mt.), neurotroch (nt.), two of the three petrochal nerves (prt.n.), one of which (n. II in subsequent figures) is associated with the eye (e.), the mouth (mo.), pharynx (phar.), stomach (st.), intestine (in.), anus (an.), anal vesicle (a.v.), one of the pair of protonephridia (neph.) and mesoblasts (mb.), and the region occupied by the suboral complex (s.cplx).

Rapid expansion of the blastocoel begins at about 16 h. Larval diameter is typically 110–115 μm at 24 h, 150 μm at 40 h and 160 μm at 48 h. The larvae appear to be capable of feeding by about 24 h. If they are fed at this time, development beyond the stage seen at 48 h begins only at 4–5 d with the resumption of proliferative activity in the apical region and later, in the trunk. By 8–9 d the larvae reach the metatrochophore stage (figure 2), and have
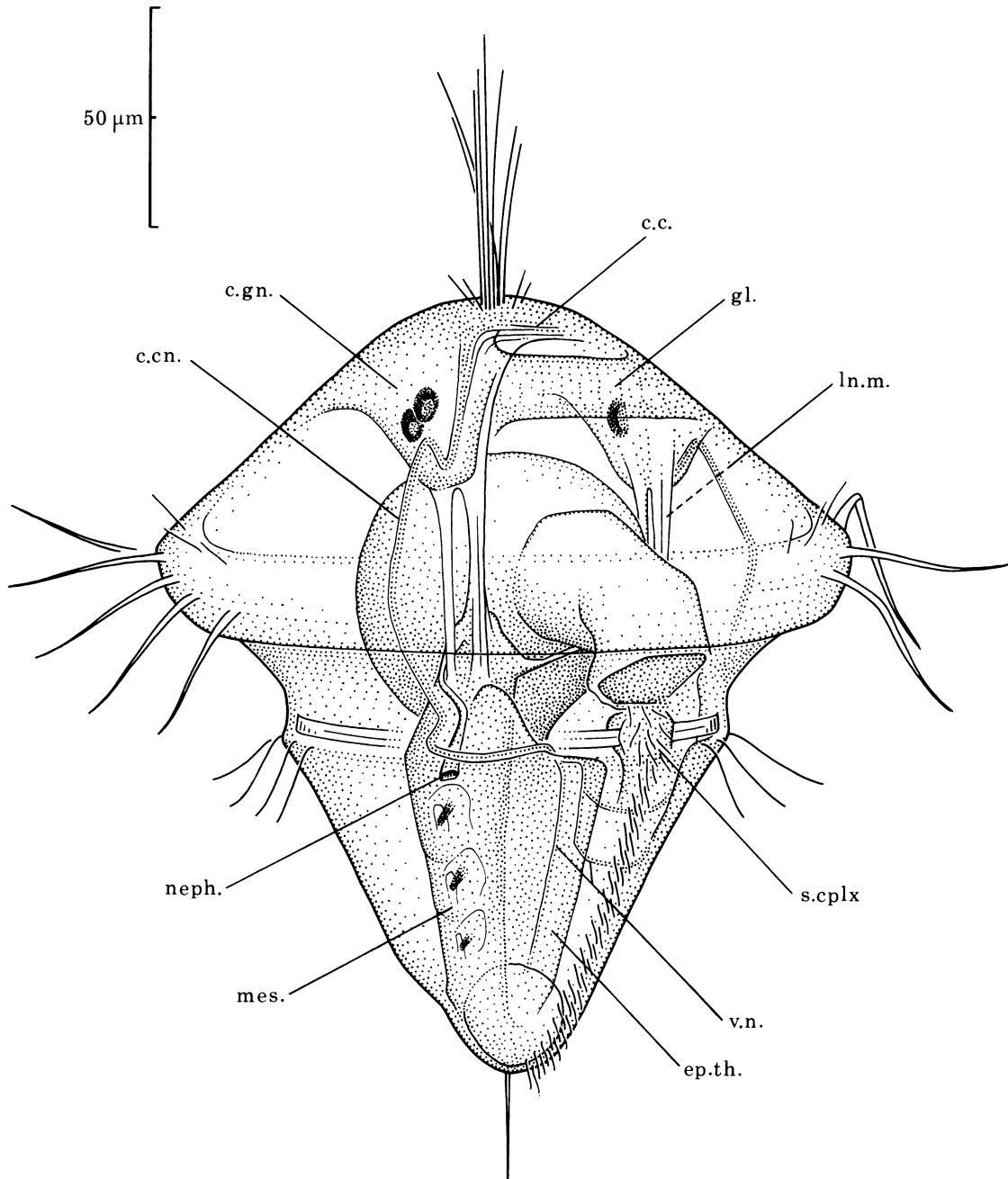

FIGURE 2. *S. polycerus*, the three-eye stage examined here in detail. Shows, in addition to the structures in figure 1, the developing cerebral ganglia (c.gn.) in which the eyes are embedded, the cerebral commissure (c.c.), circumoesophageal connective (c.cn.), ventral nerve cords (v.n., figure 74*a* shows their arrangement in detail), and the rudiment of the trunk, showing one of the pair of epithelial thickenings (ep.th.) and mesodermal bands (mes.). There are three larval segments with rudimentary setae. Note the apical row of gland cells (gl.), and the pronounced shift in the position of the nephridiopore (neph.) in comparison with the trochophore.

large cerebral ganglia and trunk rudiments with three larval segments. Three additional eyes appear at about this time, the second on the left side, and the third and fourth on the right and left, respectively. Setigerous, bottom-seeking stages develop by 18–20 d, but about another week is needed before the first settled juveniles appear. The larvae examined here as representative of the metatrochophore were three-eyed stages from a 9 d culture.

(a) *The* 48 h *trochophore*

Spirobranchus trochophores (figures 1, 4) have a spacious blastocoel and a thin, domed episphere whose only obvious thickening is the apical organ. The episphere epithelium is transparent, and the thread-like pretrochal nerves crossing it can be seen in some cases in live larvae (e.g. as in figure 6). *S. polycerus* has three such nerves, the most prominent of which passes through the single larval eye, located on the right side.

The body is unciliated except for specific ciliary organs, e.g. the tuft and trochal bands. There are three bands. Two are circumferential, the prototroch and metatroch, and pass, respectively, above and below the mouth. A ciliated food groove lies between these (figure 13). The metatroch is interrupted just below the mouth (figure 10) by the third band, the neurotroch, which runs down the ventral midline to the posterior pole of the body, occupied by the terminal pore of the anal vesicle (figures 13, 14).

The mouth leads to a thin-walled, tapered vestibule that joins to a thicker-walled, tubular pharynx (figure 3). Both structures are supplied with muscle cells. The circumoral muscle cell lies near the front of the vestibule. This cell produces a single band of muscle that encircles the vestibule and effectively defines the mouth. The pharynx has two muscle cells that form a reticulum of narrow bands over its surface, investing its front and back halves separately. The nuclei of all three cells have quite characteristic locations. These are: below the vestibule for the circumoral cell, half way up the left side of the front part of the pharynx for the front pharyngeal cell, and a corresponding position at the back on the right side of the pharynx for the back pharyngeal cell. Internally (figure 3*b*), both vestibule and pharynx are lined with cuticle and packed with cilia. A shallow ridge in the floor of the pharynx divides its lumen into front and back halves. The cells of the basal pharyngeal complex lie beneath this ridge. The back half of the pharynx, which forms a somewhat rounded chamber (figure 5), then communicates with the oesophagus through a flap-like one-way valve (figure 12). Most of the cilia in the vestibule and pharynx are much longer than the cross-sectional diameter of the lumen of either structure. Cilia in the vestibule and the front half of the pharynx form a compact bundle that projects forward toward the mouth (some that extend through it are visible in figure 10). Those in the back chamber of the pharynx form a coil pointing in the opposite direction. The majority of the cilia have thin, tapered ends that come together at the centre of the bundle to form a continuous channel through it from the mouth to the pharyngeal valve. The cilia clearly cannot beat freely in such a situation, but the undulatory motion of the bundle as a whole appears to be responsible for drawing particles up the channel and depositing them in the back chamber of the pharynx where a food bolus is formed (figures 8, 9) that can then be swallowed as described in §3.7.

Behind the pharynx lies the stomach, which connects through a narrow channel to the tubular intestine and thence to the small, ciliated anal chamber. The stomach is a thin-walled, bag-like structure whose cells, from sections, appear actively secretory. The front part of the stomach is encircled by muscle bands arising from a single cell whose nucleus sits directly on
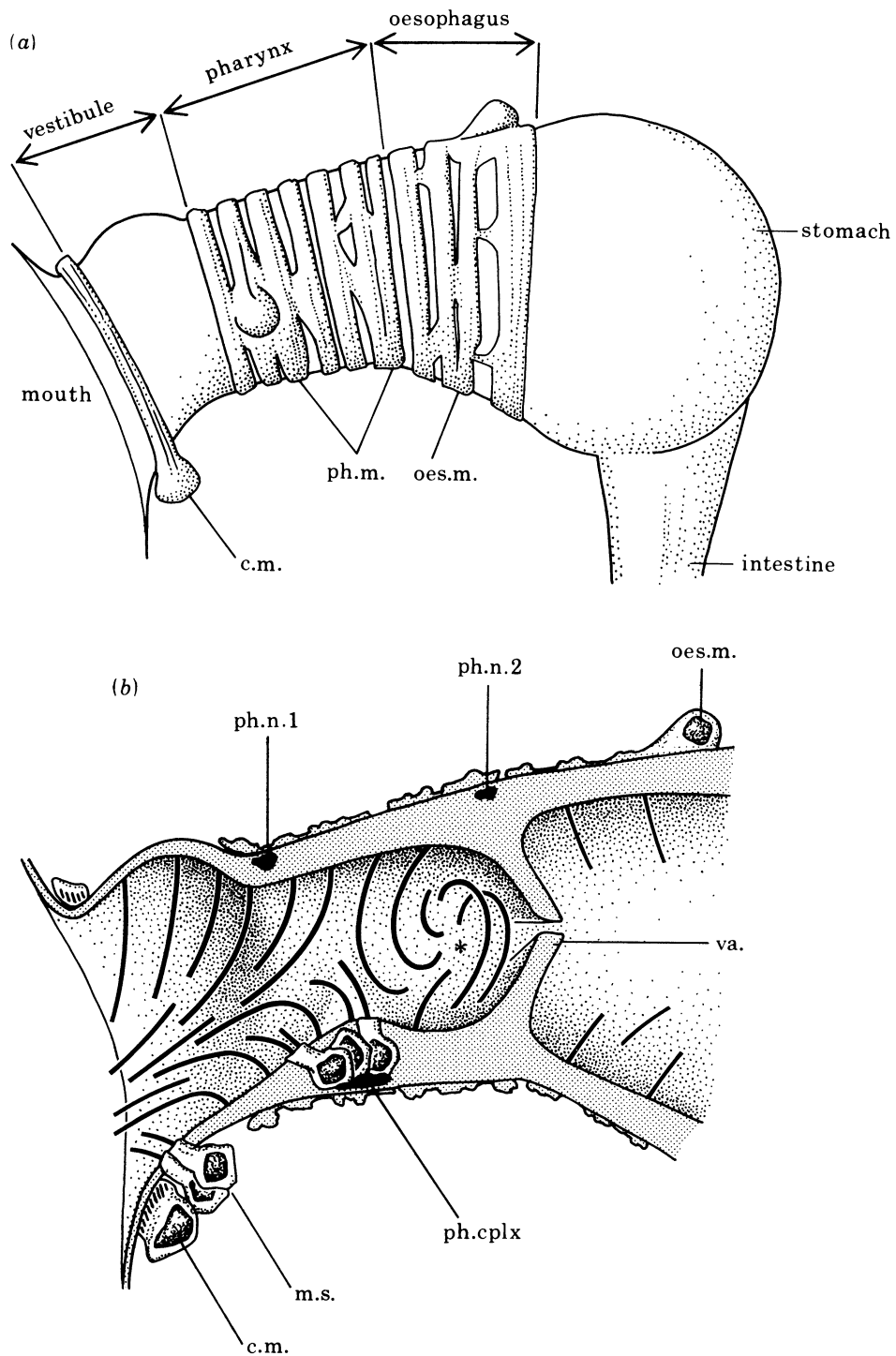
FIGURE 3. Digestive tract of a 48 h trochophore. (a) External view from the left side showing the principal parts of the system and their muscles: the circumoral muscle cell (c.m.), the two pharyngeal muscle cells (ph.m., the nucleus of the front cell, ph.m. 1 in subsequent figures, is visible from this side), and the oesophageal muscle cell. (b) Cut-away view of the vestibule and pharynx showing the arrangement of their cilia (representative cilia shown as heavy lines), positions of the nerves (solid profiles) and nerve cells, and the pharyngeal valve (va.). The plane of section is medial, and cuts both pharyngeal nerves (ph.n. 1 and 2), the basal pharyngeal complex (ph.cplx) and its nerve, the two median suboral cells (m.s.), and the back chamber of the pharynx (*). The arrangement of these structures in three dimensions is shown more clearly in figure 43a.

top of the gut (figure 11). This muscular region is referred to here as an oesophagus, though it differs from the stomach only in its muscular investment and is not divided internally from the stomach. The pharyngeal valve, which separates the pharynx and oesophagus, is formed by vesicle-filled cytoplasmic flaps arising from a ring of four cells located at the junction of the two organs. Immediately in front of the flaps, on the pharynx side, lies a single ring of cilia belonging to the valve cells. These are unusual in that they are entirely encased in a thin layer of cytoplasm that also extends between individual cilia to form a delicate cilium-supported sheet. The presence of cuticle in the vestibule and pharynx, its absence in the oesophagus and stomach, and the unusual nature of the valve cilia, which resemble those seen at the boundary of the ectoderm and endoderm in other spiralian larvae (e.g. in Müller's larva, Ruppert 1978), clearly identify the vestibule and pharynx as stomodeal derivatives, and the valve as the junction between stomodeum and endoderm. Oesophagus, stomach and intestine are then endodermal; the anal chamber is, presumably, a proctodeal derivative. There are no muscles associated with stomach, intestine or anus, or with the junctions between these, and it is not clear what controls the passage of food through these parts of the digestive tract.

The region just beneath the mouth is a centre of considerable developmental and functional importance. Besides the circumoral muscle mentioned above, nuclei of two other muscle cells are located here. These are: the ventral muscle cell, which spans the ventral region of the larva at about the level of the metatroch, and the metatrochal muscle cell responsible for the circumferential band of muscle located just above the metatroch. There is also a suboral complex of nerve cells whose fibres innervate these muscles and the ciliary tracts that converge in the suboral region. Flanking the suboral region, also at the level of the metatroch, lie clusters of cells associated with the right and left protonephridia. Each protonephridium has one terminal cell and one accessory cell at this stage. These are attached to the body wall behind the metatroch, and are also attached, by means of slender cytoplasmic strands (figure 45), to the underside of the pharynx just behind its junction with the vestibule. The two cell clusters also contain the precursors for the longitudinal muscles in larvae in which these have not as yet formed (e.g. as in figure 45, which shows an unfed 48 h larva). These cells migrate laterally along the metatroch and establish axial processes that become the muscles. This process is one of the few morphogenetic events of any significance that can be followed in detail in live larvae. It occurs late in development, when the blastocoel is fully expanded and transparent, and is complete by about 48 h in feeding larvae and 60 h in unfed ones.

From the terminal protonephridial cell, each nephridial channel crosses the blastocoel, attaches to the body wall beside the anal vesicle, and traverses the epithelium to the anal chamber. On each side of the intestine is a large, dense mesoblast cell, and the nephridial channels open into the anal chamber just beneath these. The underside of the mesoblasts in fact form the top half of the nephridiopore on each side (figure 17). Each mesoblast also encapsulates a smaller dense cell (figure 18). These are presumably primordial germ cells which are known to segregate early from the mesoblast in other serpulids (Malaquin 1934).

The terminal structures of the larva are the anal vesicle, produced by a single cell (figure 15), and a spine of several cilia arising from a cell just behind the anal vesicle (figure 16). The anus is situated behind and to the left of these structures (figure 14), and has no obvious or very close structural association with them.

Trochophores of *S. spinosus* (figures 4–9) differ from those of *S. polycerus* in their generally more conical form and their thicker and less transparent episphere. There appear also to be

differences in the arrangement of the pretrochal nerves (§3.3). With regard to all other features visible at the light microscopical level, trochophores of the two species are identical.

(b) The metatrochophore

Development to the metatrochophore stage (figures 2, 7) involves little change in size, except for elongation of the posterior part of the body as the trunk develops. Most of the larval structures are also largely unchanged, including the trochal bands, anal vesicle, digestive organs, and the various structures of the suboral region. What new structures do appear are mainly associated with the developing brain and trunk.

The brain arises from paired ectodermal thickenings that become the cerebral ganglia. Associated with these is an extensive mass of apical gland cells, also of ectodermal origin. The cerebral ganglia communicate via a cerebral commissure that passes through the larval apical organ. They also give rise to two circumoesophageal connectives that cross the episphere, prototroch, and food groove to the metatroch, where they are diverted forward and pass down the inside of the epithelial part of the trunk rudiment to form the ventral nerve cords and a segmental series of commissures.

The trunk has both ectodermal and mesodermal components. The mesodermal part, consisting of two narrow, lateral bands, is the less extensive of the two at this stage. The thickened ectodermal part of the trunk rudiment extends ventrally to the neurotroch from both sides (see figure 85). Only the post-metatrochal ectoderm participates in the formation of the trunk, but epithelial folds from this proliferative zone project into the blastocoel above the metatroch where they and adjacent mesodermal structures are attached, by means of the longitudinal muscles, to the underside of the cerebral ganglia. This presegmental part of the trunk rudiment eventually forms the collar. As reported by Segrove (1941), the nephridiopore migrates during trunk development so as to retain a presegmental position as well.

DESCRIPTION OF PLATE 1

FIGURES 4–9. Live *S. spinosus* larvae. Most structures can be identified by referring to figures 1 and 2. All other figures in §3 except figure 89 are of *S. polycerus*.

FIGURE 4. Side view, from the left, of a feeding 7 d trochophore. Shows the apical tuft, apical organ, trochal bands, anal vesicle, and the main parts of the digestive tract. Magn. × 280.

FIGURE 5. Apical view of a 5 d trochophore showing the position of the pharyngeal valve (between arrows) and the back chamber of the pharynx (*). Magn. × 280.

FIGURE 6. Front view of a 5 d trochophore showing the pretrochal nerve (arrow) associated with the eye (e.) as it descends toward the prototroch. Magn. × 215.

FIGURE 7. Metatrochophore with four eyes, a slightly more advanced stage than that shown in figure 2. The eyes are embedded in the cerebral ganglia, a thickened commissural region lies between these, and longitudinal muscles connect the ganglia with the trunk. Magn. × 380.

FIGURE 8. A 6 d trochophore fed for 10 min on Aquadag. The stomach and intestine are filled with particles, and a bolus of compacted particles is visible in the back chamber of the pharynx (arrow). Magn. × 280.

FIGURE 9. As in figure 8, from above. Magn. × 280.

FIGURE 10. Scanning micrograph of the oral region of a 48 h *S. polycerus* trochophore. Magn. × 2110.

FIGURE 11. Scanning micrograph of a fractured 48 h trochophore showing the digestive organs in external view. Arrows indicate the position of the pharyngeal valve. The oesophagus is longer than in figure 3a because its muscle has contracted on fixation. Magn. × 1300.

FIGURE 12. Section through the junction of the pharynx (phar.) and oesophagus (oes.) in a 48 h trochophore showing the pharyngeal valve (va.). Some of the cilia on the pharyngeal side are enveloped into a thin layer of cytoplasm (examples circled). These arise from the valve cells and form a single row just in front of the valve. Magn. × 8040.
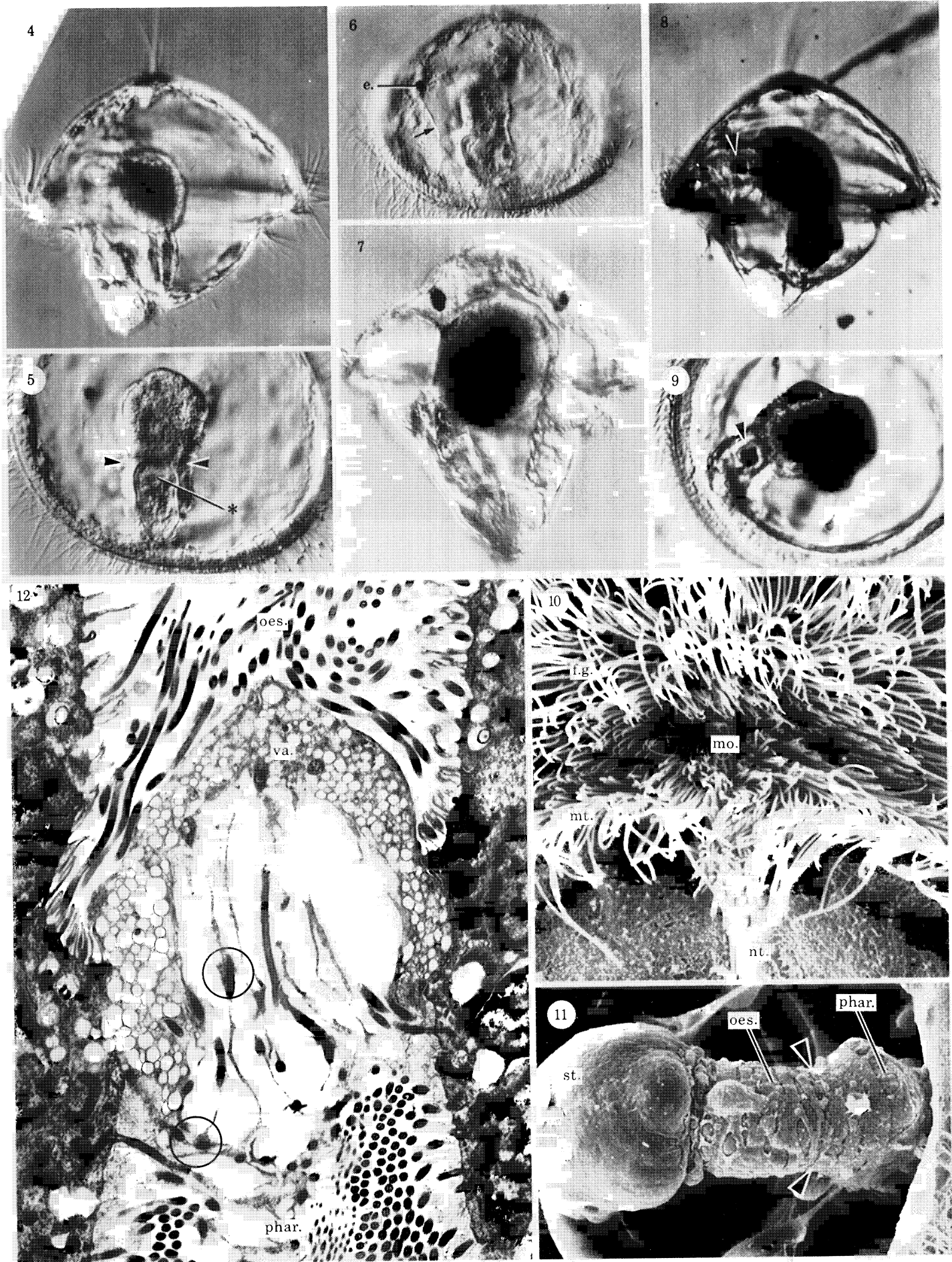
FIGURES 4–12. For description see opposite.

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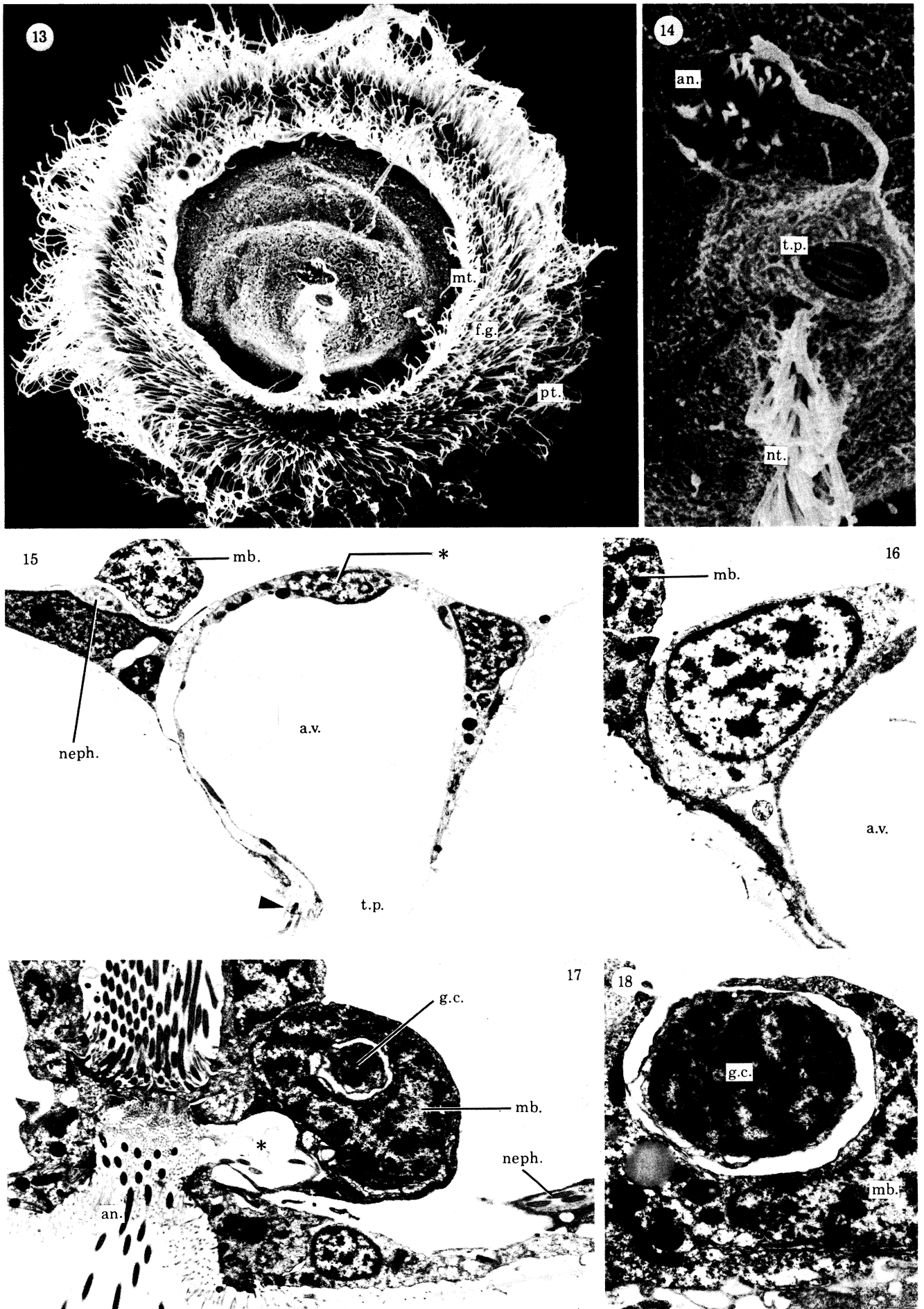
FIGURES 13–18. For description see opposite.

(c) *The larval nervous system*

The arrangement of nerves and nerve cells in the 48 h trochophore is summarized in figure 19. The nervous system as a whole is divisible into two parts: (1) a system of trochal and pretrochal nerves restricted to the episphere and linked to the apical organ, the function of which is locomotory (i.e. innervation of the prototroch), and (2) an interconnected system of pharyngeal, suboral and trochal nerves supplying the various structures involved in feeding, which includes the ciliary structures used for food collection (metatroch and neurotroch) and
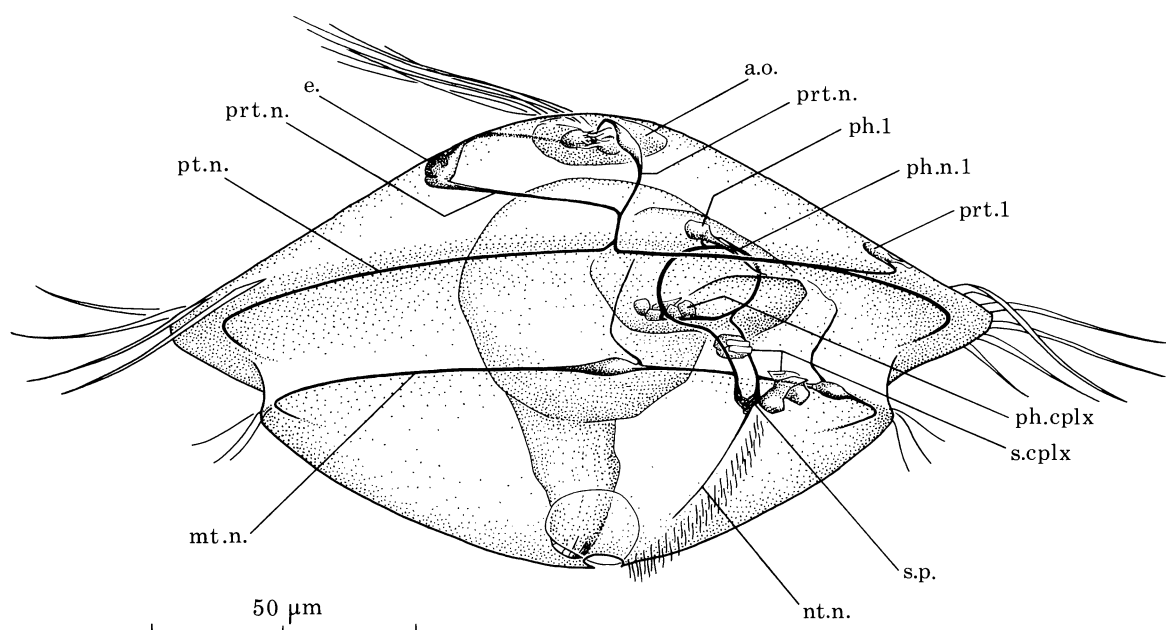
FIGURE 19. Summary diagram of the nerves and principal nerve cells of a 48 h trochophore. Shows three organizational centres: the apical organ (a.o.), basal pharyngeal complex (ph.cplx), and suboral complex (s.cplx), the last including the suboral plexus (s.p.). Nerves are: the prototroch nerve (pt.n.), metatroch nerve (mt.n.), neurotroch nerve (nt.n.), two of the three pretrochal nerves (prt.n., for the third see figure 31a), and the primary pharyngeal nerve (ph.n. 1). Two cells contributing to these that are of special interest, pretrochal cell 1 (prt. 1) and the suprapharyngeal cell (ph. 1), are also shown.

DESCRIPTION OF PLATE 2

FIGURE 13. Scanning micrograph of the posterior half of a 48 h trochophore. The mouth is visible as a depression in the food groove (f.g.) at the bottom of the figure. The neurotroch runs from the mouth to the posterior pole of the larva, shown also in figure 14. Magn. × 685.

FIGURE 14. Detail of figure 13 showing the anus (an.) and terminal pore (t.p.) of the anal vesicle. Magn. × 3375.

FIGURE 15. Section through the anal vesicle of a 48 h trochophore. Shows the base of the cilium (arrow) forming the anal spine in this specimen, the right mesoblast (mb.), the channel (neph.) of the right protonephridium and the nucleus (*) of the anal vesicle cell. Magn. × 4860.

FIGURE 16. A more medial section of the anal vesicle in figure 15 showing (*) the cell responsible for the anal spine. Magn. × 8600.

FIGURE 17. Junction of the left protonephridial channel (neph.) with the proctodeum (an.). A small chamber (*) is formed lined partly by the left mesoblast cell (mb.), and a portion of the left primordial germ cell (g.c.) is visible. Magn. × 6780.

FIGURE 18. Detail of the primordial germ cell shown in figure 17 in another section. Magn. × 14470.

the muscles responsible for swallowing and food rejection. While one point of connection between the two systems was identified in some larvae (via a small apically-directed process from the right metatrochal nerve cell, as shown in figure 19), this is a minor feature in structural terms, in both the trochopore and the metatrochophore, and does not appear to be of major functional importance.

The first of the above subdivisions, responsible for locomotory control, is discussed in detail in §§3.2 (the apical organ) and 3.3 (the prototroch and pretrochal nerves). Briefly, its main features are organized as follows. The prototroch is innervated by a circumferential prototroch nerve. There are, however, no nerve cells in the trochal band. The nerve's fibres arise, instead, from one cell in the pretrochal episphere (pretrochal cell 1, prt. 1 in the figures) and three pretrochal nerves, which themselves develop as bipolar outgrowths of three additional pretrochal cells. Two of the nerves enter the apical plexus, which lies at the centre of the apical organ, thus linking the apical organ and the prototroch nerve. One of these, pretrochal nerve II, passes through the eye, thereby establishing the structural basis needed for a locomotory light response.

The second of the above subdivisions, the system of nerves that innervates the feeding apparatus, is discussed in detail in §3.4. Briefly, its main features are organized as follows. The pharynx is innervated by two nerve rings that encircle the pharynx and join together at its base beneath a distinctive cluster of neural and non-neural accessory cells referred to here as the basal pharyngeal complex, which appears to act as an organizing centre. The primary pharyngeal nerve, which is the closer of the two ring nerves to the front of the pharynx, is also the larger and apparently more important of the two, and develops first. One particular nerve cell, the suprapharyngeal cell (ph. 1 in the figures), appears to be the main source of fibres for this nerve. The pharyngeal nerves are then linked to the rest of the system by two lateral vestibular nerves that extend along the sides of the vestibule to the suboral region and unite there to form a U-shaped suboral plexus. Four precisely placed suboral sensory cells contribute to and assist in the formation of this part of the system, and one of these is responsible for the fibres of the tiny neurotroch nerve. The metatroch nerve is also linked to the suboral plexus, joining it laterally on each side, but its fibres arise from two cells lying within the metatroch itself, one on either side of the oral region. The oesophagus, stomach, intestine and anus are apparently without direct innervation at this stage.

Later development of the nervous system was not followed in detail. It is clear, however, that the larval system changes very little after 48 h and is involved to only a limited extent in the development of the adult nervous system. The structure and arrangement of the various rudiments of the adult system are discussed in §3.5 with, in §3.6, a consideration of the morphological and ultrastructural characteristics of the various larval and adult cells encountered. Briefly, a distinction is made between cells that, on morphological grounds, are apparently either non-sensory neurons, sensory neurons, or are entirely non-neural even though they contribute fibres of various sorts to the nerves and plexuses. The developmental events responsible for establishing the basic organization of at least the larval part of the nervous system involves cells of all three types, and there is no clear evidence for the existence of cell types exclusively or primarily concerned only with development that are not also involved functionally in the operation of the nervous system.

3.2. *The apical organ*

The apical organ is visible as a well defined thickening at the apical pole of the larva throughout the trochophore stage. It is incorporated into the brain as the cerebral ganglia

develop in the metatrochophore, and becomes progressively less conspicuous as a discrete structure. In section, however, the cluster of cells that makes up the main part of the apical organ in young stages is easily identifiable in more advanced ones owing to the cells' characteristic number and arrangement, which changes very little, and their distinctive ultrastructural appearance in comparison with other neural structures.

(a) *Basic organization*

The apical organ in the 24 h trochophore has been described previously in some detail (Lacalli 1981, summarized here in figure 20). While it is not fully developed at this stage, its basic organization is evident, connections with the pretrochal system are established, and the cells of its central core are relatively well differentiated. For this study, reconstructions were prepared of the apical organs of two 48 h trochophores (figures 21, 22 and 24–26) and two metatrochophores (figures 23, 27 and 28) to determine the eventual fates of the various apical cells. The results demonstrate how very little the overall organization of the apical organ changes during later development as the more peripheral cells of the apical region complete their differentiation.
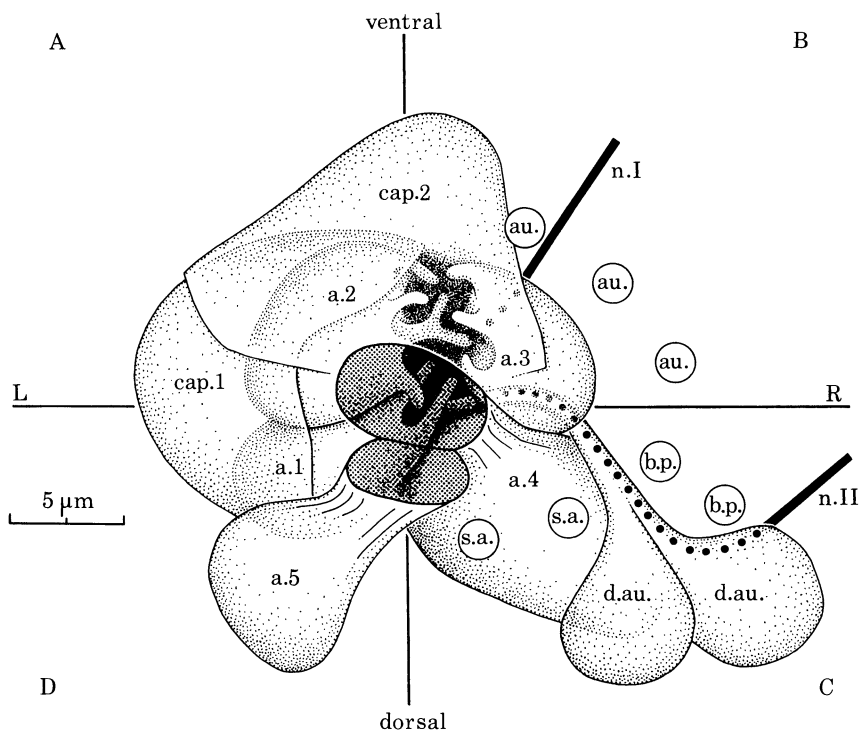
FIGURE 20. The apical organ of a 24 h *S. polycerus* trochophore, based on previous work by the author (Lacalli (1981), compare with figure 2 of that paper for changes in terminology). Shows nine of the 16 principal cells and, as labelled circles, the positions of the nuclei of the remaining seven. Lines representing the mid-sagittal and median frontal planes divide the apical organ into quadrants that roughly correspond to embryonic quadrants A–D as indicated. Conventions: exposed edges of cells are drawn as continuous lines, and as dotted lines or stipple where covered by encapsulating cells or otherwise obscured. The nerves, drawn as heavy lines, are likewise dotted where obscured by overlying structures. The projecting surface processes of the two tuft cells are included. Their ciliary fields are shown by uniform stippling through which some underlying detail (for example, the plexus) is visible. Figure shows the apical cells (a. 1–5), including the tuft cells (a. 4 and 5), capsular cells (cap. 1 and 2), auxiliary cells (au.), dense auxiliary cells (d.au.), basal process cells (b.p.), the secondary apical cells (s.a.), which are not fully differentiated at this stage, pretrochal nerves I and II (n. I and II), and the apical plexus (centre of figure, not labelled).

The central feature of all apical organs examined is the apical plexus, a mass of interdigitating, vesicle-filled processes (figures 29 and 30) arising mainly from three apical cells, a. 1–a. 3. Of these, two large, multipolar cells, a. 2 and a. 3, are the main contributors. The apical tuft arises from two cells, a. 4. and a. 5, with the majority of the tuft cilia coming from a. 4. which lies adjacent to and imediately behind the plexus. The rest come from a. 5, a smaller cell lying behind and to the left of a. 4. The plexus and cells a. 1–a. 3 are encased by flattened, sheet-like extensions from two capsular cells, cap. 1 and cap. 2. In the trochophore, both of these lie ventrally on the left side of the apical organ, in quadrant A (see figure 20 for designation of quadrants) and encapsulation of the left side, i.e. cells a. 1 and a. 2, is nearly complete. Cap. 1 encloses these two cells directly to form an inner capsule, while cap. 2 wraps over the outside of cap. 1 as shown in figures 20 and 22 to form a second layer. On the right, where various cell processes and nerves enter the plexus, encapsulation is less complete. Two capsular cells are found in the apical organ of the metatrochophore as well, but the cell forming the outer of the two layers, which is presumably cap. 2, is shifted to the right side and surrounds a. 3, so as to give the apical organ as a whole a more nearly bilateral symmetry (figure 23). Cell a. 3 is the most variable in position of the apical cells in the trochophore, however, and it is not unreasonable to suppose that a. 3, along with cap. 2, moves or is shifted during the reorganization of apical cells that accompanies the development of the cerebral commissure since this affects, almost exclusively, the cells on the right side.

Between 24 and 48 h, processes arrive in the apical plexus from two additional apical cells, referred to here as the secondary apical cells. These develop from the two undifferentiated, flask-shaped cells reported in the 24 h stage (Lacalli 1981) to lie behind a. 4. They do not change position as they differentiate, so their processes must pass around the surface process belonging to a. 4, which they do on the right side. The cells then resemble a. 1 to some extent, since a. 1 squeezes between a. 2 and a. 4 in a similar fashion on the left side. They differ, however, in developing much later than a. 1 and, unlike a. 1, they are not enclosed by capsular cells.

Two pretrochal nerves enter the apical organ in the trochophore, both from the right side.

The ventral nerve, pretrochal nerve I, enters basally into the plexus after passing along the underside of one of the three auxiliary cells that lie in a row in quadrant B. It is difficult to distinguish among these three cells as to function because of variability between larvae. In several cases, the cell associated with the entry of nerve I extended a basal process toward the plexus and appeared, in this way, to be involved in assisting the passage of nerve I into the plexus, but this was not observed in all larvae examined. In addition, in several of the larvae, a second auxiliary cell, somewhat larger than the other two, loosely encapsulated the exposed side of the plexus. Such cells have previously been identified by the author as capsular cells (as cap. 3 in Lacalli 1981), but this appears to be an inappropriate designation. Encapsulation of the plexus by auxiliary cells in quadrant B is a variable feature in the trochophore, and the cells involved differ ultrastructurally from the two main capsular cells, which are generally of type 1 ultrastructure as defined in §3.6. The above suggests that quadrant B auxiliary cells are not essential participants in the early events of apical organ development or, if essential, have at most a transient involvement.

The dorsal pretrochal nerve, pretrochal nerve II, enters the apical organ in the C quadrant, and is invariably associated with two very dense cells referred to here as dense auxiliary cells. One or both of these send thick dense processes of a very distinctive type (figure 29) into the plexus along which the processes of nerve II also run. This suggests their function may be to pioneer a path along which nerve II is guided into the plexus (Lacalli 1981). Two cells, the

main characteristic of which seems to be the production of flattened basal processes, termed here basal process cells, are also associated with the entry of nerve II. Cells in this category are of somewhat variable ultrastructural appearance (e.g. figure 24) and also vary in position. They lie in front of the dense auxiliary cells in some larvae (figure 20) and behind them (figure 21) in others. Their processes usually wrap around or under various parts of the dense auxiliary cells and cell a. 4, and appear thereby to restrict the path open to nerve II.

The reconstructions therefore identify 16 cells that are reliably associated with the apical
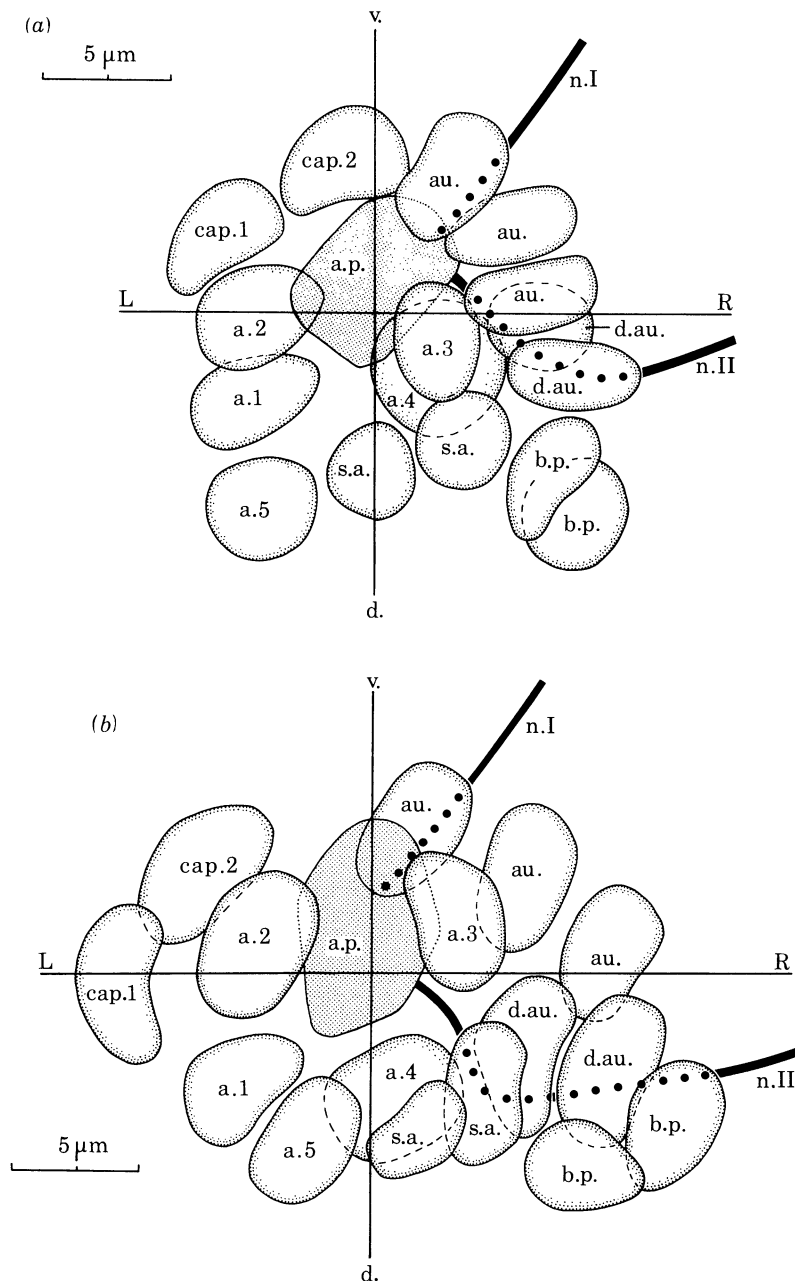
Figure 21. The arrangement of cells in the apical organs of two 48 h trochophores, showing the positions of the nuclei of the 16 apical cells. Figure orientation and labelling follows that in figure 20 with, in addition, the apical plexus (a.p., uniformly stippled). Where obscured by overlying structures, the edges of nuclei are shown by broken lines, the edges of the plexus by small dots, and the nerves by large dots.

organ, all of which are assignable to well defined roles within it. This may preclude a role for any of these 16 cells as precursors for other apical structures, i.e. for the ventral gland cells or cells of the cerebral ganglia. If the gland cell precursors and neuroblasts lie elsewhere among surrounding epithelial cells, they are not distinguishable at 24 h, as the cells surrounding the apical organ are all of a similar epithelial type at this stage. By 48 h, smaller dense cells have appeared in the epithelium that probably are the neural and gland cell precursors. Their appearance is not correlated with any obvious disturbance to the organization of the apical
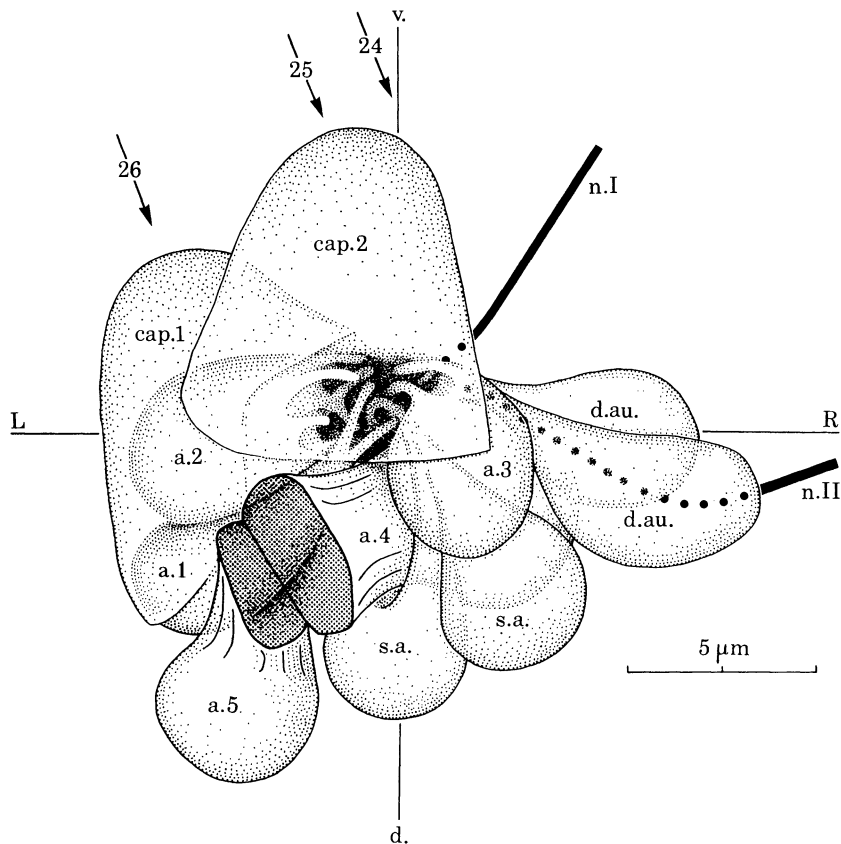
FIGURE 22. Reconstructions showing the principal cells of the 48 h apical organ in figure 21 a, following conventions and labelling used in figure 20. Sections of this particular apical organ are also shown in figures 24–26, and arrows at the upper left indicate the approximate planes of section by figure number.

organ, and they appear therefore not to be derived from it. Except for the tuft cells, the apical cells are shown in the figures (figures 20, 22 and 23) without surface processes. In fact, all except the dense auxiliary cells have surface processes. Usually these are slender and tapering, and bear a cilium or cilium-like stub. These processes are particularly prominent in the metatrochophore, where they form a ring around the apical tuft. Their cilia (one or two per cell) are visible near its base as shown in figure 2.

(b) Relation to the cerebral commissure

The apical organ of a metatrochophore is shown in figure 23. A second organ reconstructed was essentially identical. The principal apical cells (a. 1–a. 5), the capsular cells, and the secondary apical cells are arranged very much as in the 48 h trochophore except for the shifting

of the cell identified as cap. 2, mentioned above. The cerebral commissure has developed by this stage and is divided into two tracts, a small dorsal tract that enters symmetrically from both sides beneath the plexus, and a larger ventral tract whose processes communicate medially with the base of the plexus, the exchange of fibres between them forming the median integrative centre (see §3.5). There are several cells ranged along the front of the apical organ with surface processes bearing cilia whose basal processes extend down between the plexus and ventral tract
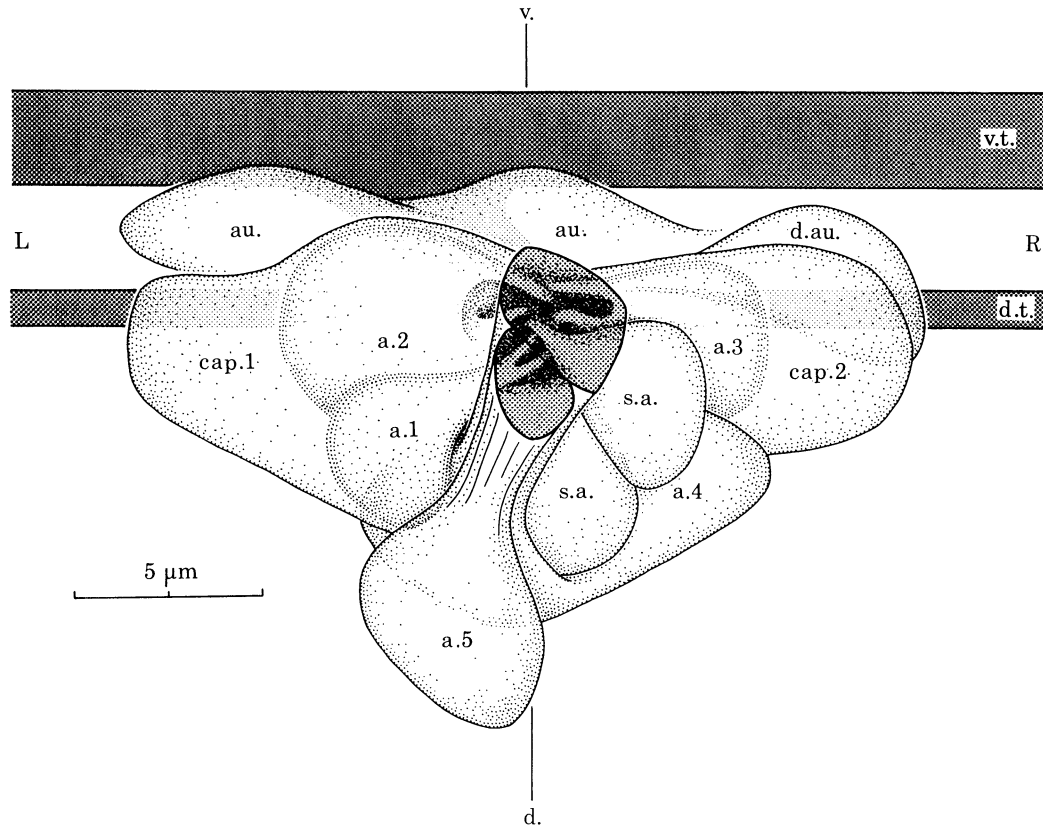
FIGURE 23. Reconstructions showing the principal cells of the apical organ in a metatrochophore. Follows conventions and labelling used in figure 20. Shows also, as stippled bands (lighter where obscured), the dorsal (d.t.) and ventral (v.t.) tracts of the cerebral commissure. Figures 27 and 28 show sections through this apical organ, which also appears in the brain surveys and reconstructions (figures 76–80).

to form a partial obstruction (figure 28). Some or all of these cells, and certainly those closest to the apical plexus, must, by location and general morphology, be derivatives of the quadrant B auxiliary cells, and are labelled accordingly in the figures (e.g. figure 23). This means that the point of contact between the ventral tract and apical plexus, the median integrative centre, is equivalent to the point of earlier entry of pretrochal nerve I, which also passes beneath the auxiliary cells and interacts with their basal processes. It seems reasonable as well to conclude that pretrochal nerve II and the right half of the dorsal tract follow comparable paths, though it is difficult to identify equivalents of the dense auxiliary and basal process cells in the metatrochophore with certainty, owing to the rearrangement of the cells that occurs on the right side. It is also entirely unclear how the left half of the dorsal tract establishes communication with the apical plexus without disturbing any of the cells on the left side of the apical organ. The shifting of the two points of entry into the plexus, which are located on

the right side of the apical organ in the trochophore, to a medial, basal position in the metatrochophore, may be of some significance in this regard.

In summary, the apical origin is a compact, well defined, and remarkably constant structure throughout the development of the trochophore. Its principal feature is the apical plexus, to which first apical cells and capsular cells, and later the more peripheral cells of the apical organ, contribute. Nerves enter first on the right, but their initial points of entry are shifted basally later in development, to accept additional nerves arriving from the left. The very stereotyped arrangement of cells on the left side of the apical organ is not disturbed during later development by these events, but cells on the right are shifted to some extent. What appears, in the end, as a comparatively symmetrical arrangement of cells and nerves, is the result of a process exhibiting considerable asymmetry from the first.

3.3. *Innervation of the prototroch*

(a) *The prototroch and prototroch nerve*

The prototroch of *S. polycerus* is composed of four tiers of cells (figure 32), numbered here from its anterior margin for purposes of reference. The cells of the second tier bear the locomotory cilia and develop first, producing a single row of cilia initially and multiple rows, usually five, by the time the trochophore is fully formed. Development of tiers 1, 3 and 4 then follows, but each produces only a single row of cilia. Development of the prototroch, metatroch and the food groove proceeds in a ventral to dorsal direction. Thus at 24 h, tier 2 is made up of a ring of five large trochal cells complete except for a dorsal gap filled by several small cells, tier 1 is of similar extent, while tiers 3 and 4 are limited to the ventral half of the larva. By 48 h, all four tiers are complete and fully developed.

Typical prototroch organization is shown in figure 32. Cells belonging to tier 2 are long and cylindrical with large nuclei (figure 35) and numerous mitochondria. The tier 1 cells form a thin sheath over the top of tier 2, their flattened, sheet-like extensions wrapping over and pressing against the inside surface of tier 2, i.e. the surface facing the blastocoel. Flattened processes from tiers 3 and 4 enclose the bottom of tier 2 in a similar fashion at 24 h, but subsequently detach, leaving behind only the delicate basement membrane laid down earlier over their surfaces. The result, at 48 h, is that tier 2 is completely encapsulated from the top, but not from the bottom, a situation that persists in the metatrochophore.

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DESCRIPTION OF PLATES 3, 4 AND 5

FIGURES 24–26. Sections through the 48 h apical organ shown in figures 21a and 22, planes of section and labelling of cells as shown in figure 22. Shows also the mesenchyme cell (mch.) that lies beneath the apical organ, nuclei of some adjacent epithelial cells (ep.), and the cilia of the apical tuft (a.t.). Magn. × 9450.

FIGURE 27. Section through the apical organ of a metatrochophore, specimen orientation and labelling of cells as in figure 23. Magn. × 10200.

FIGURE 28. Subject and orientation as in figure 27, but a more basal section through the apical plexus (a.p.) where it widens to accept the dorsal tract (not distinguishable from the plexus at this point) and the ventral tract (v.t.) of the cerebral commissure. Cell processes belonging to the auxiliary cells that lie along the front surface of the apical organ are indicated by *. Magn. × 9530.

FIGURE 29. Detail of the apical plexus at 48 h, from a section near that shown in figure 25. Processes belonging to the lower of the two dense auxiliary cells in figure 24 are indicated by *. Magn. × 21280.

FIGURE 30. Detail of the basal part of the apical plexus at the metatrochophore stage, from a section near that shown in figure 28. Magn. × 40820.
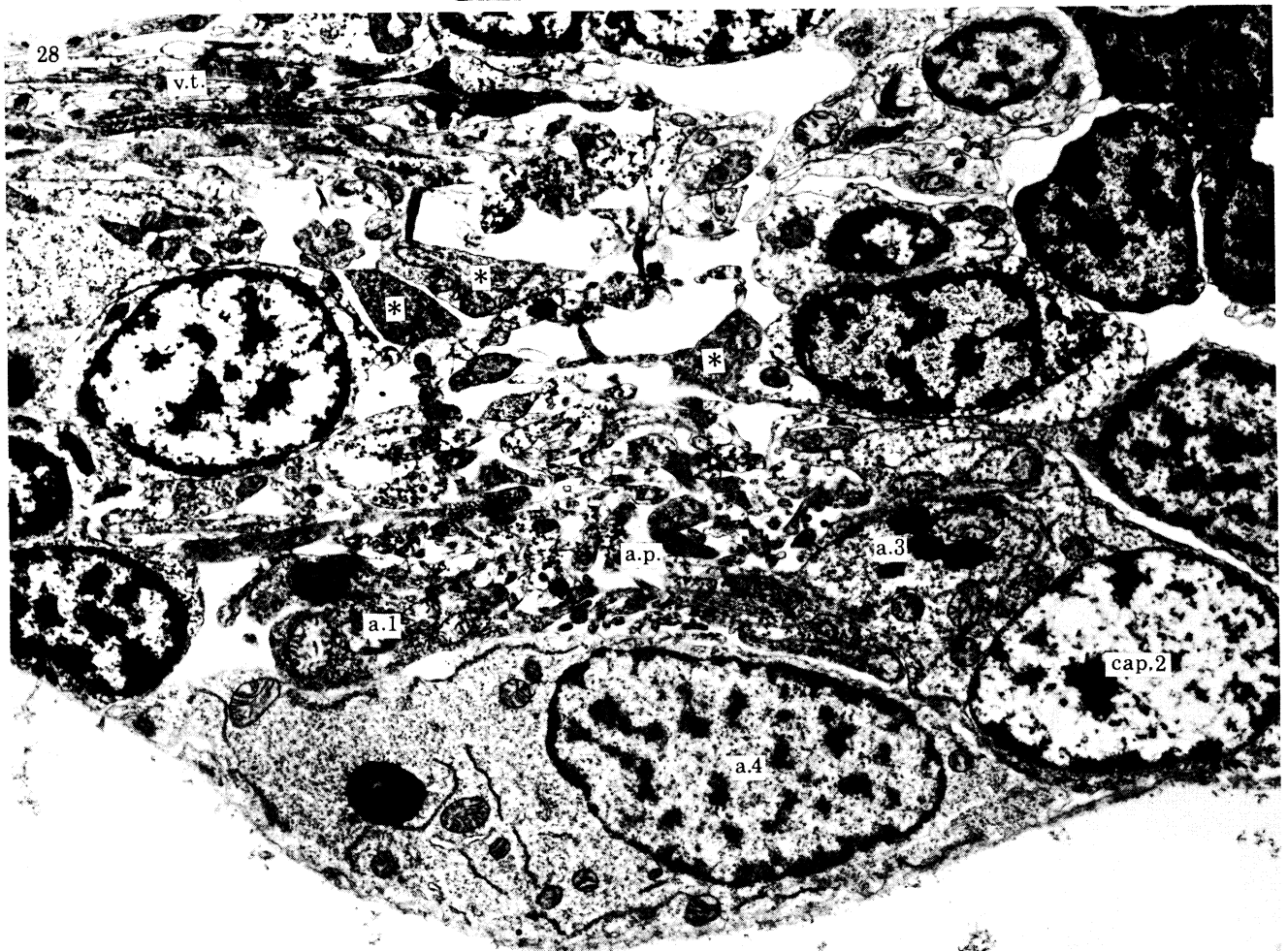
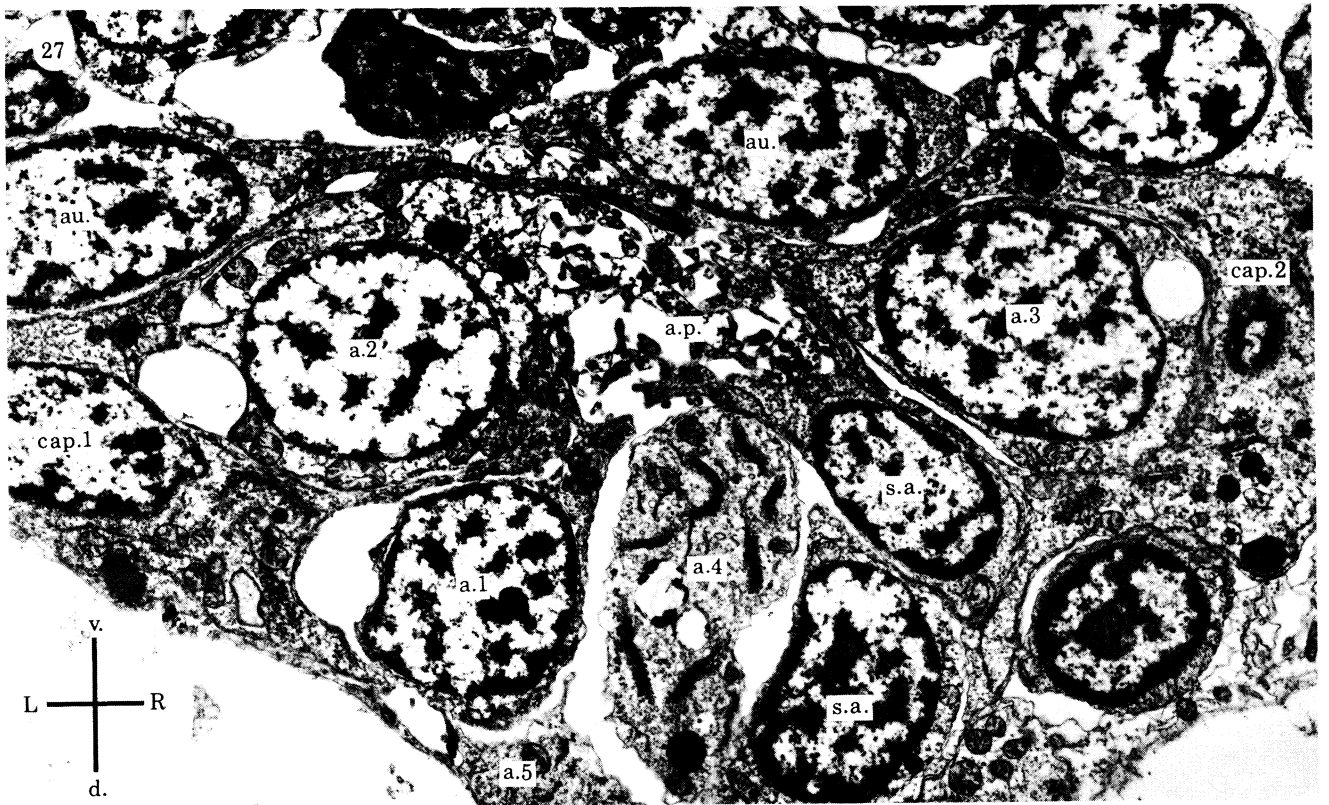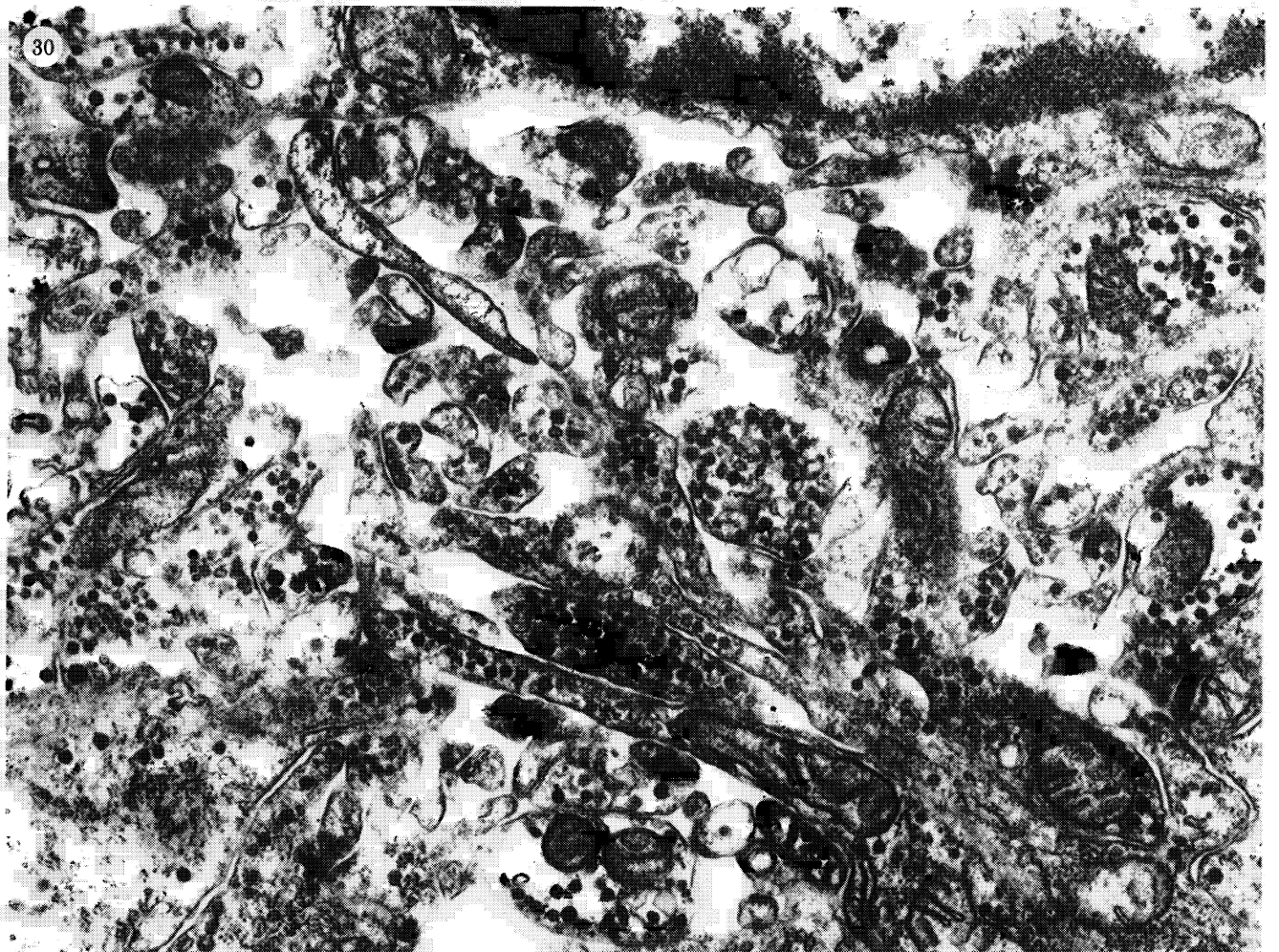FIGURES 24–26. For description see opposite.

FIGURES 27 AND 28. For description see p. 98.

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Lacalli, plate 5

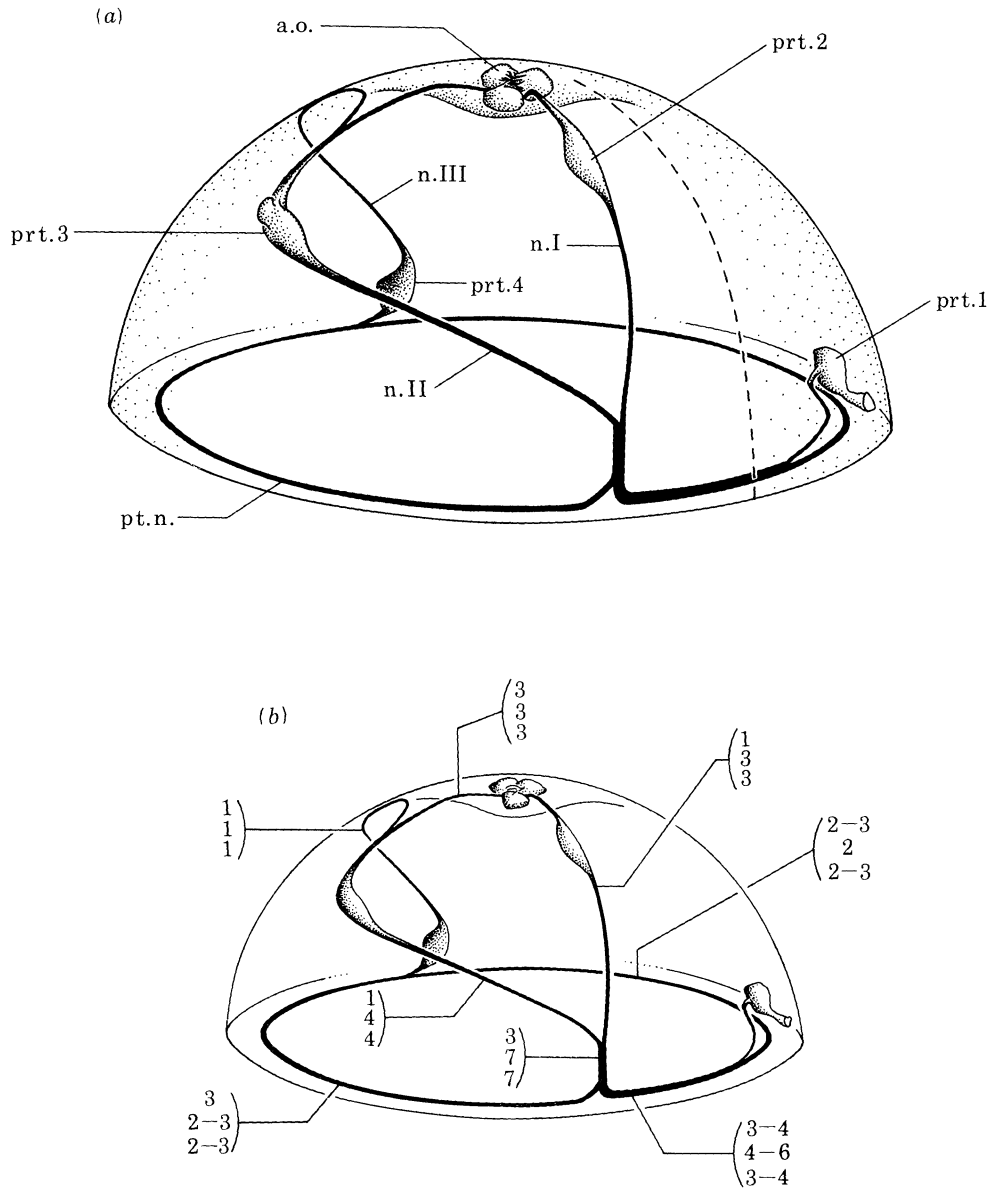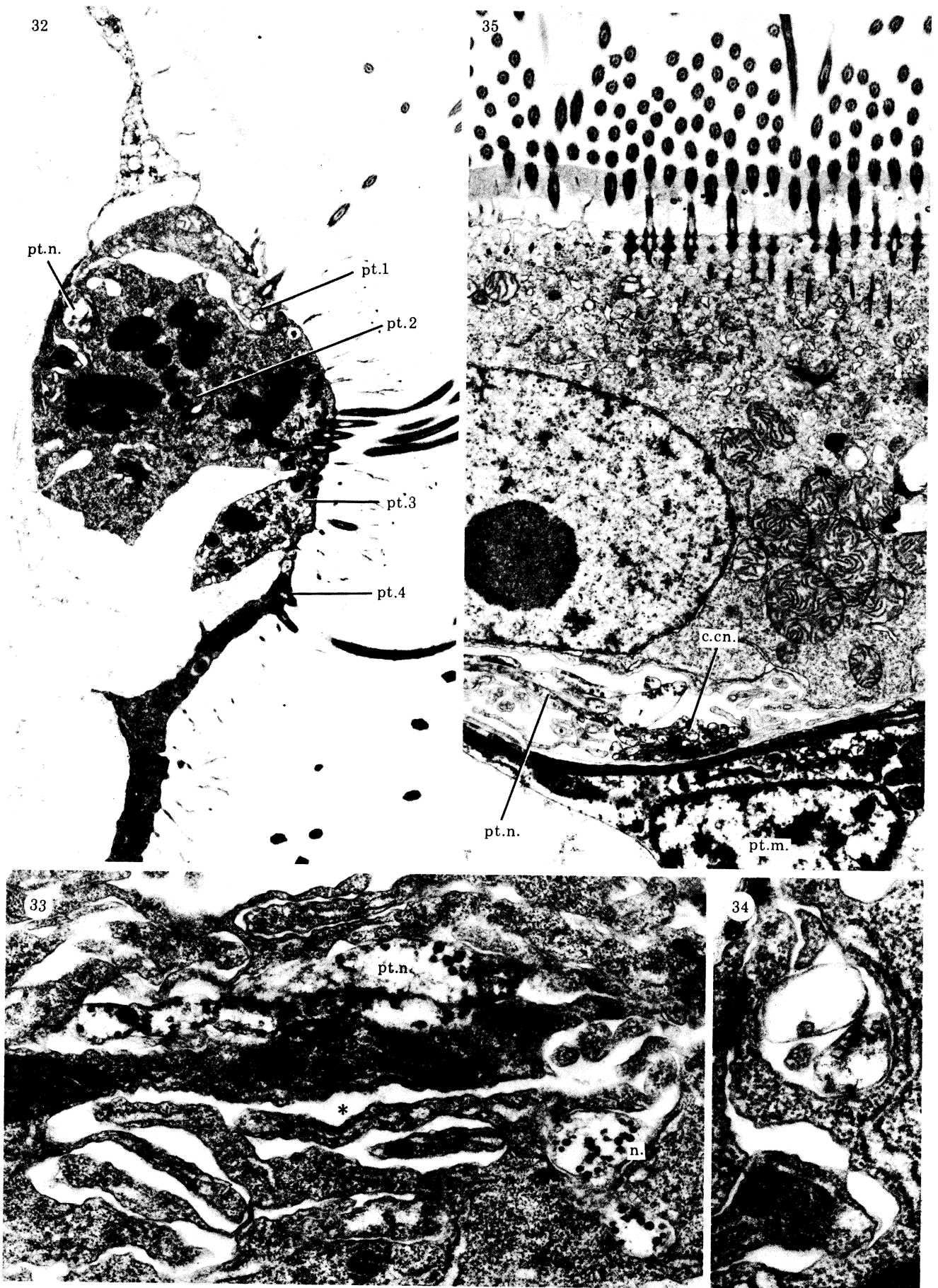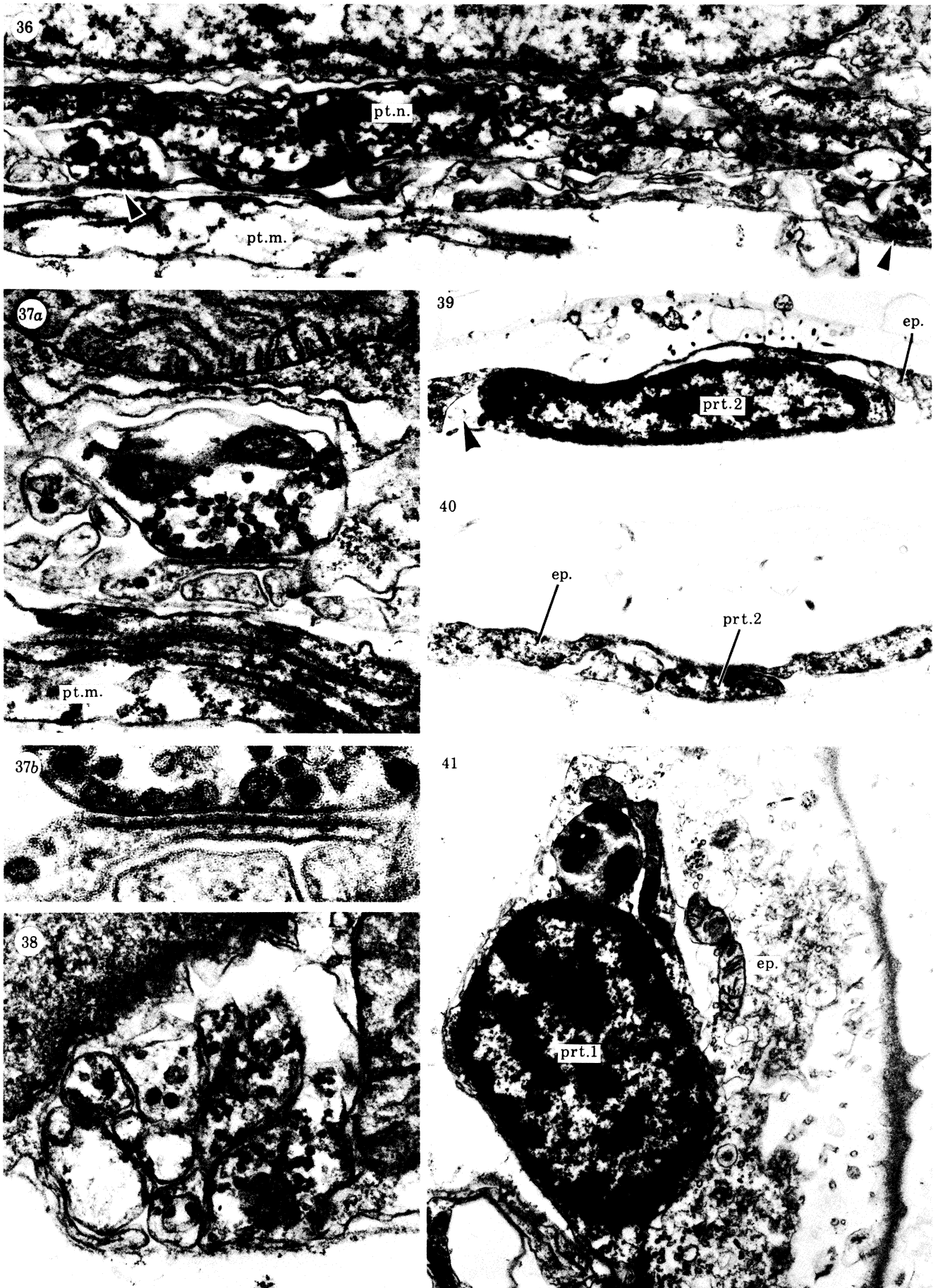FIGURES 29 AND 30. For description see p. 98.

FIGURE 31. (a) Summary diagram showing the neural structures of the episphere of a 48 h trochophore. Includes the apical organ (a.o., shown with three cells), the pretrochal cells (prt. 1–4) and their nerves (n. I–III), and the prototroch nerve (pt.n.). The ventral midline is indicated by a broken line. (b) Diagram as in (a) with fibre counts at strategic points giving (top to bottom) the number of neurites in the 24 h, 48 h, and metatrochophore stages.

FIGURES 32–35. For description see p. 99.

FIGURES 36–41. For description see opposite.

The prototroch nerve is associated primarily with tier 2, and runs along a small channel in the back of the tier 2 cells (figure 34) covered secondarily by the flattened processes from tier 1. In young trochophores, during the period of blastocoel expansion, tier 2 cells are spindle-shaped and may wrap partially around the tapered ends of neighbouring cells of the same tier, which are themselves drawn out along the axis of the nerve and wrapped around it. It is therefore not unusual to find the nerve wrapped by two concentric layers of trochal cell cytoplasm, the two layers belonging to two adjacent cells. By 48 h, a complex system of ridges and folds develops at the back of the tier 2 cells in the area around the nerve (figure 33), and this is the arrangement that persists through to the later stages (figures 36, 37).

Neurites of the prototroch nerve are highly irregular in outline, which makes it difficult to trace individual fibres. At 48 h the neurites are only sparsely supplied with vesicles (figures 33, 34) except at one location, at the junction with pretrochal nerves I and II, and no synapses or synapse-like cell junctions were encountered. In the metatrochophore, the neurites have far more vesicles (figure 36), and synapses occur at regular intervals between the neurites and flattened profiles belonging to the layer of cell processes closest to the basement membrane (figures 36, 37), which almost certainly belong to tier 1 cells. The fact that synapses are found nowhere else among the maze of folds and processes produced by the tier 2 cells suggests that the synaptic interaction is exclusively with tier 1. Two additional features of the trochal band of interest in the metatrochophore are its muscle, and the junction between the trochal nerve and circumoesophageal connective. The prototroch muscle runs around the inside of the prototroch at the level of tier 2 (figures 35–37) and comprises two cells whose nuclei lie on either side of the larva, at the two points where the circumoesophageal connectives cross the prototroch nerve (figure 35). With regard to the two intersecting nerves, the prototroch nerve

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DESCRIPTION OF PLATES 6 AND 7

FIGURE 32. Section through the prototroch at 48 h. Shows the four tiers of prototroch cells (pt. 1–4), each with cilia, and the prototroch nerve (pt.n., detail in figure 34). Magn. × 12150.

FIGURE 33. Tangential section through the prototroch nerve (pt.n.) at 48 h as it passes among the folds and ridges on the inner surface of tier 2. The figure also shows the deep cleft (*) that develops in the tier 2 cells, that separates the upper and lower parts of the cells. It may be significant that the prototroch nerve is always restricted to the upper part of tier 2 while, in the one specimen in which a process from the metatrochal nerve cell was traced into the prototroch, the process (n.) associated preferentially with the lower part of tier 2. Magn. × 20540.

FIGURE 34. Section through the 48 h prototroch nerve, a detail of figure 32. Magn. × 49500.

FIGURE 35. Section in the plane of the prototroch of a metatrochophore showing the large nucleus of one tier 2 cell and a typical distribution of organelles with, in sequence from the apical surface, regions containing predominantly rootlets, golgi bodies, vesicles, and mitochondria, with the nerve (pt.n.) at the basal surface. Also shows the prototrochal muscle (pt.m.) and the circumoesophageal connective (c.cn.). Magn. × 12260.

FIGURE 36. Section in the plane of the metatrochophore prototroch, orientation as in figure 35, showing an enlarged view of the prototroch nerve and its neurociliary synapses (arrows). Magn. × 30410.

FIGURE 37. (*a*) One of the synapses in the metatrochophore prototroch. Magn. × 52000. (*b*) A detail of (*a*). Magn. × 118400.

FIGURE 38. Section through pretrochal nerves I+II just above their point of entry into the prototroch in the metatrochophore. Magn. × 50850.

FIGURE 39. Pretrochal cell 2 at 48 h. The two other fibres of nerve I (arrow) pass alongside. Magn. × 13075.

FIGURE 40. Pretrochal nerve I at 48 h showing its three fibres. Magn. × 31080.

FIGURE 41. Pretrochal cell 1 in a metatrochophore. Magn. × 16300.

passes immediately adjacent to the connective on both sides, and at least one of its neurites makes direct contact and swells en passant at that point. There was, however, no indication of any exchange of fibres between the prototroch nerve and either of the two connectives.

(b) The pretrochal system

Though its principal function appears to be the innervation of the prototroch, the prototroch nerve is not an independent neural entity, but is, rather, only a part of an interconnecting system of trochal and pretrochal fibres that cross the episphere and pass into the apical organ (figure 31 *a*) and that originate entirely outside the prototroch. A comparatively complete and consistent picture of the composition of nerves in this system has emerged from tracings and counts of
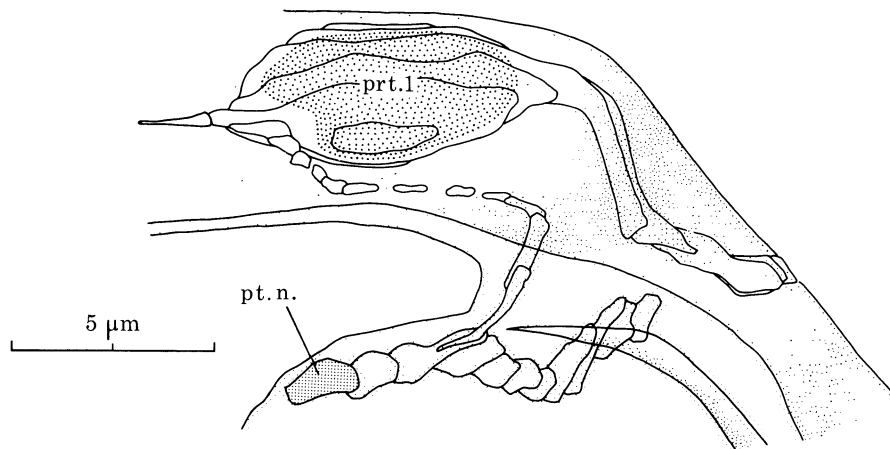
FIGURE 42. Reconstruction from sections of pretrochal cell 1 at 48 h showing the entry of its fibre into the proto-troch nerve. The cell nucleus is heavily stippled, the front surface of the reconstruction is lightly stippled.

the fibres in each (figure 31 *b*), and four cells, the pretrochal cells (prt. 1–prt. 4), appear to be responsible for most of the fibres. All four cells and their fibres are basiepithelial, that is, they lie beneath the epithelium adjacent to the thin basement membrane (figures 39–41). They are the only cells in the larval nervous system that do not develop as an integral part of (i.e. buried within) the epithelium with which they are associated. This raises at least the possibility that these cells migrate to some extent along the inside surface of the episphere during their development, but cell migration appears otherwise not to be involved in the development of the nervous system.

Beginning in quadrant A, the first cell, prt. 1, is a flask-shaped cell with a single surface process, apparently with no cilium, that emerges just above tier 1. Prt. 1 contains large granules (figure 41) that make it somewhat refractile, and it can be seen fairly easily in live larvae. Its principal process wanders to the midline and there enters the prototroch nerve. In two larvae, an additional small process was detected leaving the cell toward the apex, but in both cases this ended within a few micrometres.

The second cell, prt. 2 (figures 39, 40), lies in quadrant B, is bipolar (see Lacalli 1981 for a tracing), and is responsible for establishing the path of nerve I, which at 24 h is made up of only one fibre. By 48 h, two additional fibres have been added (figure 40) that evidently originate in the prototroch nerve and travel along prt. 2 to the apical organ.

The third cell, prt. 3, lies on or just in front of the boundary between quadrants B and C, and functions as the receptor cell for the single larval eye. It bears a cluster of microvilli enclosed

by a cup-shaped mass of pigment granules formed by the adjacent pigment cell. Nerve II consists of the processes of the bipolar receptor cell, two processes that appear to originate in the apical organ (Lacalli 1981), and a fourth process derived from nerve III. Nerves I and II join near the prototroch (figure 31 a) and then enter it as a unit. In most larvae, and in the young trochophore in particular, it is at this region of the prototroch that the largest number of fibres and greatest concentration of vesicles occur.

The fourth cell, prt. 4, is bipolar, lies in quadrant D, and is responsible for the single fibre found in nerve III. The nerve approaches the apical organ, but then bypasses it (an early stage in this process is shown in Lacalli 1981, figure 3) and enters nerve II instead. In the metatrochophore, this same fibre can be traced into the brain, which it enters on the right side.

If we assume that the neurites of the prototroch nerve are derived from the pretrochal nerves, and probably from one or more of the pretrochal cells, it remains to identify the specific cells involved. Based on the number of neurites in the protroch nerve, it seems unlikely that all four pretrochal cells contribute equally. A major role for the processes descending from the apical organ via nerve II is also unlikely since the prototroch nerve appears to have a full complement of fibres long before these have reached it, i.e. at 24 h. Two of the pretrochal cells, prt. 1 and 3, are judged the most probable source of prototrochal neurites on the basis of their ultrastructural characteristics. These two cells, and their processes, are pale and nerve-like in appearance in both the trochophore and the metatrochophore. In contrast, prt. 2 and 4, and their processes, are comparatively dense and rather unlike the prototrochal neurites in general appearance.

In summary, the prototroch and pretrochal nerves form a single network or system whose fibres are derived chiefly from four cells. The system supplies the prototroch and links it, by several routes, to the apical organ and the eye. It is important to note that all four of the cells involved lie outside (i.e. anterior to) the prototroch, and that none of the cells of the trochal band itself can be ascribed any neural function. Further, though the nerves appear to be better developed and more active in older larvae (e.g. in the metatrochophore), the number of fibres in the system changes little if at all during larval development. There are two features of potential functional importance: (i) the concentration of vesicles on the right side of the prototroch nerve at early stages associated with nerves arriving from the eye and apical organ, and (ii) the presence of neurociliary synapses in the metatrochophore, but not in the young trochophore.

An additional point of interest is the apparent difference in the arrangement of the pretrochal nerves in *S. spinosus* as compared with *S. polycerus*. In the latter, nerve II and at least the lower portions of nerve I are always visible in live larvae, and are particularly noticeable in older larvae. In *S. spinosus*, nothing corresponding with nerve I is ever clearly visible and, instead, several cells and a slender nerve-like thread appear just above the cell corresponding in location to prt. 1. The thread appears to pass into the apical organ. This suggests that a ventral pretrochal nerve may be present in *S. spinosus*, but if so, it probably lies to the left of the midline rather than on the right as in *S. polycerus*.

3.4 *Innervation of the pharynx and associated feeding structures*

(a) *Pharyngeal nerves*

The pharyngeal nervous system is entirely intraepithelial. Its nerves generally run beneath the epithelium (e.g., figures 53, 62–64), but in many places are enclosed wholly or in part by flattened extensions from adjacent epithelial cells. The nerves are easily traced and follow

generally predictable paths, shown here in two reconstructions (figures 50, 61) and summarized in figure 43. The nerve cells are more difficult to deal with in a comprehensive fashion. This account focuses on the 48 h pharynx because the cells responsible for the nerves are conspicuous and easily identifiable at this stage. There are nine such cells in the two 48 h larvae examined (ph. 1–ph. 9 in the figures) whose appearance and arrangement at this stage in treated in detail (figures 44, 47–54 and accompanying text). The pharynx continues to develop after 48 h, becoming increasingly complex and difficult to interpret as a variety of new cell types differentiate. With regard to the nervous system, later development involves primarily the completion of the secondary pharyngeal nerve and differentiation of a small number of additional nerve cells at points along the existing pharyngeal nerves. The basic organization of the system and its identifiable core elements remain essentially unchanged. The 48 h stage is therefore a suitable subject for a cell by cell analysis of the type employed elsewhere in this study, but it is not fully developed at this stage, and the figures (e.g. figures 19, 43) must be interpreted with this limitation in mind.

When complete (e.g. in the metatrochophore, figure 43 b), the pharyngeal system consists of two nerves that encircle the pharynx, one at its front end and the other at its back, that join basally beneath the basal pharyngeal complex. The primary pharyngeal nerve, which constitutes the front half of the system, develops first and is complete by 24 h. Very few if any additional fibres are added to this nerve during later development (figure 43 b). This is initially surprising, since only two of the pharyngeal cells identifiable from later stages are evident at 24 h, and only one of these is a nerve cell. This one cell, the suprapharyngeal cell (ph. 1 in the figures) lies buried in the top of the pharynx just behind the primary pharyngeal nerve (figures 47, 48, 50). It is elongate and flask-shaped, with a surface process bearing a single cilium that projects into the pharyngeal lumen. Its neural processes pass along the midline of the pharynx and via several branches into the primary pharyngeal nerve. Another group of processes, including some apparently also from ph. 1, continue along the top of the vestibule and end at the circumoral muscle, thus forming the median vestibular nerve. At 24 h, the circumoral muscle and prototroch lie very close to one another, and the terminus of the median vestibular nerve is in contact with both of them. It is therefore not clear which structure is the initial target for the nerve during its development. As the food groove develops after 24 h, the circumoral muscle and prototroch becomes progressively separated, and it is then apparent that the nerve innervates the circumoral muscle only (figures 49, 50), and not the prototroch.

The only other cell clearly identifiable at 24 h is a flask-shaped cell filled with densely stained vesicles located just to the left of the basal midline of the pharynx. The cell has a tapering surface process with microvilli, but no cilium, and the basal part of the primary pharyngeal nerve passes just beneath it. It is clearly the 24 h equivalent of the dense vesicle cell (ves. in most figures, but designated ph. 3 in figures 43 and 44) identified at 48 h (figure 54) and in the metatrochophore (figures 63, 64). This cell is unique in the organism. Its apical cytoplasm is filled with the dense vesicles (figure 63), and basally there are distinctive dense profiles of endoplasmic reticulum (figure 64). In the 48 h stage and later, it is responsible for several slender, dense processes that enter the pharyngeal nerve and travel for some distance in it (figure 65). These are evidently not neurites, at least not of a conventional type. They contain no vesicles, instead being filled with a fine reticulum of dense strands, and closely resemble processes belonging to the dense auxiliary cells of the apical organ (compare figures 29 and 65). One or two profiles of this type are typically found in most sections through nerves in the pharynx and suboral region, but it is not clear what proportion of these arise from the dense vesicle cell.
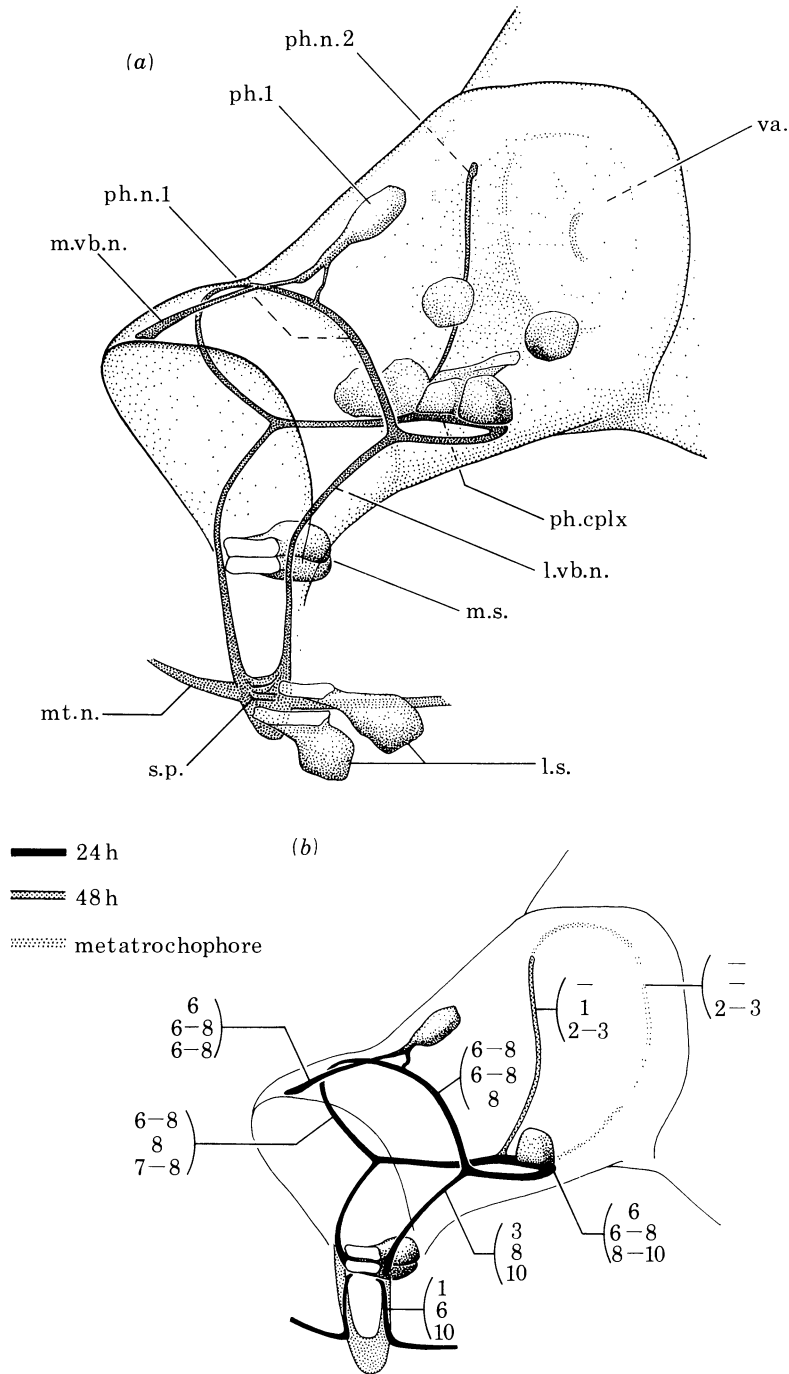
FIGURE 43. (a) Optical section through the pharynx and suboral region of a 48 trochophore from the left side showing the pharyngeal and suboral nerves, and the cells associated with them. Nerves are: the median and lateral vestibular nerves (m.vb.n. and l.vb.n.), the primary pharyngeal nerve (ph.n. 1), which passes through the basal pharyngeal complex (ph.cplx), the partially developed secondary pharyngeal nerve (ph.n. 2), the suboral plexus (s.p.), and the metatroch nerve (mt.n.). Cells include: the median and lateral suboral cells (m.s. and l.s.), the suprapharyngeal cell (ph. 1), and cells in the pharyngeal complex and the lateral walls of the pharynx (ph. 3–9, arranged as in figure 44 but with ph. 2 deleted since it hides the other cells). With further development, the loop of the secondary pharyngeal nerve is completed as in (b). (b) Diagram showing the sequence of development of nerves in (a). Fibre counts are given at strategic points for (top to bottom) the 24 h, 48 h, and metatrochophore stages. The system as a whole is well developed at 24 h, and lacks only the lower portion of the lateral vestibular nerves, the suboral plexus, and the secondary pharyngeal nerve. The four cells shown are cells that appear to be particularly important in the early development of the system (see text).
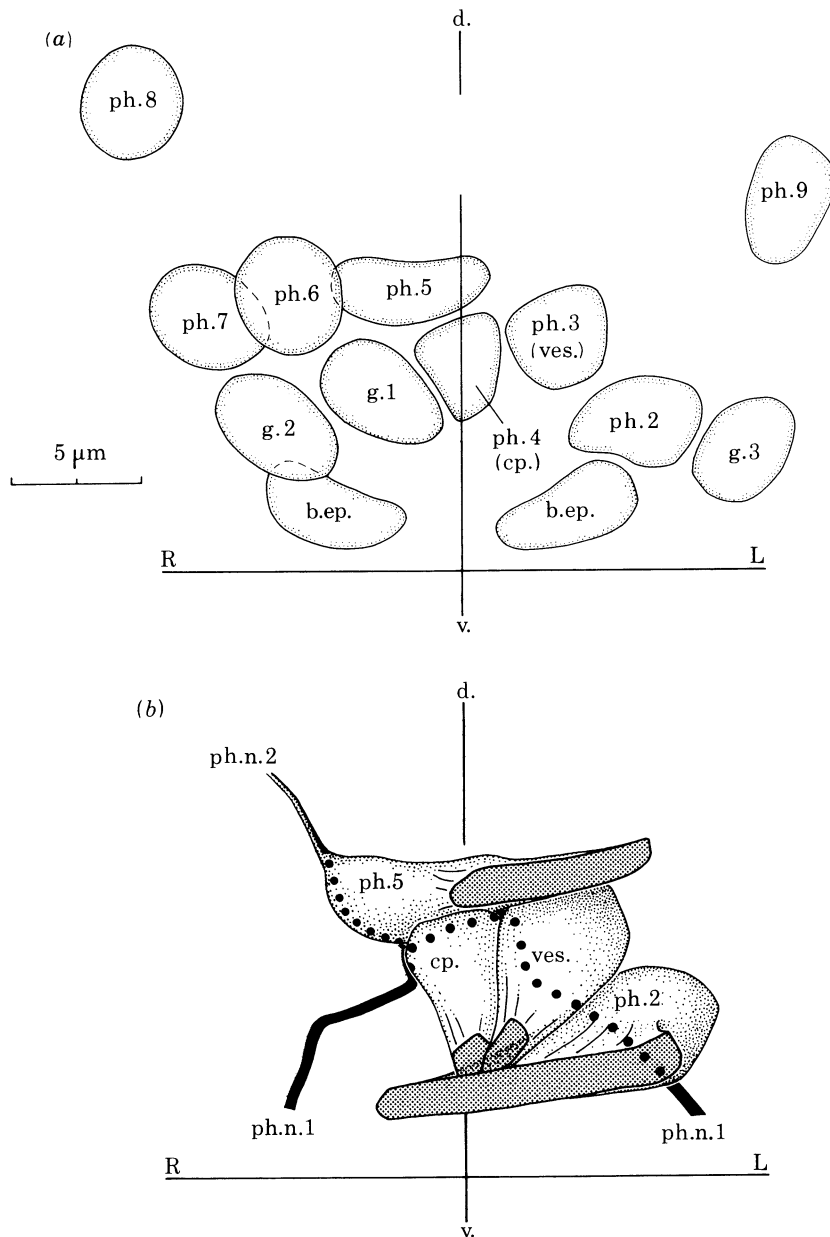
FIGURE 44. Reconstruction of the basal pharyngeal complex of a 48 h trochophore. Shows the basal half of the pharynx laid out flat and viewed from above. The horizontal line is the front margin of the pharynx, the vertical line is its basal midline. Additional reconstructions and details of the pharynx in this specimen are shown in figures 47–51, 53 and 54. (a) Shows cell nuclei of eight of the nine pharyngeal nerve cells in this specimen, the three non-neural glial-like cells (g. 1–3) associated with the basal part of the primary pharyngeal nerve, and two basal epithelial cells (b.ep.). Two of the cells, ph. 3 and 4, are given separate designations. As indicated, they are, respectively, the dense vesicle cell (ves.) and its companion cell (cp.). (b) Shows cell outlines for the four cells that form the core of the basal pharyngeal complex at this stage, ph. 2–5, and the path of the basal part of the primary pharyngeal nerve as it passes beneath them. Follows the conventions used in figure 20.

If there are only two cells to contribute to it, the neurites of the pharyngeal nerve at 24 h clearly cannot be accounted for on the basis of one or two fibres per cell. Good fixation is difficult to achieve at 24 h, however, and though ph. 1 is the only unequivocally identifiable nerve cell at this stage, the existence of other such cells in the pharynx cannot entirely be ruled out. But by 48 h, when the nerve cells and their neurites are clearly distinguishable, there is still an

insufficient number of cells with well developed neurites to account for the fibres of the primary pharyngeal nerve. Ph. 1 may then be responsible for most or all of the fibres at 24 h. If so, its fibres must either wrap repeatedly around the pharynx, or several branches must form and run in parallel along the same path. In fact, several branches do emerge from the cell body of ph. 1, but their individual paths could not be traced once they entered the pharyngeal nerve.

The arrangement of pharyngeal cells in the basal half of the pharynx of a 48 h larva is shown in figure 44. A second larva examined was identical except for minor variations in cell position, principally the shifting of ph. 5–7, as a group, to the left, which places ph. 5 behind ph. 3 and ph. 4 and 6 in direct contact.

Of the eight identifiable pharyngeal cells shown in figure 44, only the four forming the core of the pharyngeal complex, ph. 2–5, are uniquely identifiable at 48 h on the basis of appearance alone. The distinctive features of one of these, ph. 3, the dense vesicle cell, are discussed above. Its immediate neighbour on the right, ph. 4 (the companion cell, cp. in the figures), is also rather distinctive. At 48 h, ph. 4 is small, dense, and angular, and several dense processes similar to those of the dense vesicle cell arise from it and enter the pharyngeal nerve. In the metatrochophore, ph. 4 still has a dense nucleus, but highly extracted cytoplasm (figure 64). Two sensory cells, ph. 2 and 5, lie immediately in front of and behind ph. 3 and 4. Both have surface processes that expand to form long, narrow ciliary fields, each bearing a single row of cilia (figure 51), that span the base of the pharynx. The pharyngeal nerve passes directly under the front cell, ph. 2, a pale nerve-like cell with basal processes that enter the nerve. The back cell, ph. 5, is of similar morphology but type 1 ultrastructure (§3.6), and looks very much like suboral cell s. 3 in the specimens examined. It is not clear whether ph. 5 makes a significant contribution to the primary pharyngeal nerve, but it produces one vesicle-filled process that passes back along the side of the pharynx (figures 43, 44*b*) that appears to be the pioneer fibre for the developing secondary pharyngeal nerve. Ph. 2 is identifiable in the metatrochophore by position and morphology, which are both unchanged. The region behind ph. 3 and 4 is occupied by a rank of five to six cells of sensory type, each with a single row of cilia and all oriented in parallel. Only the first one or two of these contribute to the pharyngeal nerve so far as could be determined.

While ph. 3 and 4 are evidently not nerve cells, and ph. 2 and 5 are probably sensory, the remaining pharyngeal cells, ph. 6–9 appear to be proper nerve cells. They are all of one type (e.g. ph. 7 in figure 53), essentially identical in type to ph. 1, and are therefore indistinguishable from one another except by position. All have a small surface process bearing a single cilium, and all appear equally well differentiated except with regard to their neurites. In the 48 h larvae examined, only the two cells nearest the pharyngeal complex, ph. 6 and 7, had neurites, and these were far less substantial than those of ph. 1. Ph. 8 and 9 were without neurites. The cells identified as nerve cells in the metatrochophore are of similar appearance (figure 66) and occupy roughly comparable positions, though one or two additional cells were encountered. All the cells at this stage have neurites that enter the adjacent parts of the pharyngeal nerve, including the two cells nearest the pharyngeal valve, whose fibres enter the secondary pharyngeal nerve. Presumably these are the metatrochophore equivalents of ph. 8 and 9.

As it enters the pharyngeal complex, the primary pharyngeal nerve passes along the underside of three rounded, moderately dense cells (g. 1–g. 3 in figure 43*a*, see also figure 53) referred to here as glial-like cells. This is not meant to imply any detailed understanding of their function, but they associate quite specifically with the nerve, they appear not to be neurons, and they are not found elsewhere in the pharyngeal nervous system; the nerves instead

simply run beneath the generalized epithelial cells of the vestibule and pharynx. Equivalent cells were tentatively identified in the metatrochophore, that were also apparently not neurons.

The pharyngeal nerves contain fibres of several types. Their neurites have irregular profiles in section and contain vesicles and occasional mitochondria. In the trochophore, the neurites are comparatively small, and the vesicles are irregularly scattered along their length. They are larger in the metatrochophore (figures 65, 68), and vesicles may be present in considerable
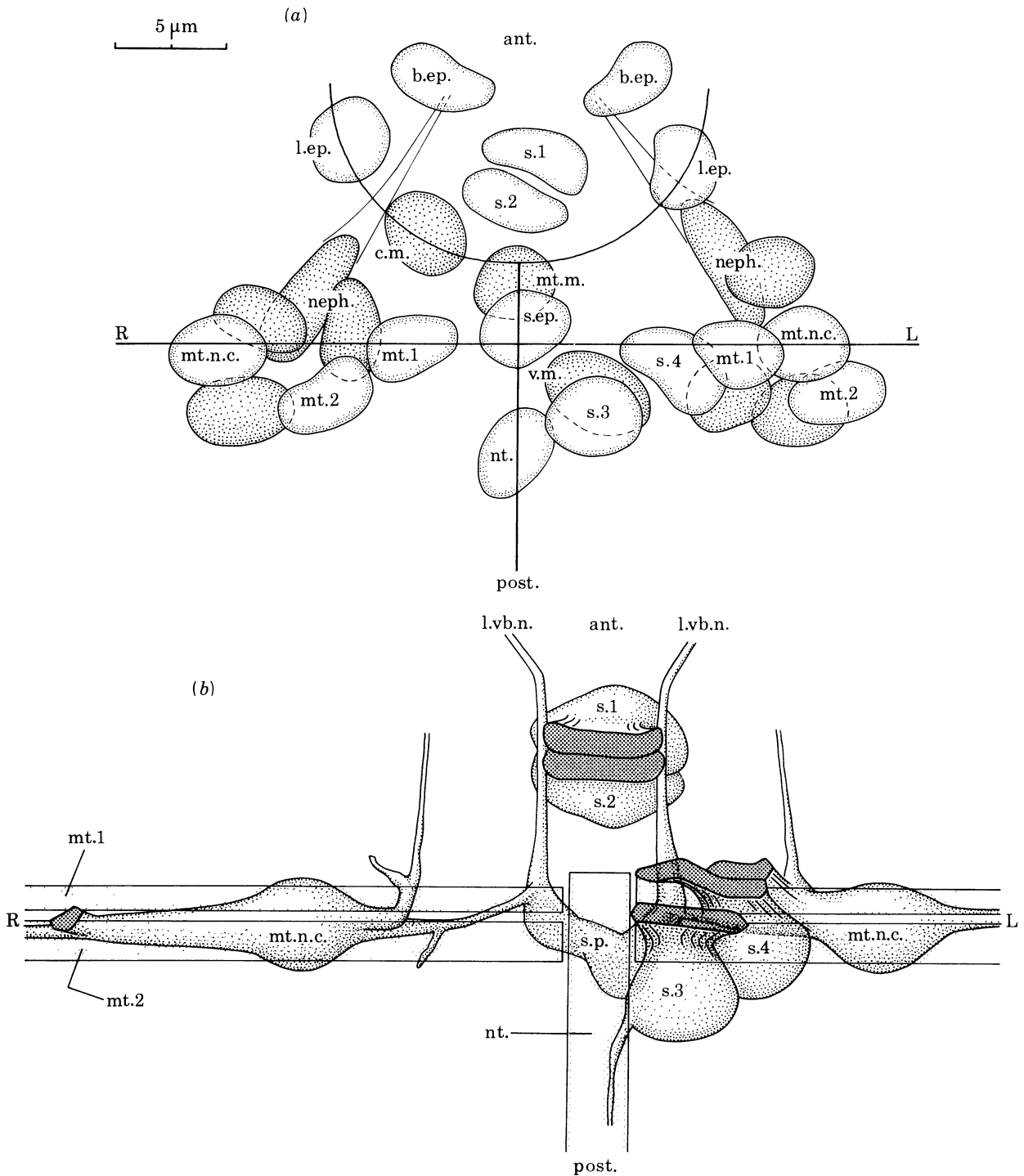
FIGURE 45. For description see opposite.

concentrations. The total number of fibres in pharyngeal nerves rarely exceeds 8–10 regardless of stage (figure 43*b*). Of these, usually 5–6 will be of a single type, with vesicles and very sparse cytoplasm, i.e. like the larger profiles in figure 68. Among the remaining fibres are vesicle-containing neurites of somewhat greater density, and dense fibres without vesicles. In the region of the pharyngeal complex, some of the latter belong to ph. 3 and 4, but elsewhere along the primary pharyngeal nerve dense fibres traceable to suboral cells (e.g. to s. 2) were also encountered.

At 48 h no synapses or similar specialized junctions were encountered anywhere along the pharyngeal nerves. Two types of specializations occur in the metatrochophore. (1) There are synapse-like junctions (figure 68) between neurites in the primary pharyngeal nerve and the adjacent pharyngeal muscle at several points. These are characterized by pre- and post-junctional membrane specializations, between which the basement membrane passes (figure 68*b*), and an accumulation of vesicles. (2) The secondary pharyngeal nerve has no such junctions in the metatrochophore, but does produce a small number of backward-projecting, vesicle-filled terminals (figure 67, also visible in figure 61). These extend into the spaces between the cells of the pharyngeal valve, generally some micrometres distant from the base of the epithelium, so they make no contact with adjacent muscle cells. Even at their point of closest approach to the tissue of the oesophagus, no contacts between these terminals and the oesophageal muscle cell could be found. If the oesophagus is controlled by pharyngeal nerves, the stimulus must come by release of transmitter at a distance and diffusion, rather than by direct contact and specific junctions. The only other possible source of innervation for the oesophageal muscle is a single multipolar cell located at the base of the stomach in the metatrochophore. This lies adjacent to the point of entry of the intestine into the stomach, which is offset to the right side, and small vesicle-containing processes from this cell wander forward to the base of the back part of the oesophageal muscle and end there. There is no connection between this cell and the pharyngeal system at the metatrochophore stage. No equivalent cell was found in the trochophore, nor were any other neural elements identified in association with the oesophagus, stomach, intestine or anus.

(*b*) *The suboral complex*

The suboral complex (figure 45) consists of two nerves, the lateral vestibular nerves, that unite to form a suboral plexus, and four suboral sensory cells. The system as a whole is

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FIGURE 45. Reconstruction of the suboral complex and associated cells of a 48 h trochophore, the same specimen as in figure 44. Additional reconstructions and details of the suboral region in this specimen are shown in figures 52 and 55–57. (*a*) Shows all of the cell nuclei, of both ectodermal and mesodermal derivatives. Ectodermal derivatives (stippled edges) include the lateral and basal epithelial cells (l.ep. and b.ep.) of the vestibule, the two tiers of metatroch cells (mt. 1 and 2), the top neurotroch cell (nt.), the median suboral epithelial cell (s.ep.), the metatrochal nerve cells (mt.n.c.), and the suboral cells (s. 1–4). Mesodermal derivatives (uniformly stippled) include the circumoral (c.m.), ventral (v.m.) and metatrochal (mt.m.) muscle cells, the terminal cell and accessory cell of each protonephridium (neph., the terminal cell nucleus is the more elongate one in each case, and the process attaching it beneath the pharynx is also shown), and two pairs of undifferentiated mesodermal cells (unlabelled) that are the precursors of the longitudinal muscles, which have yet to develop in this specimen. The horizontal line in the figure is the junction between the two tiers of metatroch cells, the vertical line is the midline of the neurotroch, and the curve traces the middle of the circumoral muscle, that is, it shows the effective margin of the mouth. (*b*) Shows cell outlines for the nerve cells in (*a*), their nerves, and the suboral plexus (s.p.), following the conventions used in figure 20. Ciliary fields of metatroch and neurotroch cells are shown by uniformly stippled rectangles. The ciliary field of the suboral epithelial cell (s.ep. in (*a*)) is not shown, but would extend from the top of the neurotroch to the bottom of the surface process belonging to s. 2. A portion of the metatroch nerve is obscured by s. 4; it passes behind s. 4 on its way to the left side of the suboral plexus.

remarkably similar from larva to larva. At 48 h (figures 45, 52, 55–57) its cells are individually identifiable and invariant in position in the specimens examined. Comparable cells occupy identical positions at 24 h (figure 46) and in the metatrochophore (figures 69–71). Indeed, with regard to the details of structure and cell associations, the suboral complex is the least variable part of the larval nervous system, and the least changed by later developmental events.
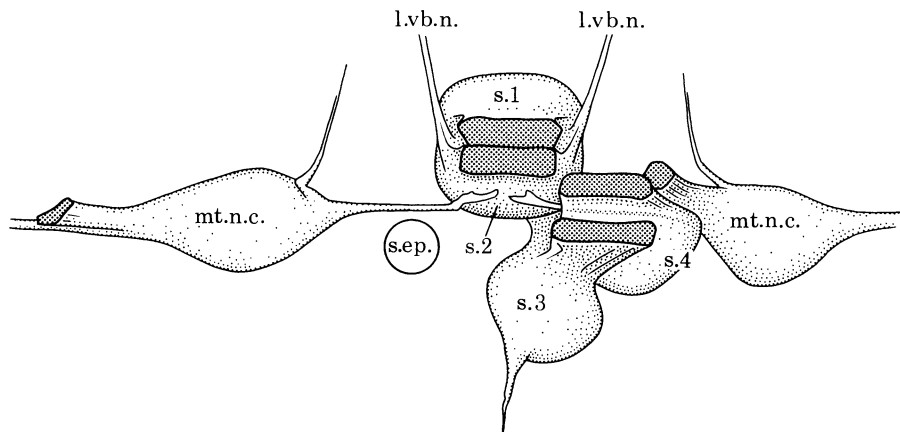
FIGURE 46. Cells and nerves of the suboral complex at 24 h, labelled as in figure 45. The circle indicates the position of the suboral epithelial cell nucleus. The metatroch nerve simply terminates beneath s. 2, and the suboral plexus is as yet undeveloped.

The suboral and pharyngeal systems are linked by the two lateral vestibular nerves, which join the primary pharyngeal nerve on either side about a third of the way up the side of the pharynx (figure 43). The two nerves enter the suboral region beneath the two flattened basal epithelial cells that form the floor of the vestibule. They then pass on either side of the two median suboral cells and the suboral epithelial cell located just below these, and join at the suboral plexus. Figure 45 shows the arrangement of nerves and suboral cells in one 48 h larva. A second larva that was reconstructed was identical in all respects at the level of detail shown in the figure. All four suboral cells have surface processes that expand to form a narrow, horizontally oriented ciliary field bearing a single row of cilia. The two median suboral cells, s. 1 and 2, lie just inside the lower lip of the vestibule. These are responsible for establishing the initial connection between the suboral and pharyngeal systems. At least one of these, and possibly both in some larvae, produce lateral processes that by 24 h extend beneath the vestibular epithelium to the pharyngeal nerve. The lower half of the system forms later, and at 24 h, the only neurites found below the level of the median suboral cells belonged to the metatroch nerve. The plexus is well developed by 48 h, and includes fibres derived from the metatroch nerve, the suboral cells, and fibres from the pharyngeal nerve that have grown down the lateral vestibular nerves into the suboral region. It is not clear whether both of the median suboral cells make equally important contributions to the suboral system. In the one 48 h stage reconstructed in detail (figure 52), s. 2 produced lateral processes on both sides that travelled

DESCRIPTION OF PLATE 8

FIGURE 47. Typical section through the pharynx and suboral region of a 48 h trochophore of the type used for preparing the reconstructions (figures 48–52). Additional details are shown in figures 53–59. Labelling of cells follows figures 43–56. Magn. × 3250.

FIGURE 48. Reconstruction of the nerves (solid lines) and nerve cells (in outline) of the pharynx and suboral region of the 48 h trochophore shown in figure 47: a key for interpreting figure 50. Magn. × 1470.

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Lacalli, plate 8

47

ph.1

phar.

ph.8

l.ep.

ph.7

g.2

s.1

s.2.

c.m.

mt.m.

s.ep.

48

ph.1

ph.n.1

m.vb.n.

ph.8

v.m.

mt.

ph.n.2

s.p.

ph.6.7

s.3

ph.cplx

m.s.

l.vb.n.

mt.n.c.

s.p.

l.s.

nt.

nt.n.

nt.n.

FIGURES 47 AND 48. For description see opposite.

FIGURES 49 AND 50. Stereoreconstructions of the 48 h trochophore.

FIGURE 49. The external ventral surface viewed obliquely from the right side. Representative cilia are included to show the positions of the trochal bands and the food groove. Also includes the inside contours of the anal vesicle, vestibule, and pharynx. Magn. × 900.

FIGURE 50. Nerves and nerve cells of the pharynx and suboral region, viewed from the same angle as figure 49, and including selected external contours. See figure 48 for a key. Magn. × 970.

FIGURE 51. Reconstruction of the region of the basal pharyngeal complex of the specimen shown in figures 47–50, viewed from the same angle. Follows the conventions used in figure 42, with the inside surface of the pharynx shown by fine dotted lines. (a) Shows the dense vesicle cell (ves., the small patch of parallel lines shows its surface process), the pharyngeal nerve (n.) passing beneath the pharyngeal complex, and one pharyngeal muscle cell (ph. m. 1). (b) Shows the dense vesicle cell and nerve from (a) in outline and, in addition, ph. 2 and 5 with their rows of projecting cilia.

FIGURE 52. Reconstruction of the suboral complex of the specimen in figures 47–50, viewed from the same angle. Shows the complex as a unit (top, nerves and plexus are shaded), and individual reconstructions of the four suboral cells. Note that both neurites of the neurotroch nerve arise from s. 3. A branching process from this same cell enters the suboral plexus and interdigitates (at *, shown in figure 57) with fibres from s. 2.

DESCRIPTION OF PLATE 10

FIGURES 53–56. Details of the pharynx, suboral region and metatroch at 48 h. Labelling follows figures 43–45.

FIGURE 53. Typical pharyngeal nerve cell with its glial-like companion. Shows the association between the latter and the pharyngeal nerve (n.). A detail of figure 47. Magn. × 15330.

FIGURE 54. The dense vesicle cell, the same cell shown in figure 51 a. Note its basal association with the pharyngeal nerve (n.), and the entry of slender dense processes (arrow) from the vesicle cell into the nerve. Magn. × 20040.

FIGURE 55. The suboral region at 48 h. Shows a typical section through s. 3 (the same cell shown in figure 52), its close association with the suboral plexus (s.p.), and the arrangement of surrounding epithelial, trochal, and muscle cells. The small surface profile just to the right of s. 4 belongs to the left metatrochal nerve cell. Magn. × 11190.

FIGURE 56. Section through the right metatrochal nerve cell, which lies between the two tiers of the metatroch. Magn. × 12470.
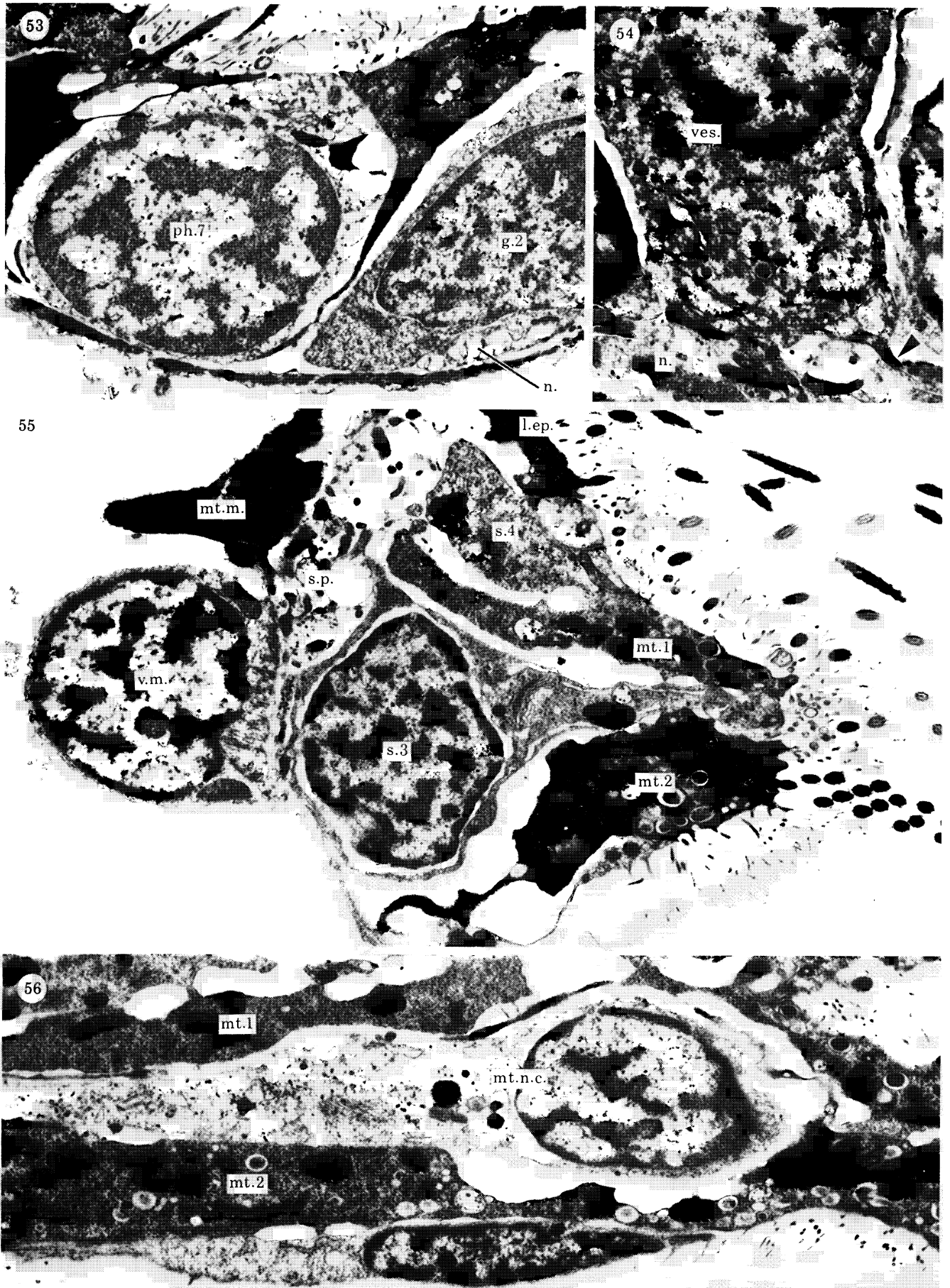
FIGURES 53–56. For description see opposite.
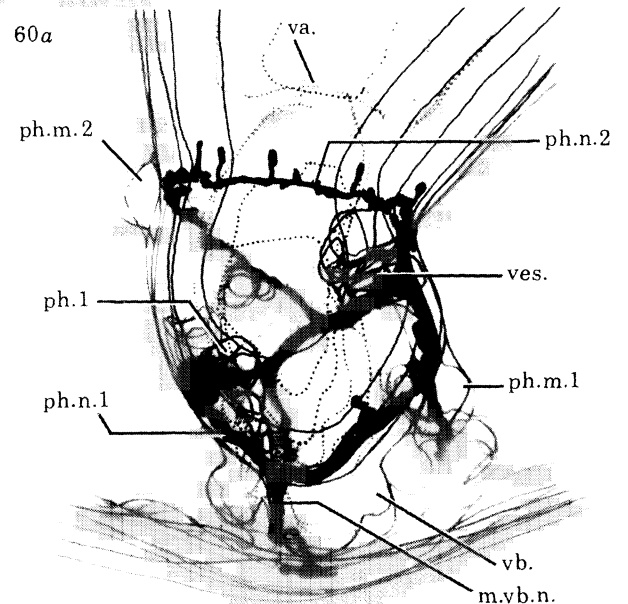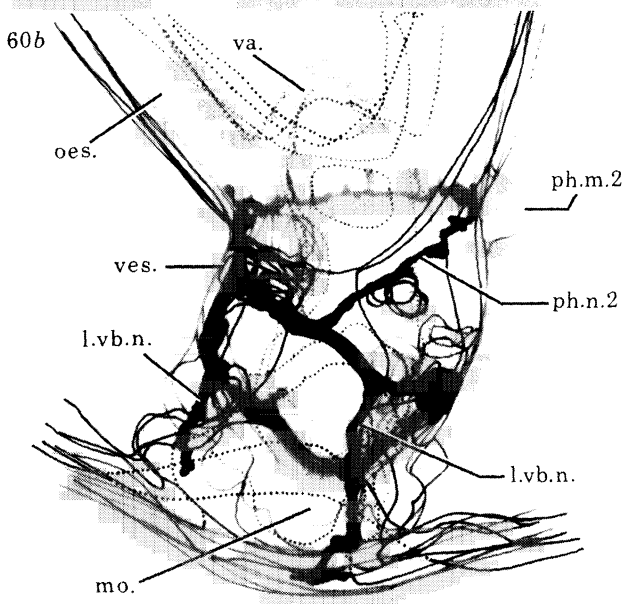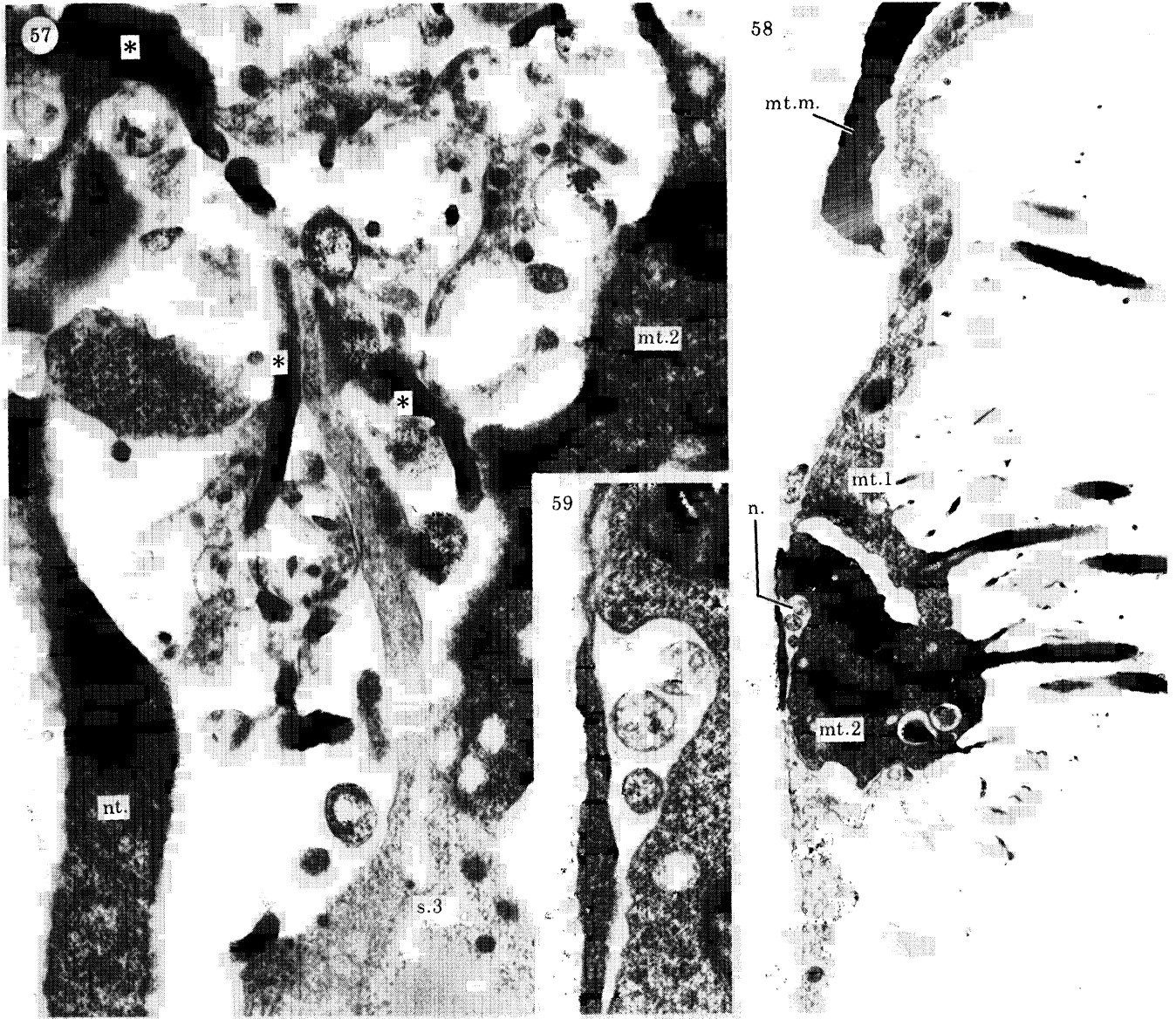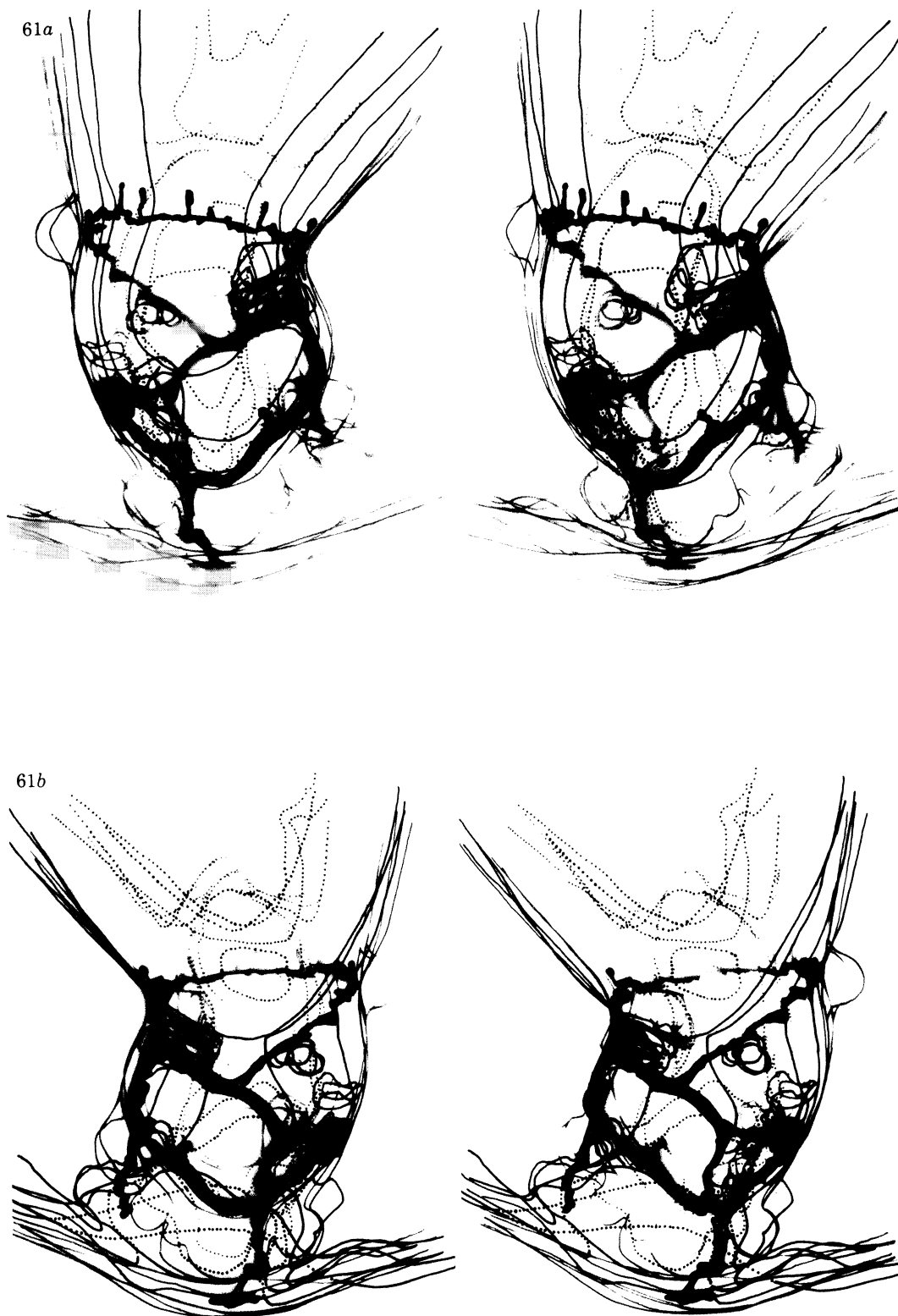
FIGURES 57–60. For description see p. 109.

FIGURE 61. Stereoreconstructions of the metatrochophore pharynx (*a*) from above, magn. × 1780; (*b*) from below, magn. × 1700. See figure 60 for a key.
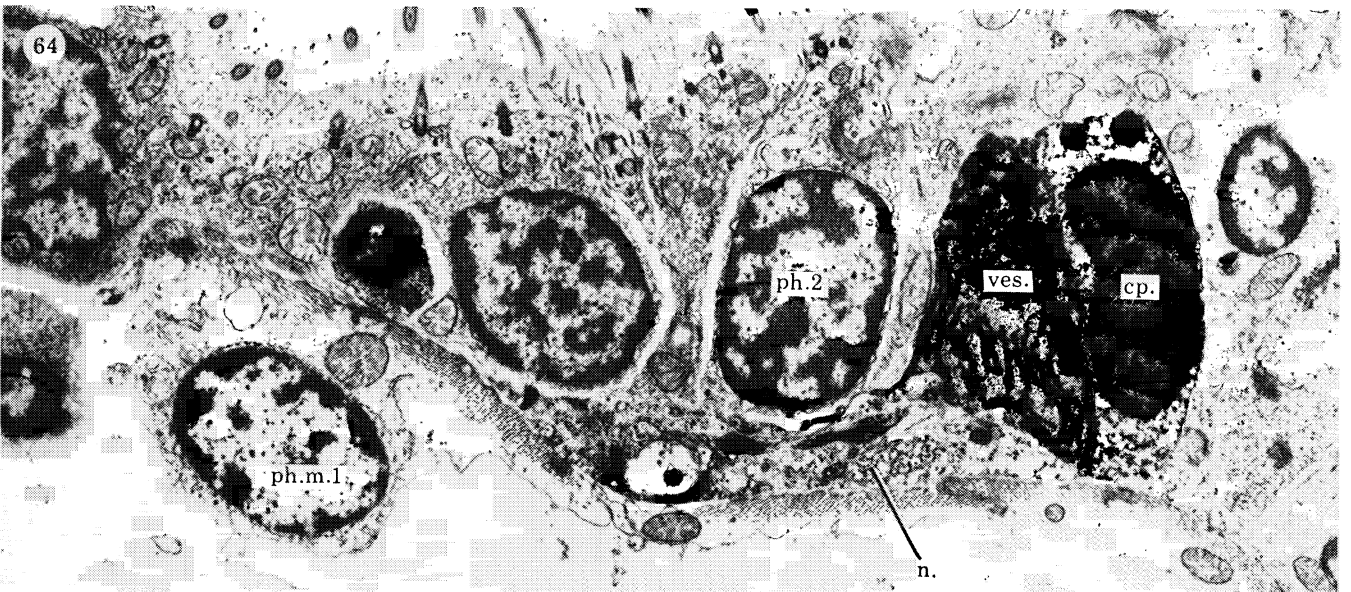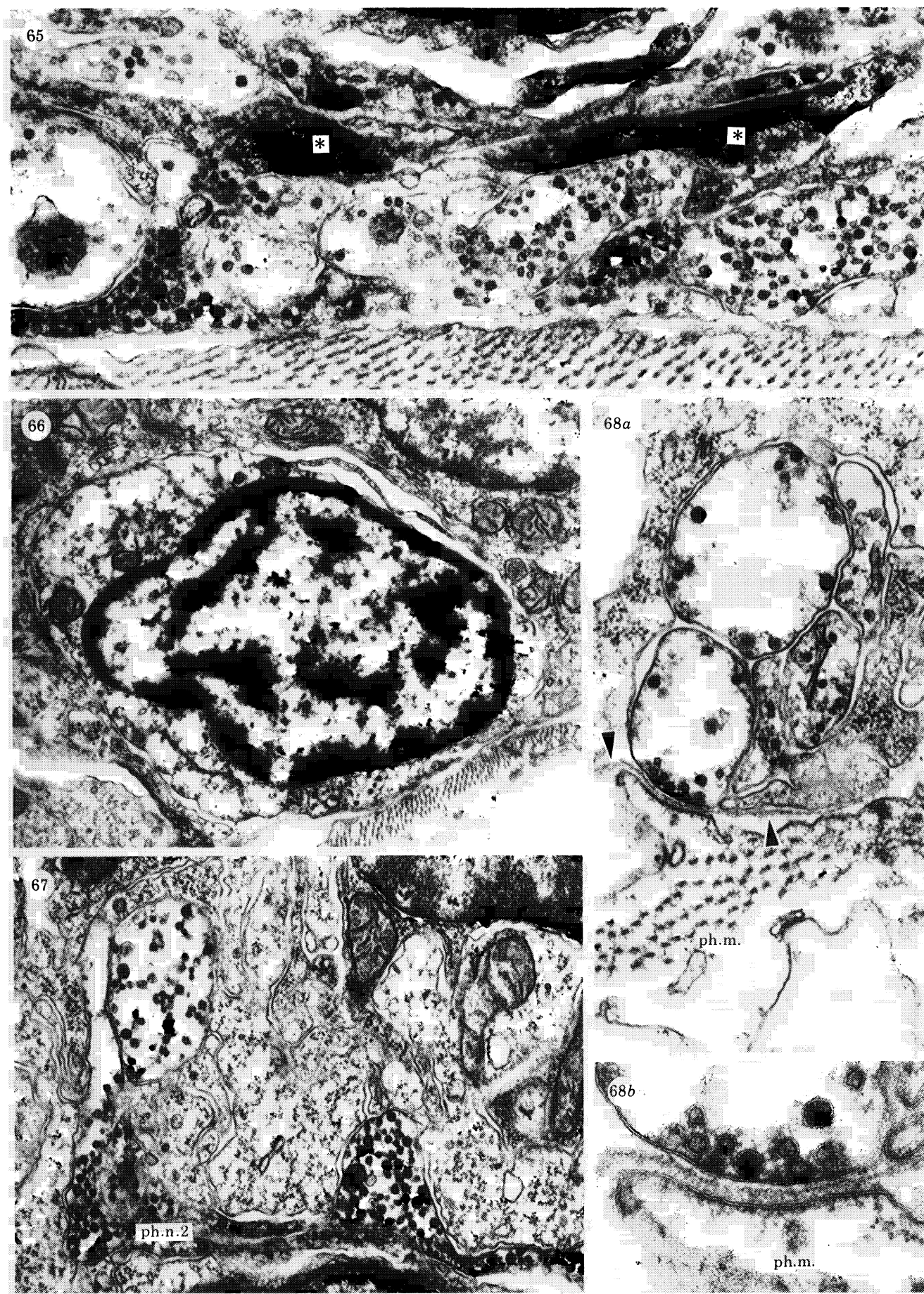
FIGURES 65–68. Details of the metatrochophore pharynx. For description see p. 109.
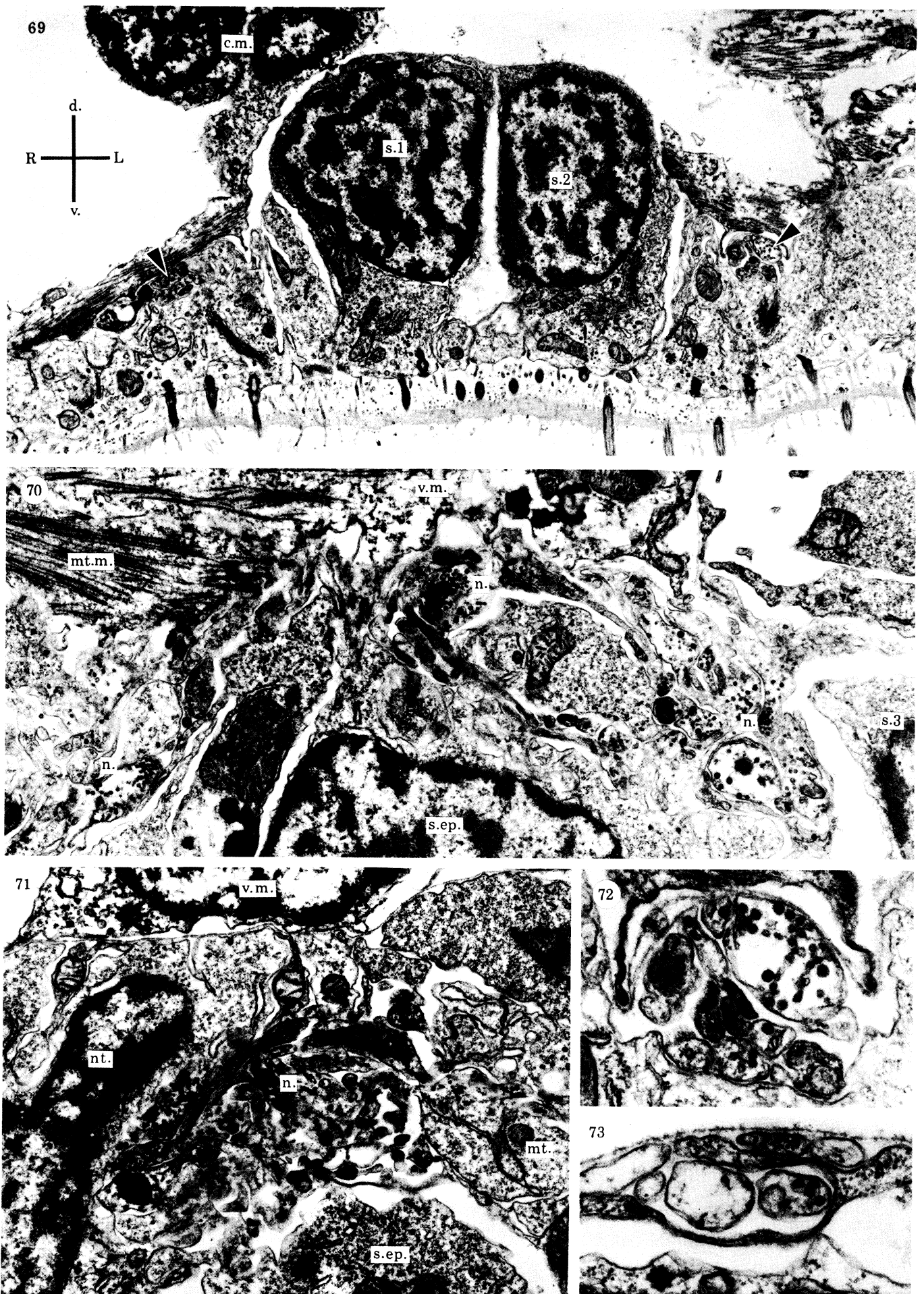
into the pharynx, and into the suboral plexus, while s. 1 was entirely without processes. The two cells were also very different from one another in appearance (figure 47). In both the 24 h and metatrochophore stages examined, the two cells were more nearly similar in appearance (e.g. figure 69), and both appeared to contribute fibres to adjacent nerves. It is not clear whether the differences observed at 48 h are a function of developmental stage, or reflect real variability between larvae.

The two lateral suboral cells, s. 3 and 4, lie on the left side of the suboral region at the edge of the metatroch, and the metatroch nerve passes behind them on its way to the suboral plexus. Their surface processes are precisely positioned relative to the main elements of the metatroch.

DESCRIPTION OF PLATES 11, 13, 14 AND 15

FIGURE 57. Detail of the suboral plexus at 48 h. Shows entry of the single process arising from s. 3 (shown also in figure 52), and its close association with dense processes (*) from s. 2 in this specimen. Some of the remaining processes belong to the metatrochal nerve cells, and some derive from the pharyngeal nerves. Magn. × 41 340.

FIGURE 58. Section through the metatroch at 48 h. Shows the two cell tiers and the metatroch nerve (n.). Magn. × 16 110.

FIGURE 59. The metatroch nerve, a detail of figure 58. Magn. × 62 060.

FIGURE 60. Reconstruction of the metatrochophore pharynx as seen from (a) above and (b) below: keys for interpreting figure 61. Outside contours of the pharynx are shown as solid lines, inside contours are dotted, heavy lines indicate nerves, and selected nerve cells are shown in outline. The axis of the reconstruction is tilted about 30° from the vertical axis of the larva because of the plane of section. To correct for this, mentally rotate the pharynx until ph. 1 is directly on top. Additional details of the pharynx and suboral region of this specimen are shown in figures 62–73. Magn. × 1700.

FIGURES 62–64. A series of sections through the base of the metatrochophore pharynx shown in figures 60 and 61, oriented as shown in figure 62. Shows the entry of the left side of the primary pharyngeal nerve (n.) into the basal pharyngeal complex.

FIGURE 62. Section at the level of the left pharyngeal mucus cell (mu.). Magn. × 9475.

FIGURE 63. Section at the level of the dense vesicle cell. Magn. × 10 510.

FIGURE 64. Section through the base of the dense vesicle cell showing its association with the pharyngeal nerve, see figure 65 for a detail. Magn. × 9950.

FIGURE 65. Section through the pharyngeal nerve at the base of the dense vesicle cell, a detail of figure 64. Processes marked * belong to the dense vesicle cell. Magn. × 49 690.

FIGURE 66. A typical pharyngeal nerve cell. Magn. × 20 690.

FIGURE 67. One of several small, vesicle-filled terminals supplying the pharyngeal valve from the top of the secondary pharyngeal nerve (see also figures 60, 61). Magn. × 28 300.

FIGURE 68. (a) Neuromuscular junction between the primary pharyngeal nerve and the front pharyngeal muscle cell. Note that the basement membrane (arrows) is continuous. This is the best defined of several such junctions encountered. Magn. × 47 460. (b) A detail of (a). Magn. × 120 720.

FIGURES 69–73. Larval structures in the suboral region of the metatrochophore. The plane of section is approximately transverse (as in figure 60), all are oriented as shown in figure 69.

FIGURE 69. The two median suboral cells flanked by the lateral vestibular nerves (arrows). Parts of both suboral cells appear in the same section because of the tilted plane of section. Magn. × 10 390.

FIGURE 70. Top of the suboral plexus at the level of the suboral epithelial cell. Parts of the plexus and the vestibular nerves entering it can be seen as clumps of neurites (n.). There is a close association between the plexus and the ventral muscle cell similar to that seen in the trochophore (figure 55). Magn. × 17 875.

FIGURE 71. Section through neurites (n.) of the suboral plexus near its base. Magn. × 19 350.

FIGURE 72. The left lateral vestibular nerve at the level of the median suboral cells, detail of a section adjacent to that in figure 69. Note the close association between the nerve and the adjacent circumoral muscle cell. Magn. × 44 340.

FIGURE 73. Section through the neurotroch nerve in the metatrochophore. The nerve consists of two fibres in most sections, wrapped by flattened processes arising from the cell immediately adjacent to the neurotroch cell. Magn. × 51 330.

The surface process from s. 3 lies between the ciliary fields of the two metatrochal tiers, and is continuous around the end of the metatrochal shelf (as in figure 52) where it is interrupted for passage of the neurotroch. The surface process of s. 4 lies just above the tier 1 ciliary field adjacent to that of the left metatrochal nerve cell. The cell bodies of both lateral suboral cells lie buried in the metatroch, however, between tiers 1 and 2. Except for a slender fibre arising from s. 3 that is apparently the beginning of the neurotroch nerve (figure 46), neither cell produces neurites or other fibres at 24 h. A flattened extension from s. 3 does, however, extend up behind s. 2 to enclose partially the metatrochal nerve, an association that is probably of some importance in the initial stages of suboral plexus development. By 48 h, the suboral plexus appears essentially complete, the only obvious subsequent change being the further folding and elaboration of the facing surfaces of surrounding trochal and epithelial cells to fill the remaining extracellular space (compare figures 70 and 71 with figure 57). Based on the reconstructions of one specimen (figure 52), s. 3 at this stage has two neurotrochal fibres, and one branched process that interdigitates with other fibres in the suboral plexus (figure 57) including those of s. 2. Cell s. 4 produces only one fibre, which runs at least to the pharyngeal nerve, and probably travels some distance in it. The two lateral suboral cells differ from one another in ultrastructural appearance in both of the 48 h specimens examined. Cell s. 4 has a comparatively pale, granular cytoplasm, rather like ph. 2. Cell s. 3 shows type 1 ultrastructure as described in §3.6.

The nerves and sensory cells of the suboral complex are surrounded and enclosed by cells that are themselves well defined in terms of type, number, and position. These include the tier 1 and 2 metatroch cells on either side of the mouth, the neurotroch cells, and the median suboral epithelial cell (figure 45). The numerous folds and ridges produced by the basal surfaces of these cells form the channels along which the fibres of the lateral vestibular nerves, suboral plexus, and the metatroch and neurotroch nerves travel. The suboral epithelial cell appears at 48 h to be a part of the neurotroch, since it forms the top part of the rejectory tract. At 24 h, this cell lies off centre to the right, and looks much more like a rudimentary part of the metatroch. Evidently the suboral complex, at least that part of it below the median suboral cells, constitutes an intratrochal system of nerves and nerve cells. The cells surrounding it, though necessarily modified and reorganized to accommodate the feeding activities of the trochophore, are all basically trochal cells or are derived from trochal cells.

With regard to the function of the suboral nerves and plexus, it may be significant that plexus neurites are repeatedly found closely associated with the muscle cells located in the suboral region (e.g. figure 55 shows the proximity of the ventral muscle cell), but specific synapses either with muscle or with other neurites are absent. This is true of the metatrochophore stage as well, and the fibres of the suboral complex remain noticeably less differentiated than fibres elsewhere in the larval nervous system at this stage. In section (figures 70–72) they consist of small fibres of several types, more types in fact than are found in the pharyngeal nerves, in which one type predominates. The suboral cells are clearly a diverse group of cells at 48 h, but this could be due to differences in the stage of differentiation each has reached by that time. While there are fewer obvious differences between them in the metatrochophore, their fibres are sufficiently diverse to suggest that there are still a number of cell types in the suboral complex at this later stage.

(c) The metatroch and metatroch nerve

The metatroch consists of two tiers of cells (figures 55, 56, 58) forming a projecting, ciliated shelf that runs along the posterior margin of the food groove (figure 49). The top (anterior) tier, mt. 1, has a single row of cilia. The bottom tier, mt. 2, has a broader band with the individual cilia arranged in diagonal columns of four or five cilia each.

The metatroch is innervated by a single tiny nerve that runs around it behind the second tier (figures 58, 59). The nerve is interrupted ventrally, in the suboral region, where it joins the suboral plexus (figure 45 b). In the trochophores examined, the nerve invariably consists of only two fibres. These arise from two metatrochal nerve cells lying within the metatroch on either side of the oral region between the two tiers of trochal cells (figure 56). These cells have a very pale, extracted-looking cytoplasm, and in this respect they resemble the pharyngeal nerve cells. Among the vesicles scattered through their cytoplasm there are, however, a number of elongate, ovoid vesicles of a type not common elsewhere in the nervous system, and these are useful markers for tracing their fibres. Both cells are elongate and bipolar. They taper gradually to form two major nerve processes which, taken together, account for the number of fibres found in the metatroch nerve. Both cells also have a surface process and a small, apically directed branch (figures 45 b, 48) that crosses the food groove toward the prototroch. The surface process of the left cell extends medially toward the oral region and surfaces adjacent to the surface process of suboral cells s. 4. In the one case traced in detail, its apically directed fibre wandered along the food groove epithelium into the region above the mouth, and ended there. The surface process of the right cell extends laterally, and surfaces some distance away along the metatroch. In the one case traced in detail, its apically directed fibre entered the prototroch and then branched to give two neurites that travelled short distances among the ridges and folds of the tier 2 prototroch cells (figure 33). It is not clear whether either branch ever joins the prototroch nerve. If they do, the connection is a very modest one at 48 h, and examination of the metatrochophore shows that nothing more substantial has developed by that stage.

The metatroch nerve apparently serves as a barrier to the circumoesophageal connectives in the metatrochophore. At their points of contact, the connectives are redirected ventrally as shown in figure 2. The fibres of the metatroch nerve pass under the connective on each side, in direct contact with it, and the two interact en passant. The metatrochal fibres and some fibres in the connective become swollen at these points, and unusually large numbers of vesicles are present. The number of fibres in the metatroch nerve increases locally to four or five, the extra fibres probably being derived from the connective. The extra fibres do not travel very far at this stage, but they may do so later. The stomatogastric nerves in serpulids arise as branches from the circumoesophageal connective (Orrhage 1980), and preliminary observations (T. C. Lacalli, unpublished) on *S. spinosus* juveniles show that the larval pharyngeal nerves, to which the metatroch fibres ultimately connect, are retained after metamorphosis as a major component of the stomatogastric system. The metatroch nerve provides a path along which the stomatogastric nerves could grow to establish their connection with the pharynx.

(d) Neurotroch

A single elongate cell is responsible for the whole of the neurotroch at 24 h. As the neurotroch lengthens during later development, additional cells are added. There are two cells at 48 h, and six in the metatrochophores examined. The median suboral epithelial cell is located at the
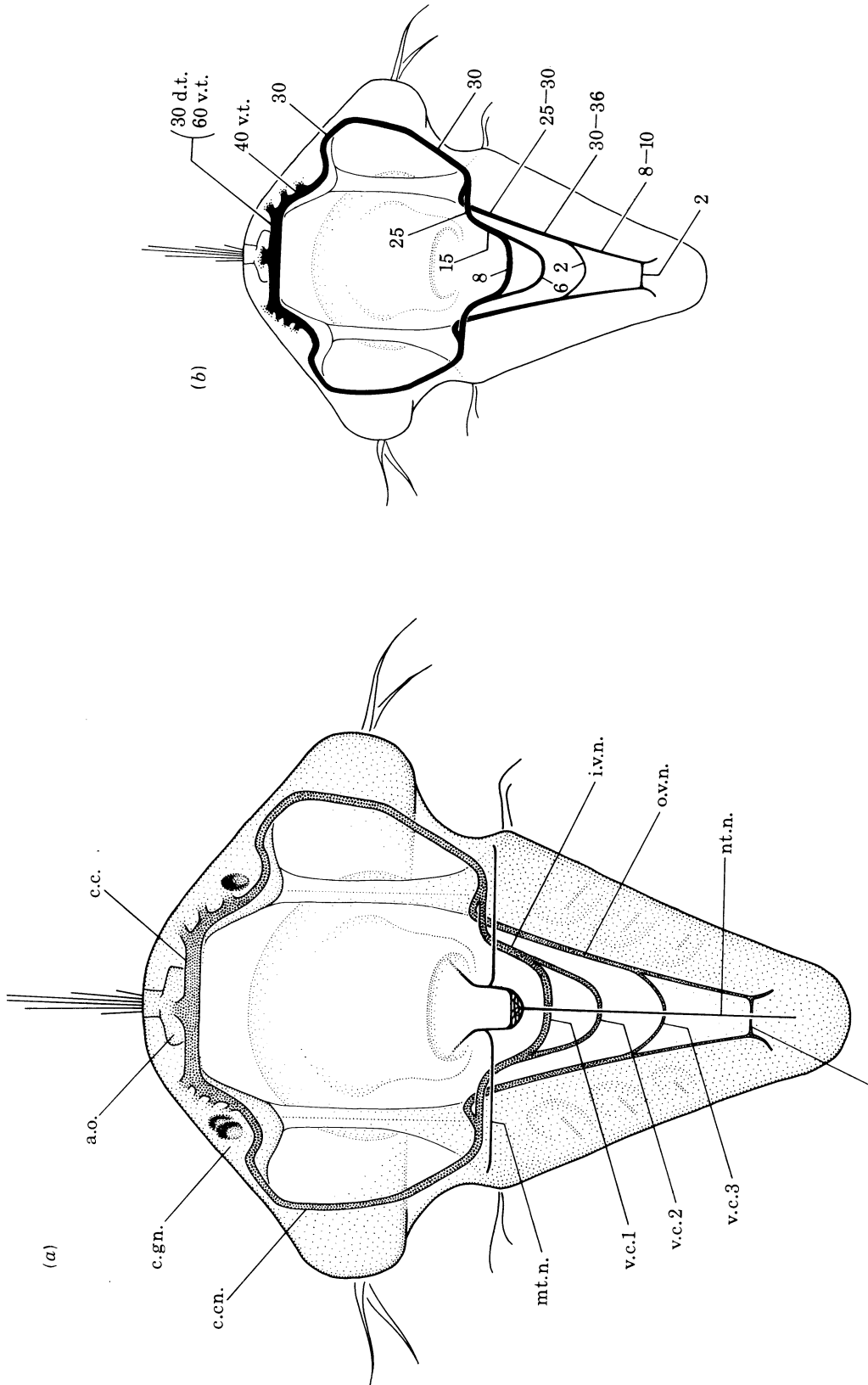
FIGURE 74. (a) Summary diagram showing the rudiments of the adult nervous system in the metatrochophore. Larval structures: apical organ (a.o.), metatroch nerve (mt.n.), neurotroch nerve (nt.n.), and suboral plexus (unlabelled). Adult rudiments: cerebral commissure (c.c.), paired cerebral ganglia (c.gn.) and circumoesophageal connectives (c.cn.), inner and outer ventral nerve cords (i.v.n. and o.v.n.), three segmental ventral commissure (v.c. 1–3), and the small terminal commissure (t.c.). (b) Diagram as in (a) giving approximate fibre counts at strategic points based on one specimen. Distinguishes between the dorsal (d.t.) and the ventral (v.t.) tracts of the cerebral commissure.

top of the neurotroch, and the entire tract, from the mouth down, including this cell, is rejectory (§3.7).

The neurotroch nerve consists of two parallel and rather irregular fibres, containing scattered vesicles, that arise from suboral cell s. 3 (figure 52). They run unenclosed down the back of the neurotroch cells at 48 h, and terminate without making further contacts. In the metatrochophore, the nerve's fibres are generally enclosed by flattened processes arising from adjacent epithelial cells (figure 73, two of the cells are visible in figure 84). These cells do not appear to be part of the developing trunk epithelium, which suggests that they and the nerve should be considered strictly larval structures. The ventral and terminal commissures, when they develop, pass behind the neurotroch nerve (figure 86), but make no contact with it. The enclosure of the neurotroch nerve by adjacent cells is presumably a factor in preventing such contact.

3.5. *Rudiments of the adult nervous system*

The main components of the adult nervous system are present in rudimentary form in the metatrochophore (figure 74 a). By this stage, the system comprises more cells (estimated 50–60 neurons) and larger nerves of greater complexity than were encountered anywhere in the larval system. A detailed description of the adult system, at the cellular level, is well beyond the scope of this account. It is treated here only in general terms, with attention to the arrangement and locations of the major groups of neurons, their fibre tracts, and the main integrative centres. This provides a sufficient basis for comparing the adult and larval systems with regard to their constituent cells and main organizational features, and for defining the structural and developmental relationship between them.

(a) *The brain*

The brain (figures 75–80) consists of two well defined but rudimentary cerebral ganglia that communicate by means of a cerebral commissure passing through the region occupied by the larval apical organ. The ganglia are rather inhomogeneous in appearance in section (figures 76–78) because of the number of cell types and different stages of differentiation that are represented. A class of comparatively well differentiated neurons can be identified that are, based on fibre traces, responsible for most of the commissural fibres. The commissure has two tracts of fibres, a small dorsal tract and a larger ventral one. Fibres in the dorsal tract arise predominantly from a layer of neurons lying against the dorsal surface of the ganglia. Fibres in the ventral tract come mainly from cells clustered at the ends of the commissure, above and in front of the ventral tract where it enters each ganglia, and scattered along its length as it passes through the ganglion. Taken together, these clusters form two continuous, U-shaped domains of differentiated nerve cells (figure 75), one on each side of the brain. The remaining ganglionic cells are largely undifferentiated at this stage, except for the eyes, and there is otherwise no obvious subdivision of the structure as a whole.

As described in §3.2, the dorsal tract of the cerebral commissure passes beneath the larval apical organ and through the basal portion of the apical plexus, where its fibres become indistinguishable from those of the plexus. The ventral tract passes in front of the apical organ, near its base, where it also makes a connection with the plexus. This takes the form of a loosely organized tangle of cell processes and vesicle-filled terminals, referred to here as the median integrative centre (figure 81), which appears to involve only a small proportion of the commissural fibres, primarily those along the back side of the ventral tract. Large scale

exchange of fibres between the ventral tract and apical plexus is prevented by a partial barrier formed by the basal processes of apical auxiliary cells, and a number of similar cells ranged with these along the front face of the apical organ (figure 28). Lateral integrative centres are formed at each end of the commissure where it enters the cerebral ganglia (figures 79, 80). These are larger and more spacious than the medial centre, but are otherwise similar in general appearance and in the types of fibres represented (figures 82, 83). Some exchange of fibres between the dorsal and ventral parts of the ganglia occurs at these points; a few fibres from cells in the ventral part of the ganglia cross to the dorsal tract, and fibres from cells in the dorsal part of the ganglia cross to the ventral tract. Most of the neurites and other processes in the lateral centres arise, however, from immediately surrounding ganglion cells or from the incoming commissure, as branches of fibres originating on the opposite side of the brain. Between the lateral and median integrative centres, the two commissural tracts are separated by a file of cells whose flattened processes form a loose curtain between them. Some of these are quite deeply embedded in the commissure. Examples are shown in figures 77, 78, 80 and 82.
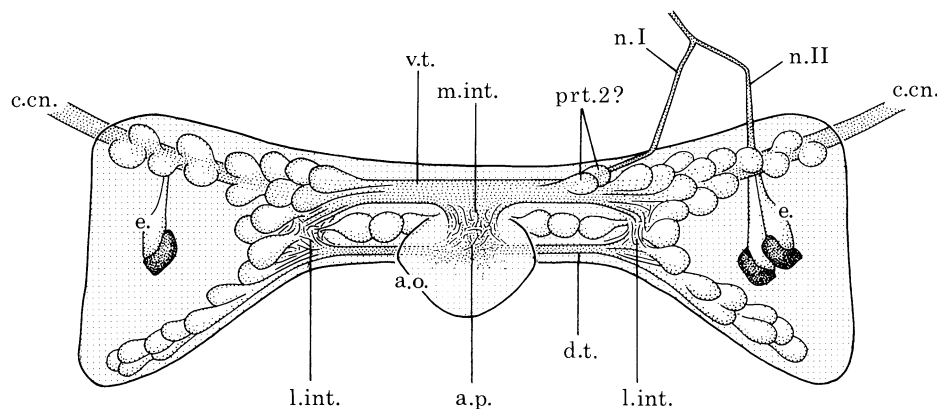
FIGURE 75. Top view of the metatrochophore brain showing location of the main clusters of differentiating neurons and (darker stippling) their fibre tracts; orientation as figures 76–79. Shows also: the apical organ (a.o.), apical plexus (a.p.), dorsal and ventral tracts (d.t. and v.t.) of the cerebral commissure, the median and lateral integrative centres (m.int. and l.int.), circumesophageal connectives (c.cn.), cerebral eyes (e.), and pretrochal nerves I and II (n. I and n. II).

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FIGURES 76–78. Sections through the metatrochophore brain of the type used for preparing reconstructions, labelled as in figure 75. The plane of section is approximately transverse. However, as in the pharynx reconstructions (figures 60 and 61), there is a degree of tilt, and the two cerebral ganglia are unequally represented: the sections cut only superficially into the right ganglion, but quite deeply into the left.

FIGURE 76. Section at the level of the apical organ (a.o.) and the upper part of the apical plexus (a.p.). Magn. × 2980.

FIGURE 77. Section at the level of the junction between the ventral tract (v.t.) of the cerebral commissure and the apical plexus (a.p.) showing the median integrative centre (m.int., see figures 28 and 81 for details). Shows also (at *) one of the cells separating the two commissural tracts. Magn. × 2920.

FIGURE 78. Section just below the cerebral commissure showing the muscle (m.) running beneath it. Magn. × 2780.

FIGURE 79. Reconstruction of the metatrochophore brain, seen from above: a key for interpreting figure 80. Labelled as in figure 75, tilted as described for figures 76–78. Shows in outline the apical organ, cerebral ganglia, and the three cerebral eyes. Remaining lines trace the paths of individual fibres in representative sections. Outlines of several of the cells separating the commissural tracts are included, but only one of these, on the left side (indicated here and in figure 77 by *) is not obscured by the fibres. Note the space above this cell, which is occupied by other cells of similar type. Magn. × 1590.

FIGURE 80. Stereoreconstruction of the metatrochophore brain. See figure 79 for a key. Magn. × 1790.
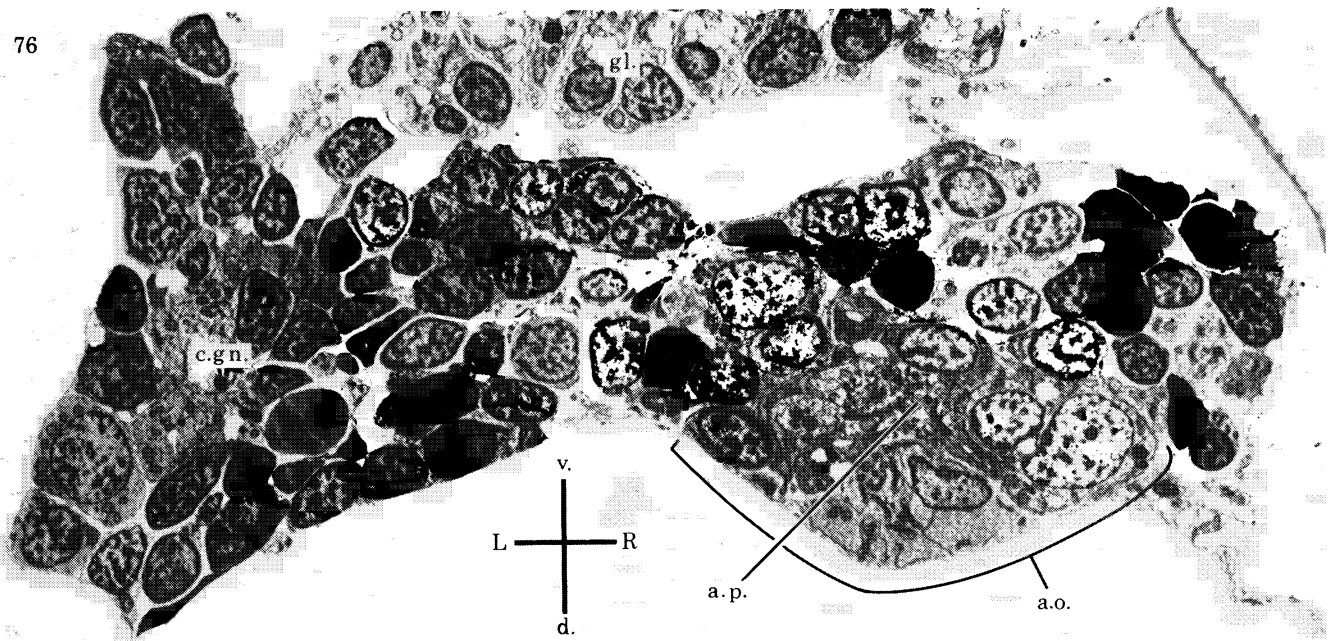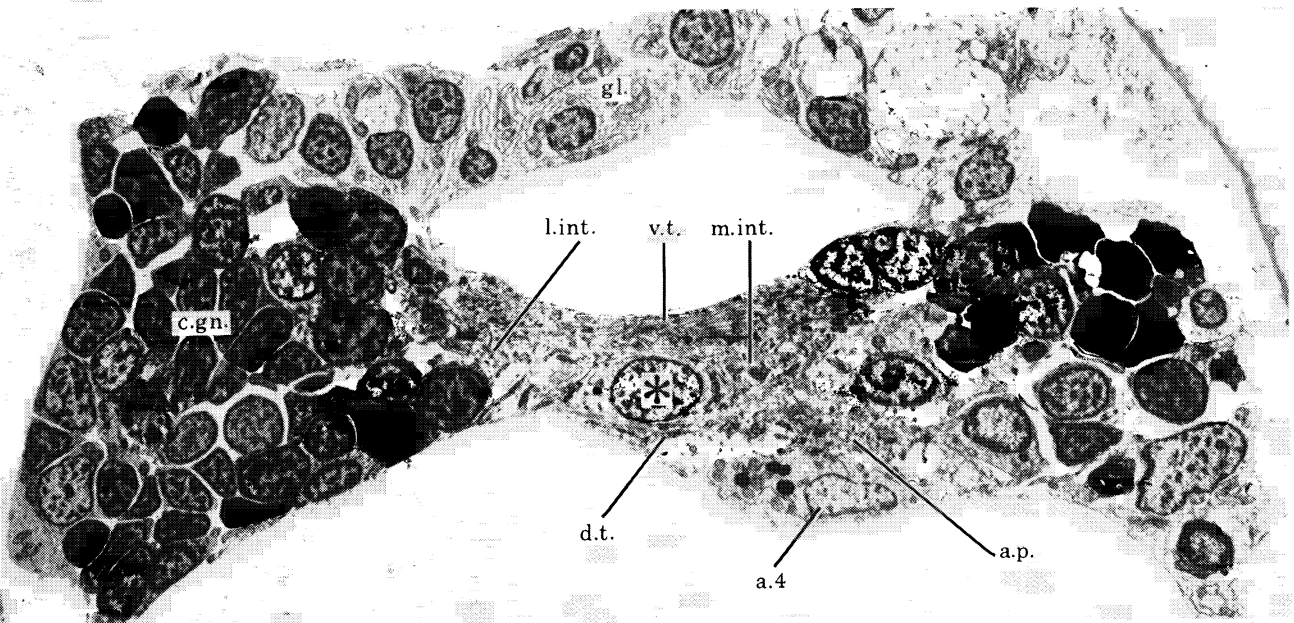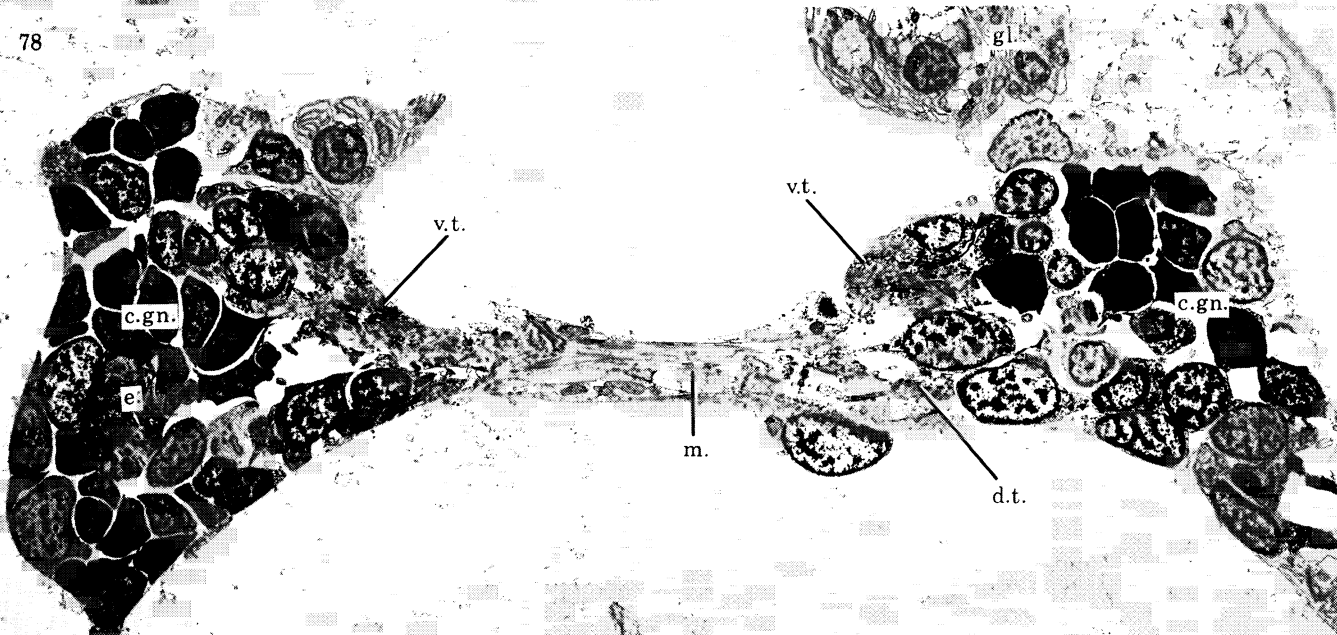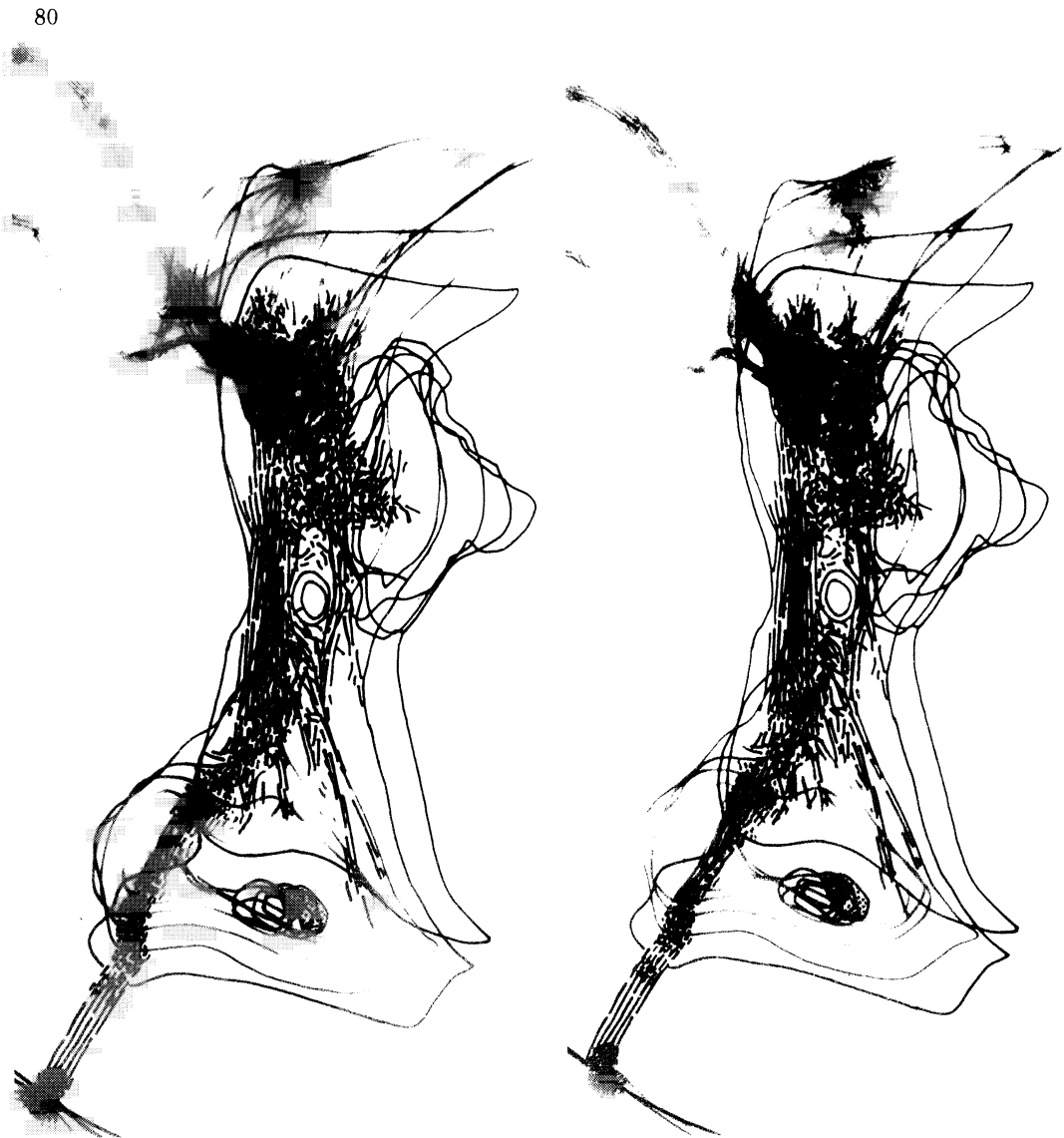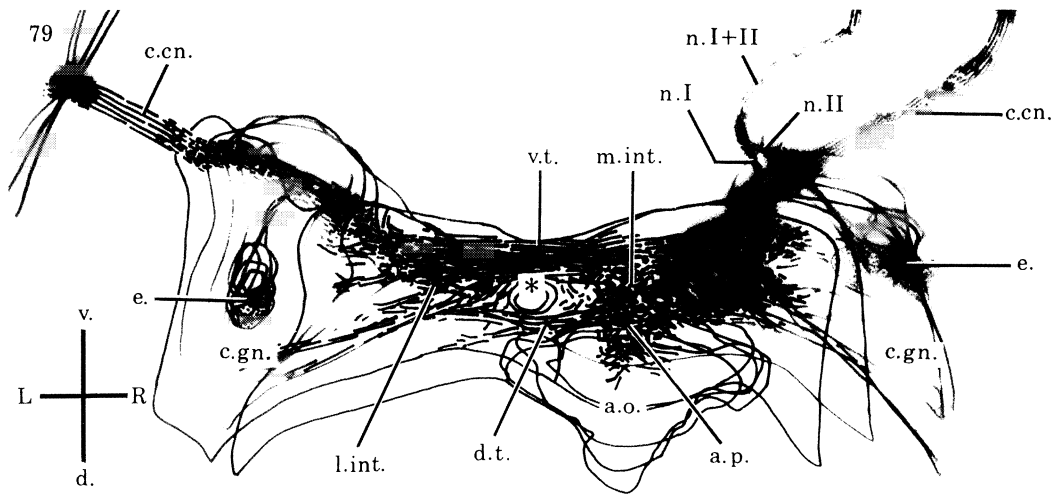
FIGURES 76–78. For description see opposite.

FIGURES 79 AND 80. For description see p. 114.
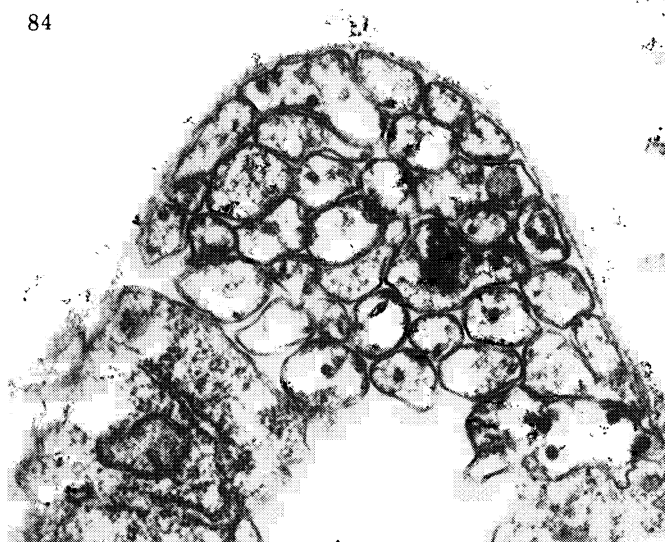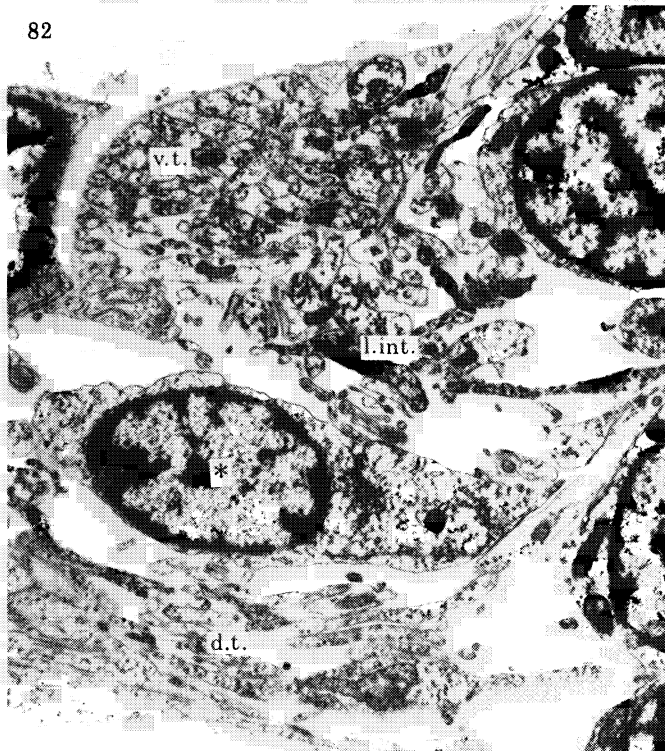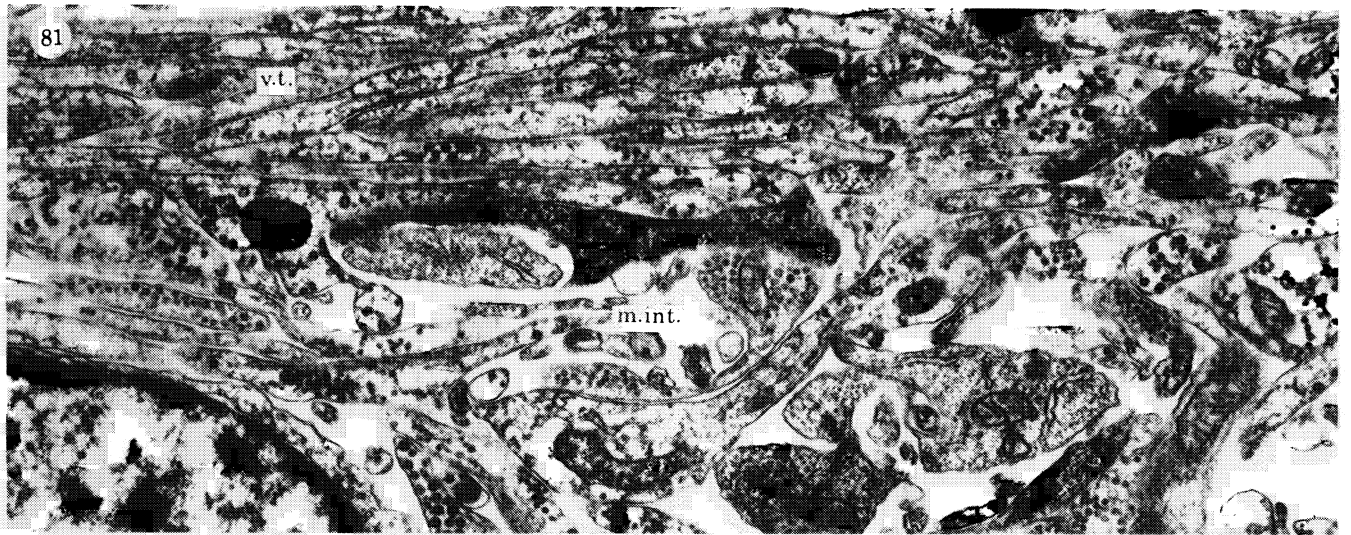
FIGURES 81–84. For description see p. 115.

FIGURES 85–88. For description see opposite.

The cerebral eyes are located approximately in the centre of the two ganglia at some distance from any nerves. The cell bodies of the receptor cells are drawn out into slender processes that extend forward and enter the ventral tracts. On the right side, at about this same point, pretrochal nerve II emerges from the ventral tract and leaves the brain, passing along the underside of the gland cell mass as it does so. The apically directed portion of nerve II, if it is retained in the metatrochophore, must then travel toward the apical organ in the ventral tract, at least initially, because there is no sign of this nerve elsewhere in the ganglion. If the entry of nerve II into the apical organ is via the dorsal rather than the ventral tract, which seems likely based on examination of the apical organ (§3.2), its fibres must be among those crossing between the two tracts at the lateral integrative centre on the right side. Pretrochal nerve I enters the brain on the right side just medial to the same lateral centre. A cell tentatively identified as pretrochal cell 2, associated very closely with the ventral tract fibres, is located at about this point.

The brain is bilaterally symmetric in most regards, but the arrangement of fibre tracts and their relation with the apical organ shows indications of an underlying four-fold plan. There are four main fibre tracts, if the right and left halves of the ventral tract are treated as separate entities, and these radiate from the centrally placed apical organ as dorsolateral and ventrolateral pairs. This represents a doubling of the arrangement seen in the young larva, in which only the right half of the system, i.e. the two pretrochal nerves, is connected directly to the apical organ.

(b) Nerve cords and ventral commissures

The ventral tracts, on leaving the cerebral ganglia, continue as circumoesophageal connectives. The connectives (figure 84) cross the episphere and prototroch to the metatroch. There is no exchange of fibres with the prototroch nerve (§3.3a), but several fibres appear to leave the connectives and enter the metatroch nerve (§3.4c). At the metatroch, the connectives are diverted forward toward the ventral midline, then inward over the top of the epithelial thickening of the trunk rudiment. Here they split to form a pair of ventral cords that travel down the inside of the epithelium well in front of the developing mesodermal bands (figures

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FIGURES 81–83. Details of the metatrochophore brain.

FIGURE 81. The median integrative centre, a detail of figure 77. Magn. × 21490.

FIGURE 82. The right lateral integrative centre (l.int.) showing (*) one of the cells separating the two commissural tracts. Magn. × 10400.

FIGURE 83. Detail of the right lateral integrative centre; * indicates the corner of the cell similarly labelled in figure 82. Magn. × 20810.

FIGURE 84. Section through the left circumesophageal connective just above the prototroch. Magn. × 40980.

FIGURES 85–88. Transverse sections through the metatrochophore trunk at about the level of the first ventral commissure. All are oriented as in figure 85.

FIGURE 85. Survey figure showing the thickened trunk epithelium (ep.th.), the mesodermal bands (mes.), and the two pairs of ventral cords (i.v.n., o.v.n.). Magn. × 2580.

FIGURE 86. Detail of the inner surface of the trunk epithelium. Shows the inner and outer ventral cords (i.v.n. and o.v.n.) and two nerve cells (*) associated with these Magn. × 13340.

FIGURE 87. Section through the first ventral commissure. Shows the median bipolar cell (*) associated with the commissure at this point. The neurotroch nerve is circled. Magn. × 12990.

FIGURE 88. The first ventral commissure, a detail of figure 87. Magn. × 48980.

85, 86). From fibre counts (figure 74 *b*) it appears that most of the fibres in the connective branch as they leave the region of the metatroch. The outer (more lateral) branch on each side then becomes the main ventral cord, travelling to the posterior end of the larva to terminate near the insertion of the developing longitudinal muscle.

The ventral commissures were examined in detail in only one larva. In this specimen there were three commissures and one tiny, terminal commissure-like nerve. The three main commissures fall roughly in register with the developing chetal bundles, and are therefore presumably segmental structures. The first two commissures were linked to the inner cords only. Fibres from the latter cross behind the neurotroch and form a small bundle of neurites (figures 87, 88). Several cells derived from the epithelium adjacent to the neurotroch associate with the commissure, and in both commissures there is one medial bipolar cell (shown in figure 87 for the first commissure) whose processes extend outward to the inner ventral cords. In the one larva examined in detail, the third commissure consisted principally of the lateral processes of a third such cell, which extended to the outer ventral cords. It is not clear whether the inner cords later develop to the level of the third commissure and contribute to it, or whether the third commissure involves only the outer cords. What is important is that cells derived from the region of the neurotroch are clearly involved in the early events of commissure development. Based on the evidence of this study, their role may be to produce lateral fibres that contact the longitudinal cords to initiate fibre outgrowth from them.

The epithelial thickenings of the trunk rudiment are a heterogeneous mass of cells of different types, most of them probably neural, in a variety of different stages of differentiation. Only a few well differentiated neurons were encountered among these in the metatrochophore, generally no more than one or two at the level of each commissure (figure 86). This is clearly an insufficient number to account for more than a small proportion of the fibres in the ventral cords, most of which must therefore derive from the brain via the circumoesophageal connectives.

In summary, the adult system develops from proliferative centres quite separate from the larval system, and its nerves also follow largely separate paths. The two systems are linked at the apical organ, which has an as yet poorly understood integrative function, and at the metatroch, where there are signs of what may be the beginning of the stomatogastric nerves.

3.6. *Nerve cell types and their distinguishing characteristics*

This section summarizes the morphological and ultrastructural criteria used to identify nerve cells and distinguish among the various types represented. While strictly morphological criteria are of only limited use in classifying cells when the significant differences between them are functional ones, governing their role in the development and operation of the nervous system, some useful morphological distinctions can nevertheless be made.

(*a*) *Sensory versus non-sensory nerve cells*

A few ciliated sensory cells are identifiable in the metatrochophore brain, but most of the nerve cells are typical unipolar, in some cases multipolar, cells that lie buried in the ganglia, and lack surface processes or cilia. In the larval system, all the fibre-producing cells associated directly with the nervous system have surface processes bearing one or more cilia except for (i) cells that are clearly non-neural and (ii) pretrochal cells. The former category includes the two dense auxiliary cells in the apical organ and the dense vesicle cell in the pharynx. All three

produce characteristic dense processes of unknown function. Of the pretrochal cells, only prt. 1 has a surface process, and this did not have a cilium.

The remaining cells of the larval system fall into two categories depending on whether their surface process has only one or two cilia, or whether it forms a well defined ciliary field. The former category includes all the cells of the apical organ except the dense auxiliary cells and both tuft cells, the metatrochal nerve cells, and the pharyngeal nerve cells (ph. 1, 6–9). In the case of the pharyngeal nerves, all of the uniciliate cells except one (the companion cell, ph. 4) are of one type and are, apparently, motor neurons. They are the main source of fibres in the pharyngeal nerves, which are clearly responsible for initiating the contractions of the pharyngeal muscles, and the nerves are supplied with a separate class of suboral and pharyngeal sensory cells. The metatrochal nerve cells are evidently effector neurons as well, but provide for neurociliary rather than neuromuscular control. Theirs are the only fibres supplying the metatroch, which has a characteristic arrest response that is probably neurally controlled. The apical organ contains a more diverse assemblage of uniciliate cells, some with numerous vesicles and some without, but none of which much resemble the metatrochal and pharyngeal nerve cells. This suggests a number of functional types may be represented in the apical organ, some of which may be sensory, but little can be said about what these different cell types may be doing without more information on the functional role of the apical organ.

The second category, cells with well defined ciliary fields, consists of cells loosely termed sensory throughout this account. Their surface processes are precisely positioned with respect to other surface features and bear multiple cilia, usually arranged as a single row. All four suboral cells are sensory under these criteria, as are two of the pharyngeal cells at the trochophore stage, ph. 2 and 5. Judging from the differences between these cells in ultrastructural appearance (see (*b*) below), it seems unlikely that they constitute an entirely homogeneous group with regard to function.

(*b*) *Ultrastructural characteristics*

Non-sensory nerve cells in the metatroch and pharynx, and in the developing brain and ventral cords, share a number of ultrastructural characteristics. All have a very limited cytoplasm that forms a thin perinuclear layer. Membranous structures, e.g. mitochondria, golgi, and transmitter vesicles, are darkly stained, but the cytoplasmic ground substance is either absent or unstained, and the cells have a generally extracted appearance (e.g. figures 53, 56, 66, 82, 86, 87). Nerve cells in the brain and ventral cords are very similar to one another except in terms of density. The nuclei are uniformly rounded with densely clumped chromatin. The spaces between clumps are filled by a fine, fibrillar matrix that varies from cell to cell, ranging from sparse or absent in the better differentiated neurons, to very dense in some of the other ganglionic cells. This range of nuclear densities accounts for much of the heterogeneity in the appearance of the ganglia at low power (e.g. figures 76–78). Cytoplasmic density also varies between cells, roughly in parallel with the differences in nuclear density, but to a lesser extent. The neurites vary even less in density, but generally the best differentiated ones are among the palest, and arise from the palest and most extracted-looking of the nerve cells. The most nearly comparable cells in the larval part of the nervous system are the metatrochal nerve cells and pharyngeal cells 1 and 6–9. These are less intensely stained than their brain and ventral cord counterparts, so they appear paler, and they are also more irregular in shape and cytoplasmic organization.

The remaining cells of the larval system have a more extensive cytoplasm than the cells just described, and are often rather large cells. Several morphological types are represented, but the ultrastructural differences do not clearly parallel the morphological ones in all cases. The two most distinctive ultrastructural types are as follows:

Type 1. Cytoplasm of type 1 cells is uniform and finely granular to the point of appearing solid, and ranges from moderately dense to very dense in appearance. The cells contain a number of mitochondria, large yolk-like granules, and transmitter vesicles, the last being quite numerous in some cells. This type of cell is most easily identified, however, by a characteristic fixation artefact that causes both the endoplasmic reticulum and the nuclear membrane to swell. Examples are shown in figures 24–26, 29, and 55. The fibres produced by these cells are similar to their cytoplasm in appearance. Figure 57 shows one such fibre, belonging to cell s. 3.

Type 2. Cytoplasm of type 2 cells is granular, moderately extracted in most cases, but not densely stained. There are generally a number of mitochondria, dense yolk-like granules, and scattered vesicles identical to those in the cells' neurites. Examples are shown in figures 27 and 28. While these features are not so distinctive as the type 1 artefact, the large volume and moderate density of type 2 cells distinguishes them from other nerve cell types. This is why, for example, the metatrochophore apical organ, whose cells are largely of this type, stands out so clearly from surrounding brain cells (e.g. in figure 76).

A number of larval cells show type 1 ultrastructure at 48 h. In the 24 h stage, the same cells have a somewhat similar appearance, but fixation at this stage is in general too poor for a meaningful comparison. By the metatrochophore stage, some, but not all, of the type 1 cells have become type 2. Type 1 ultrastructure therefore appears to represent a phase in the differentiation of type 2 cells, but if so, it is a rather prolonged phase, lasting to the metatrochophore in some instances. To be more specific: in the apical organ, cells a. 1–3, cap. 1 and 2, and the secondary apical cells are usually type 1 at 48 h (figures 24–26) and type 2 in the metatrochophore (figure 27). However, type 2 cells do occur at 48 h (a. 2 in one specimen), and type 1 cells are encountered in the metatrochophore (a. 3 in one instance and some secondary apical cells). Among the sensory cells, two are type 1 at 48 h, cells s. 3 and ph. 5. Of the remaining sensory cells, two are rather nondescript pale cells (s. 4 and ph. 2), while the two median suboral cells are of variable density (figures 47 and 69), but are also rather nondescript. Of the six sensory cells, it is these last two that appear least likely to be neural.

Type 1 and 2 cells both contain characteristic transmitter vesicles, and must therefore have some neural or related role in the larva. It may also be significant, however, that the occurrence of type 1 cells in a given structure generally correlates with periods of morphogenetic activity involving that structure, and that some, though not all, of the fibres the author would identify as pioneering fibres (for example, of ph. 5, s. 3) belong to type 1 cells. The evidence is at least consistent with the idea that type 1 cells are morphogenetically active, but convert to type 2 ultrastructure when their developmental role is complete.

(c) *Neurites*

Neurites in the larval nerves differ substantially from those in the developing adult nerves. Only the adult commissure and connectives have fibres that in any way resemble axons. These are long, of uniform diameter, and are packed in comparatively orderly bundles (e.g. figure 84), but scattered vesicles and mitochondria occur along their length in at least some cases. Neurites in the larval nerves are, in contrast, of diverse types and far more irregular in profile in section (figure 72). Whether from bipolar or multipolar cells, the larval neurites have

a distinctly dendritic appearance, regardless of stage, which is perhaps to be expected in a system in which diffuse conduction and plexus-like organization appears to be the rule. It may be significant that the pretrochal fibres are, by the metatrochophore stage (figure 38), the most regular and least 'larval' in appearance of all of the larval nerves.

(d) How many nerve cell types are there?

This account of the larval nervous system is facilitated by the existence of individual, identifiable larval cells that serve as reliable landmarks. Some of these are unique cells; the dense vesicle cell, its companion cell, and the receptor and pigment cells of the eye, for example, which are distinguishable from all other cells in the larva by type, morphology and position. It is more common, however, for there to be several cells of any one type distinguishable from others of the same type only by differences in position and morphology. In some cases these differences are of developmental origin, but are probably of no great significance in terms of their effect on the cells' differentiation. The tuft cells, a. 4 and a. 5, are an example. Cell a. 5 always lies behind and to the left of a. 4 and has fewer cilia, a consequence of its cleavage position and the suppression of development on the left side of the apical organ, but the two cells are otherwise identical with regard to all ultrastructural criteria and presumably belong to a single differentiated type. Some of the other cells pose more of a problem. Consider, for example, apical cells a. 1–3 and their surrounding capsular cells. The main difference between these two cell types is that a. 1–3 produce slender neurites, while the capsular cells have flattened processes. Both contain similar vesicles and have otherwise similar ultrastructure. All five cells could well be of one type if neurites and flattened processes represent equivalent structures whose morphology is subject to variation as conditions change, for example, are flattened when the plexus is accessible and slender otherwise. All five cells could also, however, be unique. The two capsular cells, for example, have rather different surface processes; the process from cap. 1 is covered with unusual swollen microvilli, which cap. 2 entirely lacks, and similar small, structural differences distinguish the remaining three cells from one another as well. In practice, it is exceedingly difficult to determine which of many such small differences between cells are significant, and which are not. This is a sufficiently serious problem, in the *Spirobranchus* larva, that it does not seem a useful exercise, at this time, to compile an exhaustive catalogue of cell types. Nevertheless, as a conservative estimate, it would appear that of the 36 cells of the larval system, including the tuft cells and the pigment cell of the eye, there are between 16 and 18 demonstrably different cell types.

3.7. Larval behaviour

Comparable behaviours were observed in both *S. polycerus* and *S. spinosus* except where specified otherwise.

(a) Ciliary effectors

The apical tuft projects forward during normal swimming and shows no signs of independent mobility. It bends passively on contact with obstacles and generates a weak but noticeable force on recoil.

The larva is propelled by the strong and continuous beat of the prototroch cilia, principally those of tier 2, which beat as compounds and in metachronal waves. Collision with obstacles at younger stages (e.g. the 24 h stage in *S. polycerus*) results in immediate rebound without noticeable change to the prototrochal beat. In older trochophores (48 h and older in *S.*

polycerus), collisions are followed by a brief pause accompanied by a visible slowing or apparent alteration to the ciliary beat, but only rarely do the cilia stop altogether. The metatrochophore exhibits somewhat more effective and frequent arrests, and its swimming is more erratic as a consequence.

As it swims, the trochophore rotates on its axis. This rotation is clockwise as seen from above, opposite to the direction of the metachronal wave. The trochophores also spiral as they swim, their axis of rotation precessing with a period matching that of the rotation. They swim in tight spirals when the angle of precession is small, and tumble in broad arcs when it is large.

The metatroch beats with variable speed, exhibiting periodic and sudden arrests. After an arrest, its cilia usually resume beating after a few seconds, but longer periods of quiescence were observed. Metatrochal arrests were not correlated with any of the other ciliary or muscular activities of the oral apparatus.

Cilia of the food groove, neurotroch, and proctodeum beat continuously and at constant speed. Food groove cilia beat toward the mouth, and neurotroch cilia away from it. The pharyngeal cilia do not beat freely, but a continuous undulatory motion is visible in the pharynx, which involves the entire bundle of pharyngeal cilia.

(b) *Muscles*

In slide preparations, both the trochophore and metatrochophore stages respond to vibration (for example, tapping the slide) by contracting the metatroch muscle. The strength and duration of contraction varies with stimulus strength, and repeated taps evoke repeated contractions. It was not clear whether this response involved only the metatrochal muscle, or the circumoral and ventral muscles as well.

The other obvious muscular response the larvae possess is swallowing. In the absence of visible food particles in the pharynx, single swallows occur periodically, usually from one to several times every few minutes. The presence of particles in the back of the pharynx (for example, in the feeding experiments, see below) induces swallowing directly. In *S. polycerus*, the pharyngeal muscles first become active between 16 and 18 h, and the front pharyngeal muscle is the first to begin contracting. By 24 h, a normal swallowing sequence is established in which an initial strong contraction of the pharyngeal muscles, which lengthens the pharynx, is followed immediately by contraction of the oesophageal muscle, which restores the pharynx to its original shape and position (figure 89). It was not clear whether the circumoral muscle has a role in the swallowing process. Weak pinching motions in its vicinity sometimes precede swallowing, but not always. During later development, the oesophageal muscle increases its capability for independent activity. By the metatrochophore stage, repeated series of oesophageal contractions are observed, which occur after a swallow or without prior pharyngeal contraction. Since the older larvae generally have fuller guts, these contractions may be a means to redistribute or mix food particles within the digestive tract, and probably occur in response to stimuli from inside the gut.

(c) *Phototaxis*

As previously reported (Lacalli 1981), *S. polycerus* larvae do not have a very reliable light response under laboratory conditions. Cultures vary in the degree to which they respond to light, and seldom do all larvae in a culture show the same response. In general, however, when the larvae do respond, they are most often negatively phototactic for a transient period. The period, in *S. polycerus*, is between 18 and about 30 h, that is, it begins at about the time the

eye and its nerve first develop. Larvae responding to the light swim directly away from it, in a tight spiral, while unresponsive larvae continue to tumble and execute broad spirals. This, together with the observed asymmetry of the pretrochal nervous system, suggests an obvious mechanism for the light response based on neural control over the angle of precession of the larva's axis of rotation. The advantage to a larva of rotating while it swims is the same as the ballistic advantage of a projectile that spins: the drag effect of small imperfections and asymmetries that would normally cause the projectile to veer off course are averaged out. A swimming larva experiencing asymmetric drag, if it did not rotate, would be turned back on itself and would swim perpetually in a circle. By rotating, this circular path is converted to a spiral whose pitch varies depending on the speed of rotation and the degree of asymmetry. Asymmetries are evidently minimized in *S. polycerus* when the larvae are swimming away from a strong light source. In the trochophores examined, the pigment cup of the eye is arranged so that the receptor cell microvilli are maximally shaded under these conditions, that is, when lighted from behind (Lacalli 1984). Since differences in the local strength of ciliary beat around the circumference of the prototroch is an obvious potential source of asymmetry, pretrochal nerve II may be reasonably supposed to act by locally altering (either stimulating or inhibiting) the prototroch on the right side when the receptor cell is illuminated, thereby increasing larval asymmetry. The eye is progressively shaded as the larva reorients, reducing the asymmetry, which results in a progressively tight, spiral path directed away from the light.
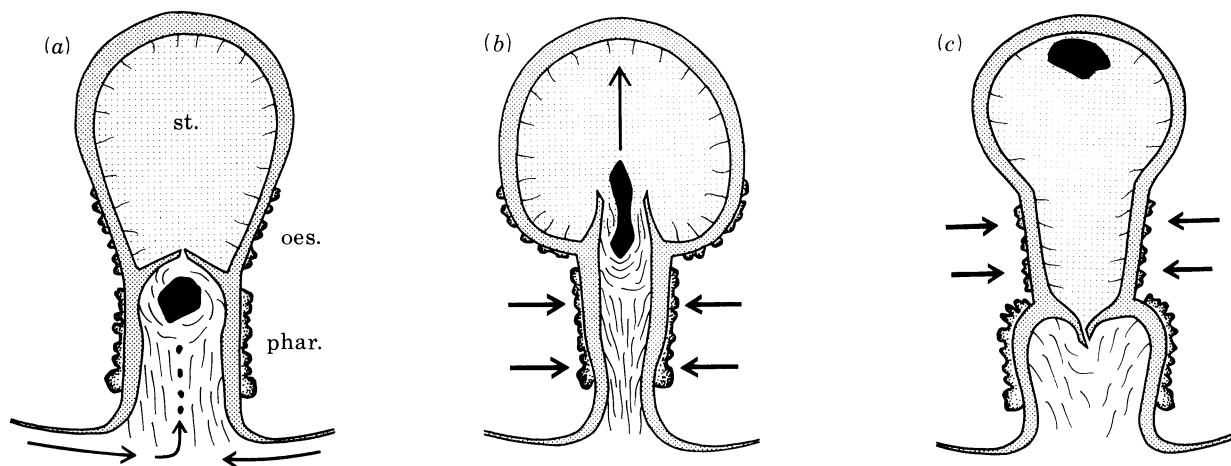
FIGURE 89. Three stages in the swallowing process, from observations on *S. spinosus*. Arrows indicate the paths of incoming particles, of the compacted food bolus and, in (b) and (c), regions of muscle contraction. See text for details.

(d) Feeding experiments

S. spinosus larvae were fed with suspensions of Aquadag particles, and these were rapidly collected and swallowed. The particles are carried by the cilia over the prototroch, into the food groove, and toward the mouth from both sides. This occurs whether or not the metatroch is beating. If the pharynx is accepting food, particles brought to the mouth will then move, evenly and in single file, through the pharynx to the back pharyngeal chamber, where they accumulate as a bolus (figures 8, 9 and 89a). When the pharynx contracts, the bolus is propelled rapidly and with considerable force to the back of the stomach (figure 89b). The valve is then closed by contraction of the oesophageal muscle (figure 89c).

Individual particles are accepted easily by the pharynx in the fashion just described. Larger

agglomerations of particles, in the 5–10 µm range, are less readily accepted. They are held at the mouth by the beating of the food groove cilia for some seconds, and eventually all or part of the mass may be drawn up into the pharynx, but usually they are carried off down the rejectory tract before this can happen. In a few instances large masses caught in the pharynx were regurgitated, and this was accompanied, and presumably caused, by a strong contraction of the metatrochal muscle. In some of the larvae observed, for reasons that are not clear, the pharynx failed to accept any particles at all. Individual particles were admitted to the front part of the vestibule, where they bounced about under the influence of the food groove cilia, but then, after a few seconds, they were carried away by the rejectory tract. It should be noted that the pharynx is so full of cilia that only a slight reduction of its diameter is needed to eliminate all the free space available between cilia. It probably requires very little, in terms of muscle contraction, to close off the central channel through which captured particles must pass, and so shut the system down completely.

It is not clear exactly how particles are drawn through the pharynx. The undulatory motion of the pharyngeal cilia may move the particles directly, but it is probably also important that excess water be removed as they are concentrated. Assuming that one function of the cilia may be to squeeze excess water out between their bases, which is the only place there is open space through which the water can escape, a pressure differential could be produced that would, at the same time, draw the particles up the pharynx, that is, the pharynx could in part be operating by suction.

(e) A functional interpretation of the larval nervous system

The larval nerve cells show a particularly low level of morphological and utrastructural differentiation. Their neurites are dendritic in appearance without regional specialization or identifiable synaptic regions except in a few instances in the metatrochophore. The circuitry of the system cannot therefore be traced in any detail and, at best, only very general conclusions can be drawn about the probable function of most of the cells.

The appearance of neurociliary synapses in the metatrochophore is correlated with a more precisely controlled ciliary arrest response. This supports the idea that the prototroch nerve is an inhibitor of ciliary activity that may act to alter the beat or cause partial arrests, which it may do even before specific synapses are present. Local inhibition of ciliary beat by pretrochal nerve II is sufficient to explain the larval light response, as discussed in §3.7 *d*, and contact with obstacles by the apical organ could cause arrests in a similar way. Either uniciliate apical cells or the tuft cells could be the receptors for the latter response, or the plexus could itself have some receptor function.

The majority of the cells in the pharyngeal system are probably motor cells responsible for the contraction of the pharyngeal muscles during swallowing, and there are sensory cells in the basal pharyngeal complex ideally placed to be stimulated in the presence of a food bolus so as to initiate this response. The median vestibular nerve provides a link to the circumoral muscle, which could be important in controlling the size of the mouth. The metatroch nerve can be assumed to be responsible for ciliary arrests in the metatroch, though the functional importance of these arrests is not clear. This leaves the rest of the suboral system, by elimination, to control the contractions of the various suboral muscles. The only obvious function for these contractions is the removal of debris from the pharynx. Presence of large particles or debris in the oral region, sensed by the suboral cells, or clogging of the pharynx, sensed by basal pharyngeal cells, presumably initiates muscle contractions sufficient to release and remove the

unwanted debris. While a strong rejectory response of this type was not observed very often under laboratory conditions, the ability to reject unwanted particles from the oral apparatus and prevent clogging is in principle as important as being able to capture particles in the first instance, and could be very important to larvae in the wild faced with a wide range of edible particles and debris. This could explain the need for something as complex as the suboral system.

The larval nervous system would seem, in summary, to be an assemblage of simple sensory and effector neurons responsible for a series of local reflexes. It remains to be seen whether these are coupled or coordinated in any significant way, and whether the system is capable of integrative activities. With regard to the cells themselves, it is remarkable how diverse they are morphologically and ultrastructurally (§3.6), and it would be surprising if there were not also some diversity as to their functional and physiological properties. As a corollary, given the obvious morphological differences between larval and adult nerve cells, it is to be expected that the former should be physiologically quite different, and probably more primitive, than the latter.

4. ORGANIZATION AND DEVELOPMENT IN THE LARVAL NERVOUS SYSTEM

4.1. *Summary*

The larval nervous system has two main parts that operate as separate functional and organizational units: the pretrochal system, which innervates the prototroch, and the system of nerves supplying the oral apparatus, that is, the pharynx, suboral region and metatroch. Nerve cells belonging to the latter are embedded in the epithelium they supply and show close associations with the larval trochal bands and structures derived from them. Some of the cells arise directly from the trochal band (the metatrochal nerve cells), others are probably modified trochal nerve cells (the suboral cells), while for the remaining cells (e.g. the pharyngeal nerve cells), a case can be made (§5) for their evolutionary origin from primitive trochal structures. The pretrochal system shows no such associations. Its cells lie entirely outside the trochal band they innervate and show affinities instead with the developing central nervous system: a central nervous system derivative, the larval eye, is an integral part of one of the nerves, and the arrangement of both cells and nerves reflects, in its symmetry, the pattern that develops later in the brain. The pretrochal nerves also differ in general appearance from other larval nerves (§3.6). It is reasonable, on this basis, to conclude that the pretrochal system is ultimately derived from the central nervous system, presumably by precocious differentiation of selected elements belonging to it. This conclusion is supported by what is known of the organization of the nervous system in related larval forms (§5.1).

The apical organ is ostensibly also a part of the pretrochal system, but is a composite structure, which makes it difficult to interpret. The apical tuft arises from it, and this is evidently an important larval structure. The principal neural associations of the apical organ are with the pretrochal nerves and the cerebral commissure, for which it acts as an organizational centre, but its early segregation, as the apical rosette, from the precursors of the rest of the cerebral nervous system establish it clearly as a separate entity in its own right. Whatever its associations or derivation, whether mainly from larval structures, the central nervous system, or both, the apical organ plays a central role in the development of the nervous system. All evidence indicates it to be an organizational centre of considerable importance and antiquity.

Although this is not primarily a developmental study, the results permit a number of inferences regarding probable events of neurogenesis. To control effectively the events of

early neurogenesis, which generally result in the establishment of a set of simple neural pathways, there are two things that must be accomplished: there must be some means available to (i) specify the cells or groups of cells that are to differentiate as neurons, and (ii) specify the pathways along which fibres produced by these cells are to travel. The first of these, in spiralia, is generally considered to depend upon cell lineage (§4.2). While there are a number of elegant classical studies of annelid lineage, some of which deal with the origin of neural structures (e.g. Wilson 1892; Child 1900), none of these are carried sufficiently far through embryogenesis to identify specific neural precursor cells with certainty. The apical organ would appear to be the most accessible of the neural structures identified here as a subject for future lineage studies, but establishing the lineage of other neural precursor cells, for example in the pharynx, is likely to be a considerably more demanding task.

However it is accomplished, neuronal precursor cells are in place in a number of larval organs by the time organogenesis has begun. Certain of these cells then differentiate, apparently of their own initiative, and produce pioneer fibres. The suprapharyngeal cell, the four pretrochal cells, both metatrochal nerve cells, and the median suboral cells all apparently belong to this category. Most of the remaining cells, if they eventually produce fibres, do so only after the arrival of pioneering fibres originating elsewhere. Most of the pharyngeal nerve cells, which add their fibres to the pharyngeal nerve in this way, and the lateral suboral cells belong to this category. This type of phenomenon, an interaction with a growing fibre followed by a response, is well documented in other invertebrate systems (Anderson *et al.* 1980; Keshishian & Bentley 1983). The pioneering fibres produced by the first cells that differentiate initially grow out either along predetermined pathways or towards identifiable targets. In the case of the oral apparatus, fibre growth appears to be predominantly of the first type, along paths formed by trochal cells or derivatives of these, but some specific targets may be important, for example, the pharyngeal complex. Fibres from the pretrochal cells ultimately encircle the prototroch, which forms an identifiable growth pathway, but their initial targets, as they cross the episphere, appear to be the apical organ and various sites along the prototroch. It is also possible that the muscle cells, which differentiate early and whose nuclei occupy invariant positions in the larva, provide guidance cues of some type.

Generally then, the earliest events of neurogenesis are initiated independently at a number of sites in the larva, and the connections between these needed to form a complete system are established secondarily. As a result, the larval nervous system is to some extent an organizational patchwork, with each part of the system retaining something of its own character in terms of cell types and the pattern of connections between cells. It is as if, in the evolution of a reduced and specialized larva, which the *Spirobranchus* larva undoubtedly is, the neural components of each larval structure have been reduced and specialized independently. To the extent that nearly every cell can be tentatively assigned an individual developmental role, each may be essential for the correct assembly of the system as a whole. While this is only the author's impression, based on a liberal interpretation of the cellular morphology, it does contrast with the impression similar criteria give of the developing adult system. The latter arises from a series of substantial cellular rudiments containing more cells, but fewer identifiable cell types. The cells in these rudiments may each have individual and essential roles to play in adult neurogenesis, but if this is the case, it is not obvious in their ultrastructure. The developing adult system is much less of a mosaic, in terms of clearly identifiable cell types, than the larval system.

4.2. *Developmental perspective*

Spiral cleavage characteristically produces an assemblage of precisely positioned identifiable blastomeres whose further development is traceable in considerable detail. This convenient fact, coupled with the results of early experimental work on spiralian embryos, led to the classical concept of mosaic, or determinate, development: the embryo was considered to be a mosaic of cells in which each cell, by virtue of its position in the cell lineage, is restricted to a particular fate toward which it differentiates autonomously (Conklin 1897; Wilson 1904). In the light of more recent experiments on molluscan embryos (Clement 1971; Cather 1971; Verdonk 1979; van den Biggelaar *et al.* 1981), this concept has been modified to emphasize (i) the importance of cellular interactions in specifying some embryonic structures, that is, some blastomeres are less than completely autonomous in their capacity for self-differentiation, and (ii) the special role of cleavage asymmetry in establishing the dorsoventral axis of the embryo. The D quadrant, or at least some of its blastomeres, exerts an organizing influence on the embryo as a whole, and is generally required for the normal expression of the structures that define the dorsoventral axis.

It is implicit in the concept of determinate development that lineage-dependent phenomena similar to those involved in cleavage and early embryogenesis could act throughout embryonic development, and even in the post-embryonic period, to control development in a variety of organs and structures. The recent work cited above on dorsoventrality tends to emphasize the importance of lineage in early embryonic events at the expense of later ones, and there remains much that is unexplained about how the majority of cell types of which the embryo or larva is ultimately composed are produced and organized into a functioning whole. Most experimental studies of necessity rely on a few readily identifiable external structures to indicate the presence or absence of particular larval organs or body parts. Ciliary structures, bristles, and various sensory organs (e.g. the eyes) have been used in the past as markers (Novikoff 1938, Clement 1971), but these constitute far less than a full inventory of the cell types and cellular structures likely to be present in a given species. While this study of *Spirobranchus* is not complete in all regards, it provides, in the author's view, a better indication of the degree of diversity of cell type and complexity of structure that can occur in spiralian larvae, and better illustrates the developmental problems these larvae face. By and large, there is little besides the gut and unspecialized parts of the external epithelium that approach a tissue level of organization in *Spirobranchus*. It is instead the rule rather than the exception to find complex structures composed of a number of disparate cell types, with no more than a single cell of any one type in some instances. It seems unlikely that such structures could develop in an orderly and reliable fashion without the repeated involvement of lineage-dependent mechanisms and considerable cell autonomy. Cell lineage has been most thoroughly studied in nematodes, and direct comparison with nematodes may be in order when dealing with highly reduced spiralian forms like the *Spirobranchus* larva. In both, the body is essentially an assemblage of individual cells that fit correctly within the whole by virtue of being correctly shaped and positioned, as well as being of the correct type and number. The cellular pieces of such an assemblage, in nematodes at least, are the result of determinate cleavage and show strict lineage-dependent cell autonomy in most instances (Sulston & White 1980). It is difficult to envisage ways in which development in *Spirobranchus* could operate very much differently.

The nervous system, more than any other structure, depends on precise and specific ordering

of its constituent elements if it is to function correctly, and in the spiralian nervous system in particular, the functional and developmental importance of individual identifiable cells is well established (Bullock & Horridge 1965; Kandel 1976; Kandel *et al.* 1980; Anderson *et al.* 1980). Cell lineage is clearly important in the development of the adult nervous system of annelids (Stent *et al.* 1982; Blair 1983), but the mechanisms involved are poorly understood. Mechanism is equally a problem in the trochophore. What is needed, at least in the initial stages of neurogenesis, is some means of causing individual cells, occupying particular and often widely separated locations, to differentiate to the correct neural cell type at the correct time. The mechanism could be the same as that regulating the fates of blastomeres in early cleavage or, since cleavage and neurogenesis are separate developmental events, the mechanisms controlling them could be separate, perhaps quite different, and independently evolved. Müller's larva serves as a useful reminder of what the ancestral polychaete larva might have been like. Each of its nerves is the result of fibre outgrowth from a considerable number of apparently identical cells, in the case of the trochal nerves, of identical uniciliate sensory cells (Lacalli 1982). Corresponding nerves in the trochophore (§5.1) consist of fibres from far fewer cells, but cells of a number of different types. All of the nerve and sensory cells of the suboral region and metatroch are, for example, probably derived from the single uniciliate sensory cell type mentioned above. Evolution of the *Spirobranchus* trochophore has apparently been accompanied by both a reduction in the number of cells innervating a given structure, and by specialization among the cells that remain. Since both Müller's larva and the trochophore have spirally cleaving eggs of a similar type, it is comparatively easy to envisage how the mechanism of blastomere determination might have been gradually refined and extended as a means of control over the process of reduction and specialization among the cells of the larval nervous system as the system evolved. An alternative is to suppose that the development of the larval and adult nervous systems are similarly controlled in ways unrelated to cleavage events. §5.1 gives examples of larval structures derived secondarily, by precocious differentiation, from the adult. This appears to be a common phenomenon in spiralian larvae, and accounts for the origin of many of their more advanced features (Jägersten 1972; Freeman 1982). Much of the larval nervous system in the trochophore is larval in origin rather than secondarily derived, but even these parts of the system are advanced, compared with Müller's larva, in terms of their complexity. One cannot rule out the possibility that a developmental mechanism evolved by the adult, as a means of controlling some aspect of its differentiation, for example neurogenesis, might appear precociously in the larva and act on larval development, for example, on its nerves.

5. PHYLOGENETIC CONSIDERATIONS

5.1. *Comparison of the* Spirobranchus *trochophore with Müller's larva*

Müller's larva, the type larva of polyclad flatworms, is generally accepted as being closely related to the trochophore. It exemplifies a prototrochophore level of organization according to some authors and, as such, is considered representative of the ancestral larval type from which the trochophore probably evolved. Embryonic cleavage is very similar in the two groups, with the segregation of the trunk rudiment proceeding in an almost identical fashion (Ax 1963). The two larvae are also similar in terms of their overall organization: both have an apical tuft and a circumferential preoral trochal band whose cilia beat toward the ventrally located mouth, and most of the internal structures, for example, brain and protonephridia, are similar in

structure and occupy comparable positions. The main differences between the two larval types relates to the more complex feeding apparatus and digestive system of the trochophore. The mouth in Müller's larva leads to a simple sac-like, endodermal gut, and cilia of the surrounding oral and trochal bands are responsible for the collection of food particles. A muscular pharynx develops later, but is not functional until after metamorphosis (Ruppert 1978). The trochophore, in contrast, has a complex, regionally differentiated digestive tract with an anus and specialized stomodeal organs (vestibule and pharynx in *Spirobranchus*) for food collection and swallowing. These operate primarily by muscle contraction, and while ciliary effectors may still function in food collection, they are relegated to a more minor, accessory role. These alterations in the feeding apparatus, and particularly the altered arrangement of the trochal bands, considerably complicates the task of identifying structural homologues in the two larvae. Larval nerves are supposed generally to be evolutionarily more conservative than the structures they innervate, so it should be possible to establish structural homologies and larval interrelations more reliably on a neuroanatomical basis (Hyman 1951; Nielsen 1979). Detailed comparison of the nervous system of Müller's larva and the trochophore, which, based on this account and previous work by the author (Lacalli 1982, 1983), are remarkably similar, strongly supports this supposition. Substantial changes to the larva are accompanied by comparatively minor changes to the organization of the nervous system, and comparable structures can be identified with some confidence.

The nervous system of Müller's larva is separable into two main parts, a central and a peripheral part, depending on whether the nerves lie inside or outside the epidermal basement membrane. The central nervous system, which lies predominantly inside the latter, comprises a brain and four longitudinal nerve cords. The brain is bilaterally symmetrical with a central plexus of fibres beneath which is a cerebral commissure. The commissure branches on either side of the brain to produce the four cords, a pair of dorsolateral cords and a pair of ventrolateral ones. These terminate at the ciliary nerve supplying the trochal band, a part of the peripheral system, which they intersect orthogonally. Equivalent central and peripheral elements in the trochophore nervous system are less easily identified on the basis of position alone since all are basically epidermal structures, at least during early development. Typically, paired epithelial proliferative centres give rise to the two cerebral ganglia. A commissure, passing through the apical organ and its central plexus, develops to link these, and only one major connective, the circumoesophageal connective, is generally reported leaving the brain. In some trochophores (e.g. *Phyllodoce*, Lacalli (1981)), a rudimentary bilaterally symmetrical brain complete with commissures and connectives is established even in the young trochophore.

The evidence provided here on *Spirobranchus* indicates a possible fourfold symmetry in the early organization of the brain, at least with regard to the arrangement of the main fibre tracts (§3.5). This raises the possibility that the basic, primitive plan is of four radiating tracts corresponding to the dorsolateral and ventrolateral cords of Müller's larva, represented in *Spirobranchus* by the paired dorsal and ventral tracts within the brain and reflected, in the larva, in the arrangement of the pretrochal cells and their nerves (§3.3). It may also be significant that in both *Spirobranchus* and Müller's larva, the cerebral eyes are mainly associated with dorsolateral nerves.

The peripheral part of the nervous system in Müller's larva consists of a number of intraepithelial nerves supplying the trochal band and oral field (figures 90*a*, 91*a*). Since the mouth in Müller's larva lies at the junction of the ectoderm and endoderm, its homologue in
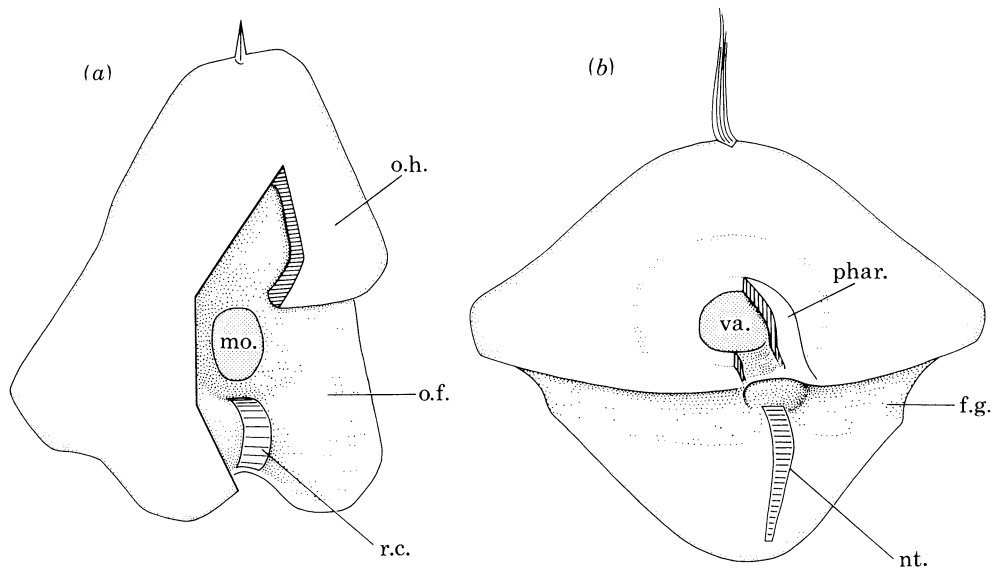
FIGURE 90. The oral apparatus of (a) Müller's larva and (b) the serpulid trochophore showing (as stippled contours) the extent of the ciliated food collecting surface in each. Portions of the oral hood and pharynx are cut away to show this surface more clearly. The inner limit of the oral apparatus is its junction with the endoderm, the mouth in Müller's larva and the pharyngeal valve in the trochophore; both are uniformly stippled. Note the substantial cavity behind the oral hood in Müller's larva. The median oral hood nerve, shown in figure 91 a, runs along the roof of this cavity.
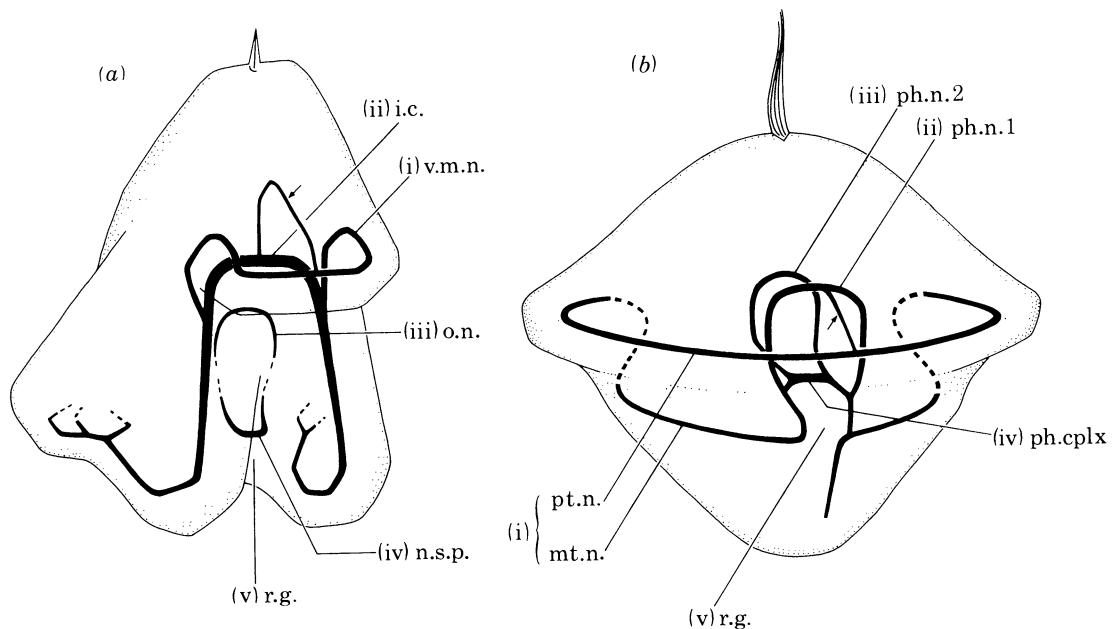
FIGURE 91. Innervation of the oral apparatus of (a) Müller's larva and (b) the *Spirobranchus* trochophore, both somewhat schematic. The five main elements considered comparable in the two systems are numbered (i–v) in the order they are treated in the text. (a) is modified from Lacalli (1982); dotted lines show the continuation of the marginal nerve in the dorsal epidermis and, between the oral and suboral nerves, a region occupied by small fibres that do not form a complete connection. The small arrow indicates the median oral hood nerve. Dotted lines in (b) show the connection between the prototroch and metatroch nerves required by phylogenetic schemes that derive the food groove, by lateral extension, from a primitive ventral oral field. The small arrow indicates the median vestibular nerve.

the trochophore is the junction between the pharynx and oesophagus, the site of the pharyngeal valve. The functional mouth in the trochophore then derives by constriction of what in Müller's larva would be the inner part of the oral field which, in the trochophore, forms the stomodeum. The outer limit of the oral apparatus in Müller's larva is the trochal band, more precisely the ventral half of the trochal band, which runs along the margin of the oral field. The comparable limit in the trochophore is the margin of the food groove, formed by the combined prototroch and metatroch (figure 90*b*). The region lying between this limit and the pharyngeal valve in the trochophore is then equivalent to the oral apparatus of Müller's larva. The nerves that supply this region (figure 91*b*) can be compared with their counterparts in Müller's larva on an individual basis as follows.

(i) *Trochal nerves*. In the trochophore, the prototroch and metatroch are separate bands with separate innervation. This is probably a secondary condition, with the two bands being derived from trochal cells at the top and bottom margins of a broad, ventral oral field that expanded progressively around the sides of the larva during its evolution, eventually fusing dorsally to form a complete ring (Nielsen 1979). This supposition is supported by the ventral to dorsal sequence seen in trochal differentiation and by the existence of dorsal gaps and a variety of middorsal anomalies in the trochal bands of various species. The most likely equivalent to the combined trochophore prototroch and metatroch, in Müller's larva, is the ventral half of the marginal trochal band in the latter. The nerve supplying this band belong to the peripheral system, and the cells responsible for it lie in the band itself. A similar intratrochal type of innervation occurs in the metatroch in *Spirobranchus*, but not the prototroch. Assuming intratrochal innervation is the primitive condition, this must have been replaced secondarily in the prototroch with external innervation by central nervous system derivatives, that is, the pretrochal nerves. Such innervation would be expected to arrive via the equivalent to the ventrolateral cord in Müller's larva, which, in the trochophore, would be pretrochal nerve I, and indeed it does. It is not clear whether the dorsal half of the trochal system of Müller's larva, as described by the author (Lacalli 1982), has any counterpart at all in the trochophore.

(ii) *Primary pharyngeal nerve*. This nerve crosses the top surface of the trochophore pharynx about midway between the outer and inner limits of the oral apparatus. Its nearest equivalent in Müller's larva is the intraepithelial commissure, which occupies a comparable position and is also incomplete basally. In both larvae, these are connected to the trochal system by a small median nerve (arrows in figure 91), the median vestibular nerve in the trochophore, which extends to the prototroch nerve in early stages only, and the median oral hood nerve in Müller's larva. These two small nerves resemble each other to the extent that both are closely associated with, and presumably arise from, large and quite distinctive medially located cells. In the trochophore there is one such cell, the suprapharyngeal cell, ph. 1. In Müller's larva there are three cells (one is visible just above the intraepithelial commissure at its centre in figure 4 of Lacalli (1982)), one of which (T. C. Lacalli, unpublished) lies behind the commissure in a position exactly comparable to that of ph. 1.

(iii) *Secondary pharyngeal nerve*. This nerve crosses the top surface of the trochophore pharynx near its junction with the oesophagus and joins basally to the pharyngeal complex. The comparable structure in Müller's larva is the oral nerve, which arises from a group of neurons along the top margin of the mouth. The oral nerve is not continuous basally in the young Müller's larvae examined, but it may link later with the nerves of the suboral plate judging from the directions the fibres follow in the oral field epithelium.

(iv) *Basal pharyngeal complex*. This structure is formed by a cluster of cells on the floor of the pharynx about midway between the primary and secondary pharyngeal nerves and is linked to those by laterals. In Müller's larva this position is occupied by the U-shaped suboral plate nerve which originates (unpublished) from three cells located beside and beneath the rejectory cell. This nerve does not connect with either the trochal nerves or the oral nerve at the stage examined, but may do so at a later stage.

(v) *Rejectory gap*. The various trochal nerves do not join beneath the mouth in a simple fashion in either larva. In the trochophore, a junction is formed by means of the suboral plexus, but this develops rather late, which suggests it may be a secondary structure. In Müller's larva, the marginal nerves connect eventually in the dorsal epithelium of the larva, but there is no direct connection along the posterior margin of the oral field at the site of the rejectory gap.

The above comparisons have some unsatisfactory features with regard to the details of innervation of the oral apparatus, but a common overall plan is evident in both larvae. Both have a system consisting basically of three supraoral components, that is, nerves that pass over the mouth and oral field or stomodeum, and one basal component associated with the inner parts of the system and located behind or under the rejectory tract. Considerable differential growth of these components, particularly of the trochal nerves, and some rearrangement of the connections between them would be needed to transform one system to the other. By and large, however, it appears that such transformation could be accomplished without the addition of any entirely new neural structures, that is, rearrangement of structures common to both larvae appears to be sufficient to account for all the major nerves.

Since Müller's larva is the more primitive of the two larvae, its oral apparatus, or something very much like it, is presumably ancestral to the trochophore system. There then remains a problem with the anus. Some authors (e.g. Beklemischev 1969; Nielsen 1979) consider both mouth and anus in the annelid to be derivatives of the primitive flatworm mouth. It is proposed that separate anterior and posterior openings to the developing gut are formed by fusion of the lateral margins of the blastopore. This process should result in the subdivision of any primitive circumoral system of nerves into separate oral, anal, and midventral tracts. The present study provides no evidence for either anal or midventral nerves of the expected type except for the neurotroch nerve, which is, however, simply an extension of the suboral system rather than a nerve tract of any consequence in its own right. The oral apparatus of Müller's larva is instead fully represented in the oral apparatus of the trochophore, and the two structures and their nerves, on this basis, would appear to be homologous.

The above similarities provide compelling evidence for a close evolutionary relation between Müller's larva and the trochophore. To carry this further, it is useful to refer to Jägersten's concepts on the evolutionary origin of larval structures. Briefly, Jägersten (1972) recognizes the existence of primary larvae in some marine phyla, which are supposed to be descended directly from the pelagic larvae of ancestral metazoa. Such larvae retain some primitive features, derived from the ancestral larval forms, and other features secondarily derived, by precocious differentiation (adultation), from the adult organism at various times in its evolution. A given larva is then, to some extent, a patchwork of primitive and secondarily derived characteristics. In the case of Müller's larva, the author has previously argued (Lacalli 1982) that the trochal bands and their nerves (that is, the peripheral nervous system) are primitive larval structures, and that the central nervous system is secondarily derived. Ciliary feeding is generally assumed to be primitive, while specialized digestive organs, like the

trochophore pharynx, are clearly secondary. Note then that in the trochophore, the major larval locomotory organ, the prototroch, a primitive larval structure, is innervated by nerves ultimately derived from the central nervous system, i.e. from an adult structure. In contrast, its principal secondarily derived adult effector, the pharynx, is innervated by nerves that are primitive and larval in origin, but are modified for this task. If this interpretation is correct, then even primitive nerves can apparently assume new functions.
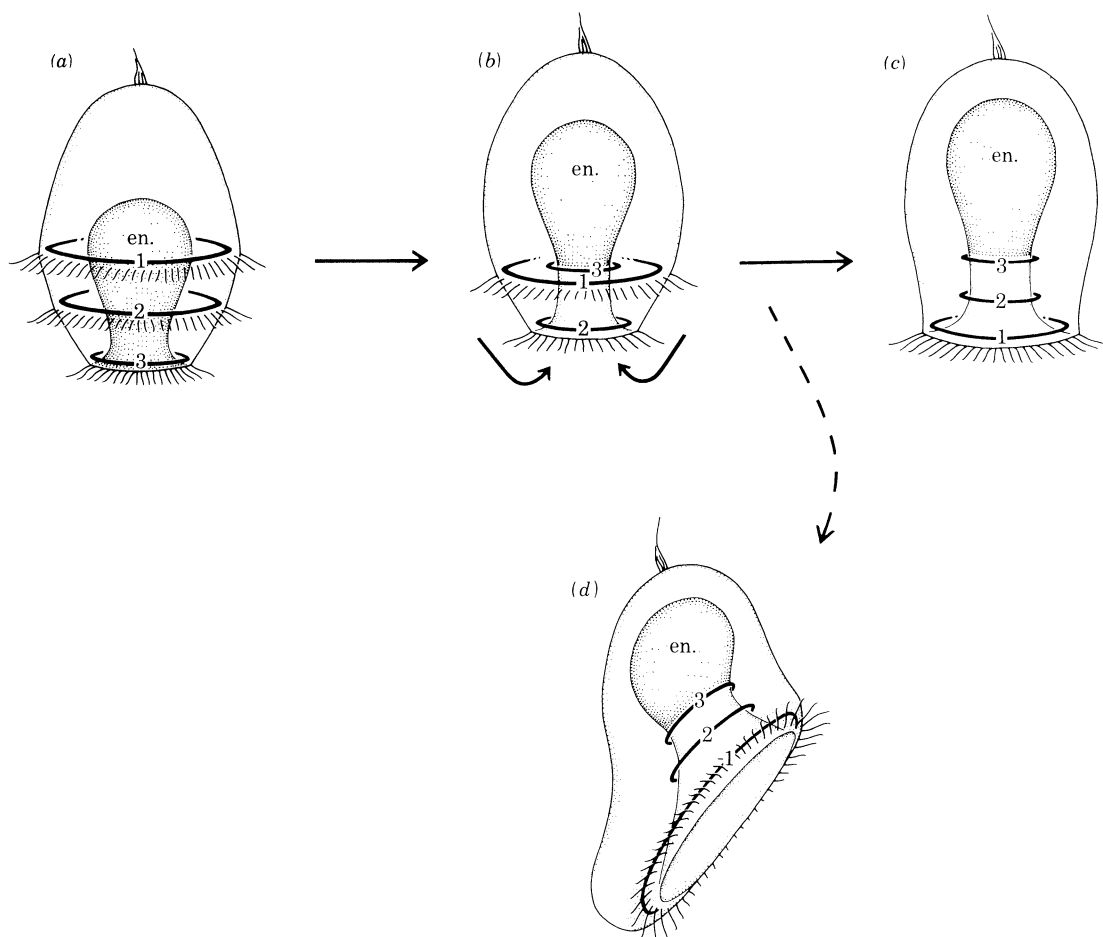
FIGURE 92*a–c*. A hypothetical scheme for the conversion of the nerves supplying three circumferential trochal bands (1–3 in anterior to posterior sequence) in a primitive polytrochal larva into one trochal and two stomodeal nerves with progressive involution of the ectoderm (as in (*b*), arrows) to form an expanded oral apparatus or stomodeal surface. Dark stippling indicates the primitive gut, that is, the endoderm. (*d*) A variant of (*c*) with a ventrally shifted mouth, which produces something approximately equivalent, in form and organization, to Müller's larva.

5.2. *Evolutionary continuity of the oral apparatus in protostomes*

The similarities demonstrated between Müller's larva and the trochophore in §5.1 have broader implications with respect to the basic plan from which both larvae may have evolved. The important point, in this regard, is that the two larvae show the differing degree to which otherwise very similar stomodeal structures and their nerves can be internalized during

blastopore. The above analysis constitutes a concrete demonstration of the phylogenetic value of structures identifiable as primitive and basically larval in origin as described by Jägersten.

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ABBREVIATIONS USED IN THE FIGURES

The organ to which particular cell types belong is given in parentheses where appropriate. Structures belonging to Müller's larva are indicated by (M.l.).

a. 1–5	apical cells 1–5	l.s.	lateral suboral cells
a.o.	apical organ	l.int.	lateral integrative centre (brain)
a.p.	apical plexus	l.vb.n.	lateral vestibular nerve
a.t.	apical tuft	ln.m.	longitudinal muscle
a.v.	anal vesicle	m.	muscle
an.	anus	m.int.	median integrative centre (brain)
au.	auxiliary cell (apical organ)	m.s.	median suboral cells
b.ep.	basal epithelial cell (vestibule)	m.vb.n.	median vestibular nerve
b.p.	basal process cell (apical organ)	mb.	mesoblast
c.c.	cerebral commissure	mch.	mesenchyme cell (apical organ)
c.cn.	circumoesophageal connective	mes.	mesodermal band
c.gn.	cerebral ganglion	mo.	mouth
c.m.	circumoral muscle	mt.	metatroch
cap. 1, 2	capsular cells 1 and 2	mt. 1, 2	tiers 1 and 2 of the metatroch
ch.	chetal cells	mt.m.	metatrochal muscle
cp.	companion cell (= ph. 4, pharynx)	mt.n.	metatroch nerve
d.	dorsal	mt.n.c.	metatrochal nerve cell
d.au.	dense auxiliary cell (apical organ)	mu.	pharyngeal mucus cell
d.t.	dorsal tract of cerebral commissure	n.	nerve or neurite
e.	eye	n. I–III	pretrochal nerves I–III
en.	endoderm	n.s.p.	suboral plate nerve (M.l.)
ep.	epithelial cell	neph.	protonephridium or parts thereof
ep.th.	epithelial thickening of the trunk rudiment	nt.	neurotroch
		nt.n.	neurotroch nerve
f.g.	food groove	oes.	oesophagus
g. 1–3	glial-like cells 1–3 (pharynx)	oes.m.	oesophageal muscle
gl.	apical gland cells	o.f.	oral field (M.l.)
i.c.	intraepithelial commissure (M.l.)	o.h.	oral hood (M.l.)
i.v.n.	inner ventral nerve cord	o.n.	oral nerve (M.l.)
in.	intestine	o.v.n.	outer ventral nerve cord
L	left	ph. 1–9	pharyngeal cells 1–9
l.ep.	lateral epithelial cell (vestibule)	phar.	pharynx

ph. cplx	pharyngeal complex	s.cplx	suboral complex
ph.m.	pharyngeal muscle cells	s.ep.	median suboral epithelial cell
ph.m. 1, 2	front and back pharyngeal muscle cells, specified	s.p.	suboral plexus
		st.	stomach
ph.n. 1, 2	primary and secondary pharyngeal nerves	t.c.	terminal commissure
		t.p.	terminal pore of anal vesicle
prt. 1–4	pretrochal cells 1–4	v.	ventral
prt. n.	pretrochal nerve	v.c. 1–3	ventral commissures 1–3
pt.	prototroch	v.m.	ventral muscle
pt. 1–4	tiers 1–4 of the prototroch	v.m.n.	ventral part of the marginal ciliary nerve (M.l.)
pt.m.	prototrochal muscle		
pt.n.	prototroch nerve	v.n.	ventral nerve cord
R	right	v.t.	ventral tract of cerebral commissure
r.c.	rejectory cell (M.l.)	va.	pharyngeal valve
r.g.	rejectory gap	vb.	vestibule
s. 1–4	suboral cells 1–4	ves.	dense vesicle cell (= ph. 3, pharynx)
s.a.	secondary apical cells (apical organ)	*	see figure description
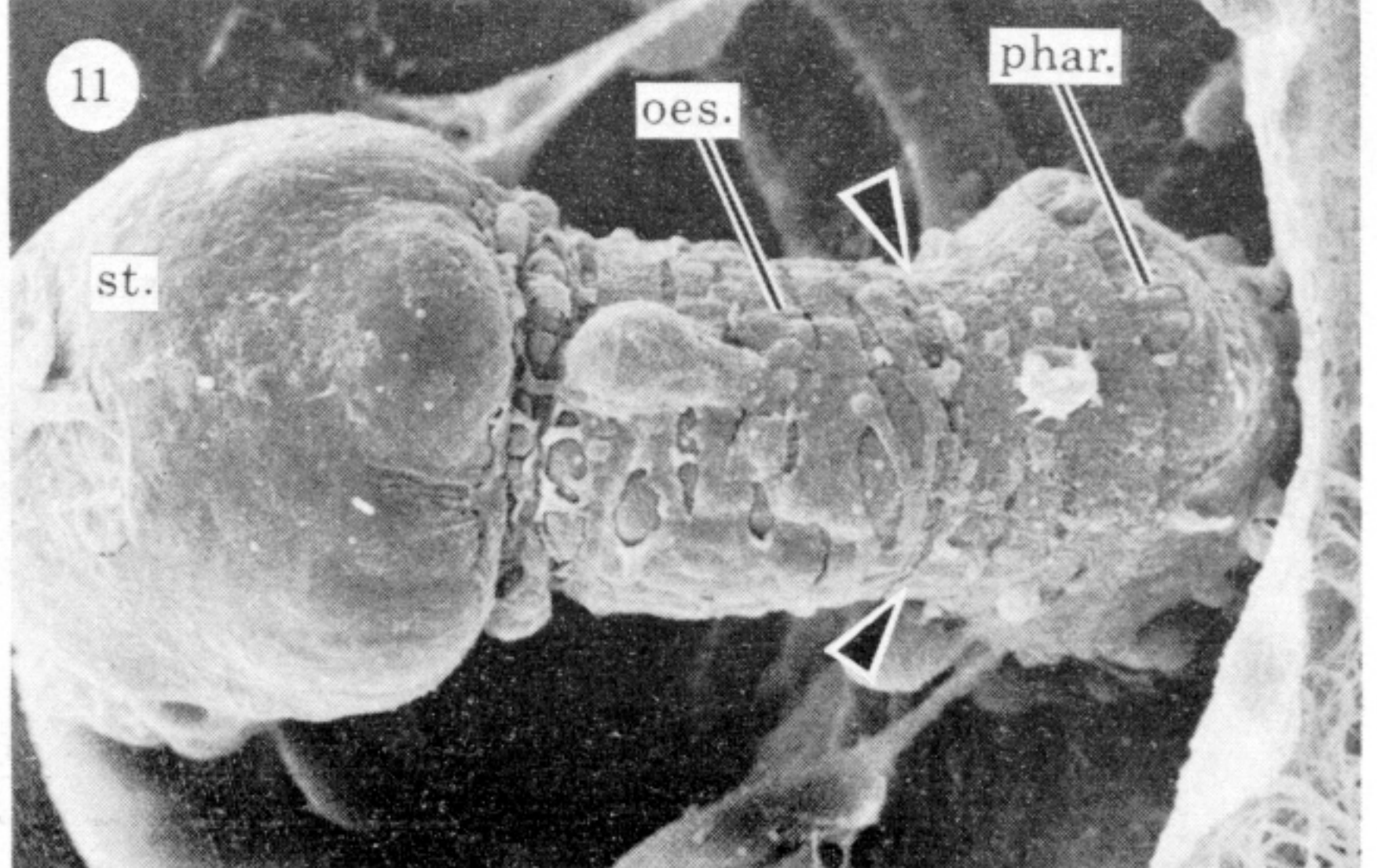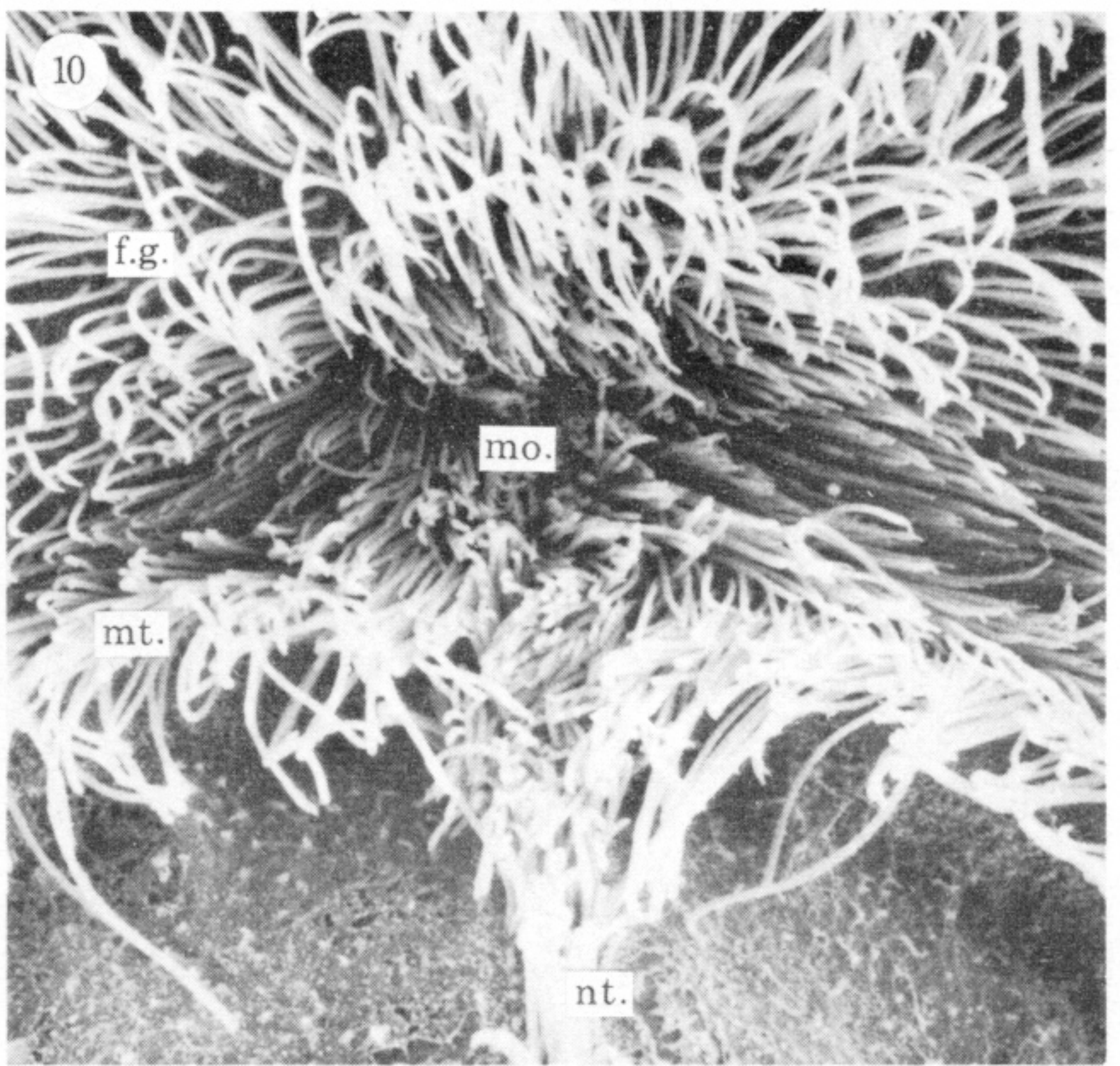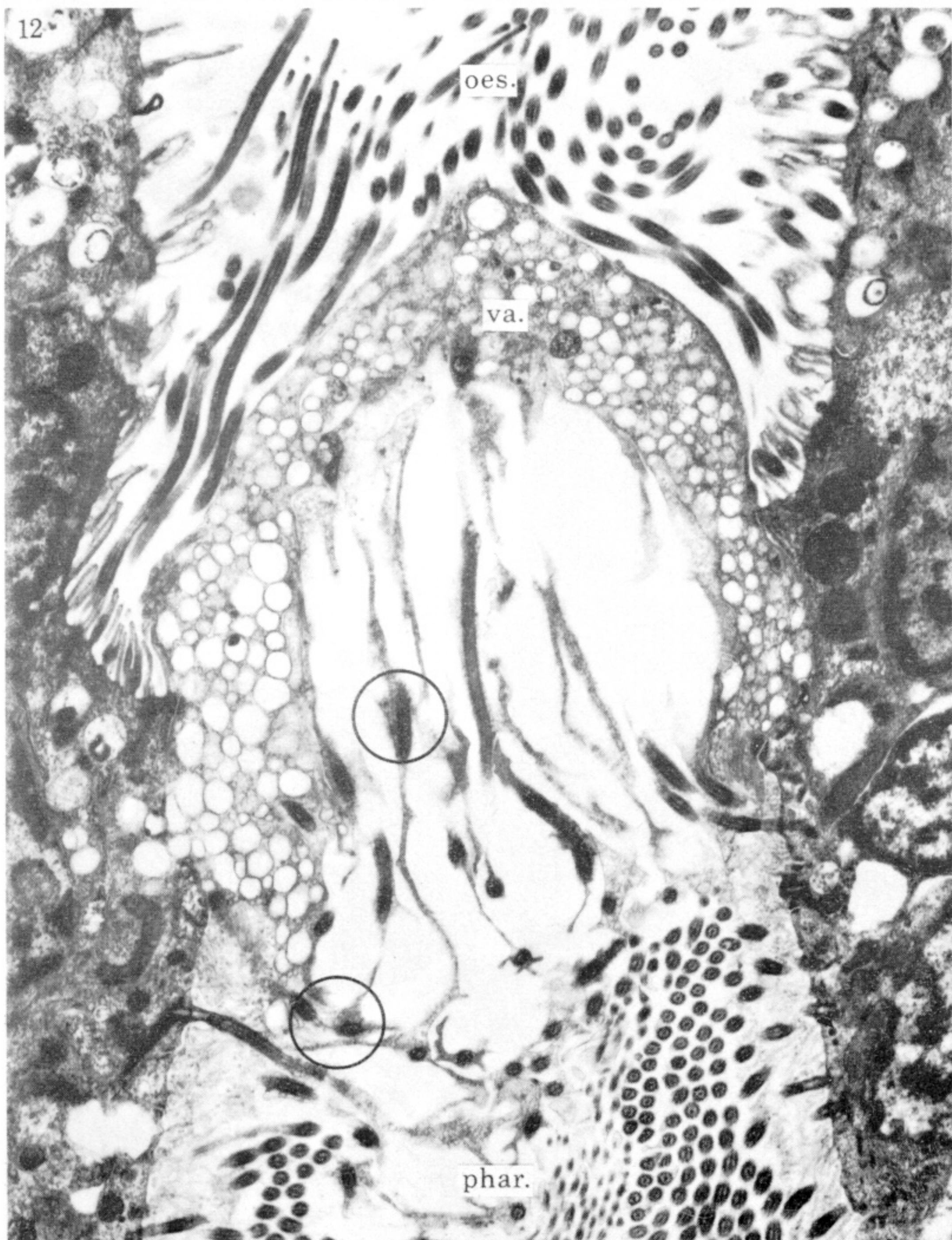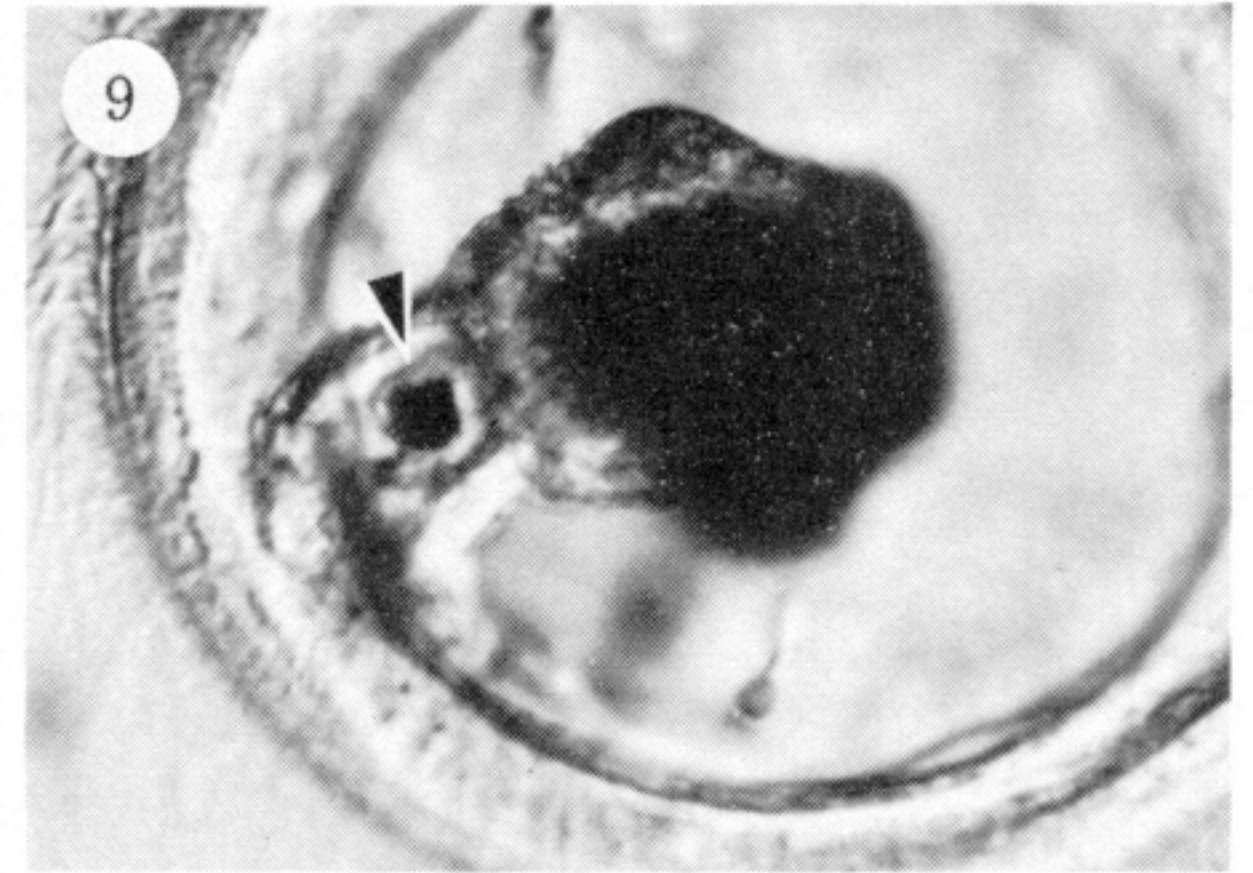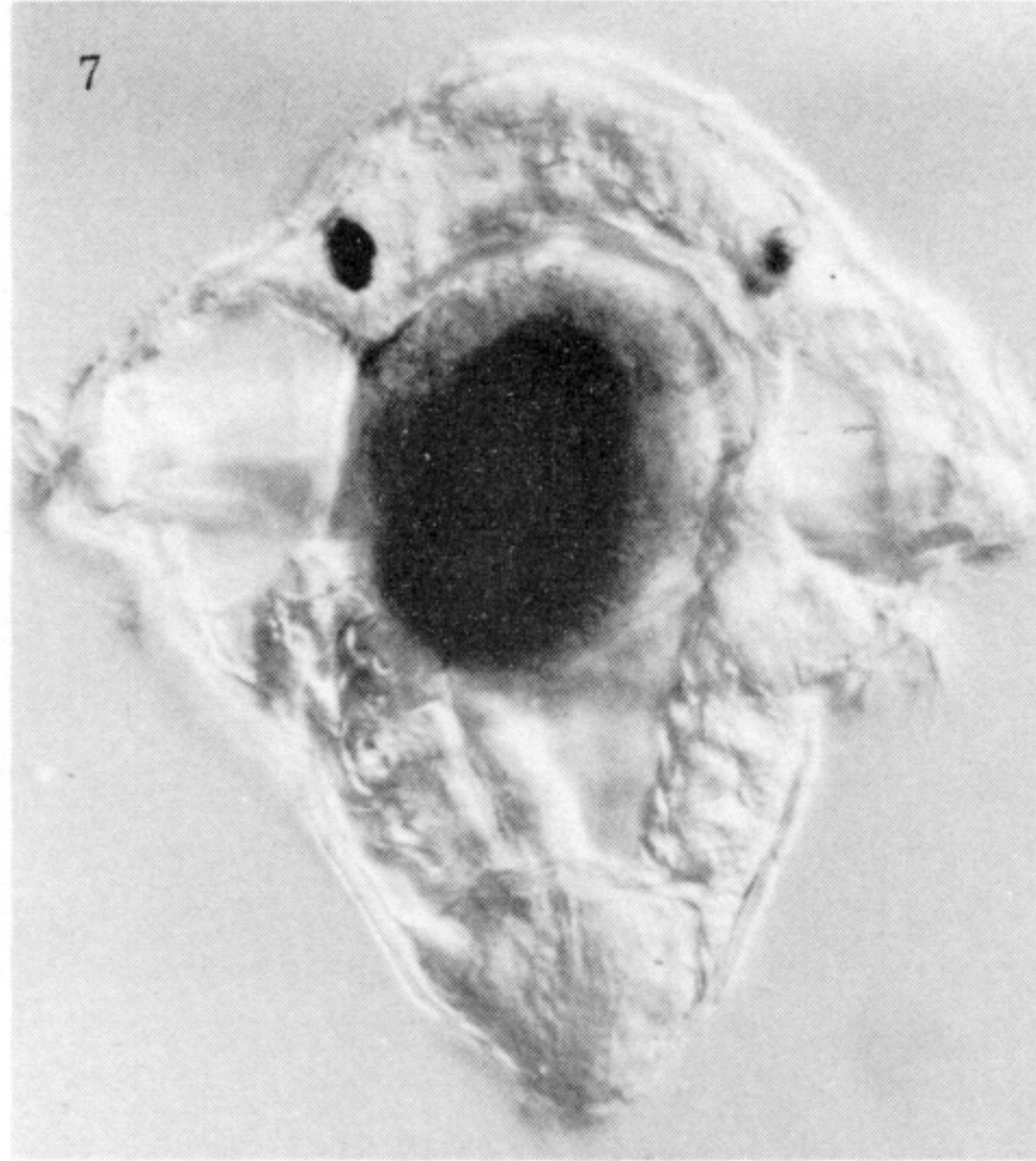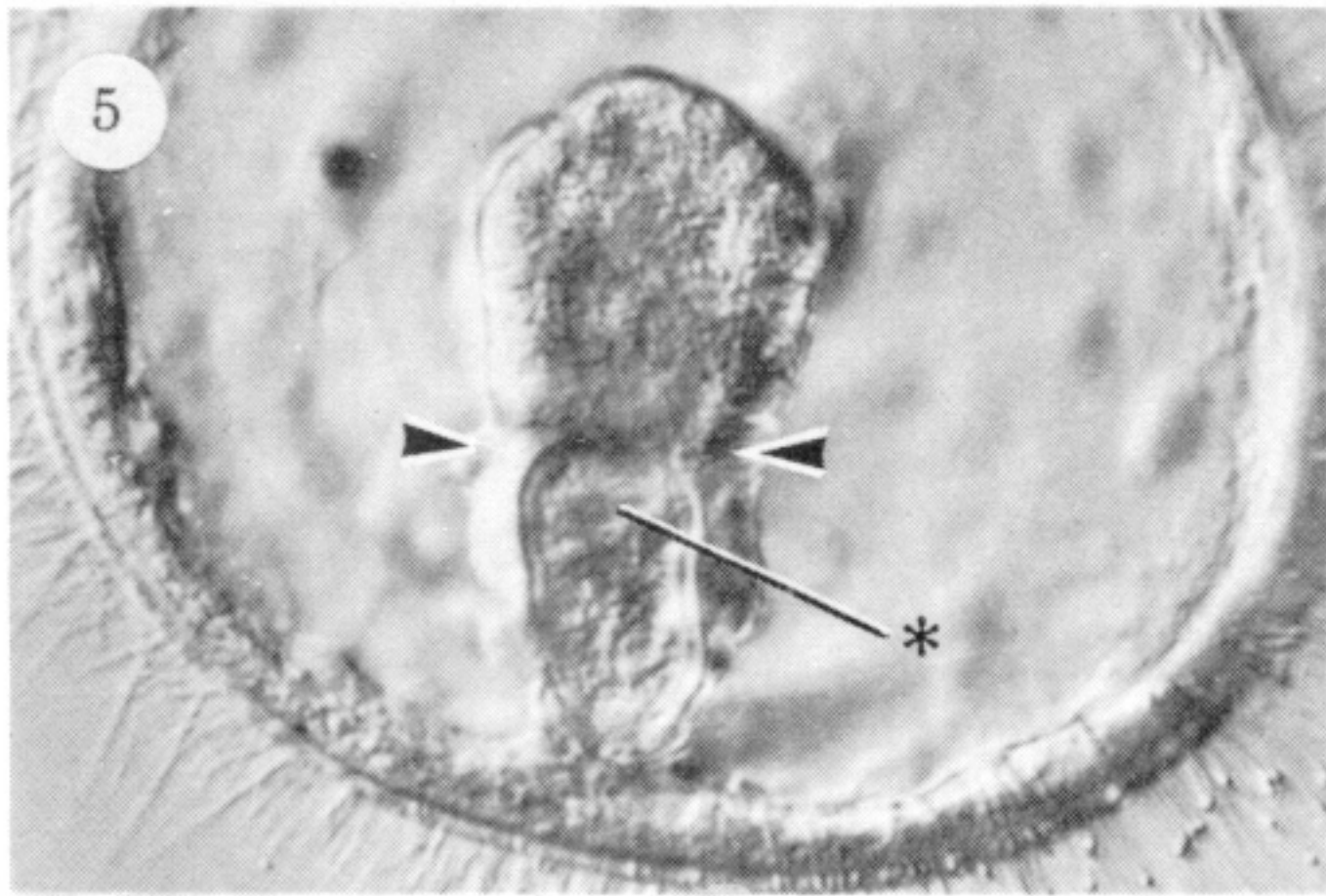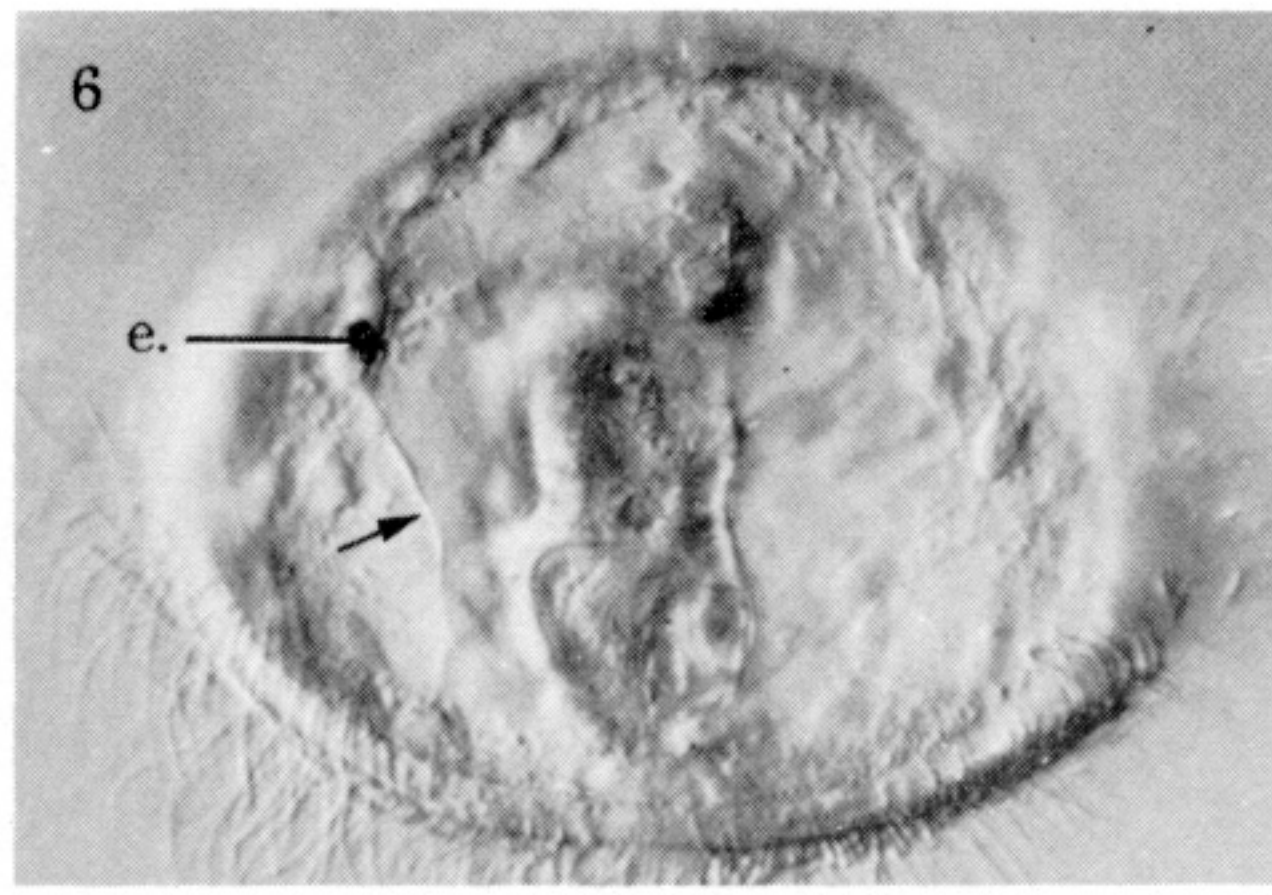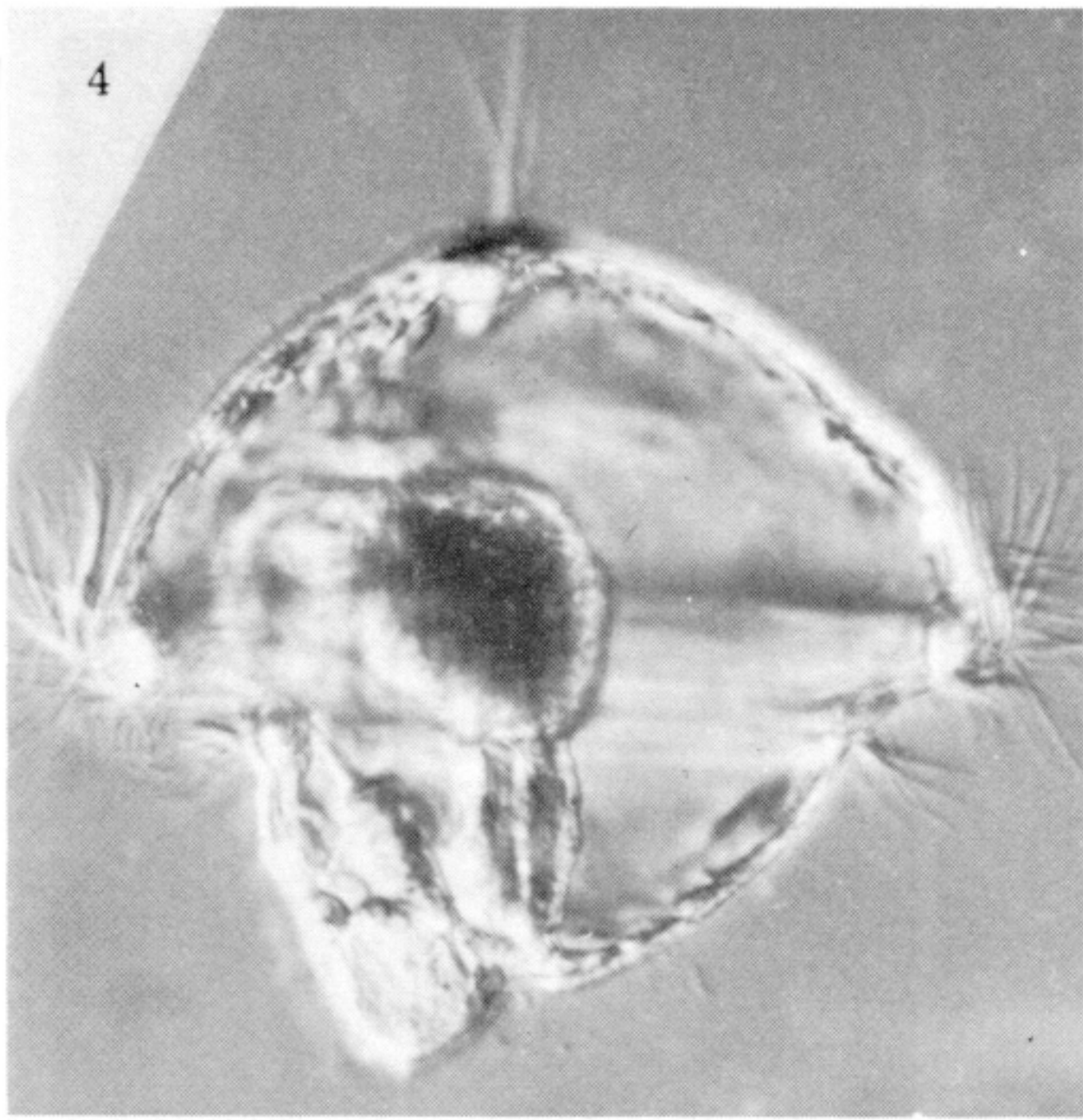
FIGURES 4–12. For description see opposite.
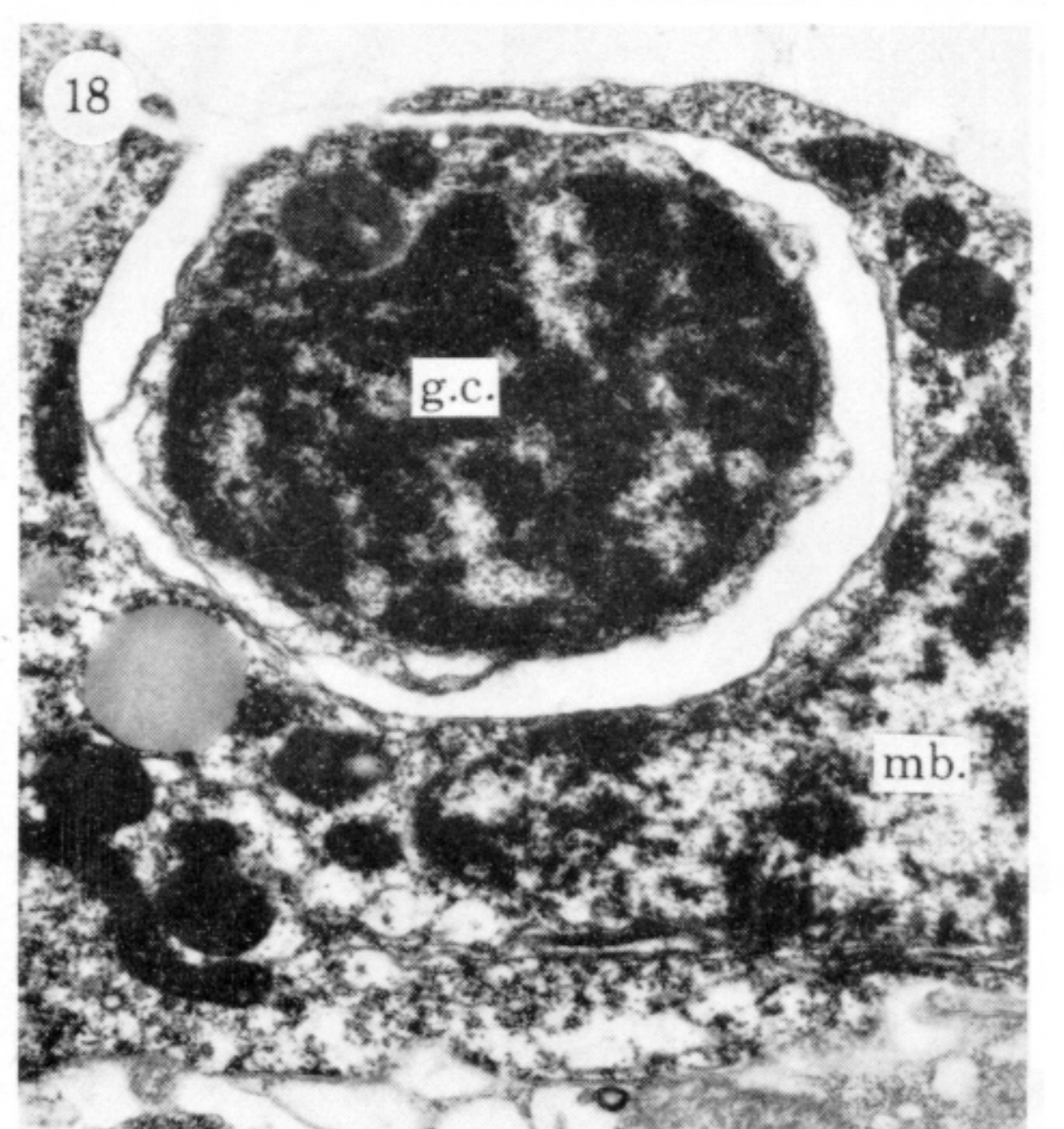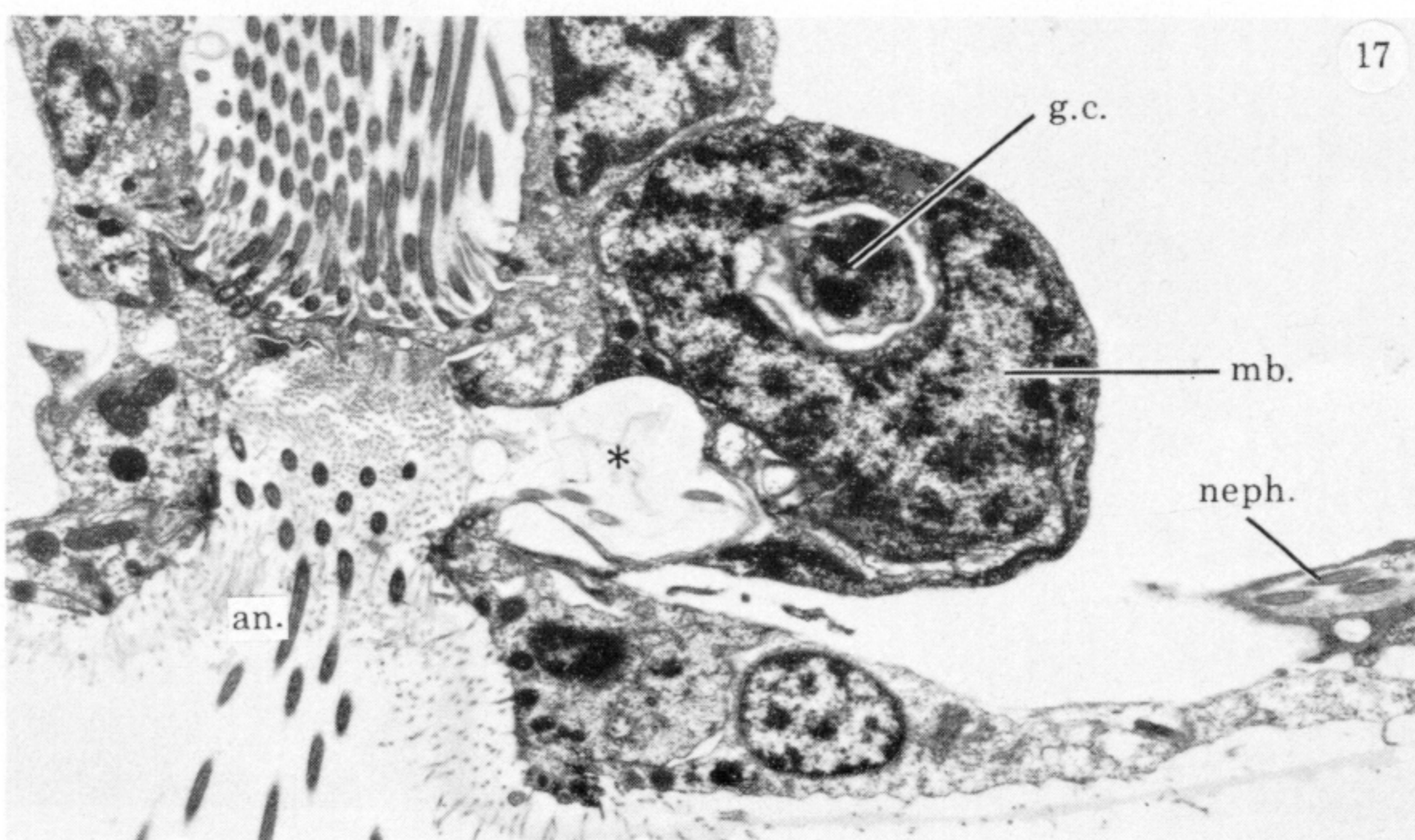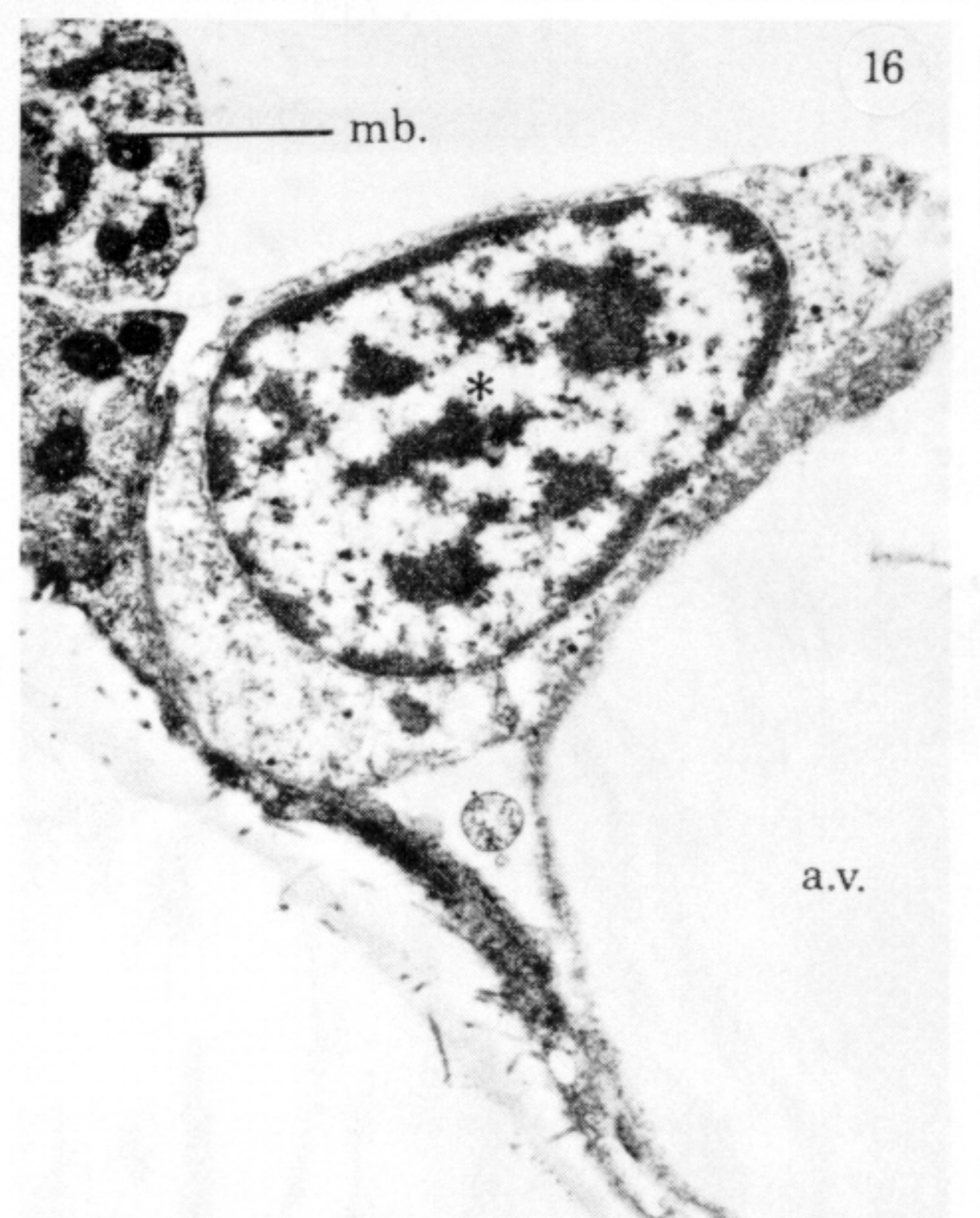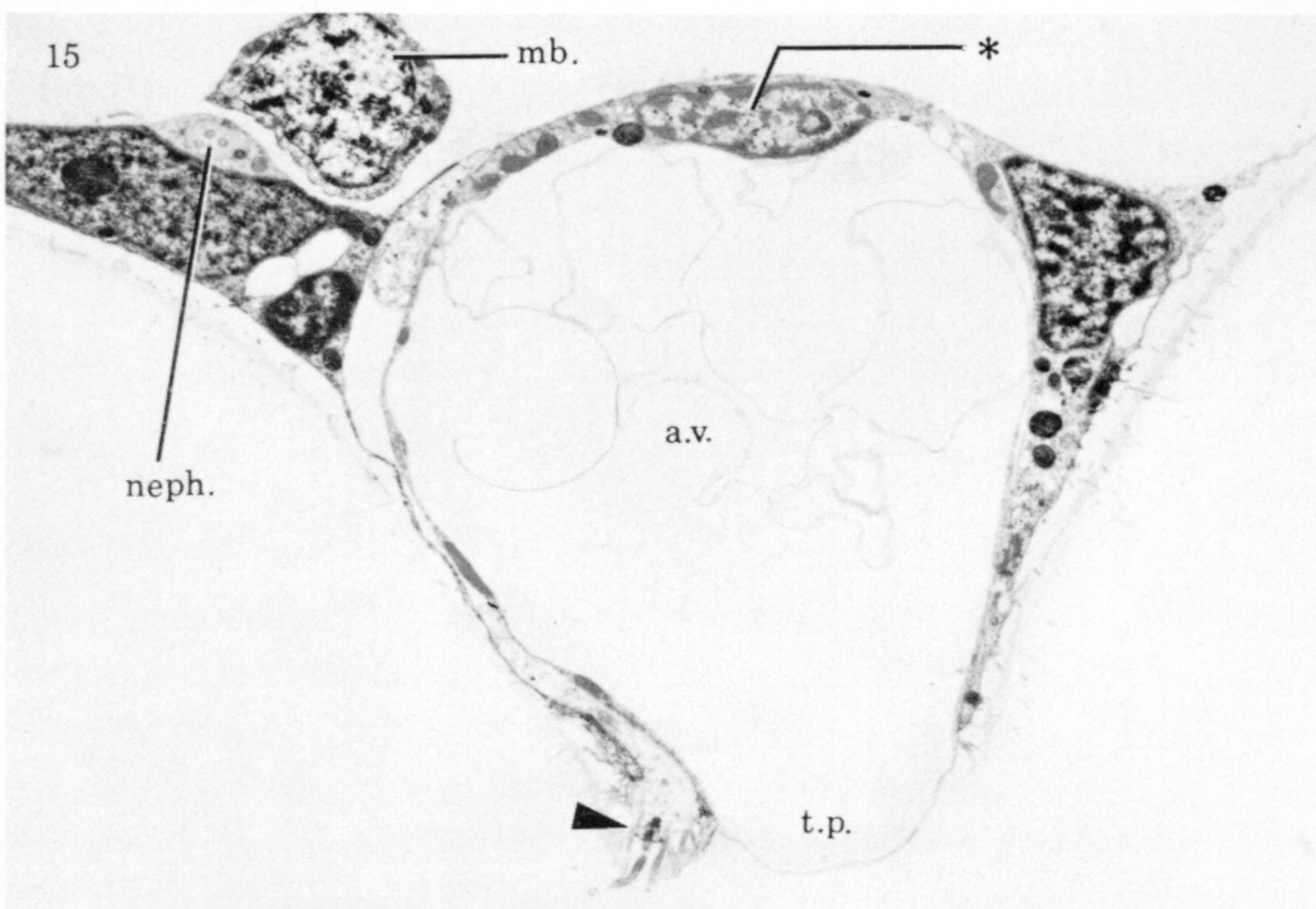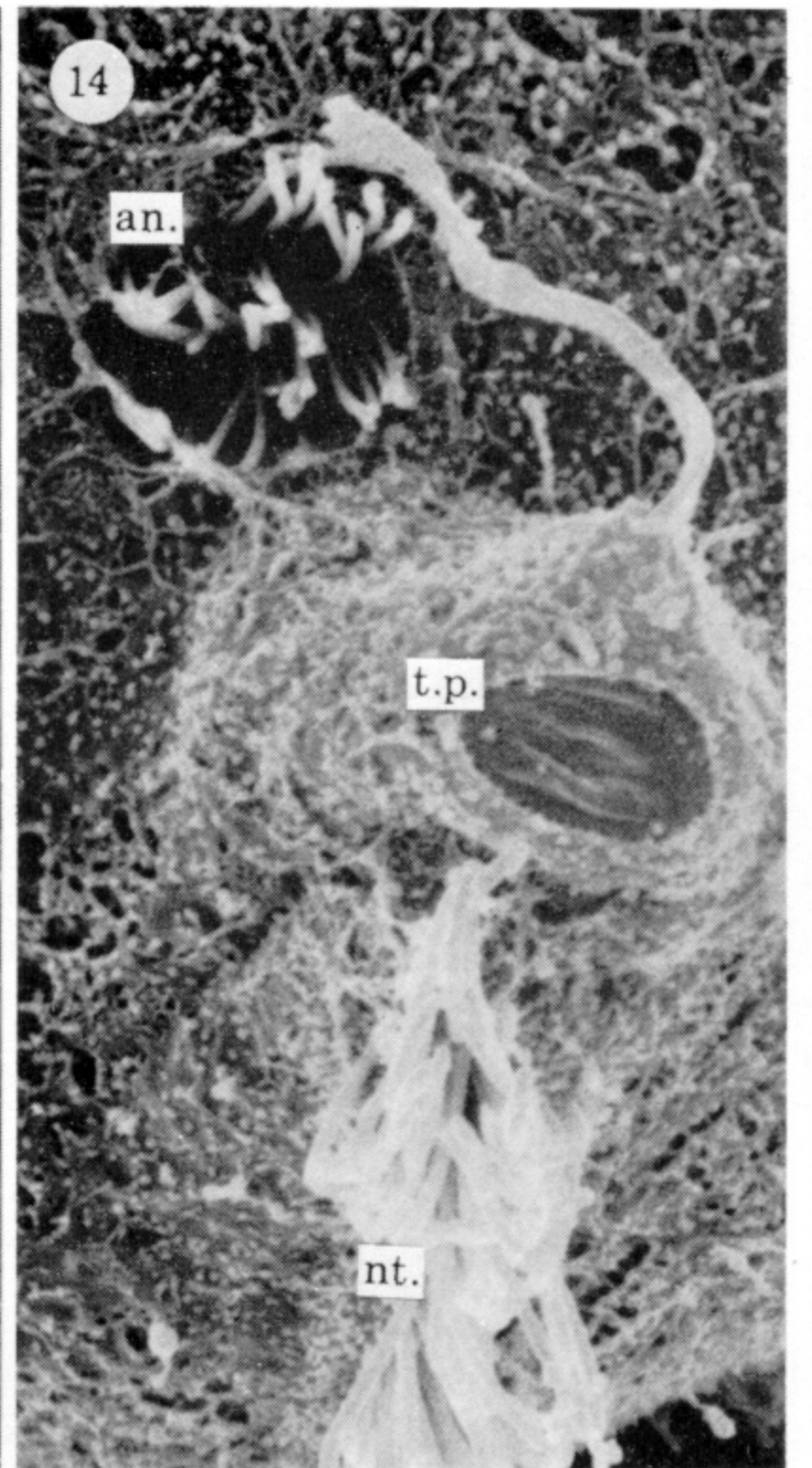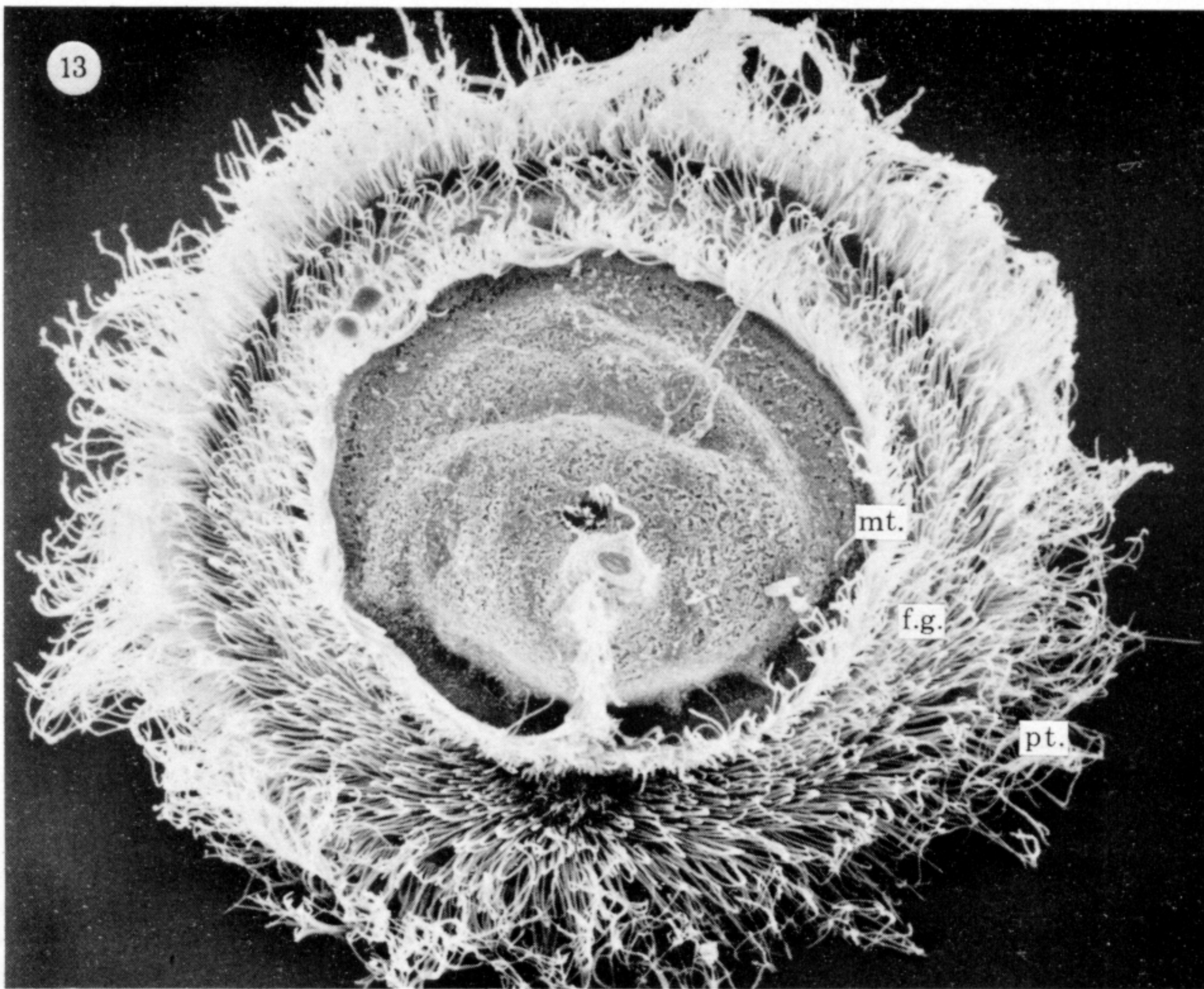
FIGURES 13–18. For description see opposite.
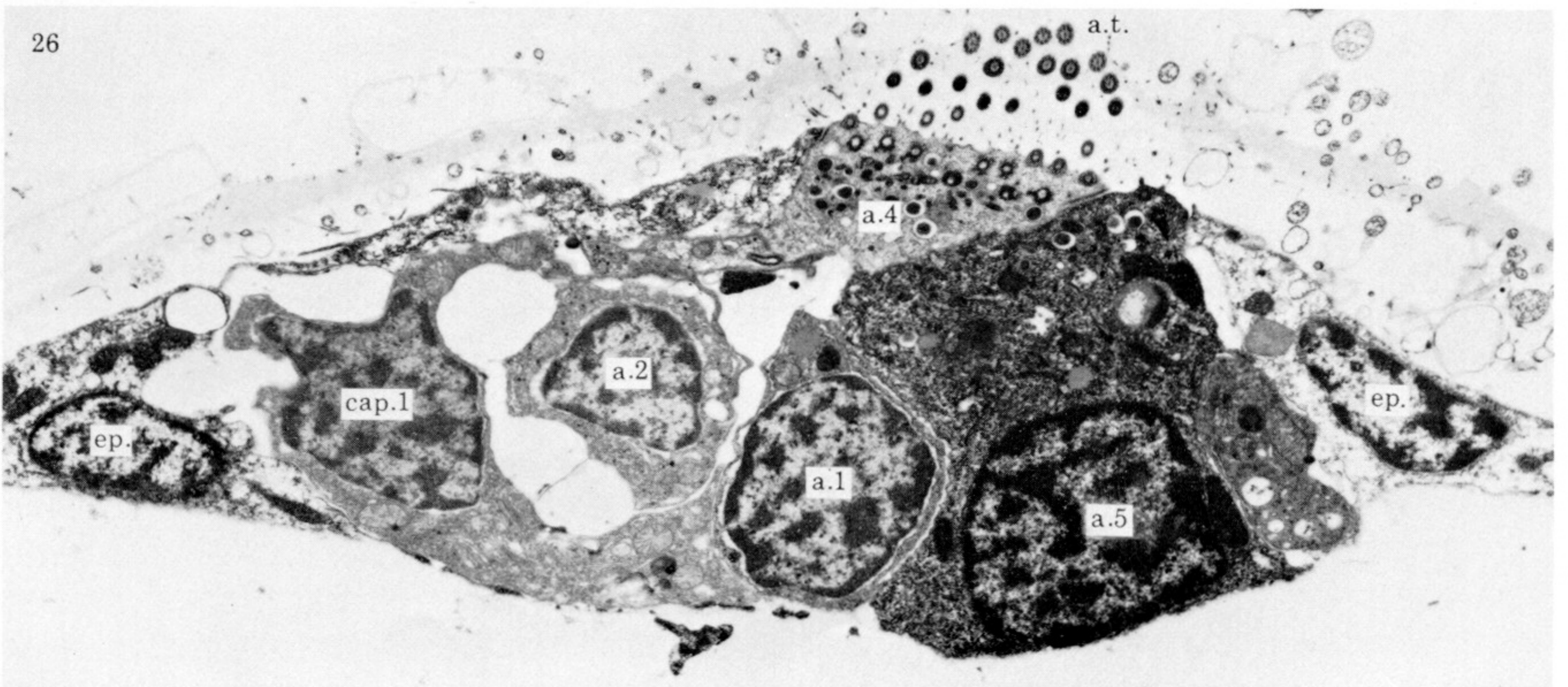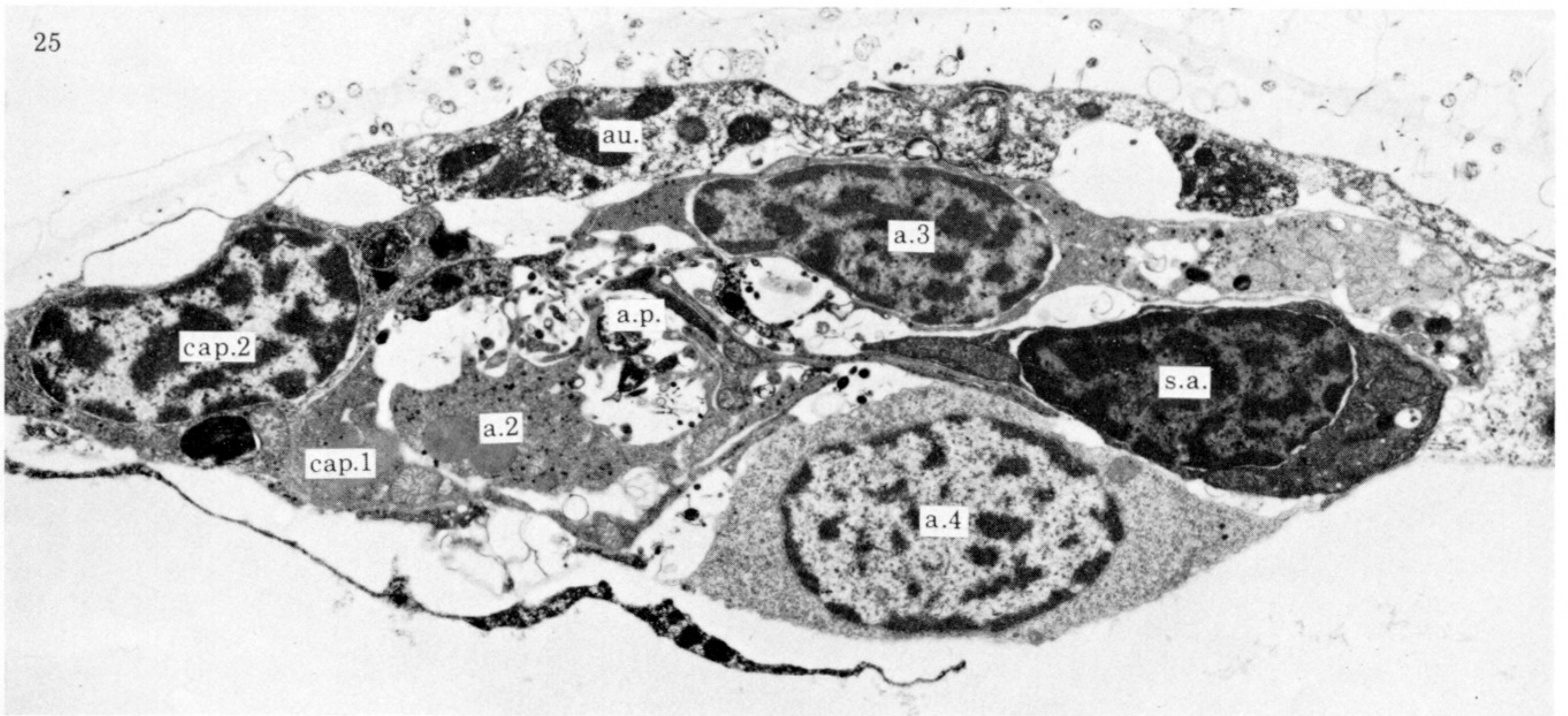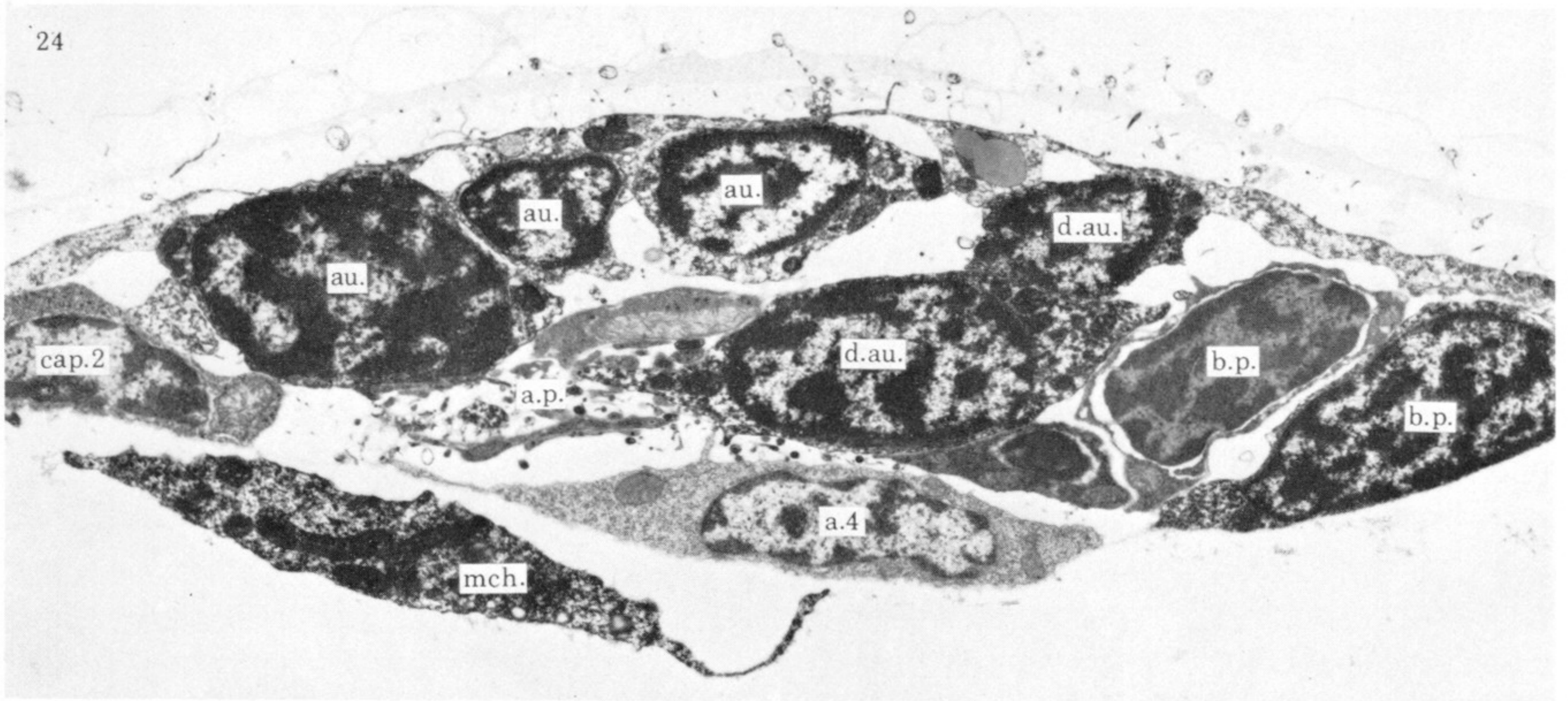
FIGURES 24–26. For description see opposite.
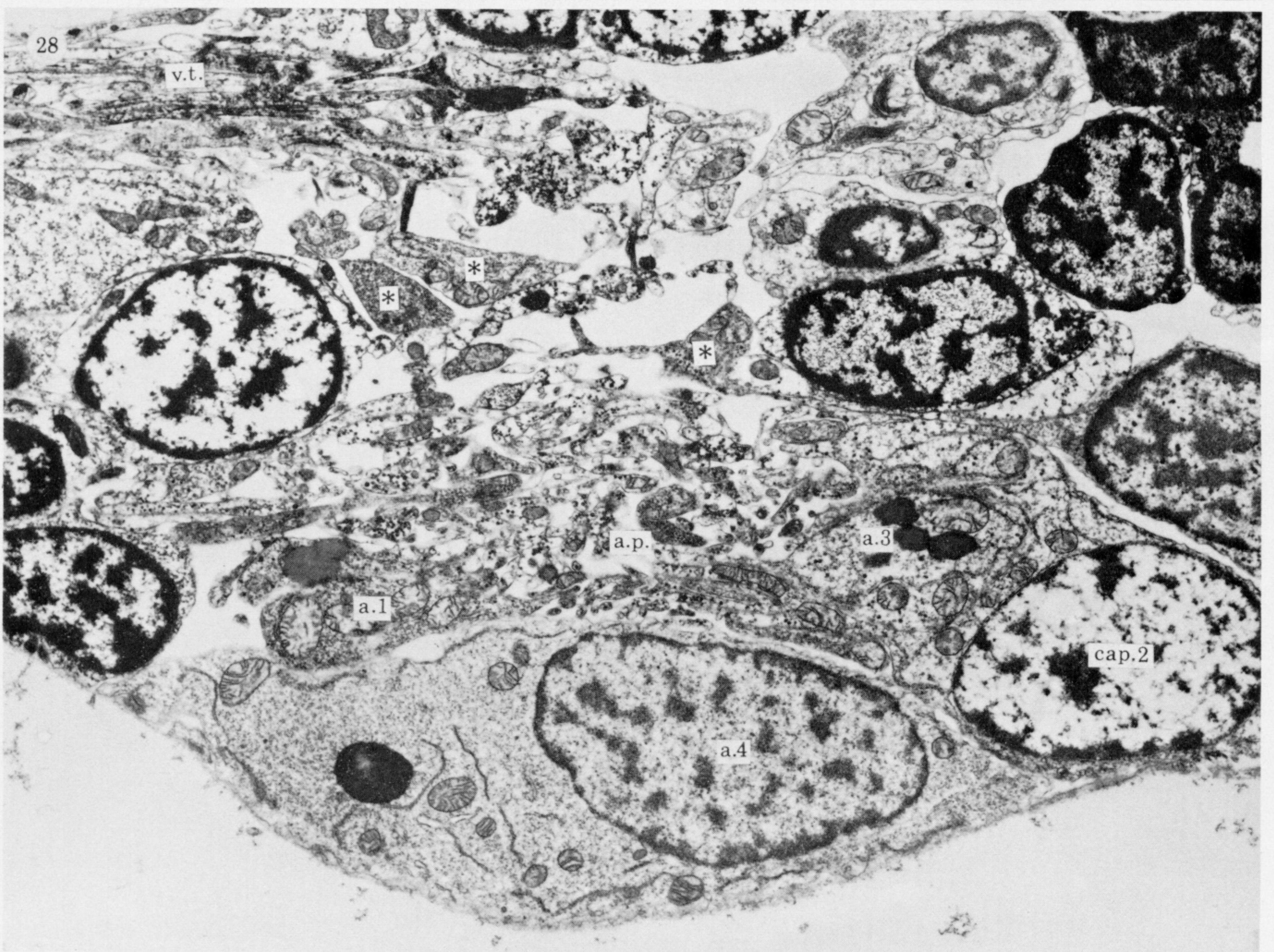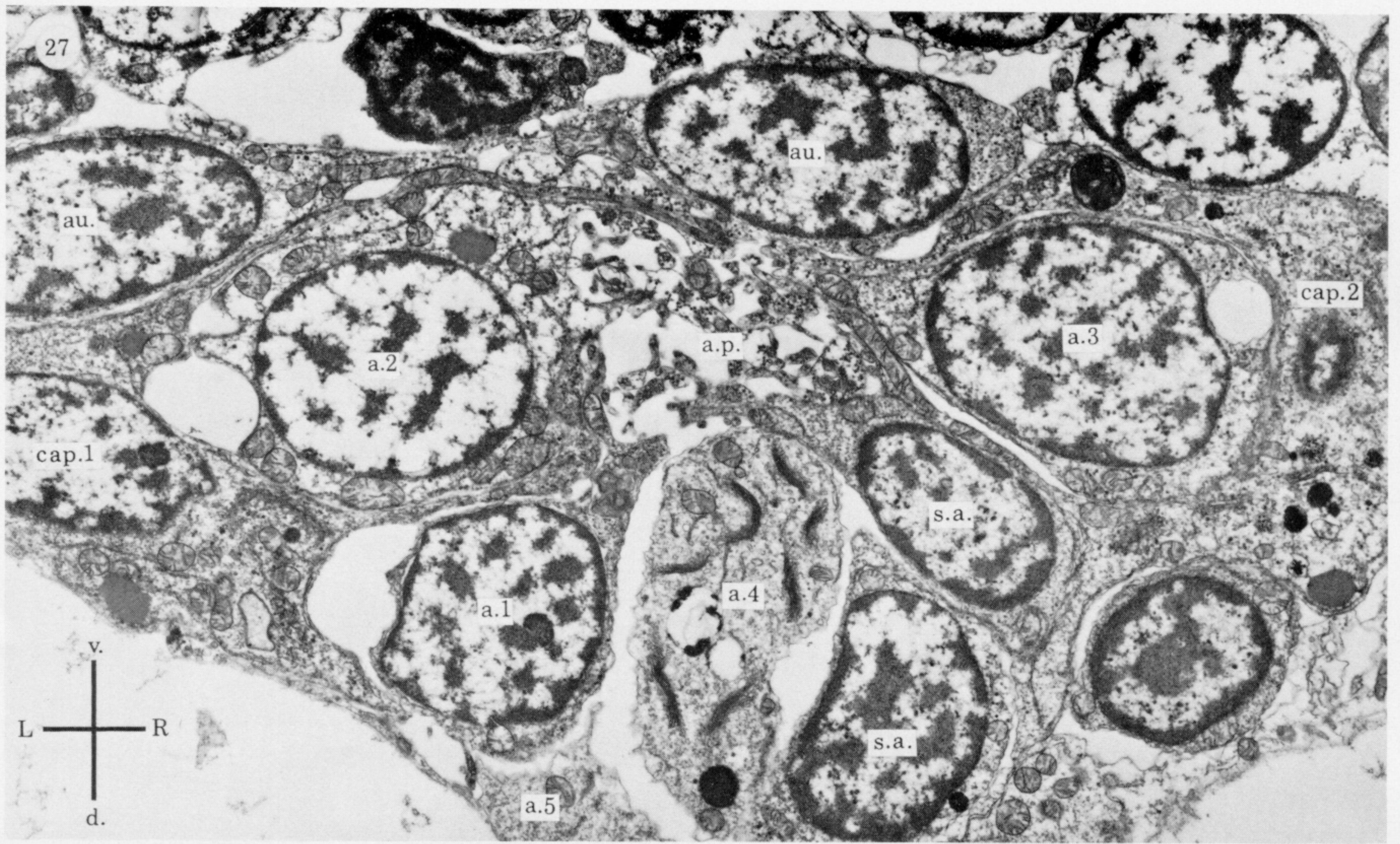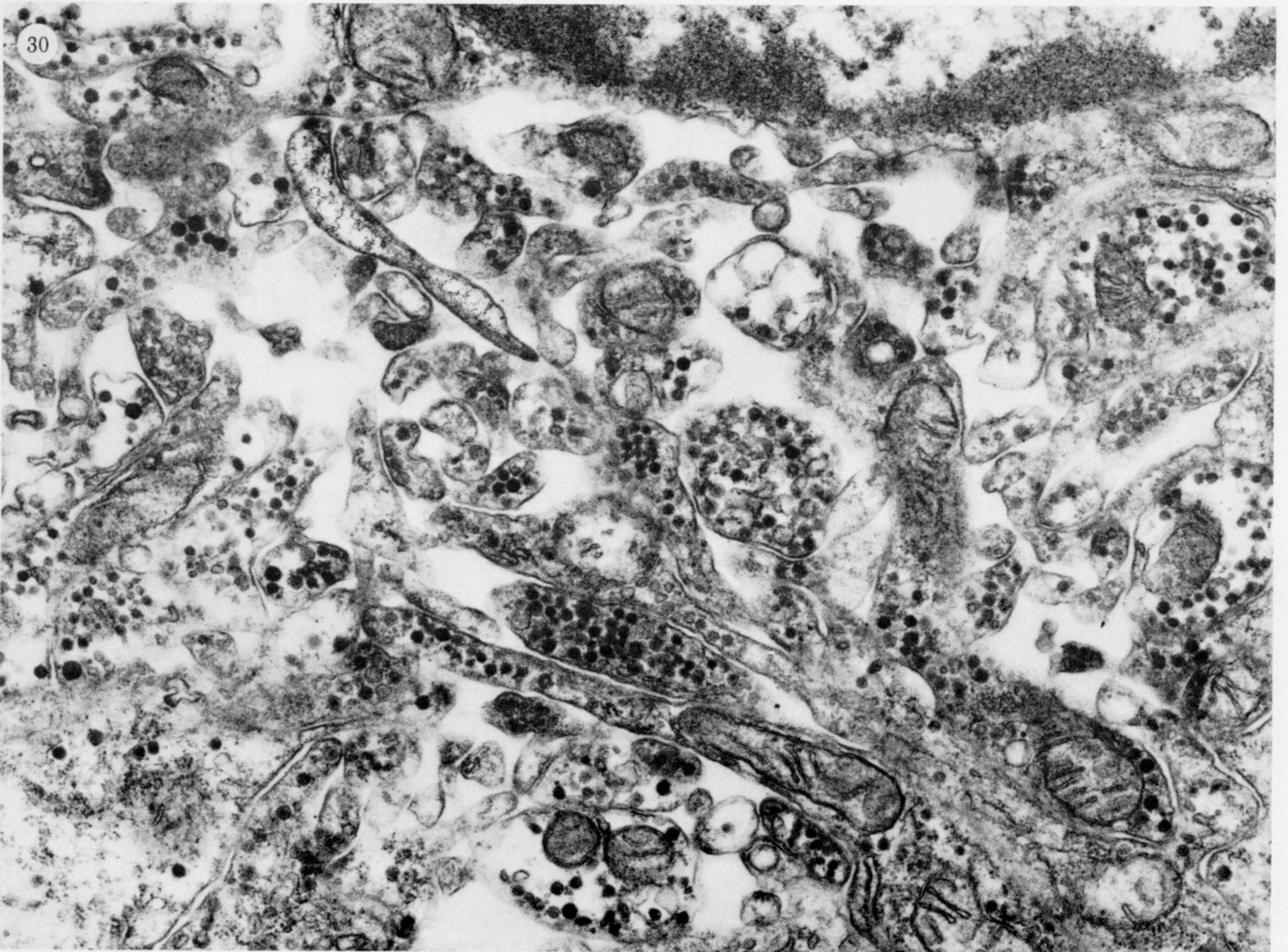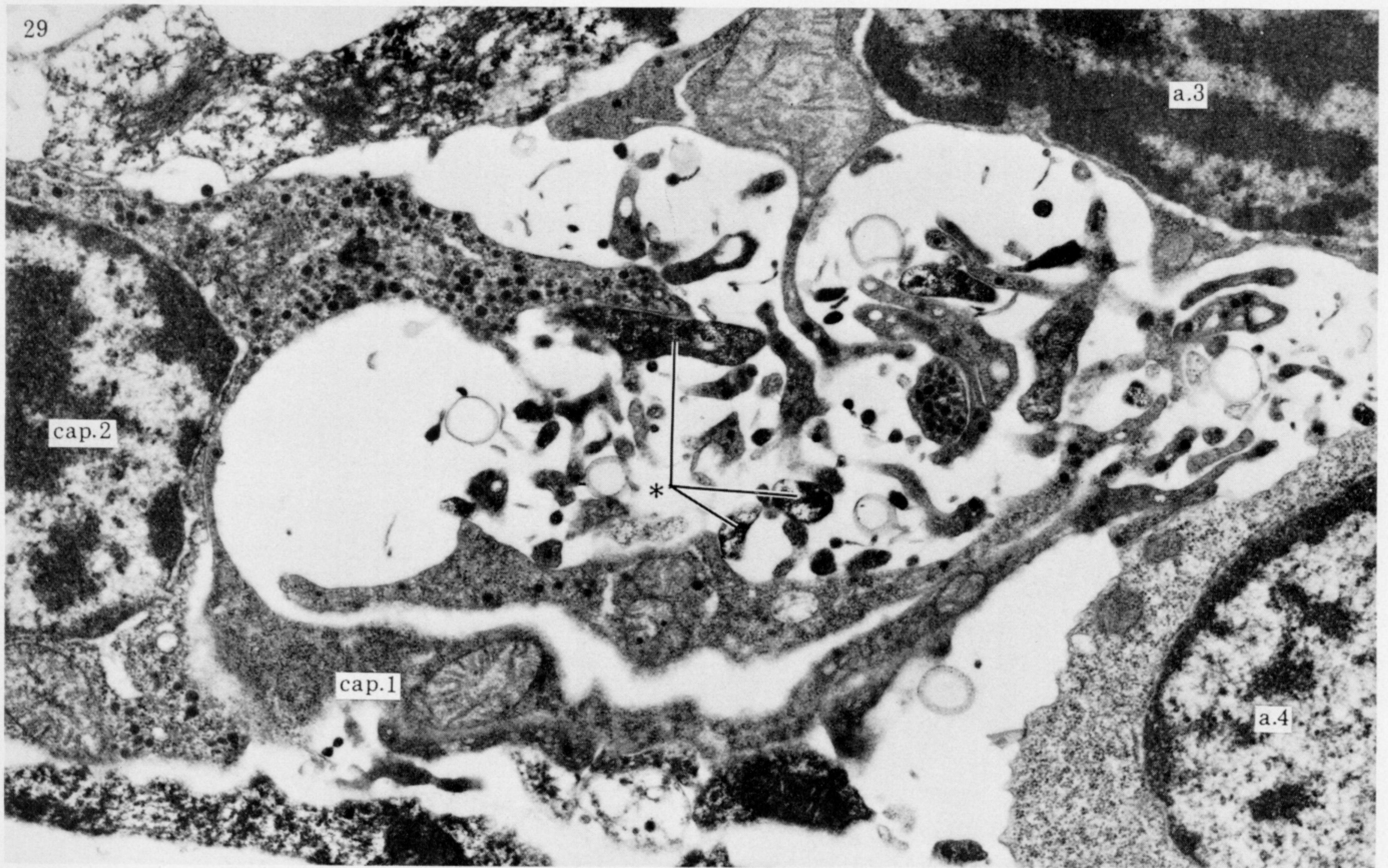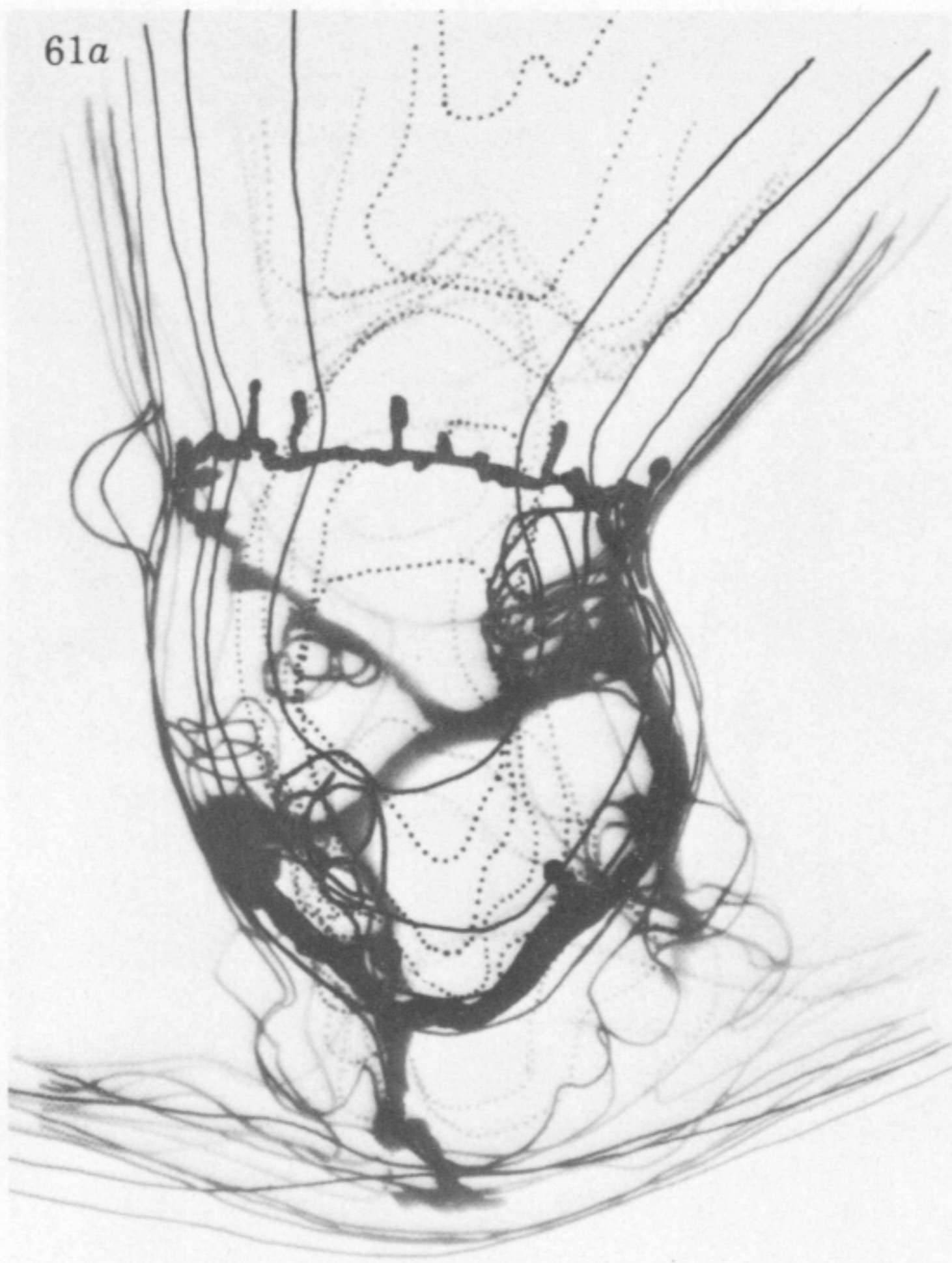
FIGURES 27 AND 28. For description see p. 98.

FIGURES 29 AND 30. For description see p. 98.
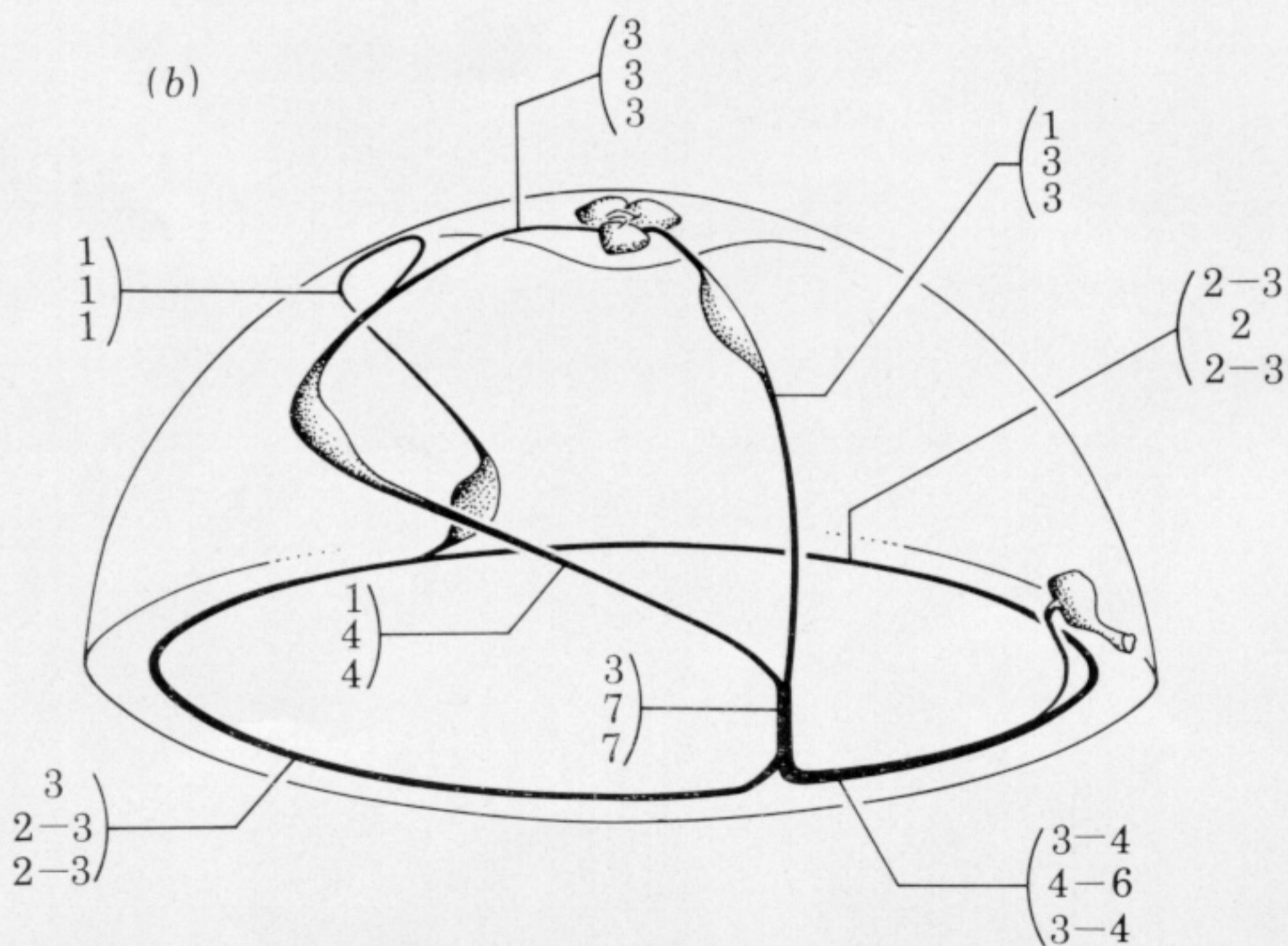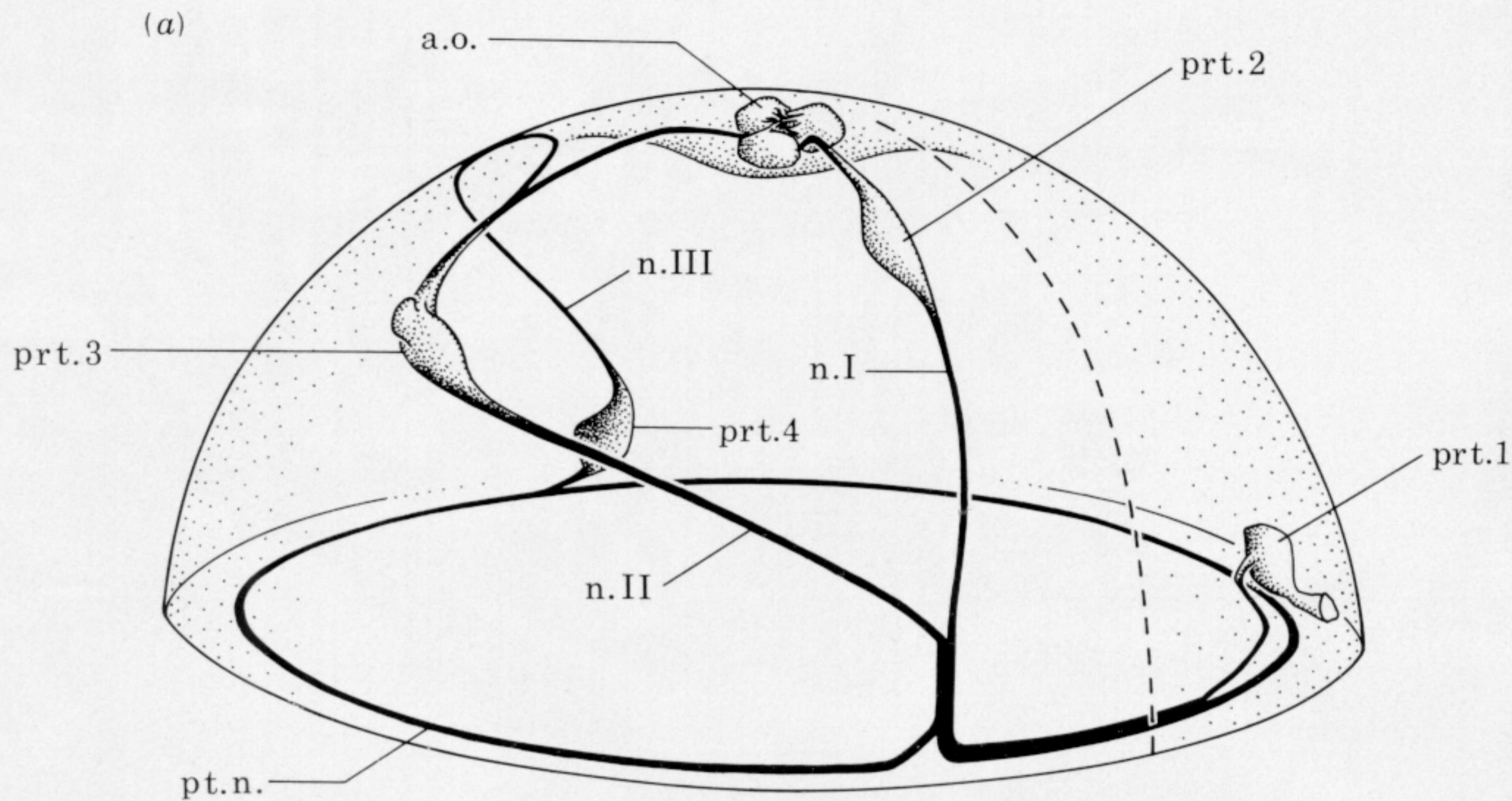
FIGURE 31. (a) Summary diagram showing the neural structures of the episphere of a 48 h trochophore. Includes the apical organ (a.o., shown with three cells), the pretrochal cells (prt. 1–4) and their nerves (n. I-III), and the prototroch nerve (pt.n.). The ventral midline is indicated by a broken line. (b) Diagram as in (a) with fibre counts at strategic points giving (top to bottom) the number of neurites in the 24 h, 48 h, and metatrochophore stages.
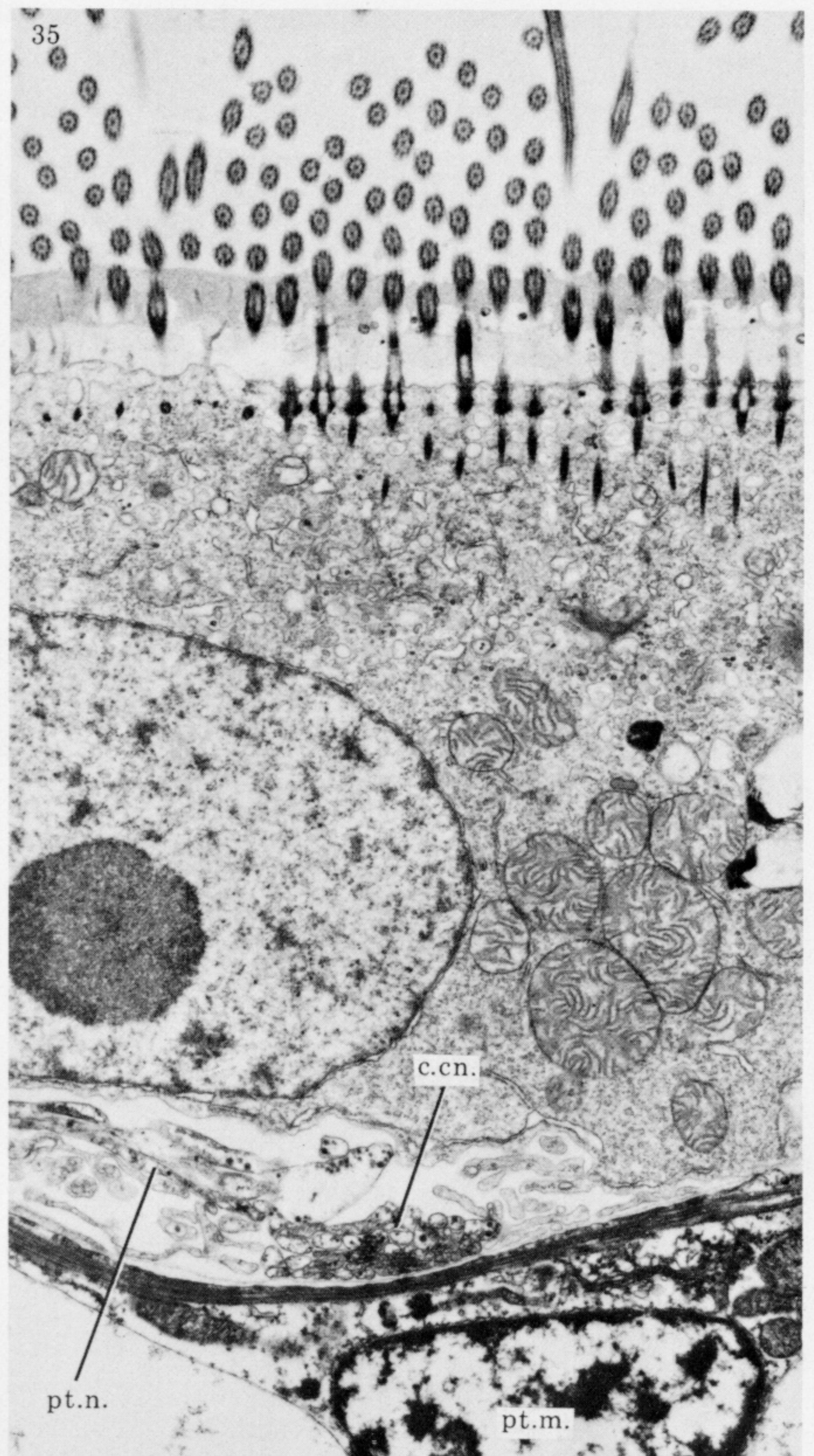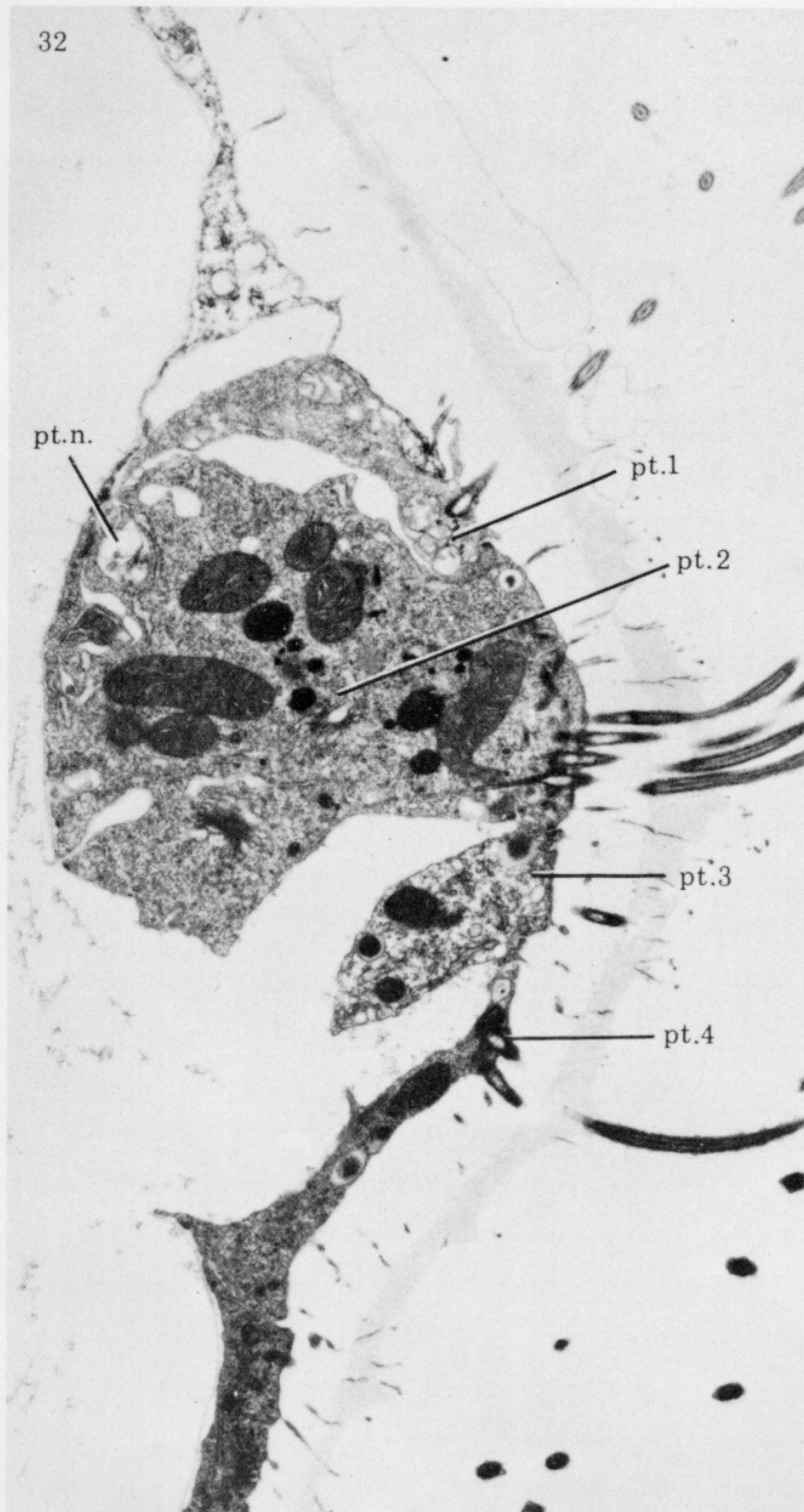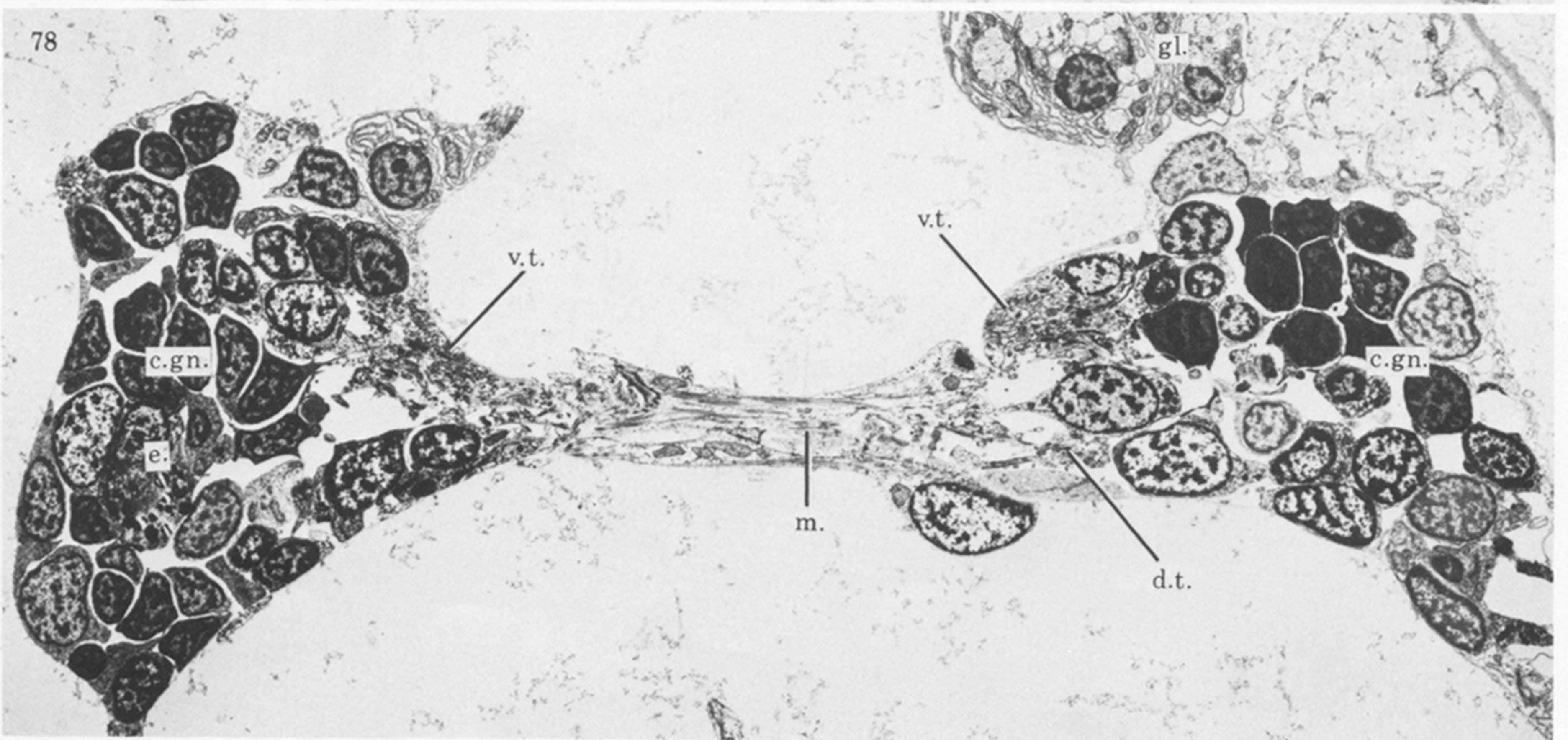
FIGURES 32–35. For description see p. 99.
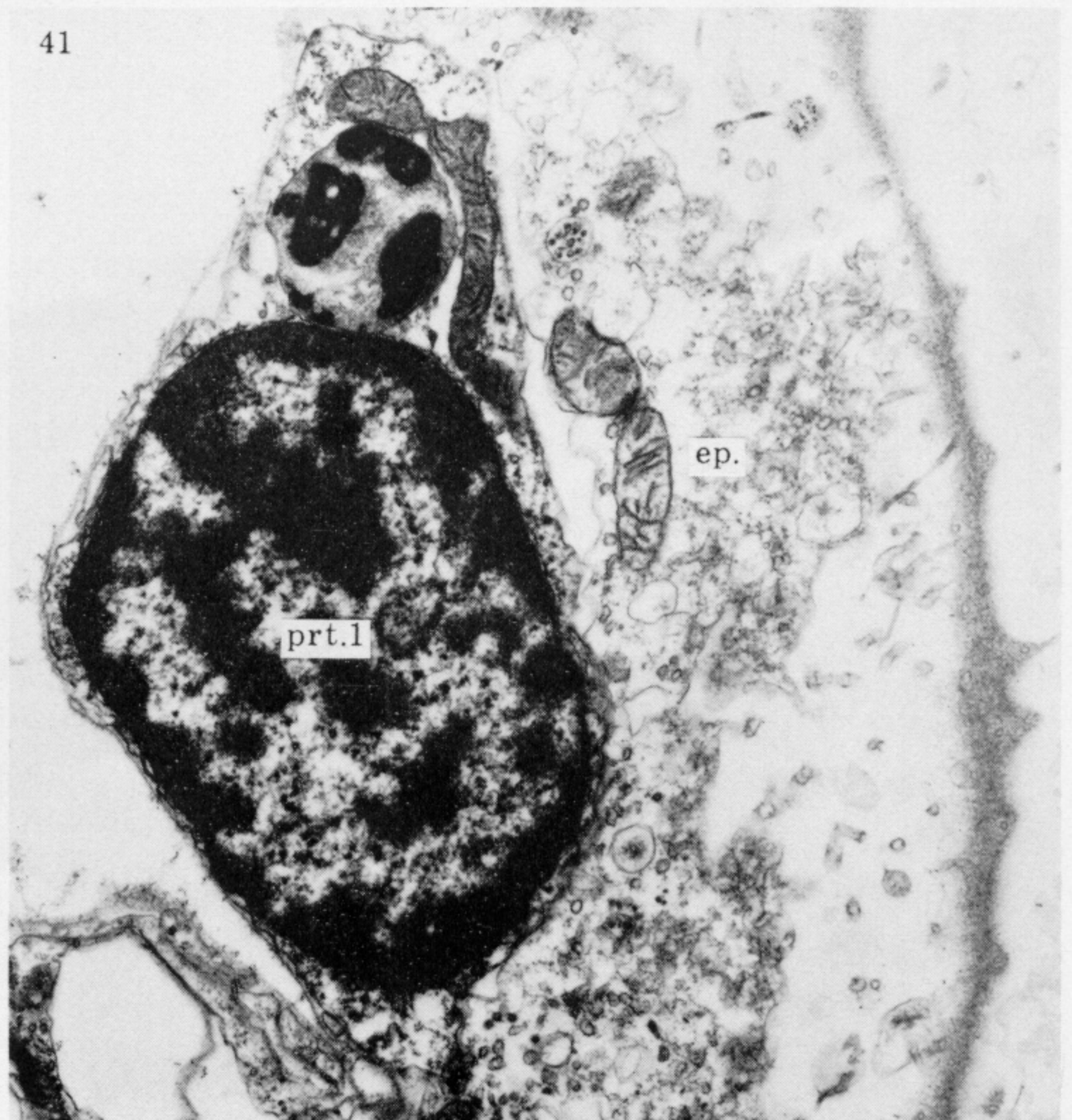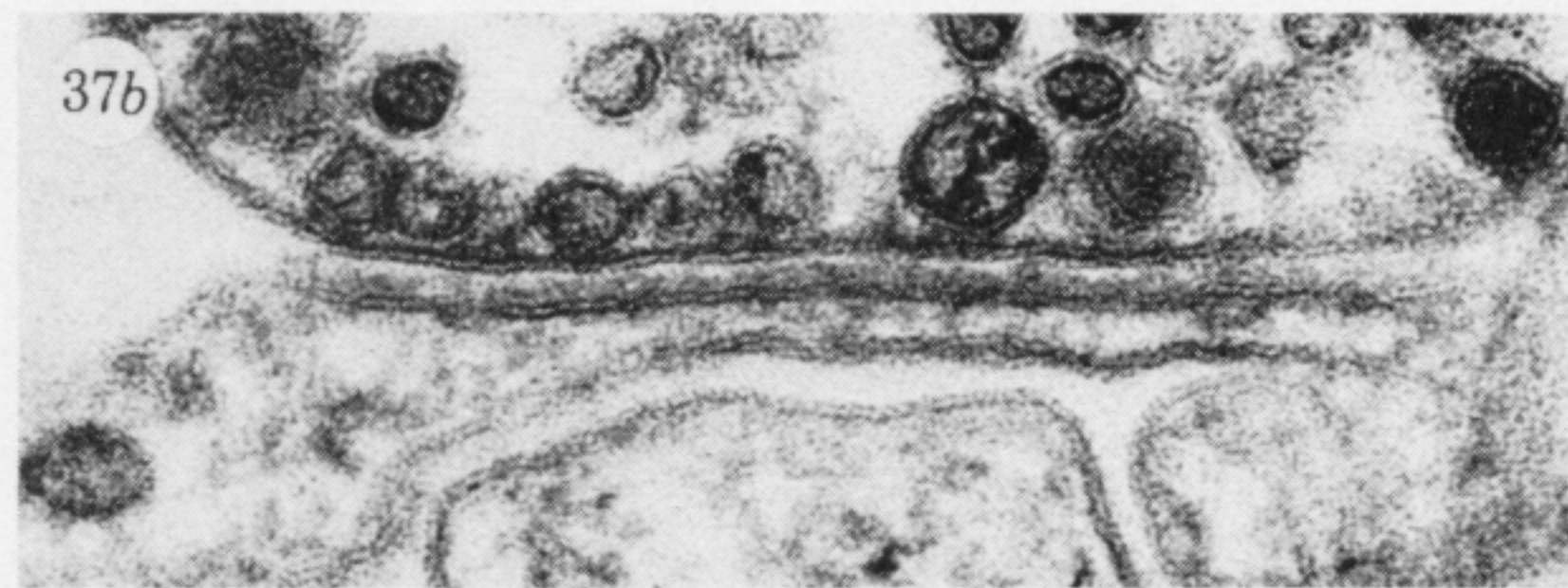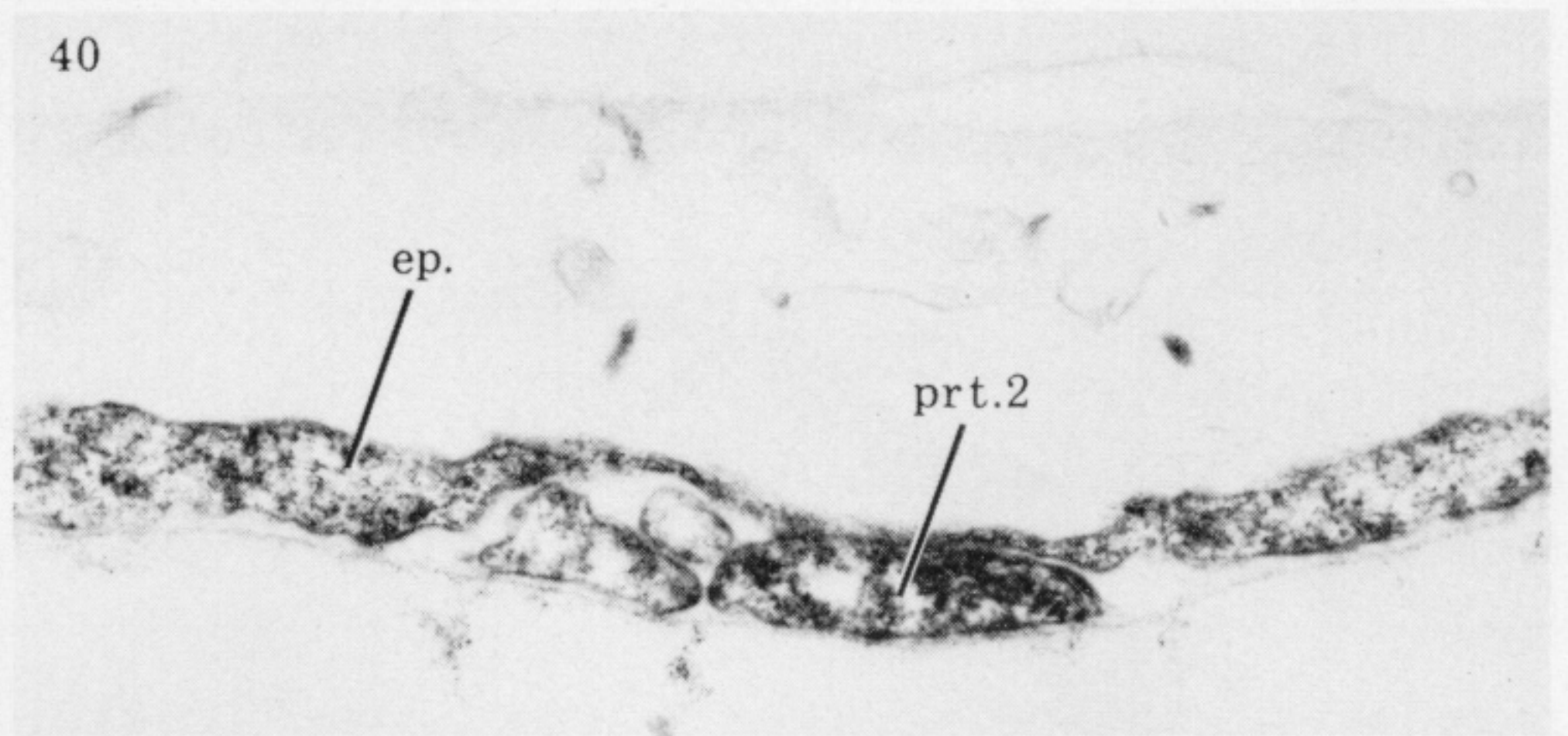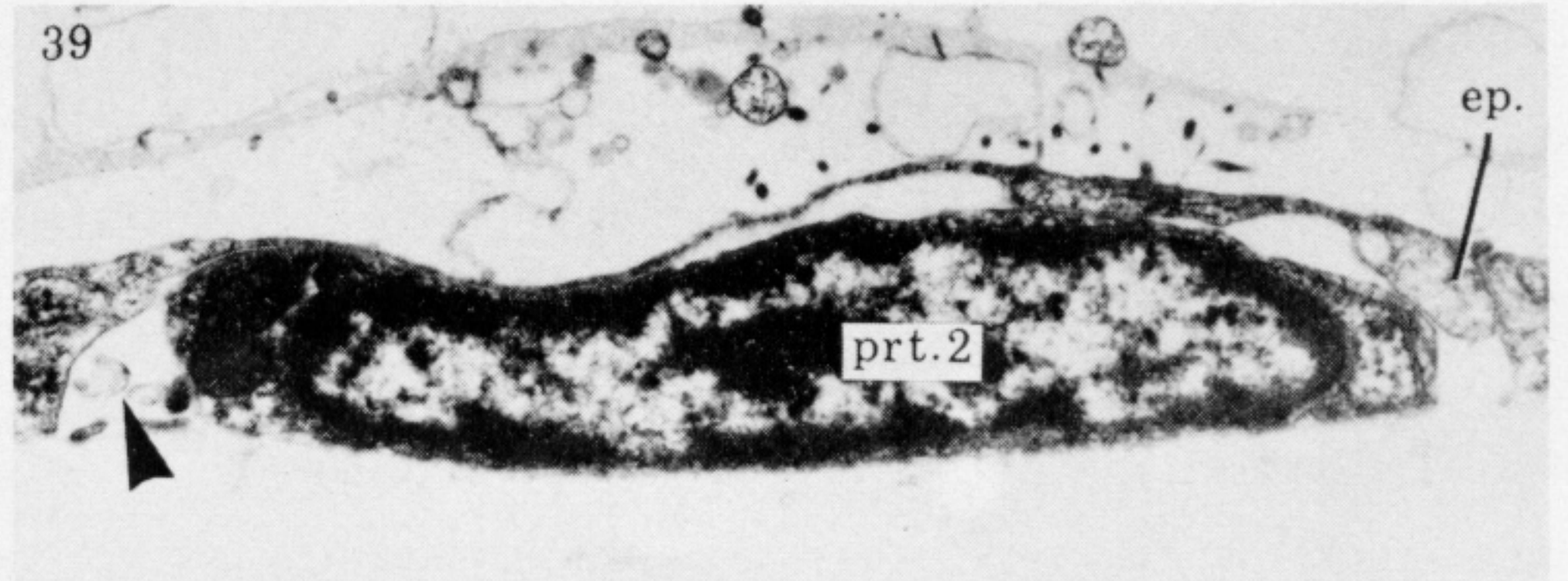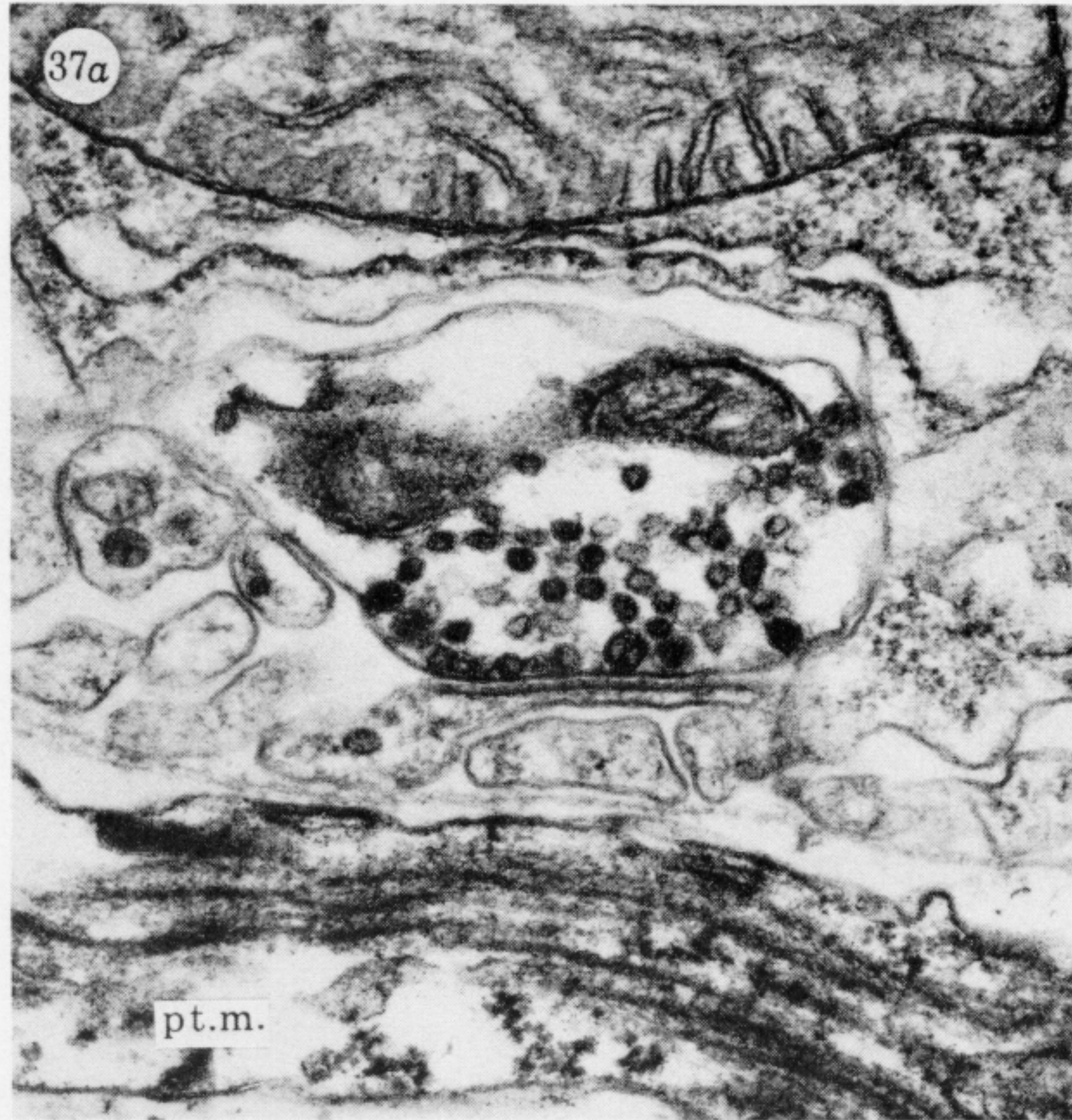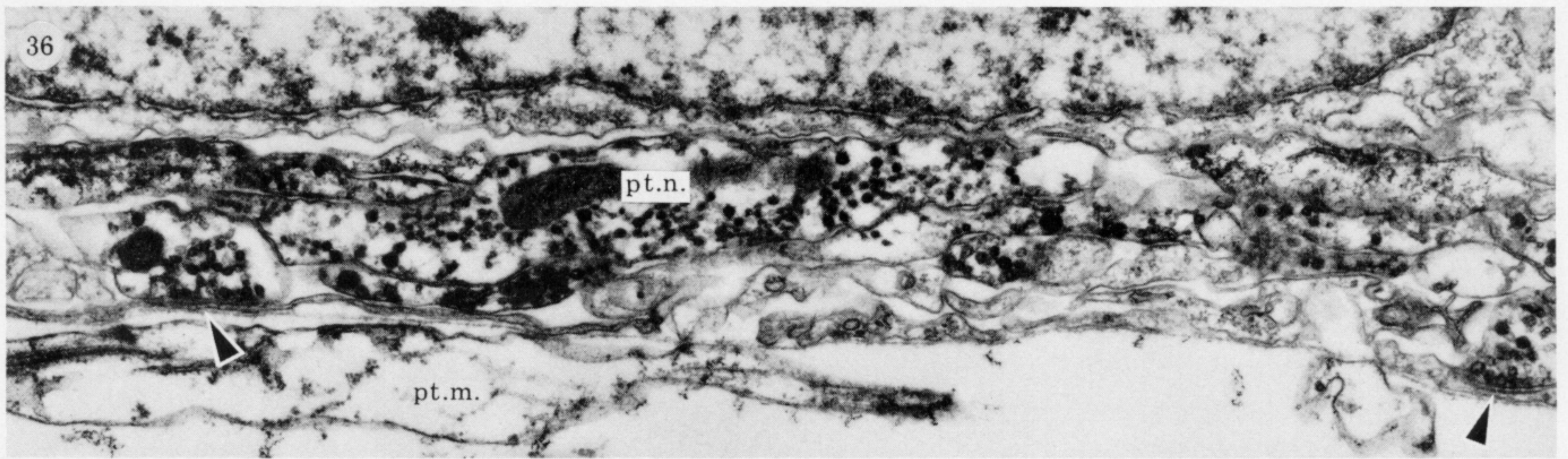
FIGURES 36–41. For description see opposite.

FIGURES 47 AND 48. For description see opposite.

FIGURES 49 AND 50. Stereoreconstructions of the 48 h trochophore.

FIGURE 49. The external ventral surface viewed obliquely from the right side. Representative cilia are included to
show the positions of the trochal bands and the food groove. Also includes the inside contours of the anal vesicle,
vestibule, and pharynx. Magn. ×900.

FIGURE 50. Nerves and nerve cells of the pharynx and suboral region, viewed from the same angle as figure 49,
and including selected external contours. See figure 48 for a key. Magn. ×970.

FIGURE 51. Reconstruction of the region of the basal pharyngeal complex of the specimen shown in figures 47–50, viewed from the same angle. Follows the conventions used in figure 42, with the inside surface of the pharynx shown by fine dotted lines. (a) Shows the dense vesicle cell (ves., the small patch of parallel lines shows its surface process), the pharyngeal nerve (n.) passing beneath the pharyngeal complex, and one pharyngeal muscle cell (ph. m. 1). (b) Shows the dense vesicle cell and nerve from (a) in outline and, in addition, ph. 2 and 5 with their rows of projecting cilia.

FIGURE 52. Reconstruction of the suboral complex of the specimen in figures 47–50, viewed from the same angle. Shows the complex as a unit (top, nerves and plexus are shaded), and individual reconstructions of the four suboral cells. Note that both neurites of the neurotroch nerve arise from s. 3. A branching process from this same cell enters the suboral plexus and interdigitates (at *, shown in figure 57) with fibres from s. 2.

FIGURES 53–56. For description see opposite.
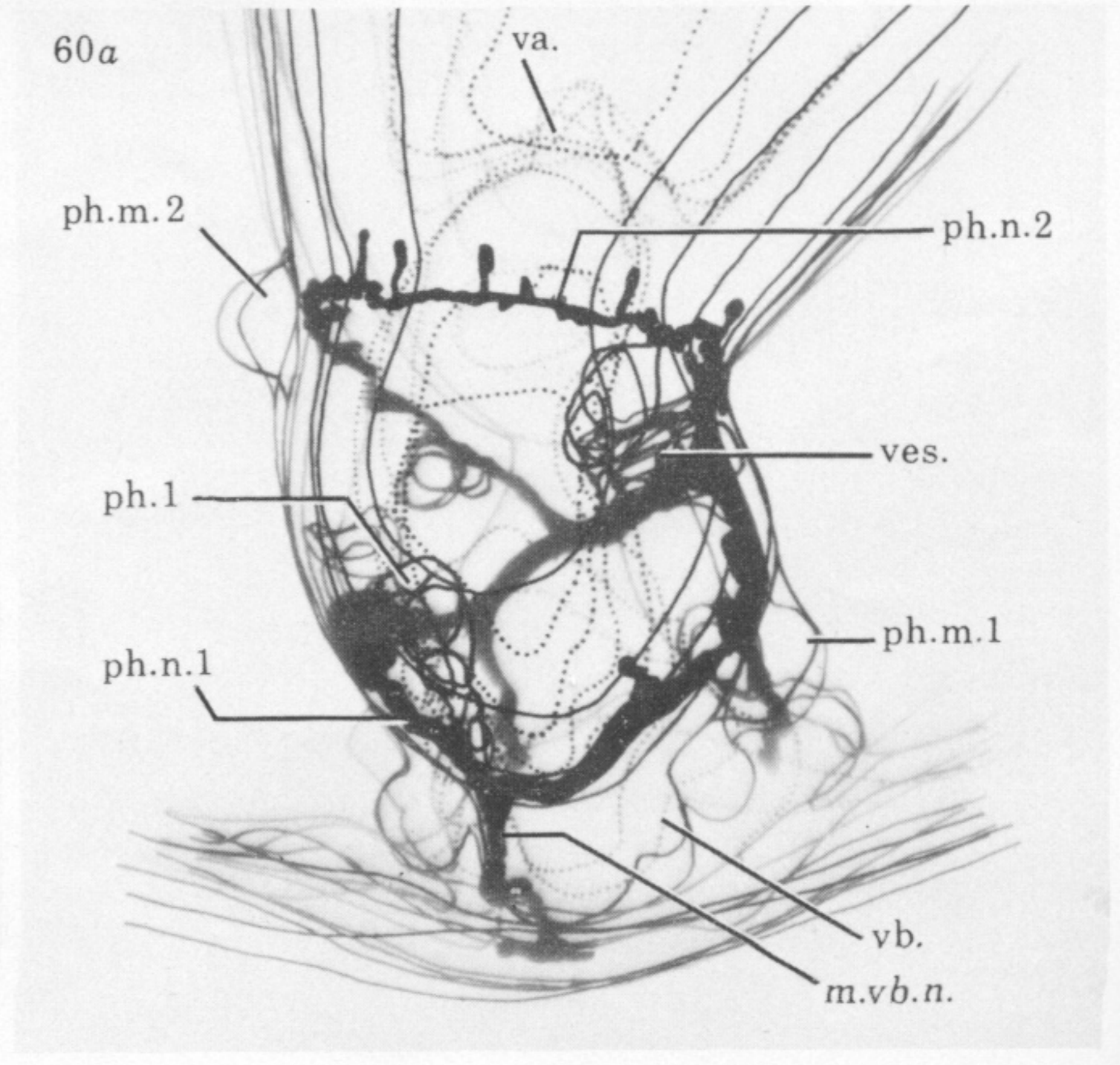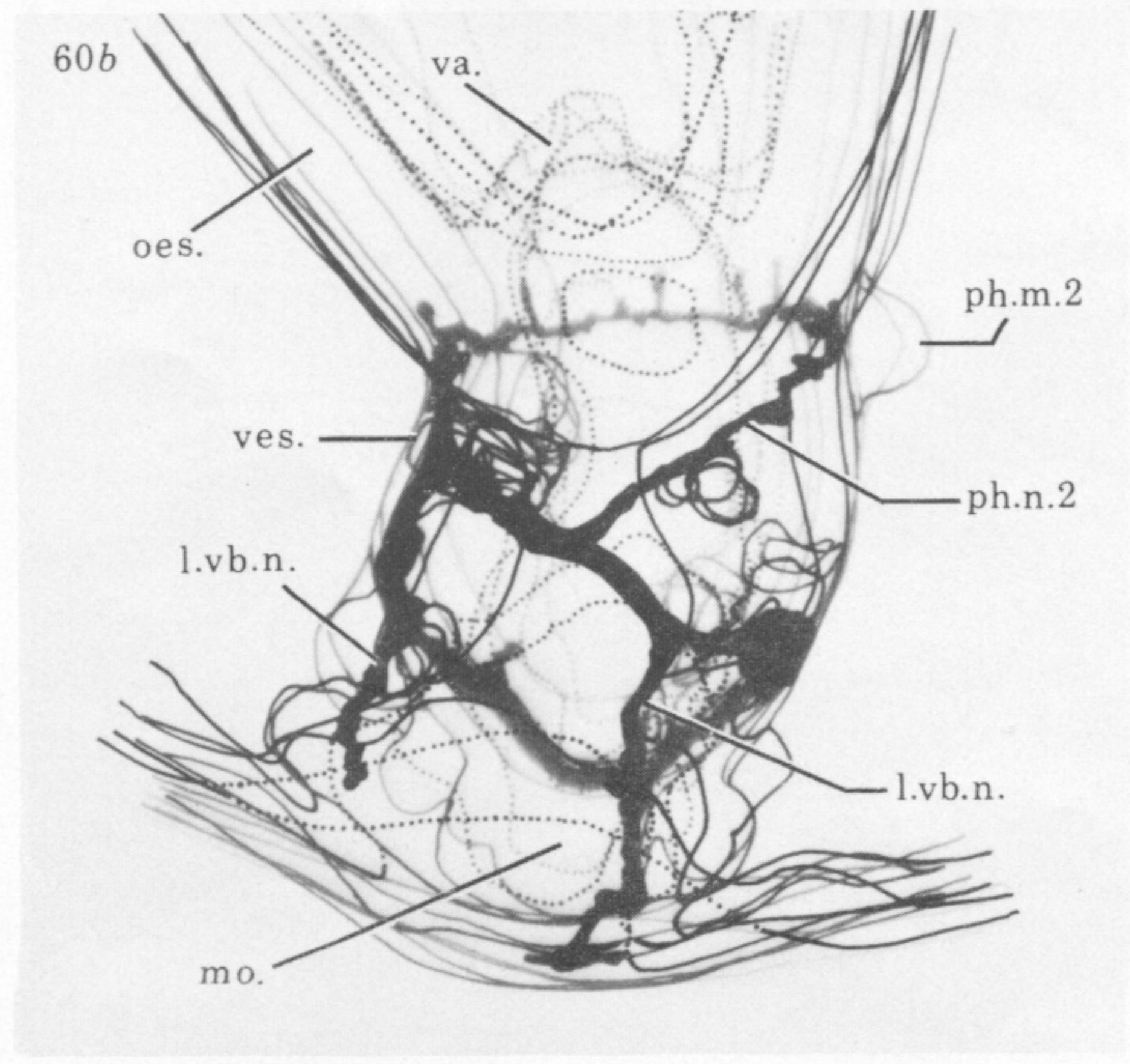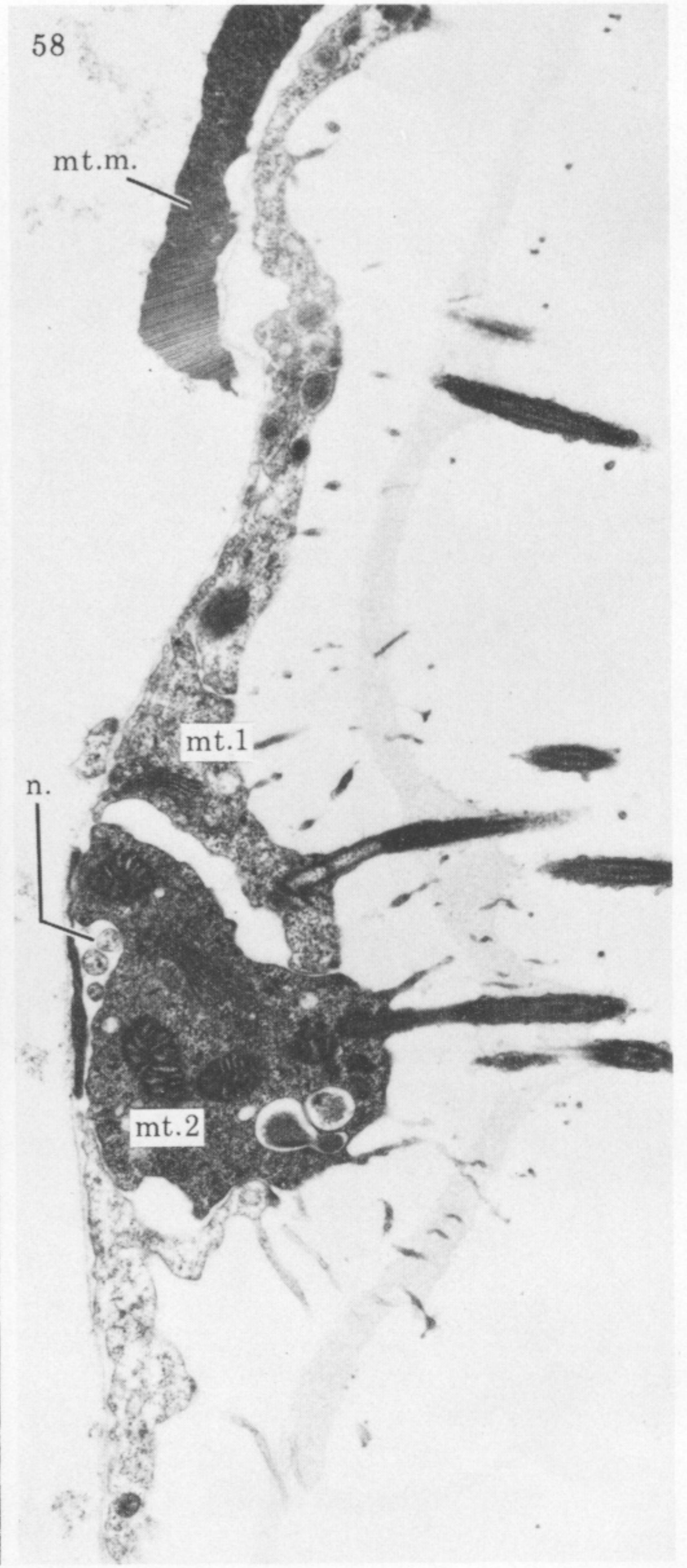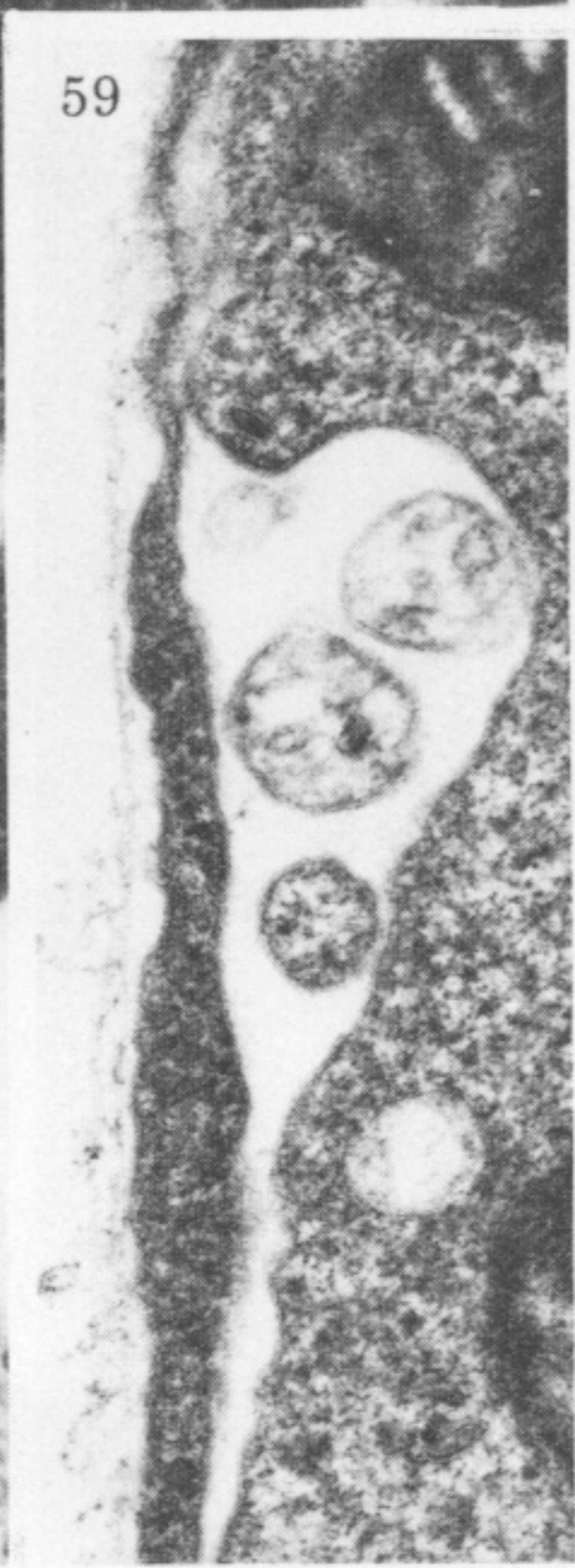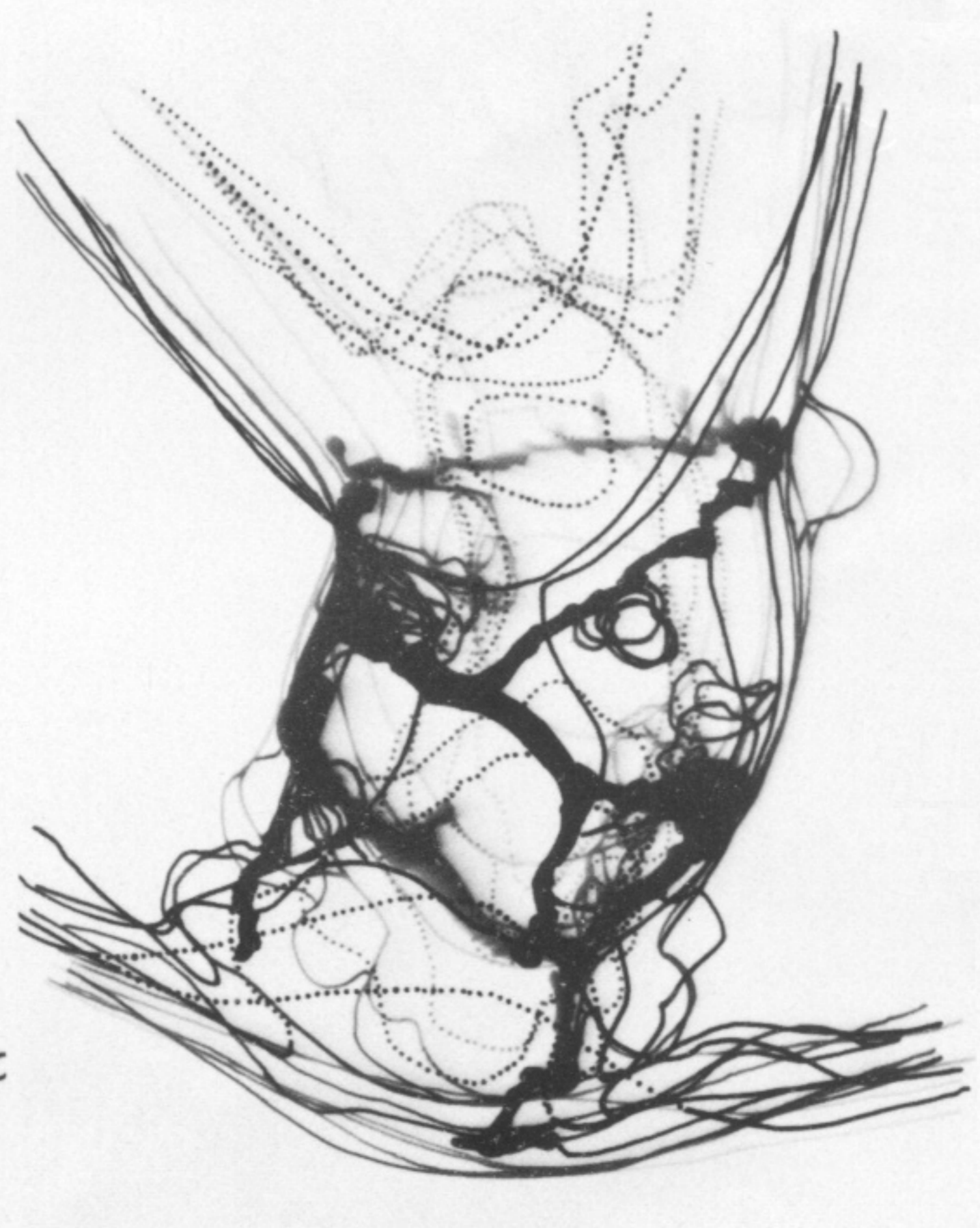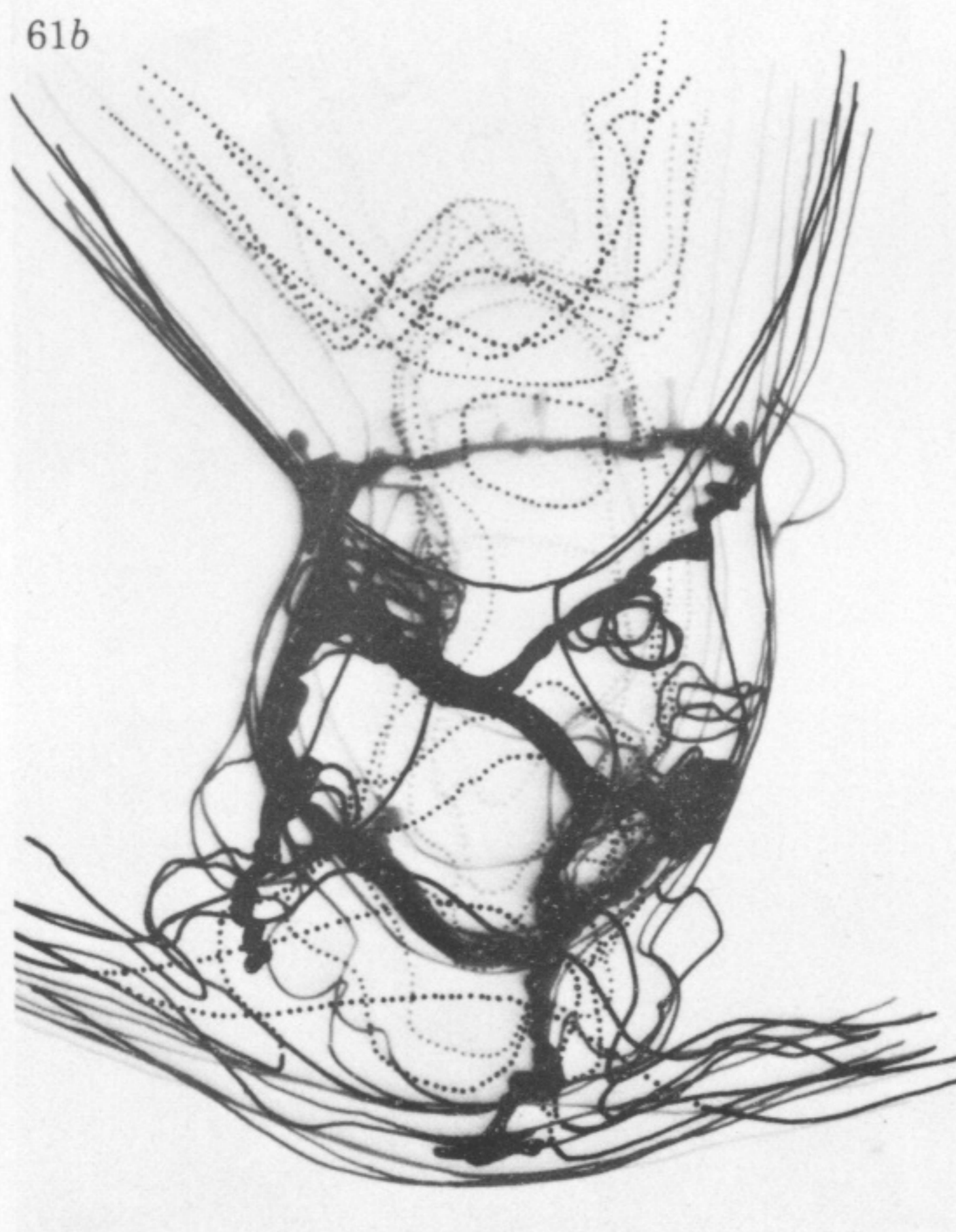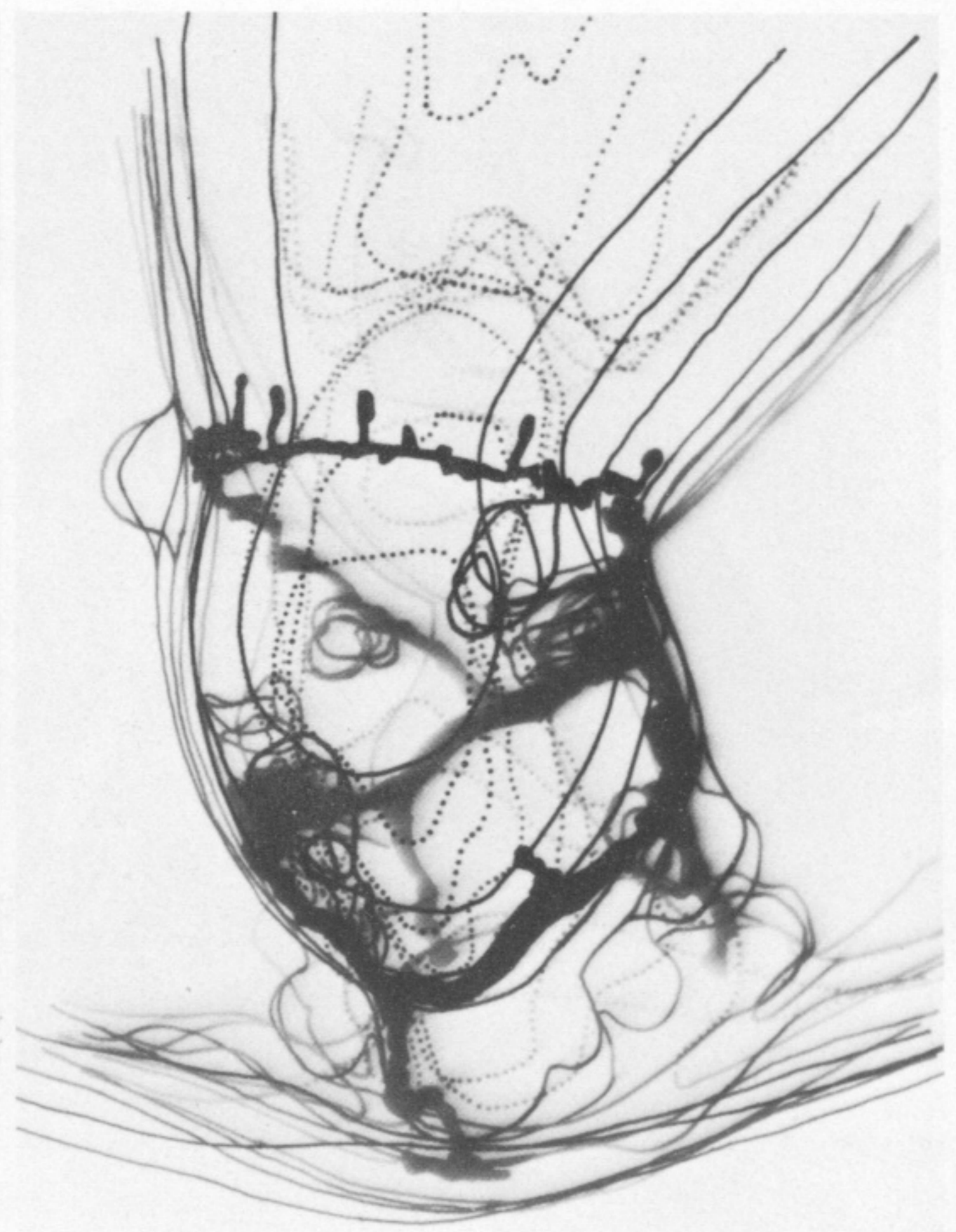
FIGURES 57–60. For description see p. 109.

FIGURE 61. Stereoreconstructions of the metatrochophore pharynx (a) from above, magn. × 1780; (b) from below, magn. × 1700. See figure 60 for a key.
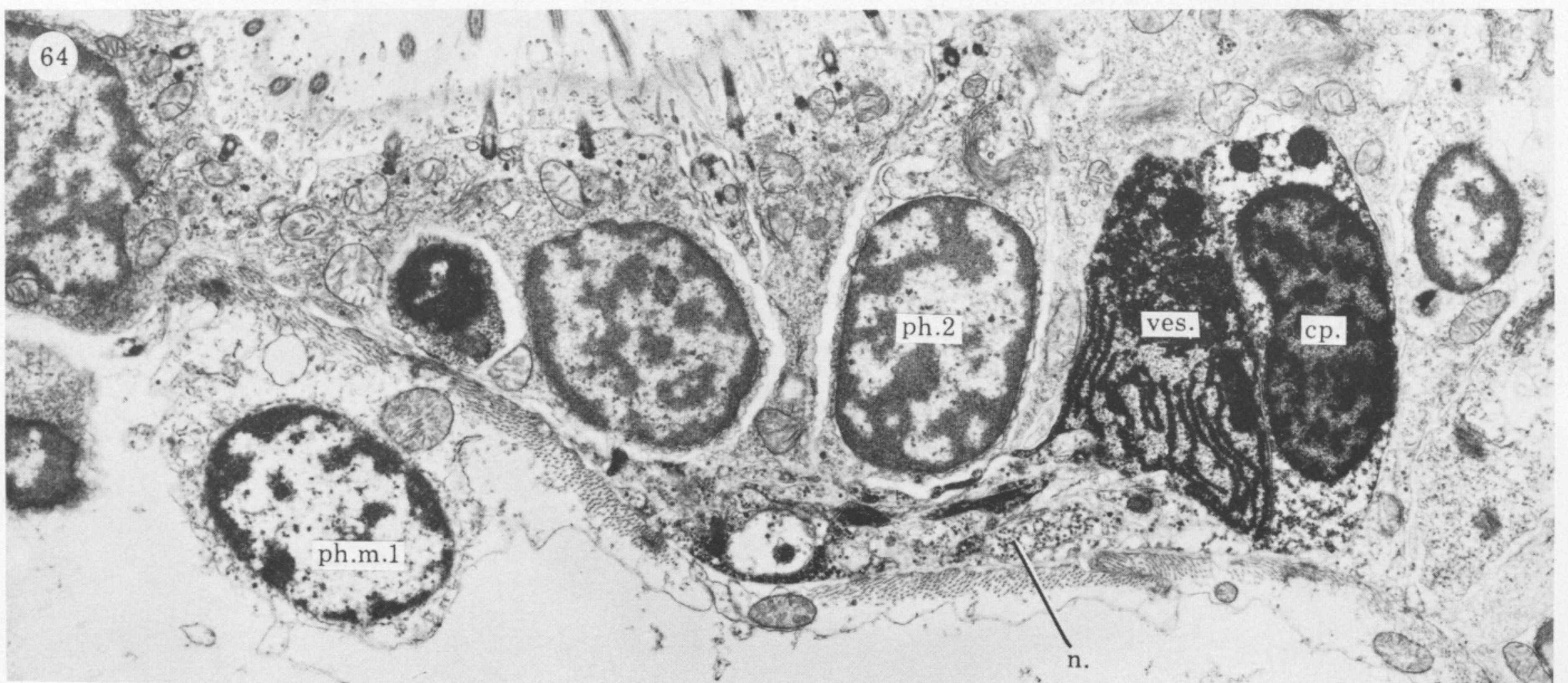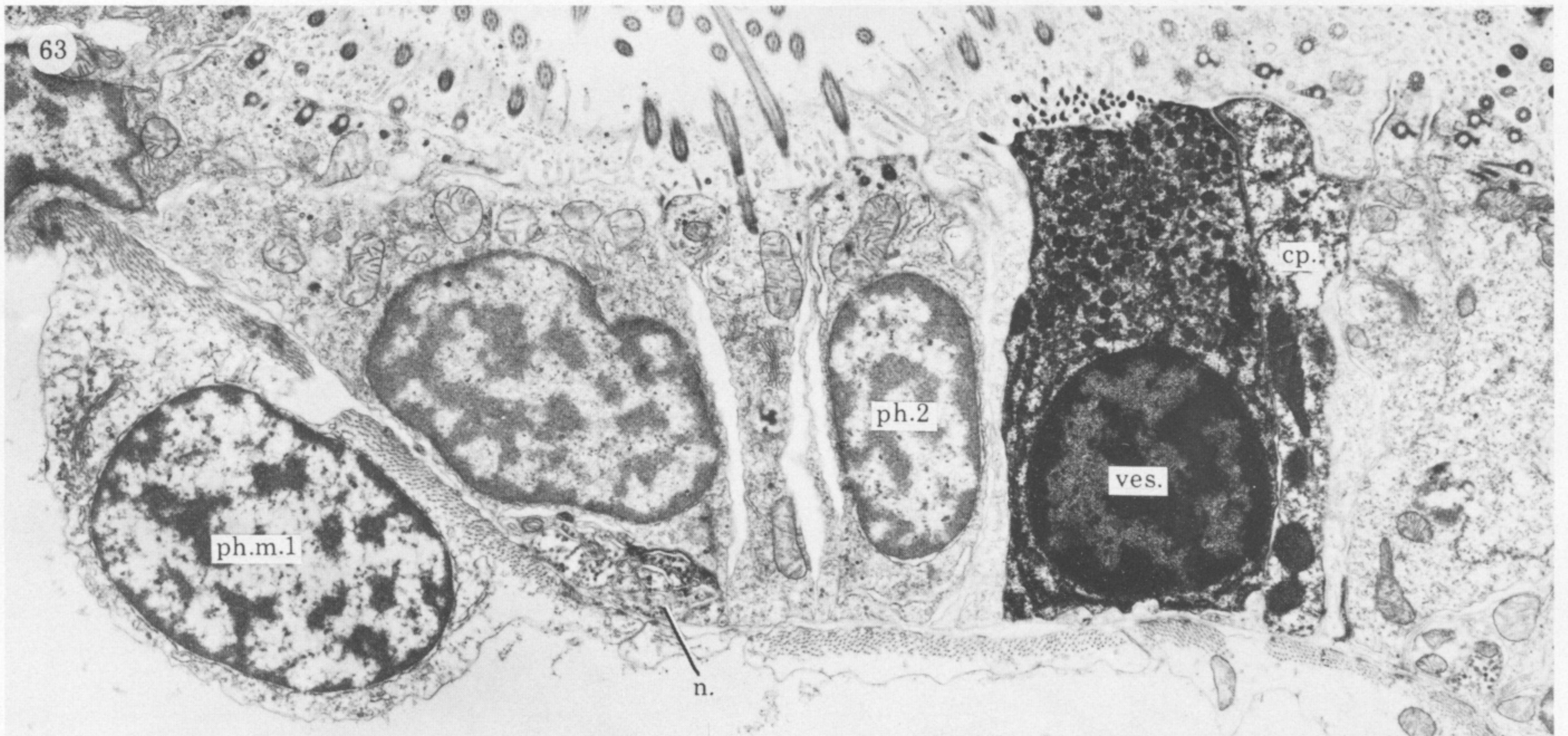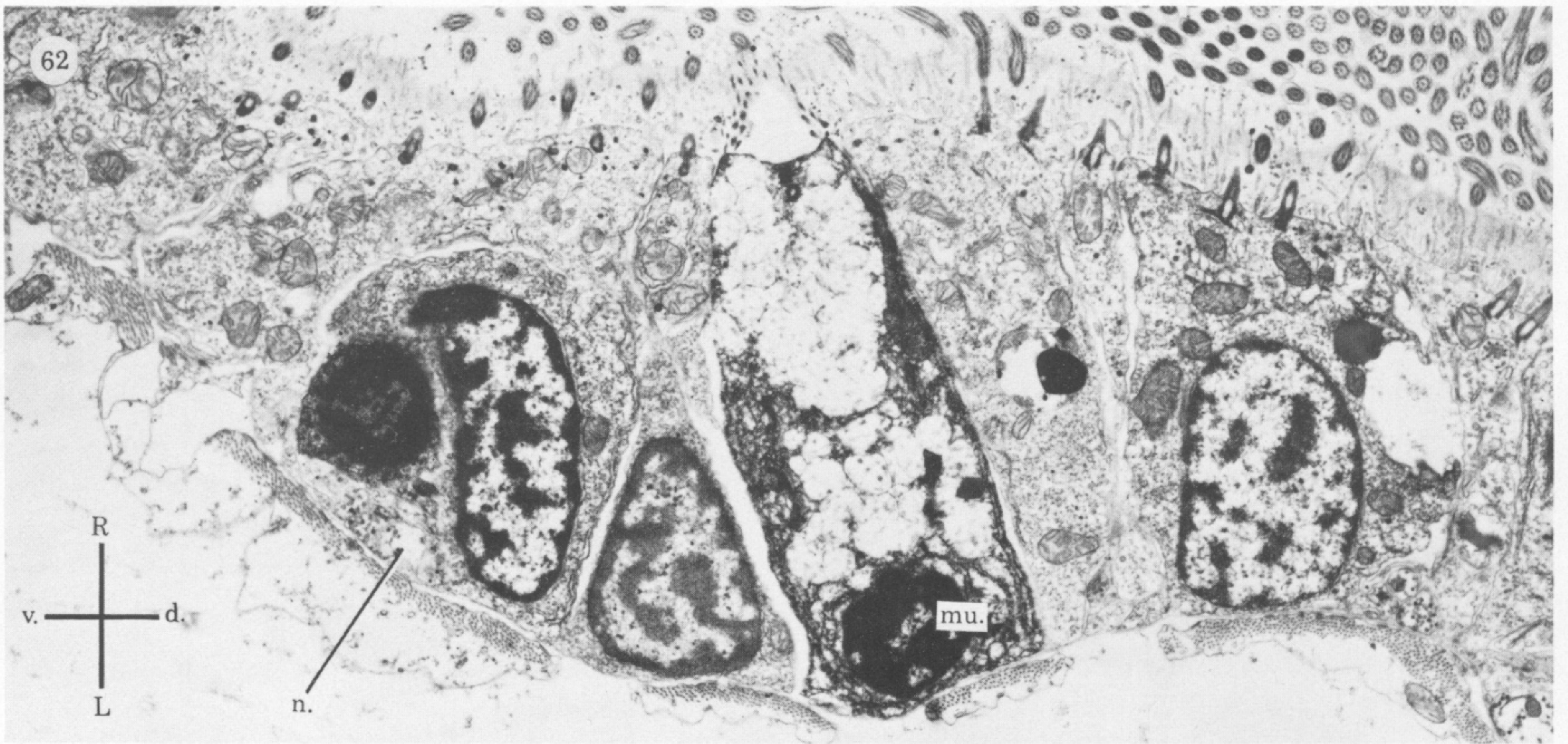
FIGURES 62–64. For description see p. 109.
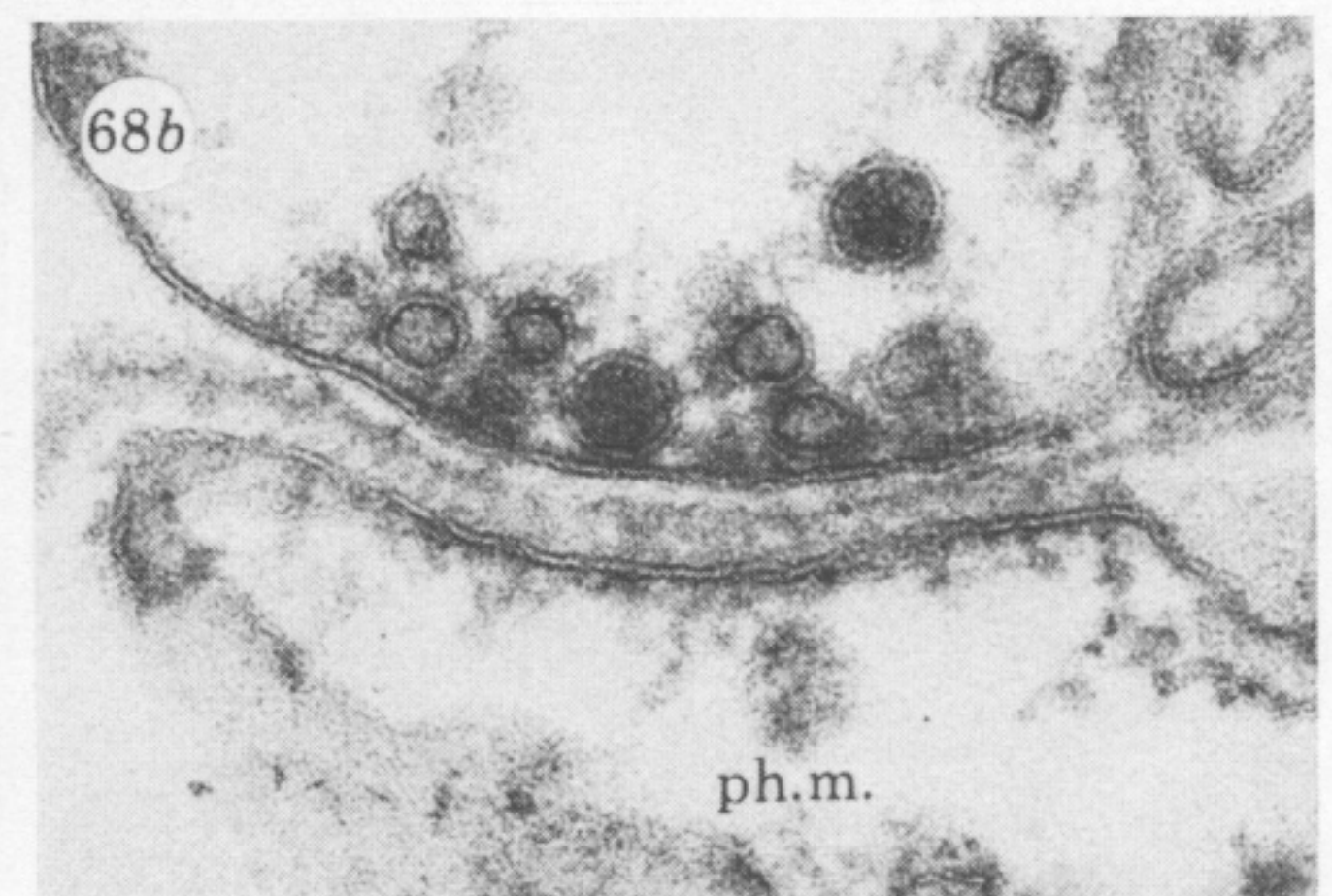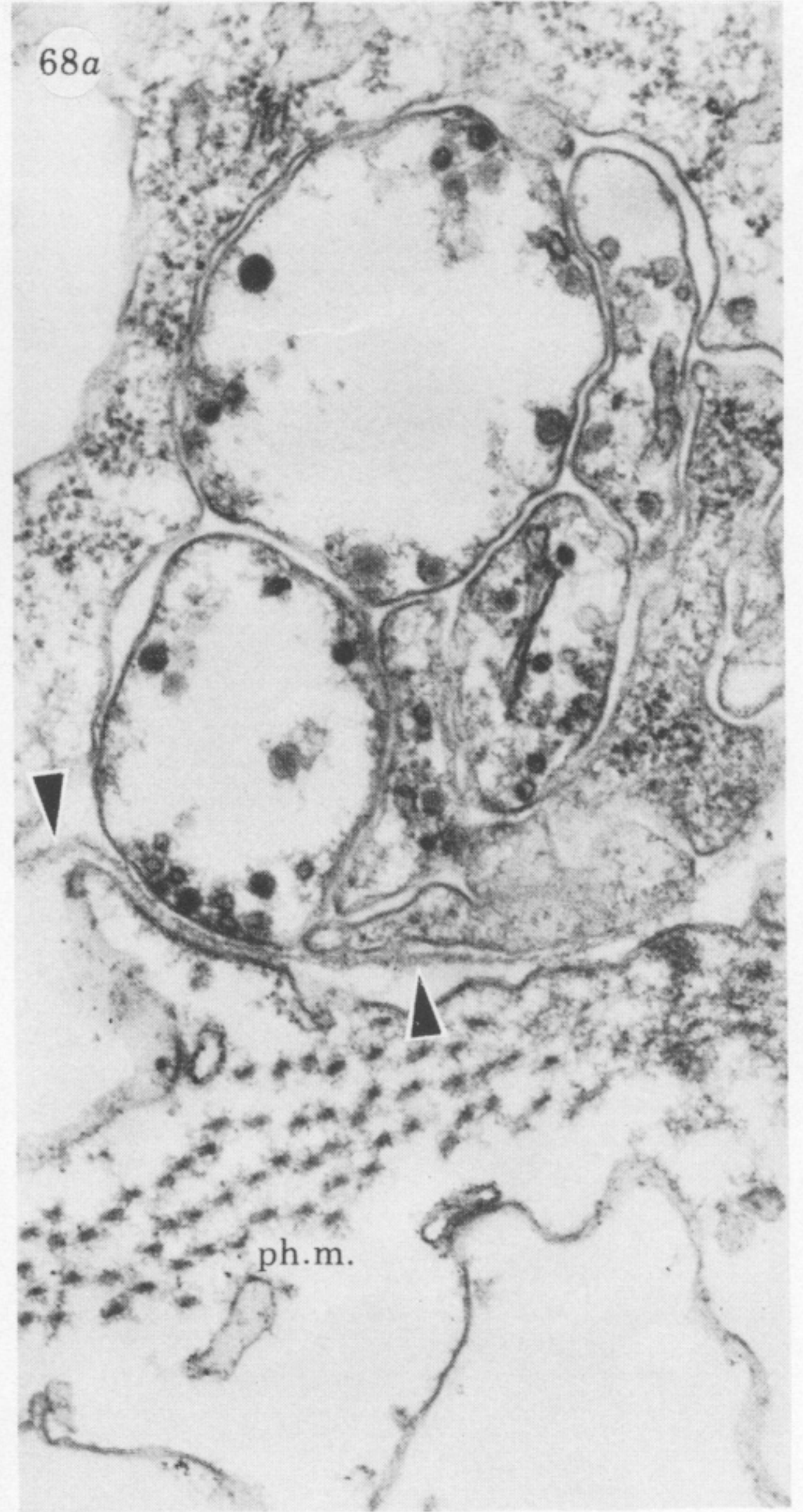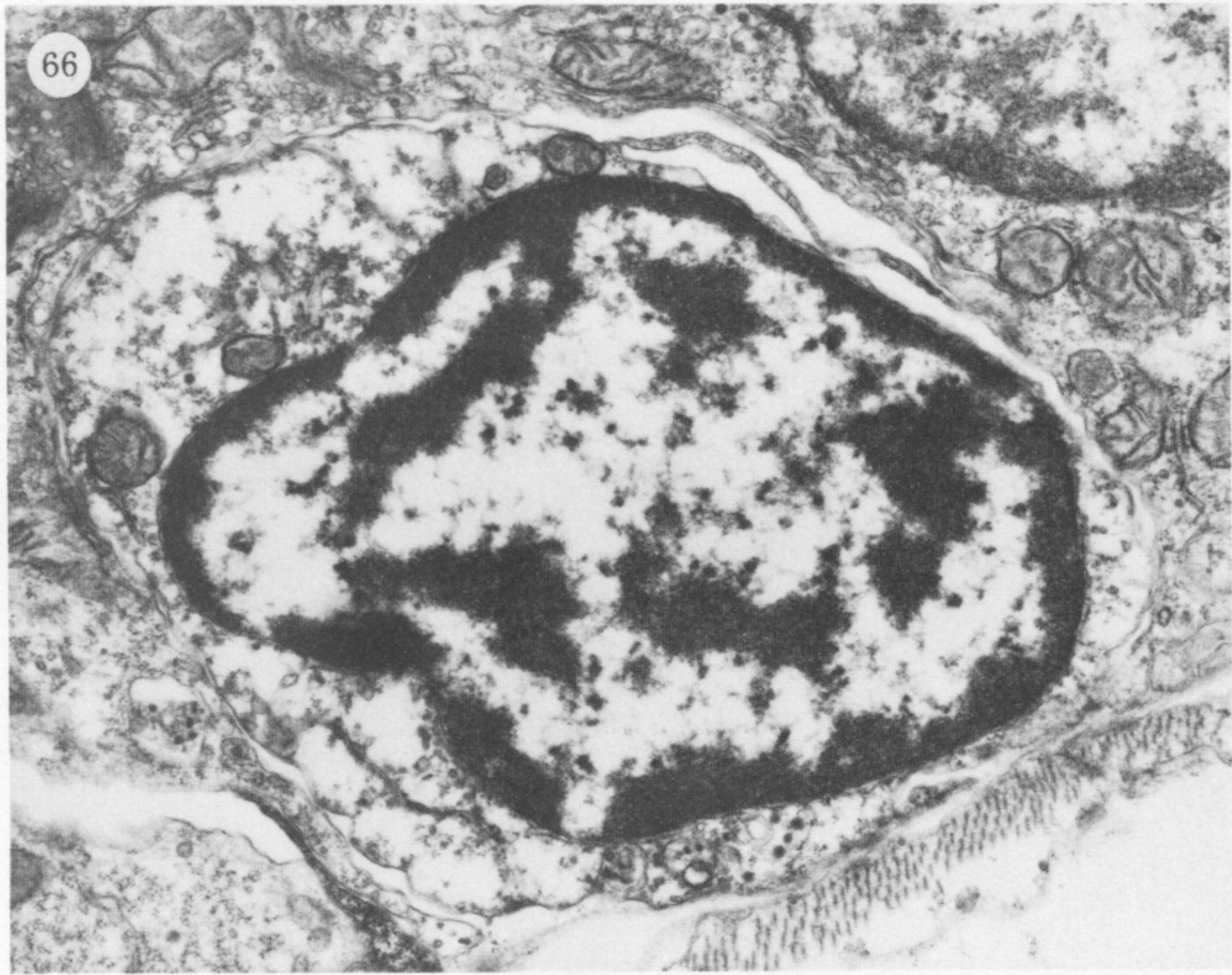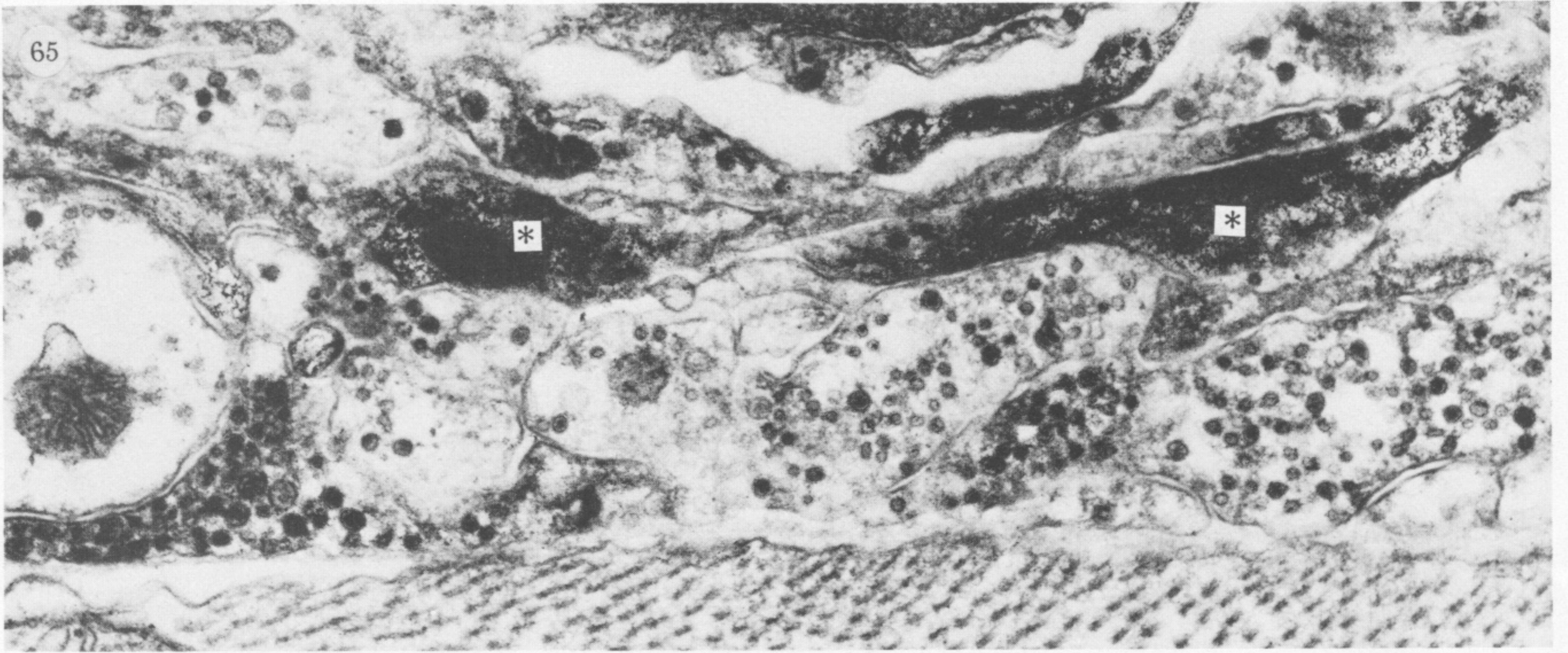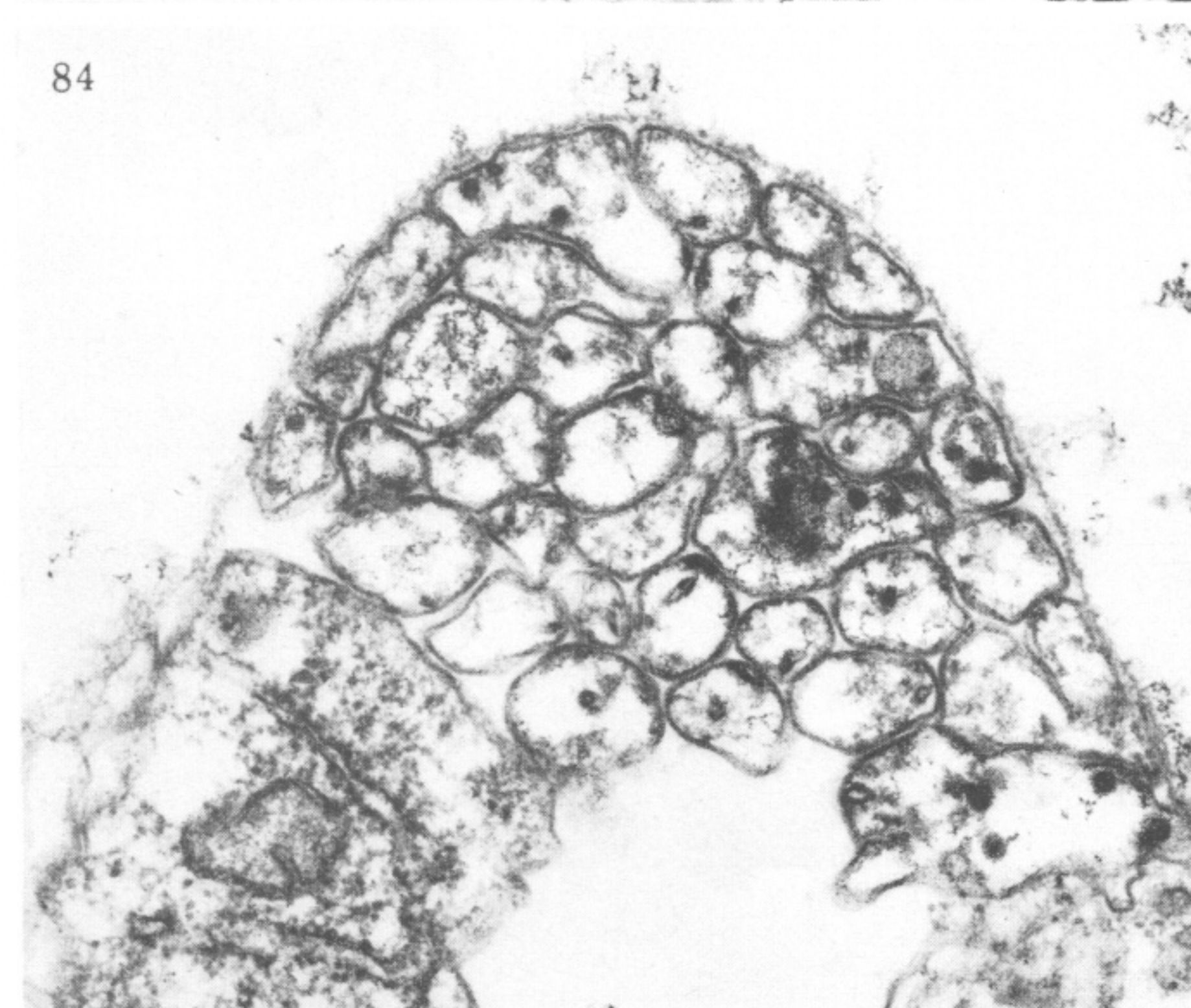
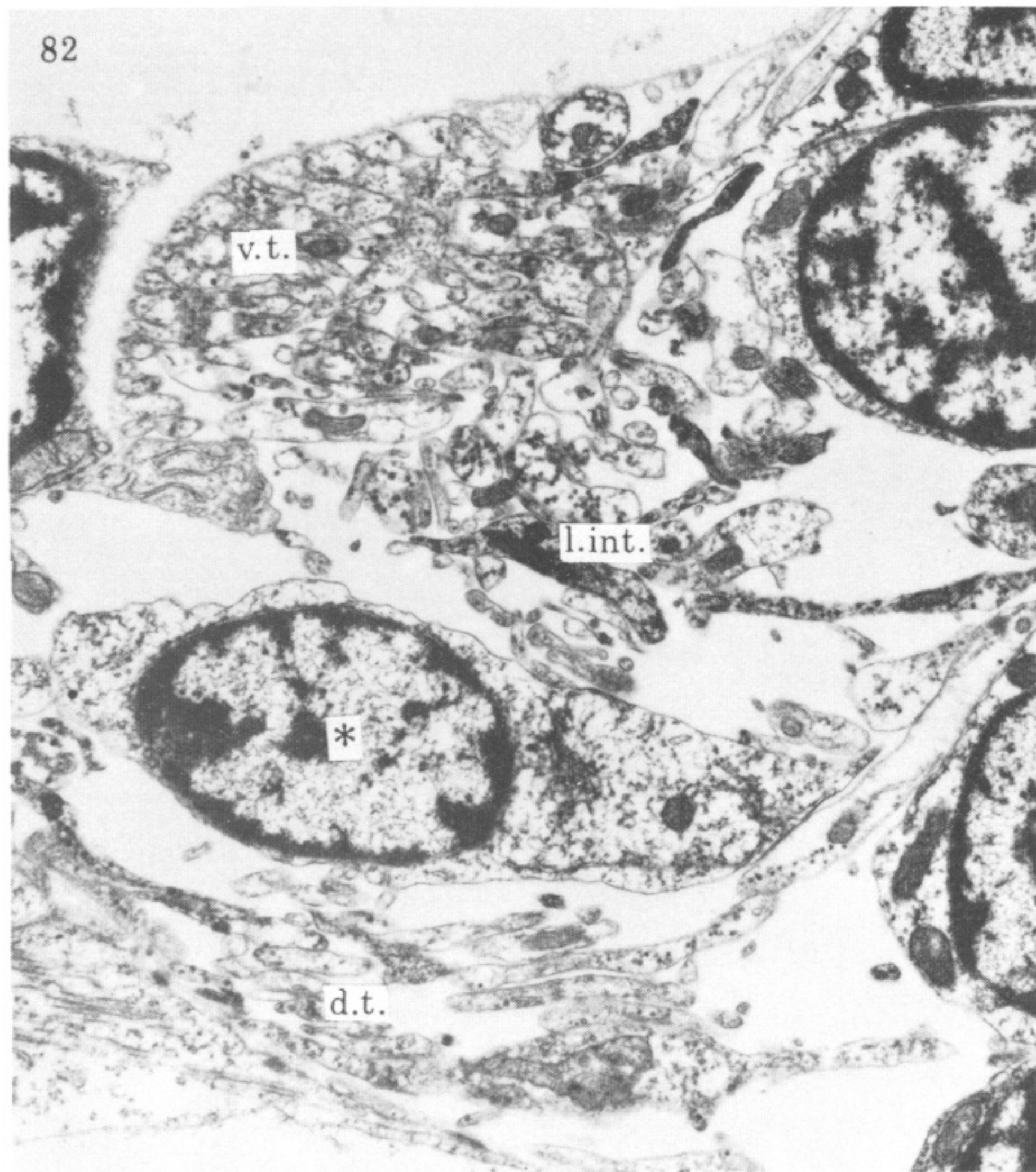FIGURES 65–68. Details of the metatrochophore pharynx. For description see p. 109.
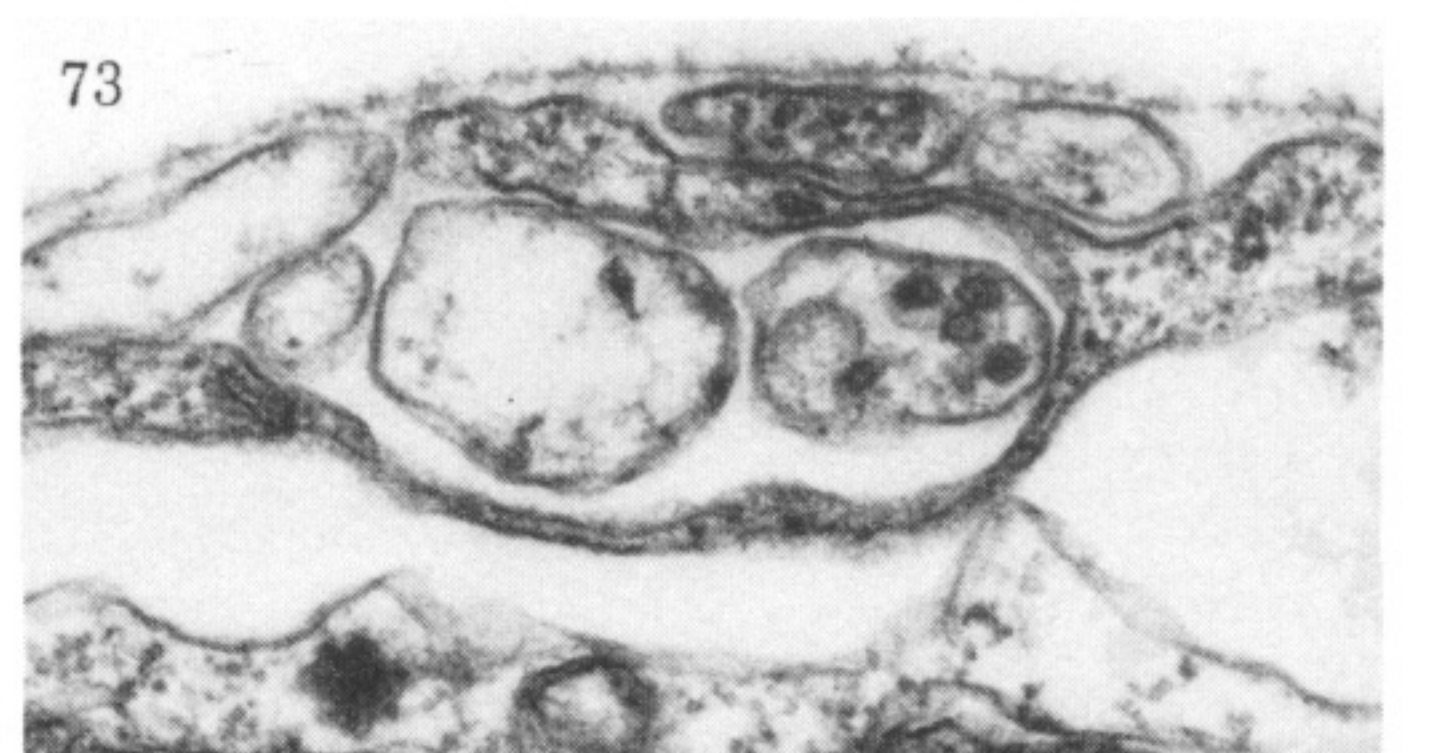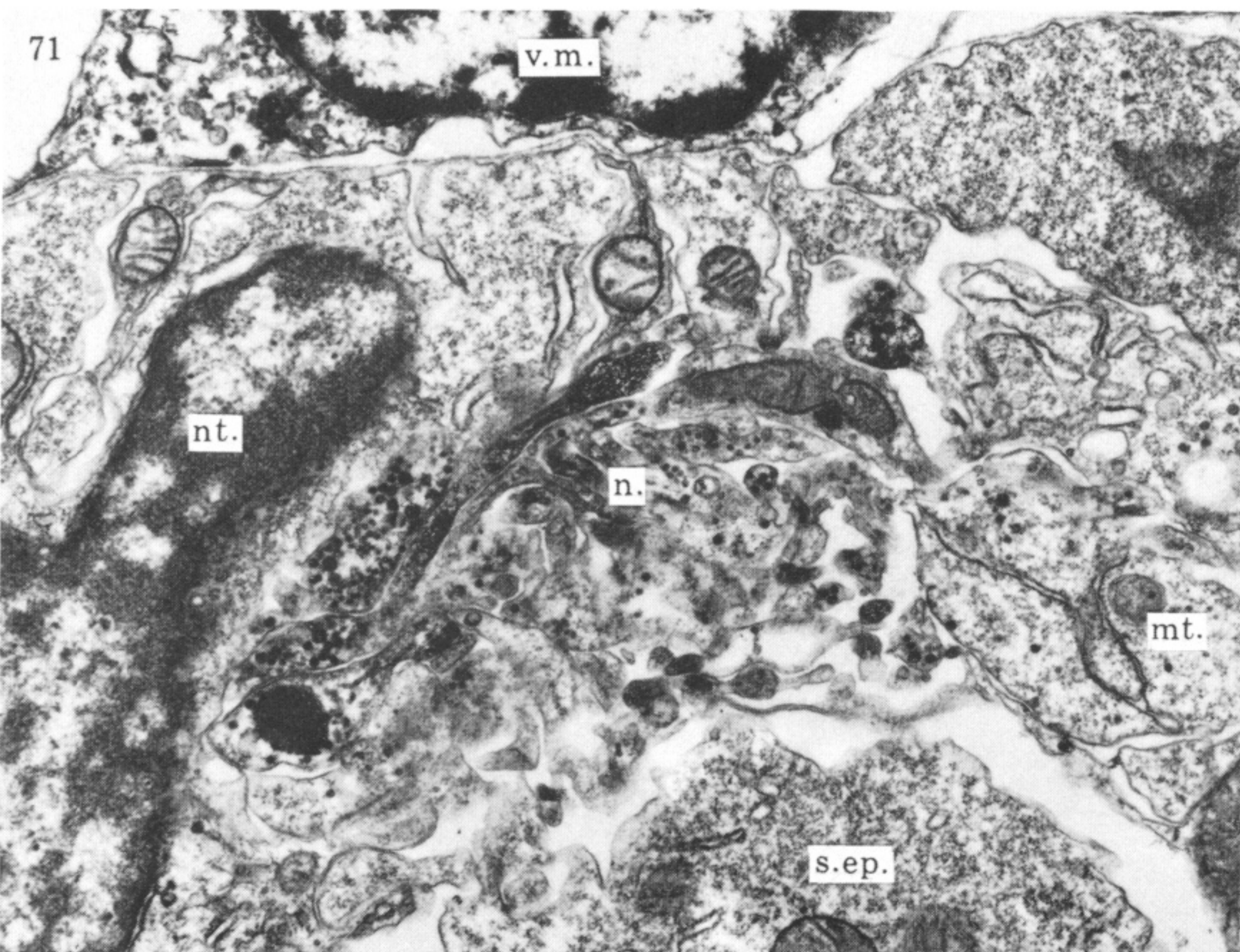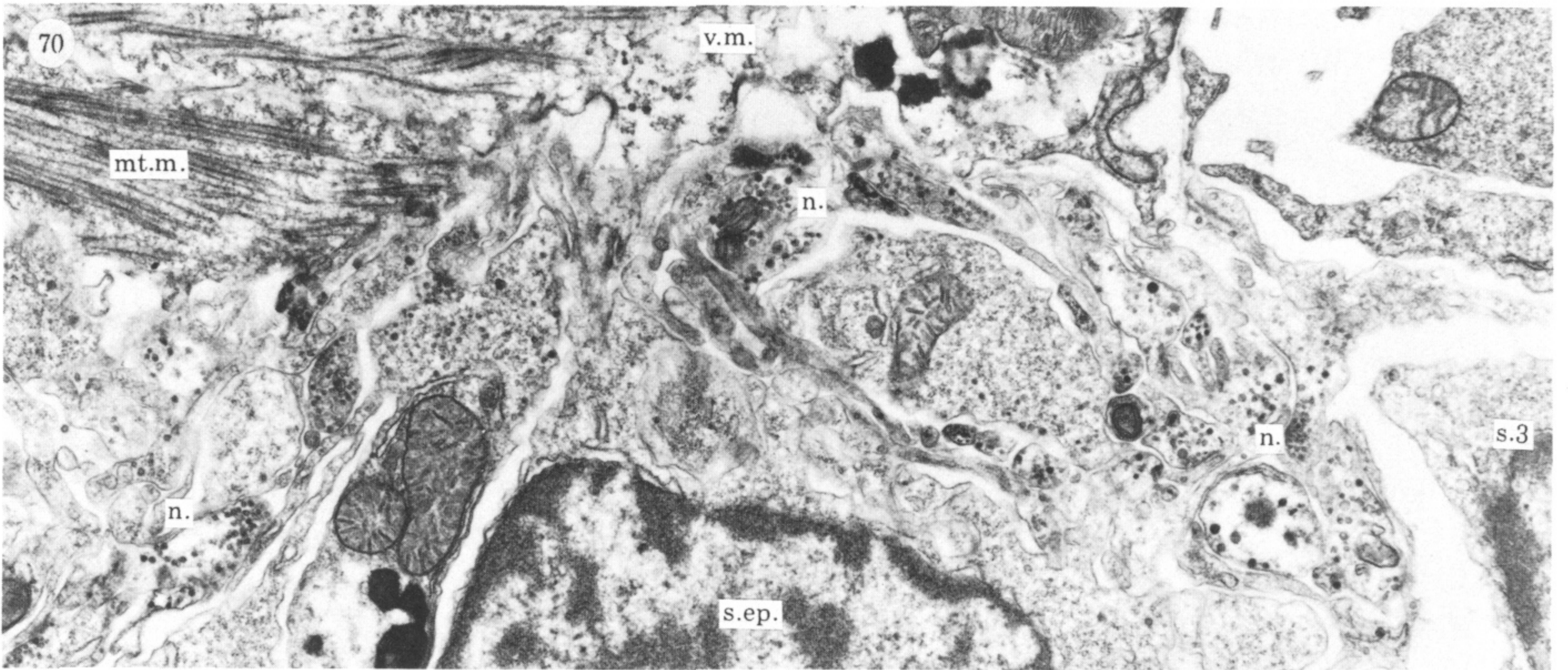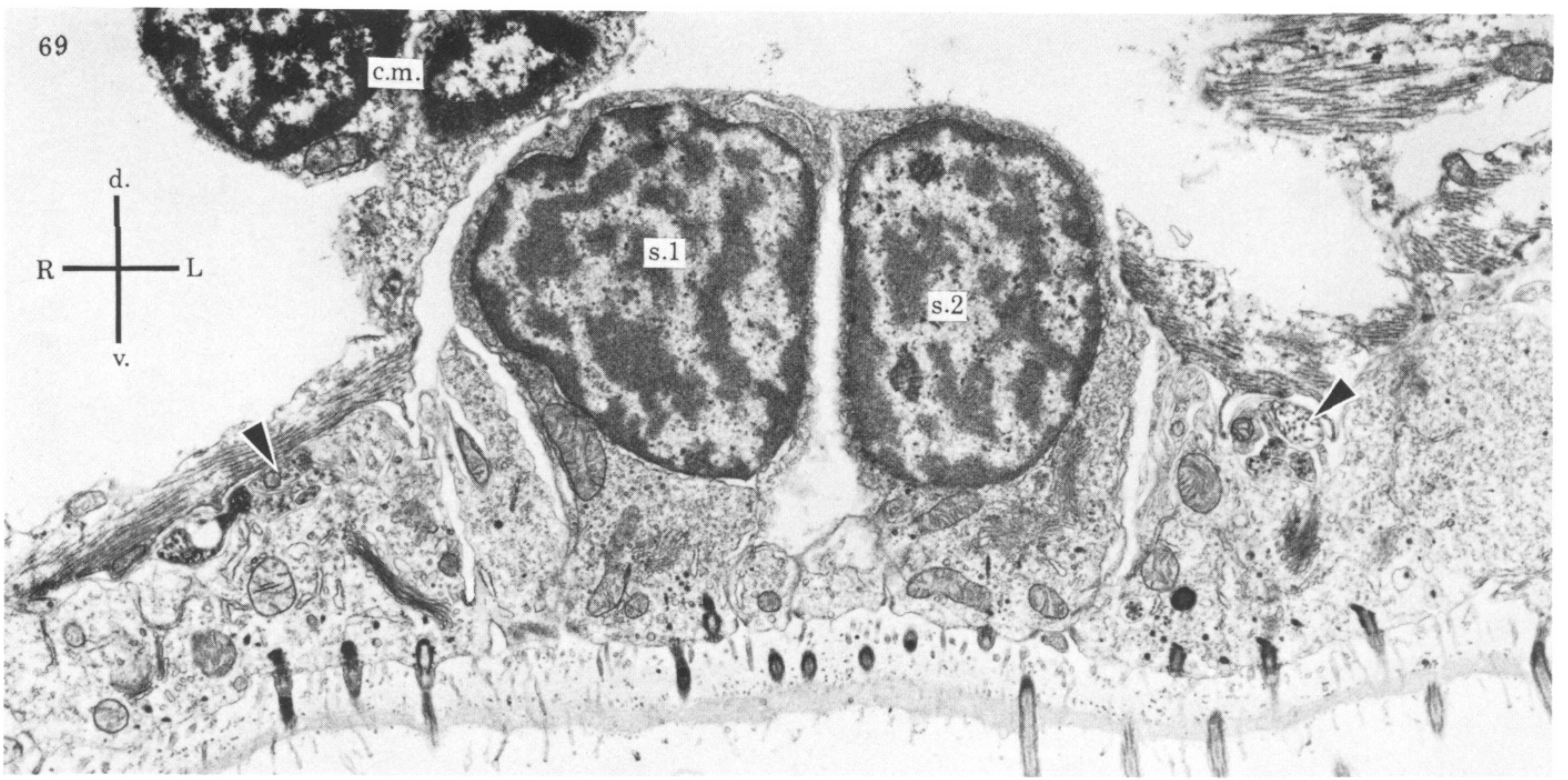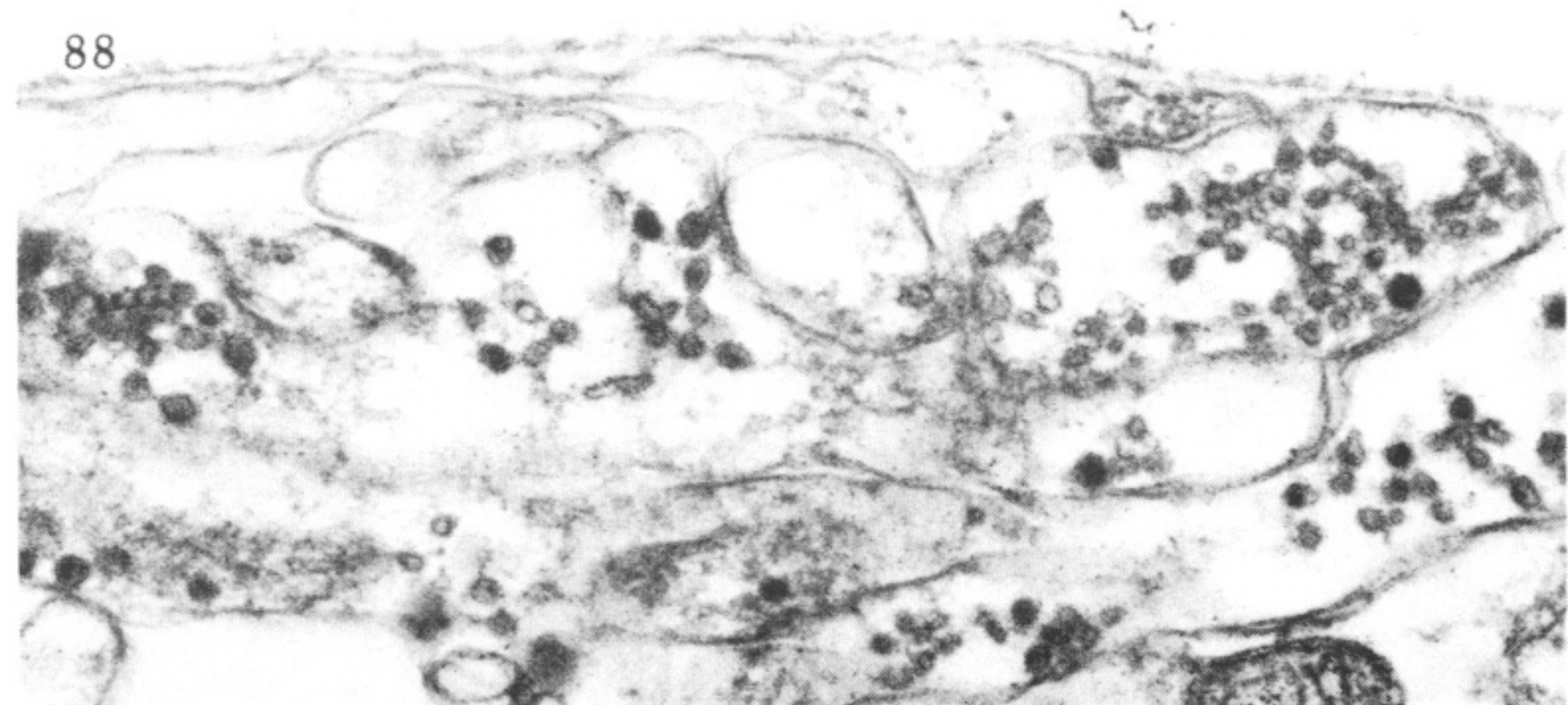
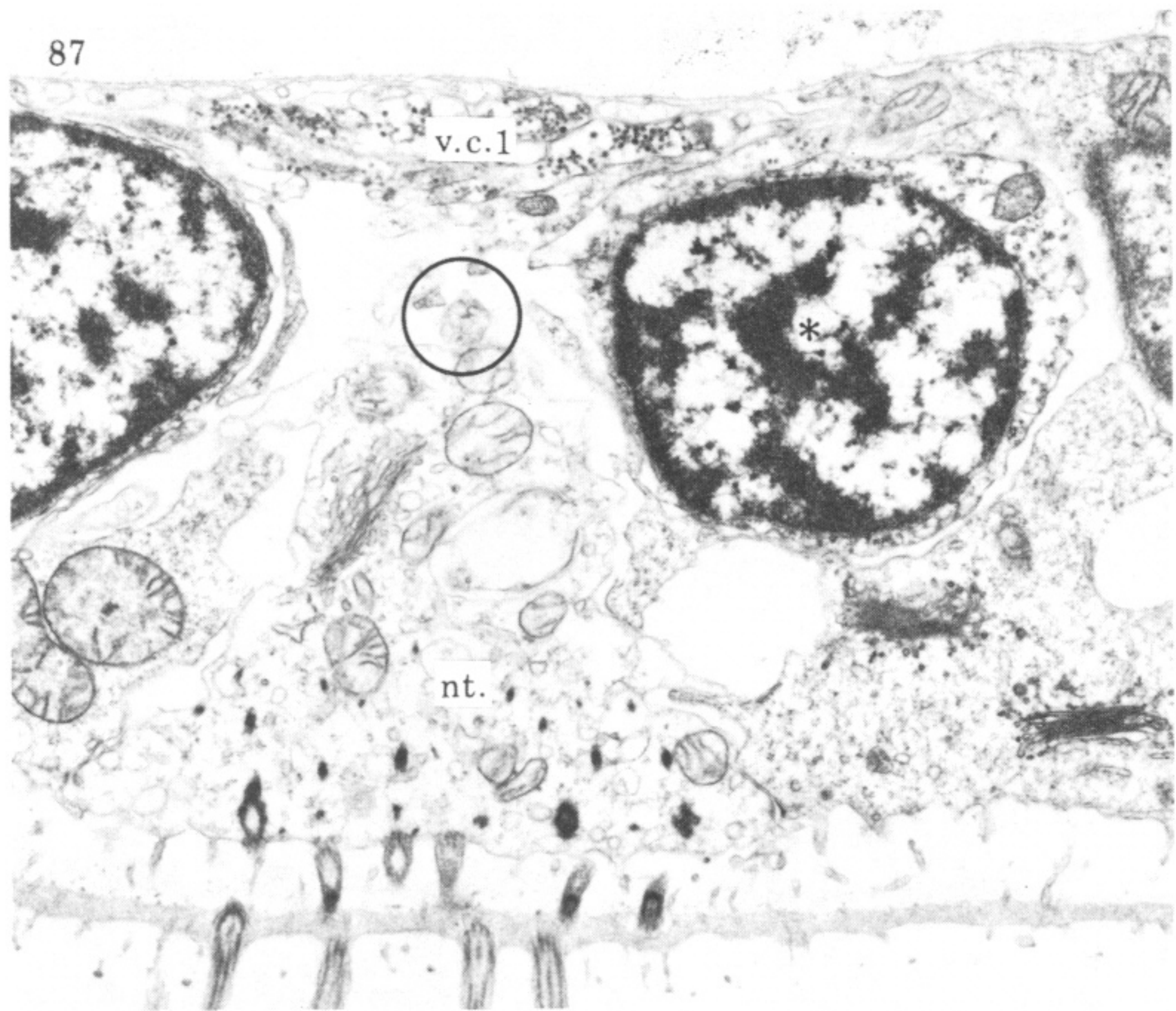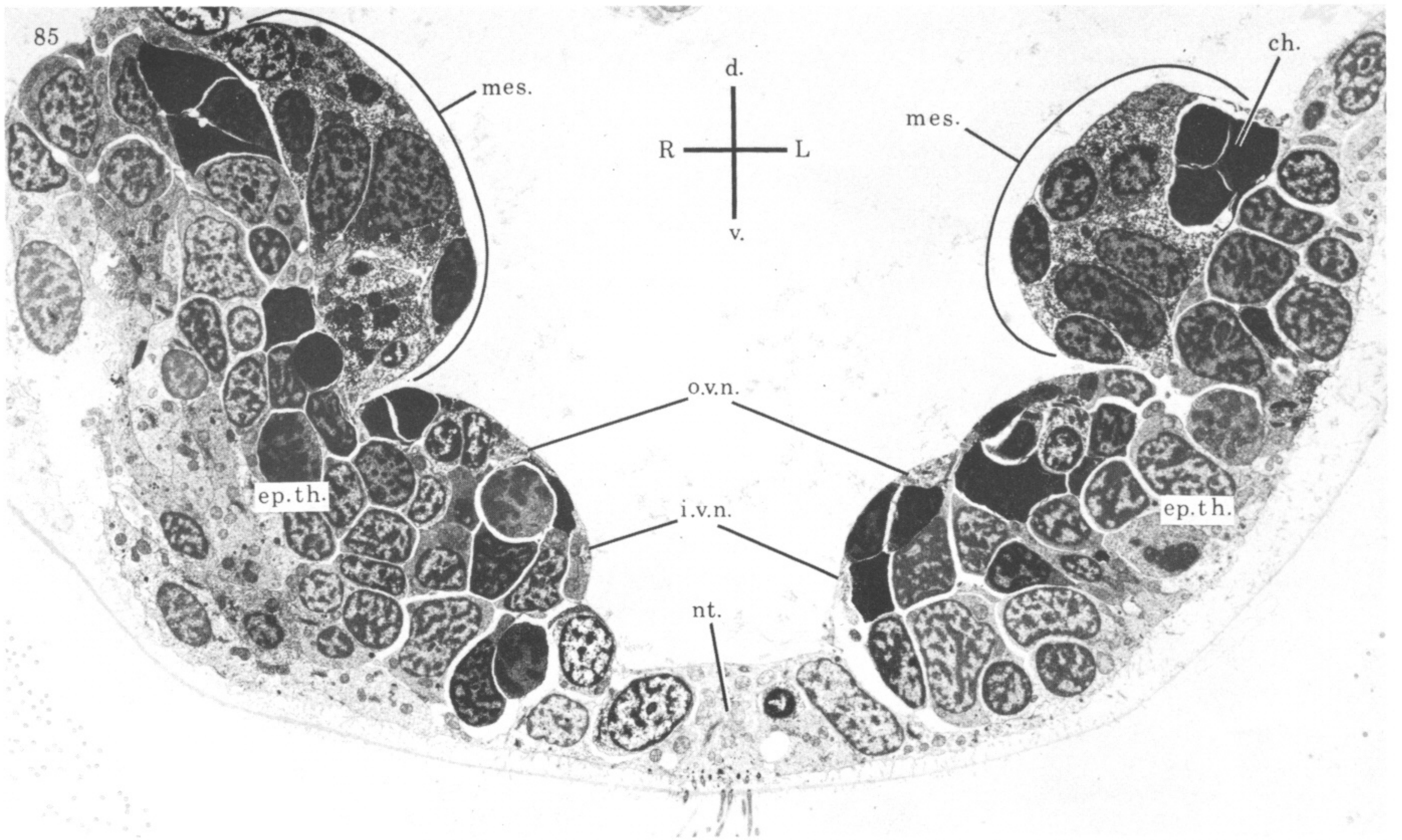FIGURES 69–73. For description see opposite.

FIGURES 76–78. For description see opposite.

FIGURES 79 AND 80. For description see p. 114.

FIGURES 81–84. For description see p. 115.

FIGURES 85–88. For description see opposite.