Ciliary band innervation in the bipinnaria larva of *Pisaster ochraceus*

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SUMMARY

Characteristic features of ciliary band organization and neurociliary innervation in *Pisaster ochraceus* larvae are described at the ultrastructural level. Serial reconstructions of selected parts of the band are used to identify the main nerve cell types and trace their fibres. The band has an intraepithelial plexus of fibres near its base and a ciliary nerve that runs along the aboral margin of the plexus. Three nerve cell types occur within the band: sensory cells, which lie along the oral margin of the band; bipolar cells, which lie along the ciliary nerve; and multipolar cells, which are more generally distributed in the band between the other two types. The sensory and bipolar cells are similar in appearance. Both contain dense-core vesicles and have slender basal processes that run lengthways along the plexus. The multipolar cells have extensive local arrays of basal processes filled with clear vesicles, and unusual apical processes that run across the apical surface of the band between the bases of its cilia. The ciliary nerve consists predominantly of a separate fibre type that originates outside the band, probably in the oral region.

Behavioural tests show that the larvae are capable of modulating ciliary beat, but coordinated reversals and arrests like those seen in other echinoderm larva do not occur. The modulatory effect operates over the long term, in response to culture conditions and nutritional state, and is involved in the larval response to contact. The latter has both neurociliary and neuromuscular aspects. There is a momentary pause in swimming and coincident backward flexure of the preoral hood. Sustained ciliary effects and flexures are induced by cholinergic agonists, notably nicotine, and transitory effects occur in response to catecholamines. Serotonin inhibits the neuromuscular response.

Assessment of the cell types, their position, morphology and contents, suggests that the sensory and bipolar cells are catecholamine-containing. The former probably have a sensory function. We suggest that the multipolar cells are cholinergic effector neurons that act directly to control ciliary beat.

There are two phylogenetic aspects of interest: (1) the echinoderm system represents an improvement on the condition seen in more primitive larval bands, which have fewer identifiable nerve cell types and more limited behavioural capabilities; (2) the bipinnaria provides an example of neural organization in a dipleurula-type larva. According to Garstang, the first chordates may have evolved from such a larva. Comparing our results on cell types, their relative positions and probable functions, with basic features of the chordate central nervous system (CNS), provides at least provisional support for this proposal.

1. INTRODUCTION

Four echinoderm classes, the asteroids, echinoids, ophiuroids and holothuroids, have planktotrophic larvae. These are remarkably diverse in overall morphological terms, but use a basically similar ciliary band system for feeding and locomotion. Current interest in the ciliary bands has several aspects. The feeding mechanism has been the subject of considerable study and some disagreement (Strathmann 1975; Gilmour 1986, 1988). How ciliary beat is controlled remains poorly understood. Neurociliary control is clearly involved, but exactly how this is achieved, e.g. the cell types involved and the precise role of each, is not known. For such simple systems, it should be comparatively easy to answer these questions. There is, in addition, a phylogenetic rationale for wanting the answers. Simple ciliated larvae occur widely among marine invertebrates, and similarities between them could provide clues to phylogenetic trends among the early Metazoa (Nielsen 1987). Although some similarities clearly reflect convergent solutions to common problems, e.g. of ciliary locomotion and feeding, there are undoubtedly important underlying homologies still to be identified. Echinoderm larvae are especially interesting in this regard because of the key position the phylum occupies at the base of the deuterostomes, a group whose early evolutionary history is very poorly understood. They are also examples of the dipleurula-type larva from which, according to Garstang (1894, 1928), the first chordates may have evolved. As such, they could provide important clues about the origin of chordates.

Too little is as yet known about band organization and innervation in echinoderm larvae to support detailed comparisons with larvae in other phyla, as we have discovered in our own attempts to compare, for example, echinoderm and phoronid larvae (Lacalli

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1990). Putative nerve cells have been demonstrated along the ciliary band in the former in stained and immunolabelled preparations of whole larvae (see, for example, Burke (1983); Burke *et al.* (1986); Bisgrove & Burke (1987); Nakajima (1987)), but it is difficult to relate these unambiguously to the cell types identified in sectioned material by electron microscopy (EM). For the echinoid pluteus larva, there are, in addition, intriguing EM reports of unusual cell types with modified cilia and peculiar apical specializations (Nakajima 1986, 1987). These observations need to be confirmed and extended. The intent of this study is to provide a better understanding of ciliary band structure and organization for the bipinnaria, with serial electron microscopy (EM) reconstruction as the principle method, and resolve, where possible, inconsistencies in previous reports. The ultrastructural results are reported in §3 and discussed in §5*a*. Observations on larval behaviour are included (§4) because they complement the ultrastructural results, and permit at least a tentative assignment of particular functions to specific cell types. Section 5*b* deals with the results in phylogenetic terms, in particular with Garstang's ideas on chordate origins. In general terms, the results support Garstang.

The bipinnaria of *Pisaster ochraceus* was chosen for detailed study for several reasons. First, previous EM work on this species clearly shows that diverse nerve-cell types occur in the larval band, and these are comparatively easy to distinguish on ultrastructural criteria (Burke 1983). Stained whole-mounts show that at least a subset of the larval neurons in *Pisaster*, the catecholamine-containing cells, are distributed regularly along the band. They are most evenly spaced in the preoral transverse band, *ca.* 25–30 μm apart. This means it should be possible to obtain a representative picture of the basic pattern of innervation from a comparatively short length of band. This is an extremely important consideration in serial EM studies, where, in practice, the length of series that can be obtained is limited. The pluteus larva is much less suitable for such work because so many of the cells involved in band innervation are located outside the band and in localized clusters, rather than being distributed along it (Bisgrove & Burke 1987). A representative section series through the band alone would provide much less information on the cells actually responsible for innervation. This is confirmed by our own preliminary studies of pluteus ultrastructure, and we have encountered difficulties with the auricularia larvae we have examined as well.

2. METHODS

Adult *Pisaster ochraceus* (Brandt) were collected at Bamfield, B.C., or obtained from commercial suppliers in Vancouver, B.C. Methods for obtaining gametes and raising the larvae have been described previously (Gilmour 1988). The larvae were kept at 16 °C and fed on suspensions of *Dunaliella tertiolecta* at concentrations of 5000 cells ml^{-1}. They were transferred to fresh cultures every 2 days to obtain optimal growth. Under this culture régime, they were competent to metamorphose beginning at *ca.* 30–36 days. We used 20-day larvae for EM and 8–26-day larvae for behavioural studies.

Larvae were fixed for transmission EM by the semi-simultaneous method described previously by Lacalli (1981). This involves initial fixation in glutaraldehyde followed, after a brief interval, by direct addition of osmium to produce a mixed fixative. An initial fixation period of 3 min proved optimal. After fixation, specimens were stained in aqueous uranyl acetate (2% by mass) overnight at 60 °C before embedding to avoid the need to stain sections, and were then embedded in Spurr's resin. Three larvae were sectioned in different orientations to obtain representative sections through different parts of the bands. The principal serial series was taken sagittally through *ca.* 25 μm of the oral region and transverse bands of one larva, and this was thoroughly analysed. For the series, sections were collected on slotted grids with a formvar support firm. Fibres were identified and traced manually in photomicrographs taken at a standard magnification. Profiles at selected regular intervals were used to prepare reconstructions for the figures.

The EM images are somewhat unconventional in appearance. The cells are unusually electron dense, and there is some shrinkage. This, is in part, unintentional, but the sections are also thicker than normal (*ca.* 80 nm) because this facilitates tracing at the magnifications we routinely use for reconstruction. Greater thickness augments differences between cells and between the different cell types, which is useful for tracing. The disadvantage is that ultrastructural detail is poorly resolved at higher magnifications. A degree of shrinkage is also useful so long as cytoplasmic integrity is maintained, because it emphasizes cell boundaries. The fixation problem to be avoided at all costs is cytoplasmic disruption. Any disturbance to cytoplasmic structures that damages cell boundaries or delicate cell processes makes serial reconstruction essentially impossible. The fixation used here is, in our experience, significantly more reliable in terms of preserving cell boundaries and delicate processes than the more conventional methods used, so far, in other studies of echinoderm larvae. The larval tissues are, in general, rather difficult subjects for transmission electron microscopy.

The behavioural observations and drug treatments were done on free-swimming larvae observed by dissecting microscope at low magnification in small dishes, and video recordings were made of both free-swimming and tethered larvae, attached to suction pipettes, using the apparatus employed by Gilmour (1986, 1989) for studies of particle capture during feeding. This provides a means of recording particle paths generated by normal ciliary beat, without an external flow, and both particle paths and dyestreams with an external flow applied. The particles in this instance were algal cells (of *Isochrysis galbana*), and the dye was Evans Blue. All drug studies were done on groups of five or six larvae maintained in small dishes, with two or three replicates at each concentration. Solutions were freshly made, with ascorbic acid added

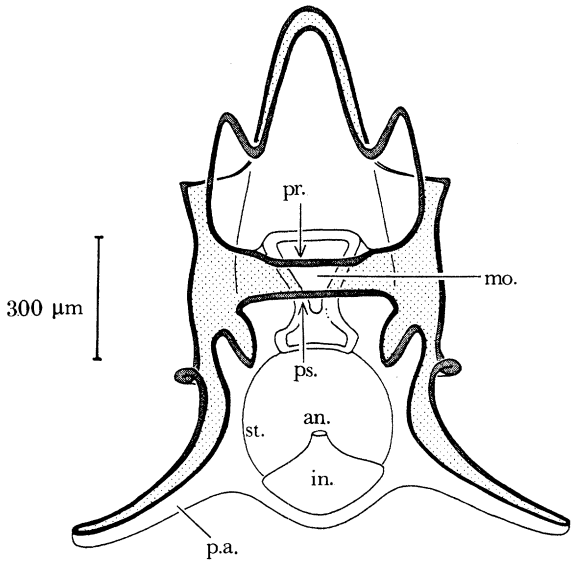


Figure 1. Bipinnaria larva of *Pisaster ochraceus* at 20 days, ventral view showing the arrangement of the ciliary band and selected structures. The ciliary band (heavy line), defines the oral field (light shading). Arrows indicate the portions of the preoral (pr.) and postoral (ps.) transverse bands sectioned for reconstruction. The mouth (mo.) leads, through an oesophagus, to the stomach (st.), intestine (in.) and anus (an.).

in equimolar concentration as an antioxidant to the catecholamines.

3. CILIARY BAND STRUCTURE AND INNERVATION

The bipinnaria larva (figure 1) resembles the other feeding larvae of echinoderms in basic plan. It develops a single circumoral ciliary band, which defines the margin of the oral field. Cilia in the band beat away from the mouth. Those in the oral field beat towards the mouth. The band becomes increasingly convoluted as the larva grows. The oral field enlarges laterally, but remains small ventrally, which leaves a narrow zone around the mouth whose upper and lower margins are formed by the preoral and postoral transverse segments of the band respectively. A loop of the band lying anterior to the mouth eventually separates from the rest of the system to form, secondarily, a roughly triangular preoral field, with the preoral transverse band at its lower margin.

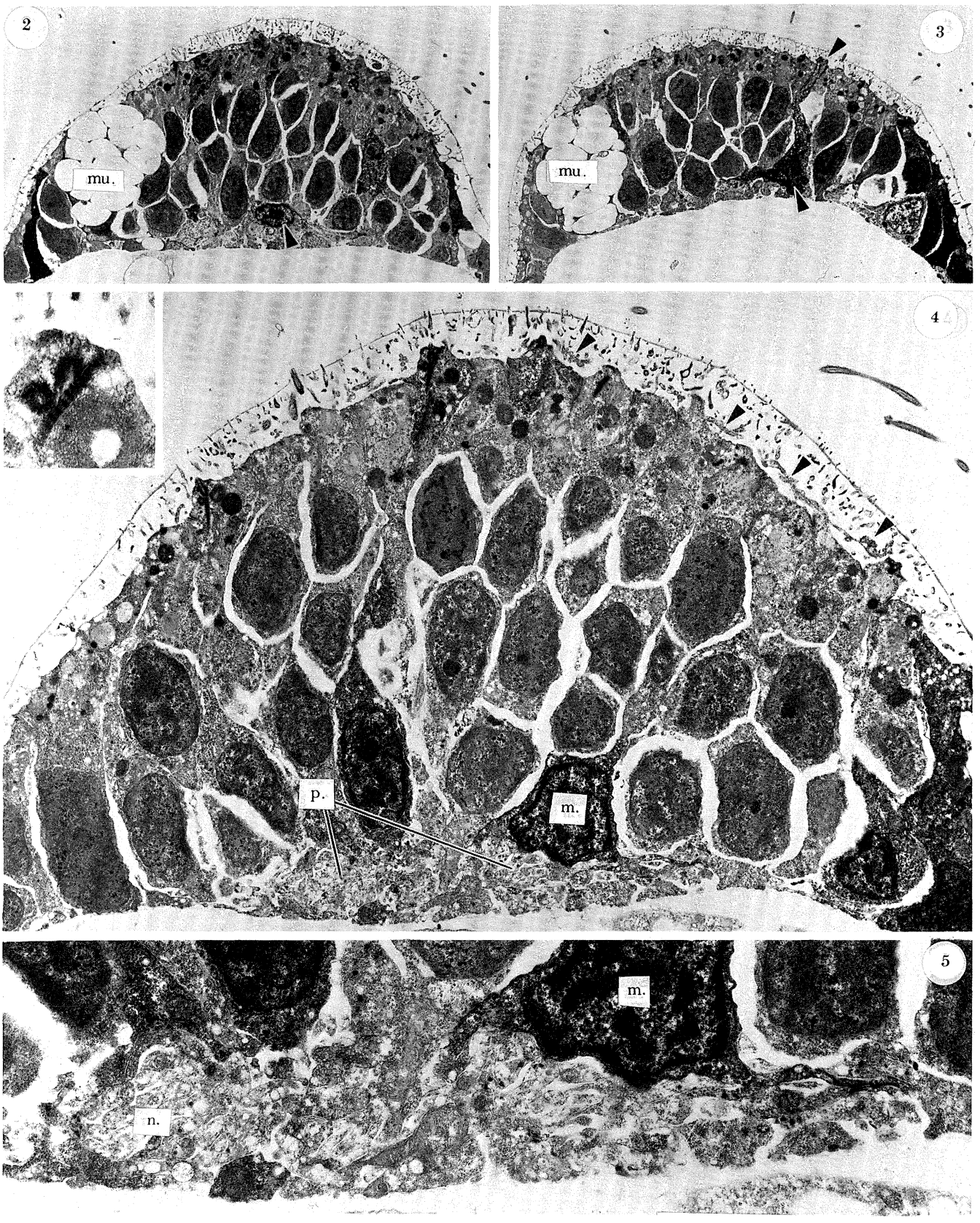
Figures 2–4 show sections through the preoral and postoral transverse bands, at points near the midline as indicated in figure 1. All the figures are oriented with the oral margin of the band to the right, so the direction of ciliary beat is from right to left. This is reflected in the orientation of the ciliary basal body and rootlet complex: in all band cells except for the specific nerve cell types mentioned below, the accessory centriole lies on the downstream side of the principal rootlet, and is oriented perpendicular to the plane of beat (inset, figure 4). The ciliary band cells are roughly columnar, expanded in the region of the nucleus, and tapered at their apices. The taper is less pronounced than in some other types of bands. In the pluteus epaulette, for example, the ciliary cells are strongly compressed to give very high-density ciliary arrays, about three cilia per square micrometre. Bipinnaria bands are comparatively broad with only a moderate cilium density, typically 5–10% of that seen in epaulettes.

Prominent mucus cells occur within the bands, usually at or near the aboral margin, but along both margins in some parts of the lateral bands. Near the base of the band, there is an extensive but irregular plexus of neurites and other cell processes (figure 5). A single distinct bundle of nerve fibres, the ciliary nerve, lies within the plexus near its aboral margin. The nerve is a prominent feature in both the preoral and lateral bands, but is poorly developed in the postoral band. Note from figure 5 that both the plexus and ciliary nerve are intraepithelial, not basal, in location. The ciliary band cells narrow near their bases to pass through the plexus layer, and then expand to form a club-like basal endfeet. The plexus lies just above the layer of endfeet, which then forms the actual basal surface of the band. Some of the band cells taper quite sharply as they pass through the plexus.

Figure 6 shows the nerve cell types identified in the band and their relative positions, based on typical sections through the lateral bands and reconstructions of the transverse bands (§3*c*, *d*). A mucus cell is also shown. Mucus cells have flattened basal processes that extend into the plexus (figures 7 and 8), but they are clearly non-neural. There are three nerve cell types whose cell bodies lie in the band. Two of these are aminergic and probably of similar type. They contain identical dense-core vesicles, and correspond, in terms of location, with the two aminergic cell types identified by Burke (1983) in glyoxylic acid-stained whole mounts (§5*a*). The first of these is referred to in this account as a sensory cell, though we have only limited direct evidence regarding its function. These cells occur at intervals along the oral margin of the band, a position suggestive of sensory function in relation to what is seen in other larvae (e.g. in phoronids (Lacalli 1990)), and in the adoral band, which is sensitive to mechanical stimulus (§4*b*). Each has a single cilium, and the cells' neurites make apparent junctional contacts with multipolar cells, the cells we judge to be effector neurons. The neurites, referred to here as type I aminergic fibres, contain dense-core vesicles and run irregularly along the band, predominantly lengthways, and are more common in the plexus than the ciliary nerve.

The second of the aminergic cell types is referred to in this account as a bipolar cell. All examples so far encountered lie directly above the ciliary nerve. They are relatively common in the lateral band, but we have not found them in the transverse bands, nor has Burke. We have only limited information concerning their morphology beyond Burke's account, and no evidence as to their function. Their fibres are indistinguishable from those of sensory cells, but appear to be restricted to the ciliary nerve.

Most of the nerve fibres in the ciliary nerve differ in appearance from the fibres just described. They have a mixed population of dense-core vesicles and clear



Figures 2–5. Transverse sections through the ciliary bands, all oriented with the oral field to the right so the direction of ciliary beat is from right to left.

Figure 2. Survey view of the preoral transverse band near the midline. There is a mucus cell (mu.) at the aboral margin of the band. The arrow indicates the cell body of a multipolar cell. (Magn. × 3400.)

Figure 3. Survey view of the postoral transverse band, as in figure 2 with a mucus cell and the basal cell body (lower arrow) and apical projection (upper arrow) of a multipolar cell. (Magn. × 3200.)

Figure 4. Enlarged view of the preoral band. Inset shows the rootlet complex of a typical cell in correct orientation, i.e. with the accessory centriole on the downstream side. The section also contains the cell body of one multipolar cell

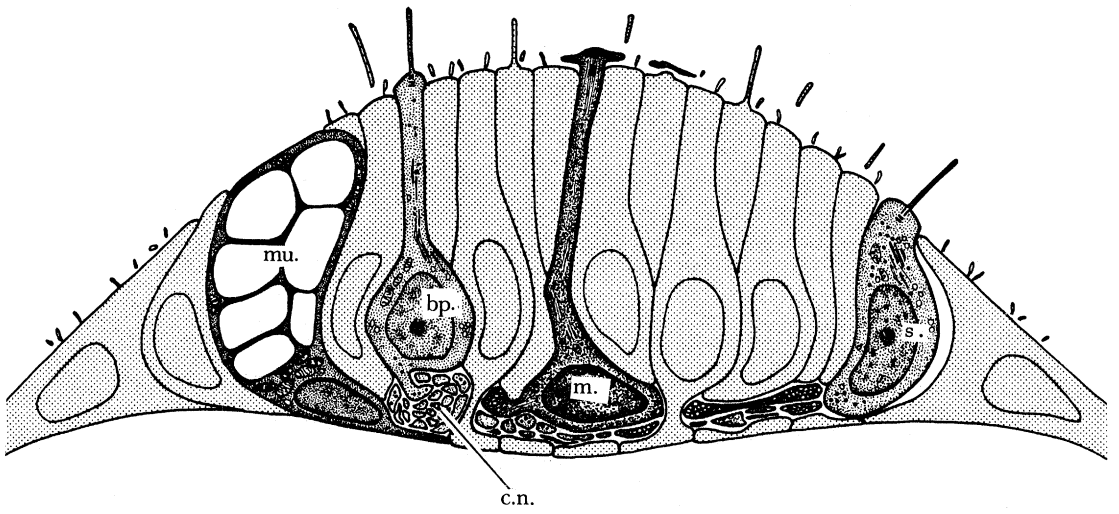


Figure 6. Transverse section through the ciliary band: a summary diagram showing the main neuronal and related cell types found in the band. Includes examples of, from left to right (aboral to oral): a mucus cell (mu.), a bipolar cell (bp.), always found just above the ciliary nerve (c.n.), a multipolar cell (m.), and one sensory cell (s.). The bipolar and sensory cells are both aminergic (§5*a*) and of similar if not identical type. The former apparently do not occur in the preoral transverse band, but one example of a morphologically similar cell was found there (§3*c*).

vesicle-like structures. They are referred to here as type II aminergic fibres, and evidently originate outside the band, in the oral region. They enter the band via nerves spaced along its oral margin.

The third nerve cell type is the multipolar cell, a type not previously reported from starfish, but similar to cells discovered by Nakajima (1986) in pluteus larvae. Each multipolar cell has an extensive, local array of basal processes, including both well-defined fibres and simple, flattened extensions of the cell body. These ramify throughout the plexus and are packed with clear vesicles. Apically, the cells have slender processes that traverse the apical surface of the band between the ciliary bases of the other band cells. The morphology of the multipolar cells, in combination with behavioural observations (§4), suggests that they may be the cells directly responsible for modulating ciliary beat, and that the apical processes may be involved in this (§5*a*).

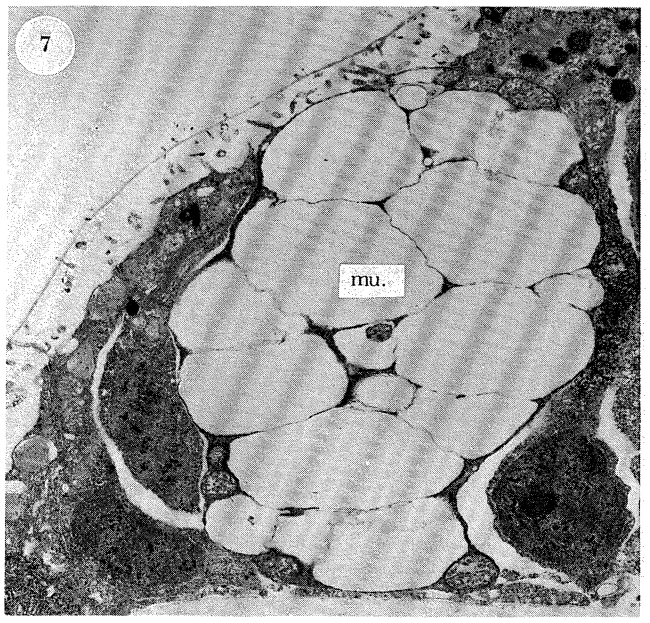
Junctions between small fibres in the plexus and mesenchyme cells lying beneath the band were encountered at several points in the reconstructions (figure 9). These may be neuromuscular junctions, but the fibres differ from all those so far mentioned, and we could not trace them to cell bodies. Further details on specific cell types and the serial EM reconstructions are given below.

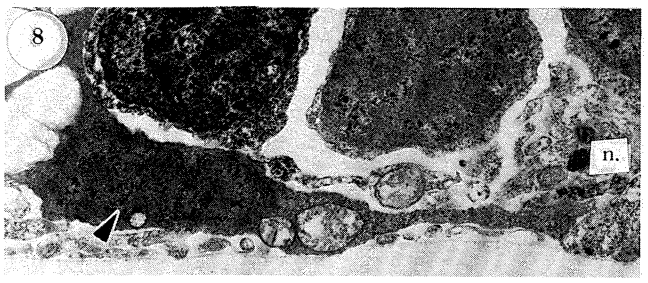
(*a*) *Sensory and bipolar cells* (*figures 10–16*)

The sensory cells are flask-shaped cells that lie at the oral margin of the band (figures 10 and 23*c*). Their nuclei and cytoplasm resemble those of surrounding band cells in terms of general appearance, but they lack basal endfeet. They also contain numerous dense-core vesicles, and the basal complex of the cilium is modified. The vesicles are *ca.* 40–65 nm in diameter, variable in shape (e.g. round or ovoid) with very densely stained cores. Bounding membranes are not always evident, and when present are often poorly defined or irregular in outline. Vesicles are found scattered in the apical region, around the golgi, which lies on the apical side of the nucleus, at the apex of the cell around the basal body, and at the base of the cell where the neurites originate. The ciliary rootlet is always reduced, typically to a slender strand, and the accessory centriole lacks a recognizable plane of alignment. In four examples examined carefully, the accessory centriole was in all cases oriented either obliquely or parallel to the direction of beat of the band cilia. They clearly differ in this respect from the non-neural ciliary cells of the band. Sensory cells have basal neurites containing vesicles identical to those found in the cytoplasm. These are the type I aminergic fibres. They are slender, and their vesicles are concentrated in irregular spaced varicosities. The latter occur most commonly adjacent to the underside of multipolar cells or their processes, as in figure 12, and often there are zones of close membrane contact. The latter were the only examples of cell contact by sensory cell processes that could be interpreted as potentially synaptic in nature. Type I fibres follow irregular paths along the bands. They travel predominantly lengthways in the examples traced (e.g. figures 22*a* and 24*c*), and singly rather than in bundles or distinct tracts. Judging from three examples examined in detail, the sensory cells

(m.) lying just above the plexus of nerve cell processes (p.). This particular cell is m2 in the reconstructions (figures 21–23). Arrows show multipolar cell processes traversing the surface of the ciliary band surface. (Magn. × 8250, inset × 32 000.)

Figure 5. An enlargement of figure 4 to show the plexus, including the ciliary nerve (n.), which lies at its left-hand (aboral) side. (Magn. × 17 050.)





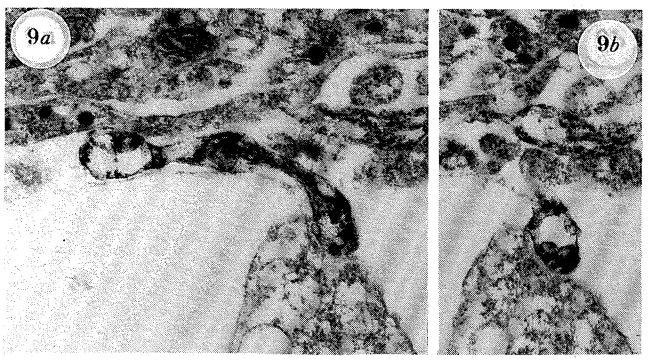


Figure 7. A mucus cell. (Magn. × 9120.)

Figure 8. Basal nucleus (arrow) of a mucus cell showing a typical flattened basal process extending into the plexus. (Magn. × 15 700.)

Figure 9*a*, *b*. Two sections through possible neuromuscular junction between a fibre from the ciliary nerve and one of the mesenchyme cells lying just under the band. (Magn. × 10 890.)

appear to be unipolar, though other type I fibres may pass near their bases, as in the reconstruction (figures 22*a* and 23*c*), which can complicate interpretation.

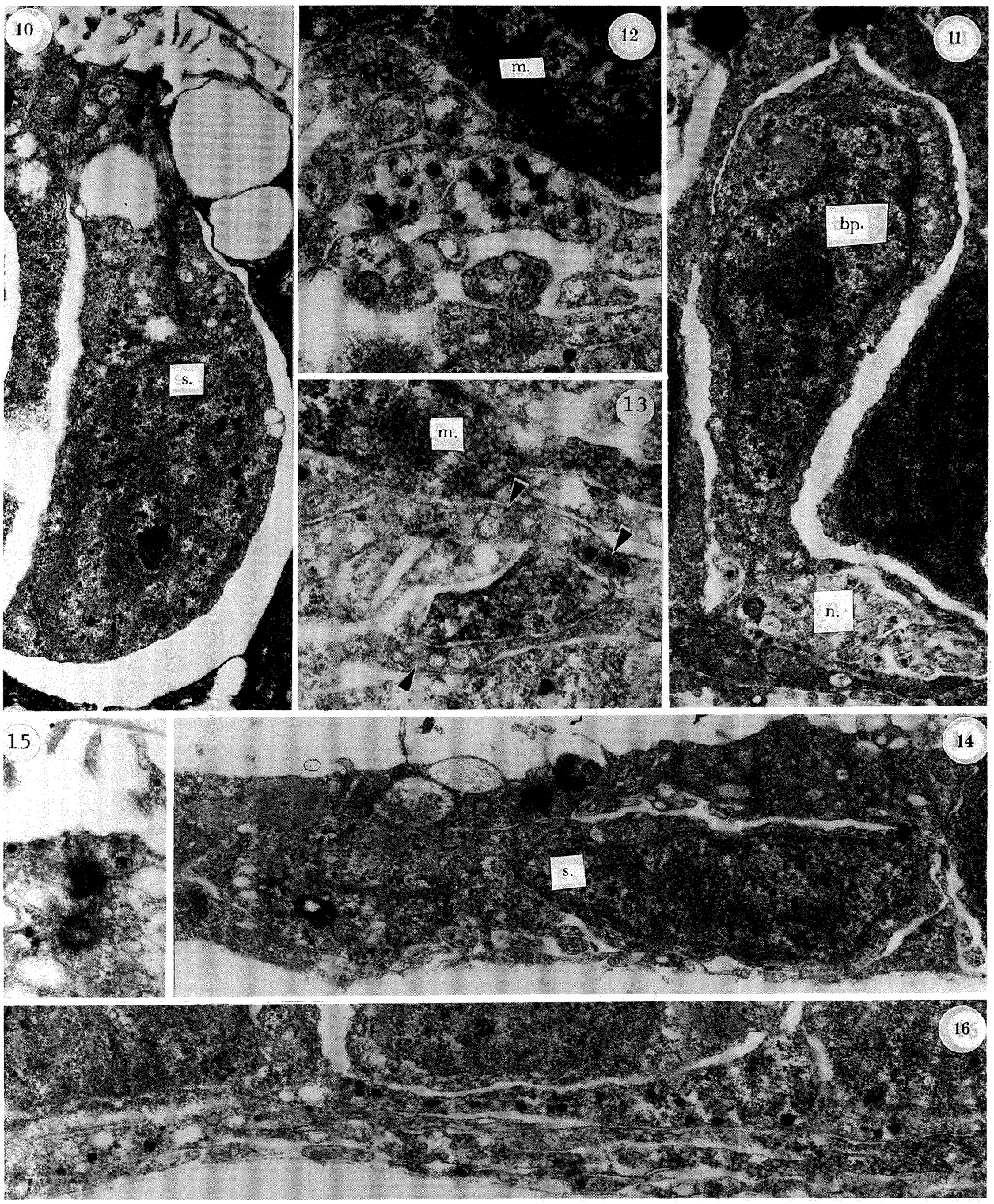
A single sensory-type cell was encountered in the epithelium adjacent to the band near its oral margin. Various features of the cell are shown in figures 14–16, and its position in the reconstruction in figure 22*b*. The cell is flattened, because of the reduced thickness of the epithelium, but is otherwise identical in all respects to other sensory cells. This part of the epithelium in fact belongs to the adoral band, which is contiguous with the preoral transverse band at this point. The adoral band is sensitive to mechanical stimulus (§4*b*), but we have not studied it in sufficient detail to know how abundant sensory cells are in it, or what other cell types occur.

The bipolar cells found associated with the ciliary nerve (figure 11) are ultrastructurally very similar to sensory cells. We have no reconstructions of them because none were encountered in our section series of the transverse bands, but they appear to be fairly common in the lateral bands. This agrees with Burke's account of their occurrence. It is clear from several examples that they send type I aminergic fibres into the nerve (e.g. as in figures 11 and 25), but these are at most minor constituents of the nerve, which suggests that they do not travel far along it. It is possible that there is more than one class of bipolar-type cells associated with the ciliary nerve. One example of a possibly related cell type was found in the transverse band (bp. in figure 21). It was similar in terms of position and morphology, but lacked identifiable vesicles.

Most fibres in the ciliary nerve are rather non-descript (figures 5, 19 and 27). Only the occasional fibre can be clearly ascribed to any of the cell types so far mentioned, and many of the rest originate from nerves that enter the transverse bands in the oral region, across the oral field. According to Burke (1983), this is the main region where nerves from the oral region enter the bands, and the latter is an important neural centre. Judging from their appearance in longitudinal section, as they cross the band, the fibres involved seem to be of a single type, referred to here as type II aminergic fibres (examples in figure 13, traced in figure 22*c*). They are cylindrical and of relatively uniform diameter. They contain scattered dense-cored vesicles that are uniformly spherical, 55–65 nm in diameter, with a spherical core, 40–45 nm in diameter, that stains less densely than the vesicles in type I fibres. Type II fibres also contain numerous empty-looking vesicles, or empty vesicle-sized spaces, up to about 100 nm in diameter, which give them a characteristic bubbly appearance.

(*b*) *Multipolar cells* (*figures 5, 17–20, 23*)

Multipolar cells are distinctive in terms of both morphology and ultrastructure, and are easily identified in sections; for example, they are visible in the survey figures (figures 2–4) even at low magnification. Both cytoplasm and chromatin show a greater degree of clumping than in most other cells in the band, and they stain very densely (figures 5, 17). Individual ciliary band cells also stained densely in some instances, but the multipolar cells have other distinguishing features as well. The cell body is basal, and gives rise to an extensive local network of cell processes (examples traced in figures 22*d*, 22*e* and 24*b*). Those from adjacent cells form the top layers of processes in the fibre plexus. Some are no more than flattened extensions of the cell body. Others are more distinctively fibre-like in that they are uniformly cylindrical and contain axial tracts of microtubules. These are typically several such fibres per cell (this feature is



Figures 10–16. Nerve cells and fibres containing dense-cored vesicles.

Figure 10. A sensory cell from the oral margin of the postoral transverse band. Dense-cored vesicles typically occur in the apical region of these cells in association with the golgi, as well as the basal region. (Magn. × 16 200.)

Figure 11. Bipolar cell from the lateral band showing its association with the ciliary nerve (n.). (Magn. × 22 900.)

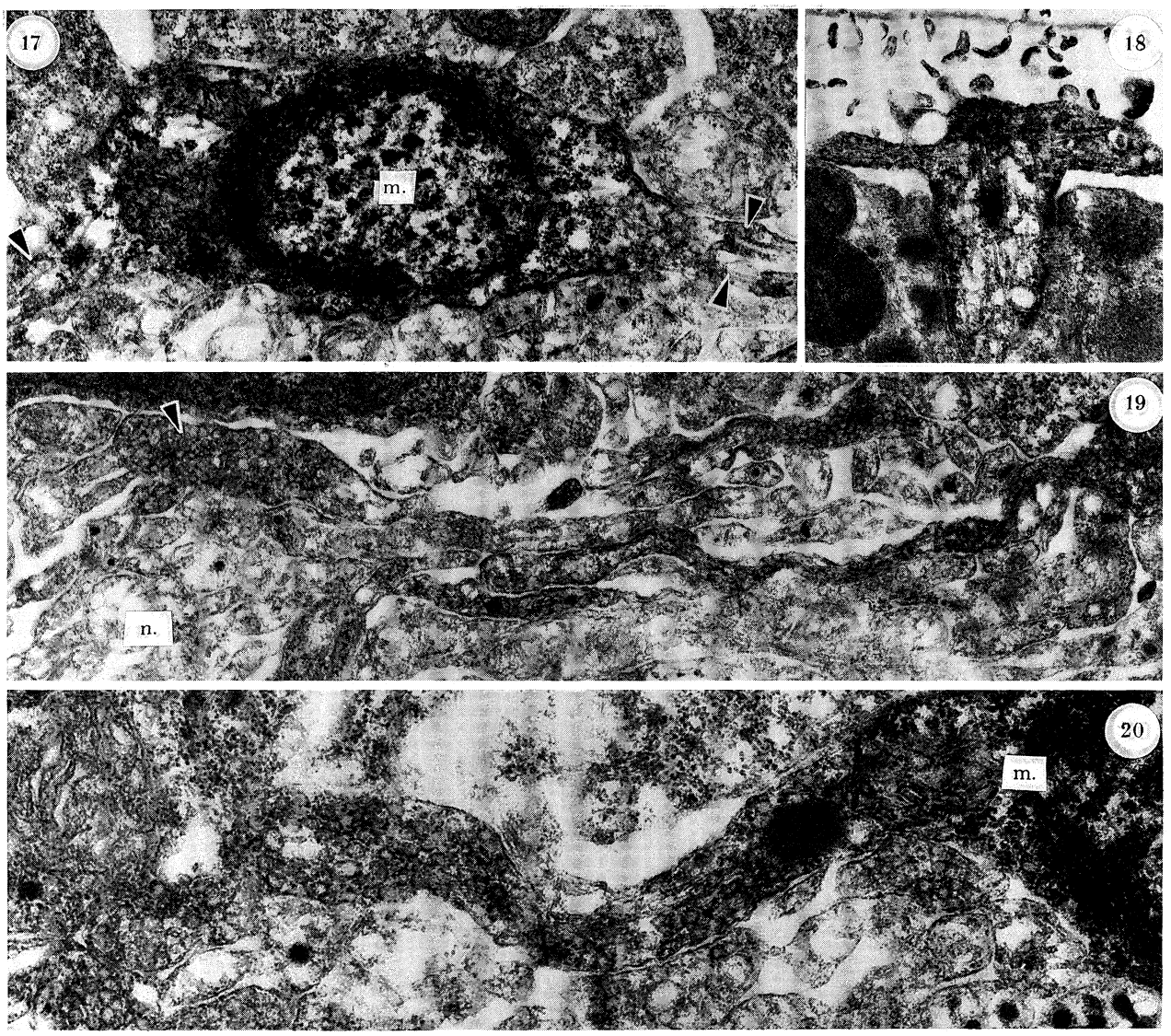
Figure 12. Detail of fibres in the plexus. Shows vesicle-containing varicosiites of two type I aminergic fibres adjacent to the underside of a multipolar cell. (Magn. × 47 550.)

Figure 13. Detail of fibres in the plexus showing examples of type II aminergic fibres (arrows). (Magn. × 44 650.)

Figure 14. A sensory cell from the oral field just adjacent to the band. This is the cell shown in figure 22*b*. (Magn. × 17 680.)

Figure 15. Basal body and accessory centriole of the cell in figure 14, for comparison with that in figure 10. (Magn. × 61 550.)

Figure 16. One of several nerves entering the preoral transverse band across the oral field. Shows a mixture of fibre types. (Magn. × 29 060.)



Figures 17–20. Multipolar cells and their processes.

Figure 17. Cell body of a multipolar cell showing its characteristic dense cytoplasm and points of origin of several basal processes (arrows). (Magn. × 32 120.)

Figure 18. Apical surface process of a multipolar cell showing the dense body from which the microtubules radiate. (Magn. × 29 850.)

Figure 19. Vesicle-filled processes belonging to multipolar cells showing an example (arrow) of contacts with fibres in the ciliary nerve (n.). (Magn. × 33 290.)

Figure 20. As in figure 19, a further detail. (Magn. × 53 820.)

also clearly shown in figures 21 and 22 in Burke (1983)). The various processes and the irregular branches they form span the width of the plexus, from the ciliary nerve on the aboral side to the bases of sensory cells on the other, and extend in some instances into the nerves that enter the band. The ends of the processes are packed with clear vesicles. These also occur in smaller numbers along the length of each process and in the basal part of the cell body. The vesicles are all of a single type, 30–45 nm in diameter and with no visible contents. Zones of junctional contact occur at various points. As in figure 19, the multipolar cell processes form a layer around the outside of the ciliary nerve in some regions, and contacts with fibres in the nerve do occur. Elsewhere in the plexus there are examples of close apposition between basal processes of neighbouring multipolar cells in which the apposed membranes stain densely. These are clearly specialized contact zones, but there is no obvious evidence that they are synaptic.

A process from each multipolar cell also projects to the surface of the band. These projections lack cilia, but produce, at their apices, small numbers (typically two to four) of slender, irregular processes, 0.1–0.15 μm in diameter. These traverse the band surface for some micrometres between the bases of the cilia. The epithelium has a surface coat, a glycocalyx, supported by microvilli, *ca.* 1 μm above the epithelium surface. The apical processes run beneath this, so they are not visible by scanning EM without special treatment. Our impression of their overall distribution, from sections, is that they travel across the width of the band rather

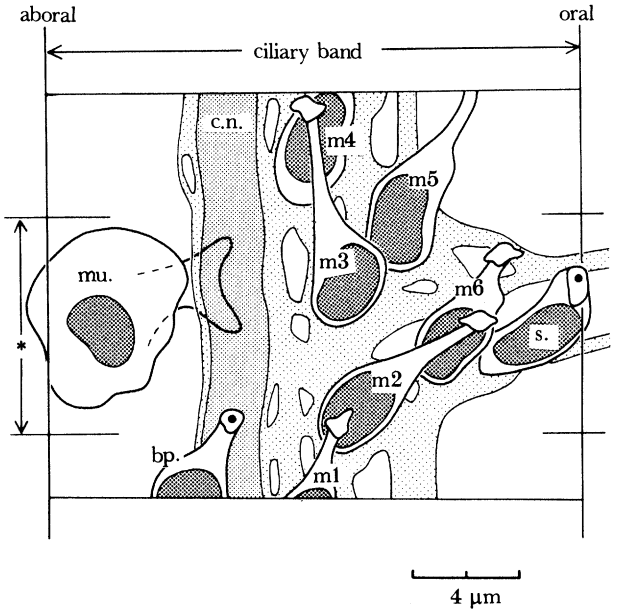


Figure 21. Reconstruction of a 15.5 μm segment of the preoral transverse band, traced at 0.5 μm intervals, oral margin to the right, and as seen looking down on the surface of the band. Shows the ciliary nerve (c.n.), the approximate extent of the fibre plexus (light shading), and various cells in outline as follows: a mucus cell (mu.), all or parts of six multipolar cells (m1–m6), a sensory cell (s.), and one bipolar-type cell (bp.) associated with the ciliary nerve. Cilia borne on surface processes are shown as filled circles. Serial reconstructions (figures 22 and 23) were prepared for selected cells and fibres from the segment of the band indicated by the asterisk.

than lengthways along it, and that they are seldom more than 5–6 μm long. They contain axial microtubules, which radiate from a central dense body located in the projecting apical tip of the cell, near the site where a basal body would be expected if it were ciliated (figure 18). The processes contact the apical surface of the ciliary cells at intervals. Regions of close membrane apposition occur, but without obvious functional specializations.

(*c*) *The pre-oral transverse band*

Figure 21 shows a reconstruction of a 15.5 μm segment of the preoral transverse band from a serial run of *ca.* 25 μm. A smaller segment of this series, comprising 8.5 μm, chosen to contain a sensory cell, was reconstructed in detail. Selected cells and processes are shown in figure 22, and in three-dimensional form in figure 23.

Shaded areas in the figure 21 show the position of the ciliary nerve and the extent of the plexus. There are gaps in the latter where basal processes from several adjacent ciliary band cells pass through. Numerous smaller gaps occur that are not shown. The following cells were identified: two sensory cells, one of which (figure 22*b*) lies in the adjacent adoral band epithelium, a mucus cell, and six multipolar cells belonging to two subtypes. From fibre tracings it is clear that the type I aminergic fibres (figure 22*a*) travel some distance along the nerve, because there were five fibres, only one of which could have originated within this particular segment of the band. The multipolar cell processes, in contrast, ramify only locally around their cell of origin. Most of those traced belonged to two cells, m2 and m3 (figures 22*d*, *e*), and a few to each of the other cells shown. None of those belonging to m1, or to unassigned processes entering the reconstruction at its lateral surface (the lower margin of the drawing in figure 21) extended through to the opposite side, and the same is true of fibres from m4 and unassigned fibres originating at the medial surface. Only four of the six multipolar cells drawn, m1–m4, were of the type described above (§3*b*). The remaining two, m5 and 6, although similarly stained, had a reduced number of both apical and basal processes, and far fewer vesicles. These two cells may be examples of incompletely differentiated multipolar cells, or be companion cells of some type. An alternative, entirely speculative but based on similarity of general appearance, is that they could be responsible for the small dense fibres involved in the neuromuscular junctions (e.g. figure 9).

We have no clear morphological evidence for synaptic junctions anywhere in the ciliary band plexus. The closest things to synapses were the neuromuscular contacts just mentioned (figure 9), and zones of contact between varicosities of type I aminergic fibres and the undersides of the multipolar cells and their processes (e.g. figures 12 and 20). The other examples of cell contact encountered, for example between neighbouring multipolar cells, could simply be adhesion plaques involved in maintaining the structural integrity of the plexus. The best morphological evidence for synaptic contact is thus that between the sensory and multipolar cells.

The cell labelled bp. in figure 21 was unlike the bipolar cells described above from the lateral bands (§3*a*), but is included as the single example from the transverse bands of a cell with some similar features. Tracing half the cell revealed one long basal process that extended some distance along the top of the ciliary nerve, but neither the cell nor its process contained vesicles. The basal body and rootlet were modified and reduced, as in sensory cells. The cell may represent a separate bipolar-type cell in a region of the band that lacks the aminergic bipolar cells found in the lateral bands.

(*d*) *Postoral and lateral bands*

For comparison with figure 21, figure 24*a* shows a segment of comparable size of the postoral transverse band. There are fewer nerve cells, specifically fewer multipolar cells, and the plexus is less extensive with fewer fibres of all three types. The multipolar cell processes and type I aminergic fibres are shown in figure 24*b* and *c* respectively. If a distinct ciliary nerve is present, it is too small at this point to be clearly distinguished from the rest of the plexus.

Elsewhere, for example along the lateral margins of the oral field, the ciliary band is more variable in size. Figure 25 shows a typical section, but the band gets much smaller in, for example, the apical region (figure

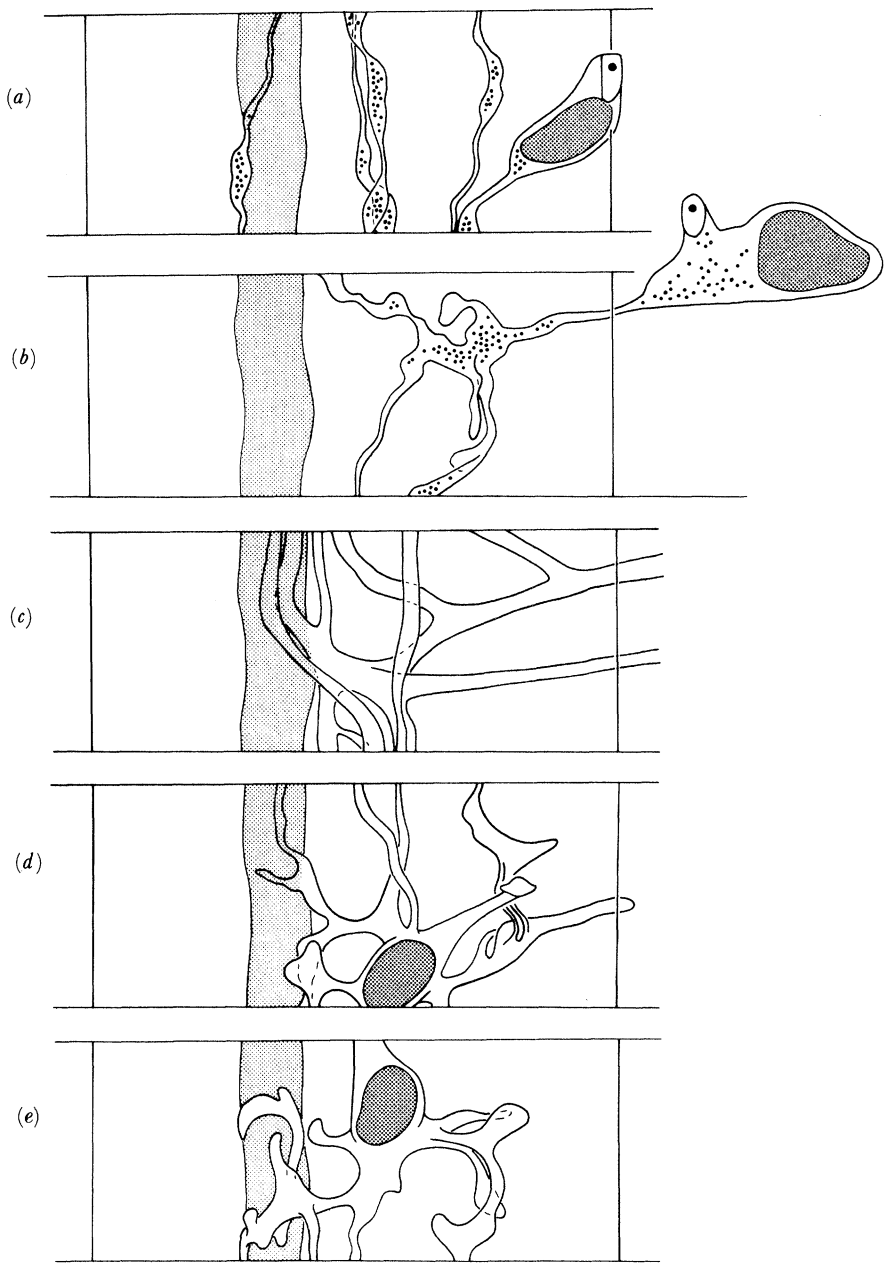


Figure 22. Individual cells and processes serially reconstructed from the segment of the preoral transverse band shown in figure 21. (*a*) The sensory cell and all type I aminergic fibres encountered except for the single sensory cell shown in (*b*), which lies in the oral field, and its fibres. (*c*) Type II aminergic fibre tracts, traced as groups consisting typically of three or four individual fibres each. (*d*) Multipolar cell m2 with its surface and basal processes. (*e*) Multipolar cell m3 and its processes.

26), and mucus cells often occupy a disproportionately large fraction of it. They also occur along the oral margin of the band in some areas, as well as in their more usual position on the aboral side. We have not examined the distribution of cells in detail, but nerve cells in general, and multipolar cells in particular, appear less common than in the preoral band. There are clearly local differences in the distribution of the various nerve cell types in different parts of the system that need more careful study. The ciliary nerve (figure 27) is always a prominent feature, regardless of the size of the band itself.

4. SWIMMING BEHAVIOUR AND DRUG EFFECTS

The 8–21-day larvae examined here represent essentially mid- to late bipinnaria stages, a period during which the convolutions of the circumoral band develop and the larva is entirely dependent on cilia for both feeding and locomotion. Observations on the feeding mechanism are reported elsewhere (Gilmour 1988) and will not be dealt with here. The posterior arms of the bipinnaria appear as small buds at 18–19 days, and lengthen rapidly (e.g. figure 1 shows a typical 20–21-day stage) before the other arms have begun to grow. By the brachiolaria stage, there are five pairs of larval arms in total, plus a tapering apical

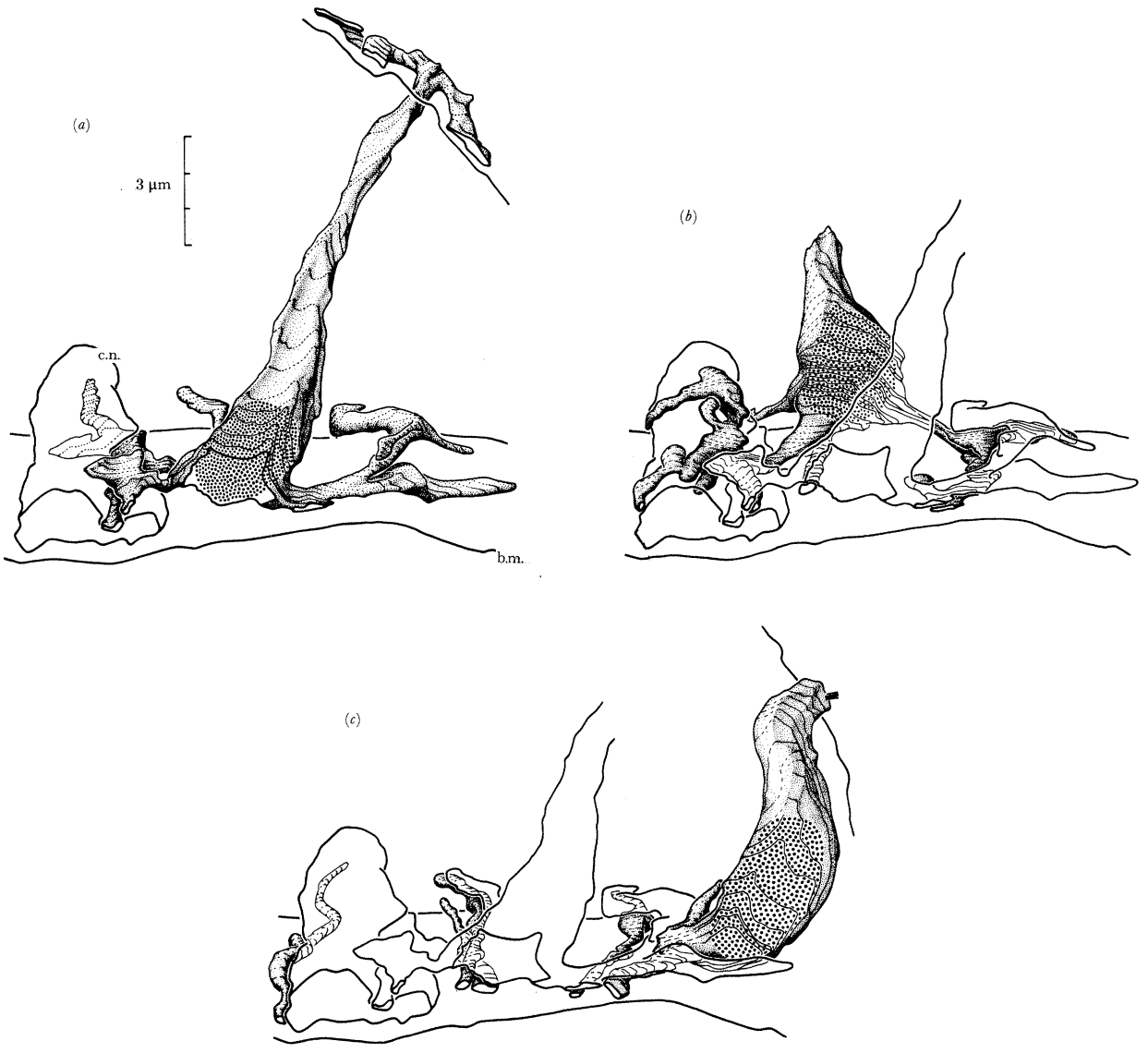


Figure 23. Three-dimensional reconstructions of selected cells from figure 22, viewed in the plane of the band as seen from the lower margin of the drawings in figure 22. Traced profiles are of every third section, with the cell surface shaded except where seen through overlying structures. Shows the outline of the ciliary nerve (c.n.) in each case, basement membrane (b.m.), and cell nuclei (dotted). (*a*) Multipolar cell m2, as in figure 22*d*. The cell has four apical processes. The truncated bases of three of these are shown. (*b*) Multipolar cell m3, as in figure 22*e*, seen behind m2, shown in outline. The cell projects to the band surface, but only the basal part of the cell lies within the reconstructed segment. (c) The sensory cell and type I aminergic fibres from figure 22*a*, showing their relation to m2, shown in outline.

projection, and three short brachiolar arms, later used as an attachment organ. The arrangement of these structures can be seen in figure 12 of Gilmour (1988). The arms are muscular, and, once they have developed, locomotion is governed by muscular activity than by cilia. Our observations conform, in general terms, with those of Gemmill (1914, 1916) and Strathmann (1971).

(*a*) *Contact response*

Echinoid and ophiuroid pluteus larvae and the holothurian auricularia are all capable of prolonged reversals of ciliary beat involving the entire band (Strathmann 1971; Lacalli & Gilmour 1990 and unpublished observations). This typically occurs on contact with obstacles, producing essentially an avoidance response, in which the larva backs away from the obstacle for a period of up to several seconds before resuming normal swimming. The *Pisaster* bipinnaria also responds to contact, but in a less dramatic way. The response involves neither reversals nor complete arrests, at least not of the sustained type seen in other echinoderm larvae, and none of our other observations on *Pisaster* suggest that the larvae are capable of either. The response to contact is probably best described as a modulation of ciliary beat. The effectiveness of the beat is in some way reduced to the point that the larvae slow or stop swimming momentarily. We were unable to determine precisely why this happens, e.g. whether the length of the effective stroke of the cilium is reduced, or its beat is slowed, and whether all the cilia are being affected or only some.

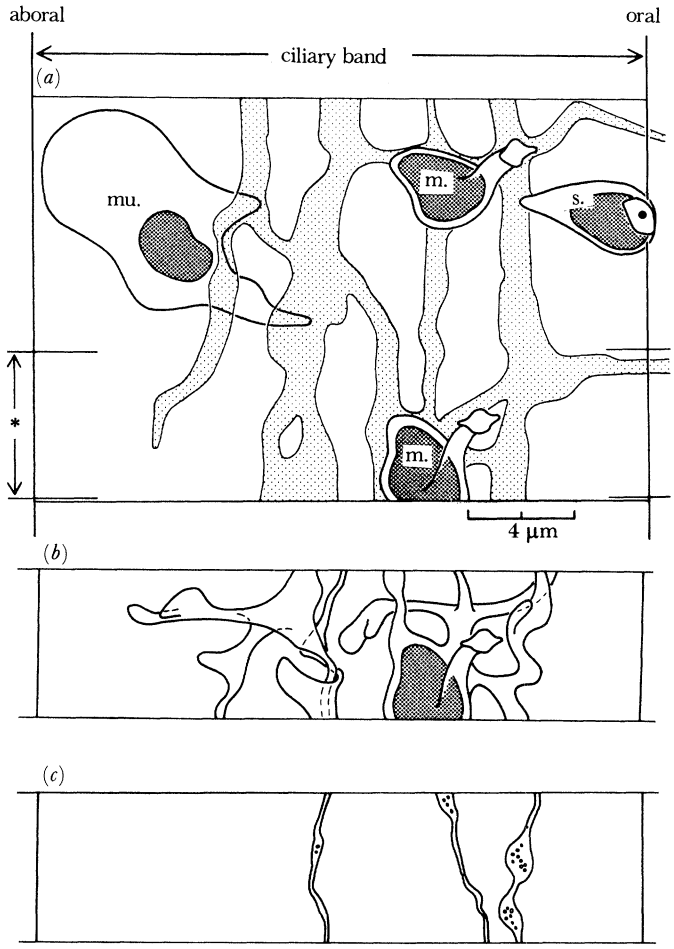
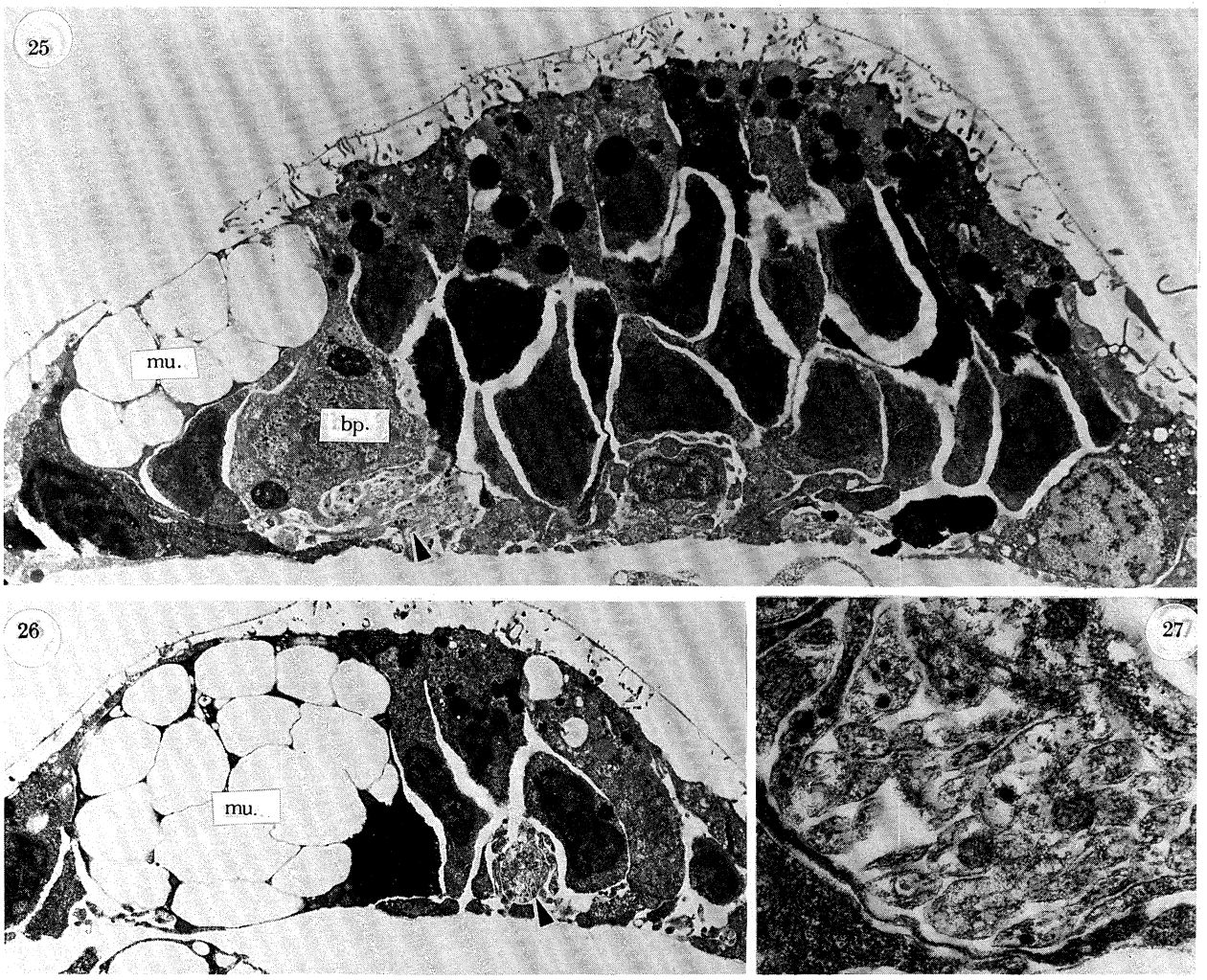


Figure 24. (*a*) Reconstruction of a 15.5 μm segment of the postoral transverse band, traced at 0.5 μm intervals, conventions as in figure 21. Shows a mucus cell, one sensory cell, and two multipolar cells. Reconstructions from serial sections for the segment of the band indicated by (*) show (*b*) the type I aminergic fibres that occur and (*c*) the cell body and processes associated with one multipolar cell.

A typical example of a contact response, involving an 8-day larva, is shown in figure 28*a* and *b*. The larva stops on contact, typically for *ca.* 1 s, and then proceeds. Usually, but not always, this is accompanied by backward flexure of the anterior part of the body, the preoral hood. Swimming typically resumes while the larva is still flexed, which causes it to turn a partial backward somersault. Flexures vary considerably in both strength of contraction and duration. The example in figure 28*b* shows a comparatively weak flexure, but larvae at this stage are capable of a stronger and more prolonged response. Two examples of this in older larvae are shown in figure 28*c* and *d*. In both cases the larvae had just been placed in a wet-mount under a supported coverglass. This usually induces sustained flexures, of 15–20 s duration. The effect on ciliary beat varies. Ciliary beat was impaired throughout flexure in the larva in figure 28*c*, so it remained almost immobile. The larva in figure 28*d* resumed normal ciliary beat after a momentary pause, so it turned repeated somersaults. Similar variability was seen under less artificial conditions in free-swimming larvae. Ciliary modulation and hood flexure are thus clearly linked in terms of the input that initiates them, but there are differences in how other aspects of each response, i.e. strength and duration, are controlled.

Large-scale, coordinated general ciliary reversals, which are relatively easy to document at high power with film and video recording, the contact response of *Pisaster* larvae is, in contrast, very difficult to study. Its occurrence can be inferred at low magnification by changes in swimming rate, and momentary changes in beat pattern are evident to the eye at higher magnification. The best examples of severely impaired beat that we have observed at high magnification are of drug treatments, which produce an abnormally severe effect. The best evidence we have as to the nature of the contact response in minimally disturbed, feeding larvae is from videotapes of tethered larvae attached to suction pipettes. In a good preparation, such larvae are capable of generating apparently normal feeding currents and of capturing particles, though usually at low rates (Gilmour 1988). Figure 29 shows an example. The larva in figure 29*a* is attached at one side and viewed from the front. A number of contact events occurred, usually in response to a particle hitting the apical region of the larva. In each, particles all along the lateral surface of the larva simultaneously either stopped or changed direction,



Figures 25 and 26. Typical sections through lateral parts of the band showing its variable size, the relative prominence of the mucus cells, and the ciliary nerve (arrow). (Magns: figure 25, ×6710; figure 26, ×6200.)
Figure 27. Detail of fibres in the ciliary nerve in a lateral part of the band. (Magn. ×32460.)

resuming their original trajectory within a second or less. The event shown in the figure occurred without flexure, which makes it easier to interpret. The larva shifted position during the first 0.2 s, owing to its rotation on the pipette, but this occurs whenever ciliary beat alters. If ciliary reversals were to occur, a quite different trace would result. To illustrate this, figure 29*b* shows a tethered auricularia larva in a similar preparation. When the lateral cilia reverse, the particle also clearly reverses direction, then reverses again when the cilia recover. We have seen the same with pluteus larvae, but never with *Pisaster*.

The above suggests that the contact response involves a coordinated modulation of cilium beat in the lateral bands sufficient to momentarily alter the current those cilia produce. At the level of the individual cilium, the event may be more like an arrest than a reversal, perhaps a partial arrest involving only a subset of the cilia rather than the band as a whole. This would disturb the beat pattern without bringing the whole band to a stop, which we would see if it occurred. The larvae also appear to modulate ciliary beat, as judged by swimming speed, on a longer time scale in response to culture conditions and nutritional state. We have not studied this systematically, but in general, both starved and satiated larvae are sluggish swimmers, which makes contact-induced modulations difficult to observe. This is presumably because the cilia are, in fact, already impaired, though whether this involves the same control system as the contact response is not clear.

There are evidently differences in the response of different parts of the ciliary band. The transverse bands are difficult to observe directly, in part because, in ventral view, it is nearly impossible to maintain the band in focus during flexure. Nevertheless, our conclusion from numerous observations is that the postoral transverse band beats continuously and strongly under most circumstances, seldom showing any sign of impairment or modulation. In contrast, the preoral band shows frequent changes in beat, and is the most sensitive part of the band to drug effects. This is consistent with out view of how feeding currents are sustained in the bipinnaria. Strong outward currents driven medially past the postoral band appear to be essential. We have seen particles swept rapidly out of the oral region during both normal feeding and flexures, which suggests that this current is maintained, regardless of what may be happening elsewhere, e.g. in the preoral band.

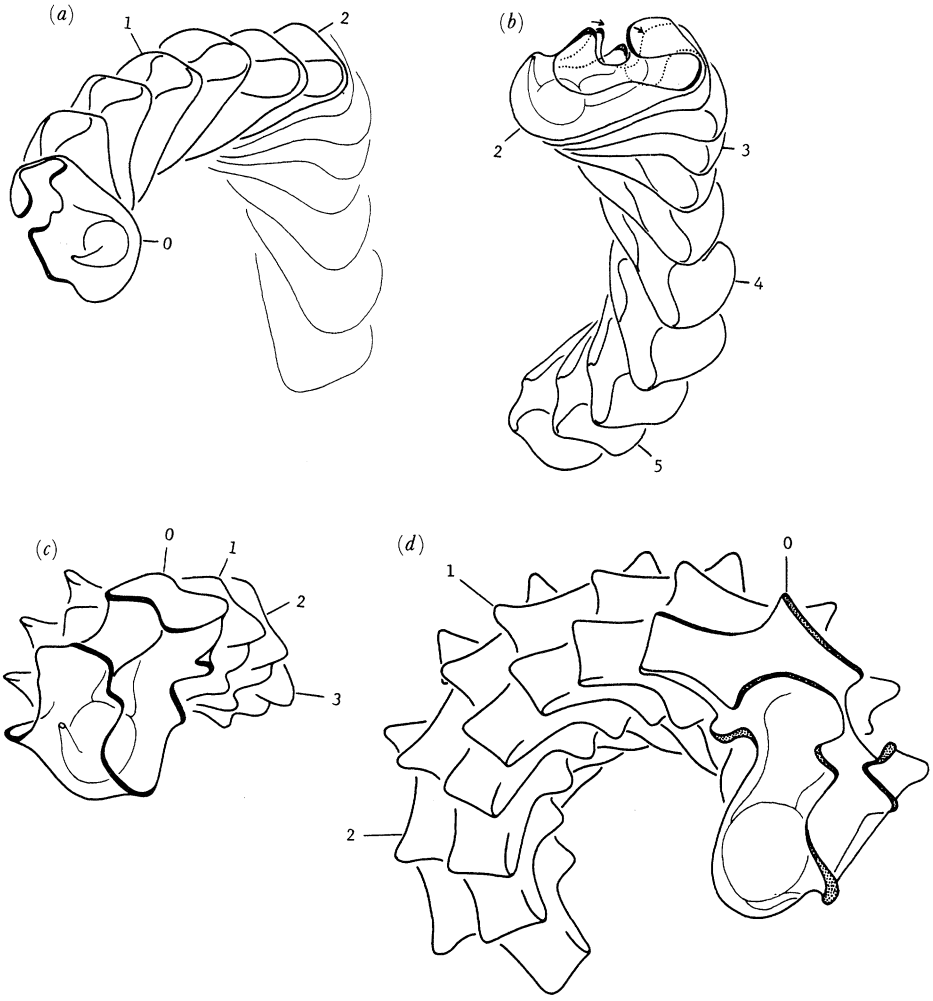


Figure 28. Swimming behaviour in *Pisaster*, tracings from video recordings of free-swimming larva. The interval between profiles is $\frac{1}{3}$ s except in (*c*). Numbers give elapsed seconds starting at zero for each sequence. Part (*a*) shows a contact sequence in detail up to the event, and the remainder, sketched in outline, is shown in detail in (*b*). Arrows in (*b*) show the flexure, a very modest one in this instance.

(*b*) *Particle rejection*

The other function of hood flexure, besides its involvement in the contact response, is in rejection of food particles and debris trapped in the oral region and oesophagus. The gap between the preoral and postoral bands is almost constant throughout growth in the bipinnaria, despite a many-fold change in body size, which suggests that maintaining a correct distance between the two is important for feeding. Flexure interrupts established feeding currents. Evidently a volume or current of water is either expelled or redirected outward from the mouth. This can be seen in dyestream experiments (figure 30). Dyestreams passing into or adjacent to the oral region are pushed away from it as the hood flexes, and return when it closes. We have examples in which flexures are initiated when debris in the oesophagus contact its upper (anterior) surface just behind the transverse band. As the hood opens, the debris slips out. The adoral band in this region is contiguous with the preoral transverse band, so sensory cells located in the adoral band are probably involved.

(*c*) *Backing up*

From their first appearance as rudimentary stubs, the posterior arms of *Pisaster* larvae are capable of muscular flexing. They flex together and in synchrony with the preoral hood. The effect is equivalent to a swimmer's backstroke: the larva backs up. It may be significant that this response appears at about the time that developing convolutions on the lateral bands are just beginning to project beyond the otherwise smooth contours of the body. Because the larva lacks a ciliary mechanism for reversing direction, clearly an important function of ciliary reversal in other echinoderm larvae, this may provide an alternative mechanism for avoiding obstacles or entanglement with debris.

(*d*) *Drug effects*

Both acetylcholine and serotonin are implicated in the control of muscle contraction in the pluteus larva (Gustafson *et al.* 1972*a*, *b*), and ciliary reversal in the pluteus epaulette appears to be under both cholinergic and aminergic control (Lacalli & Gilmour 1990). An equally detailed study of ciliary response in the bipinnaria was not attempted because it was so difficult

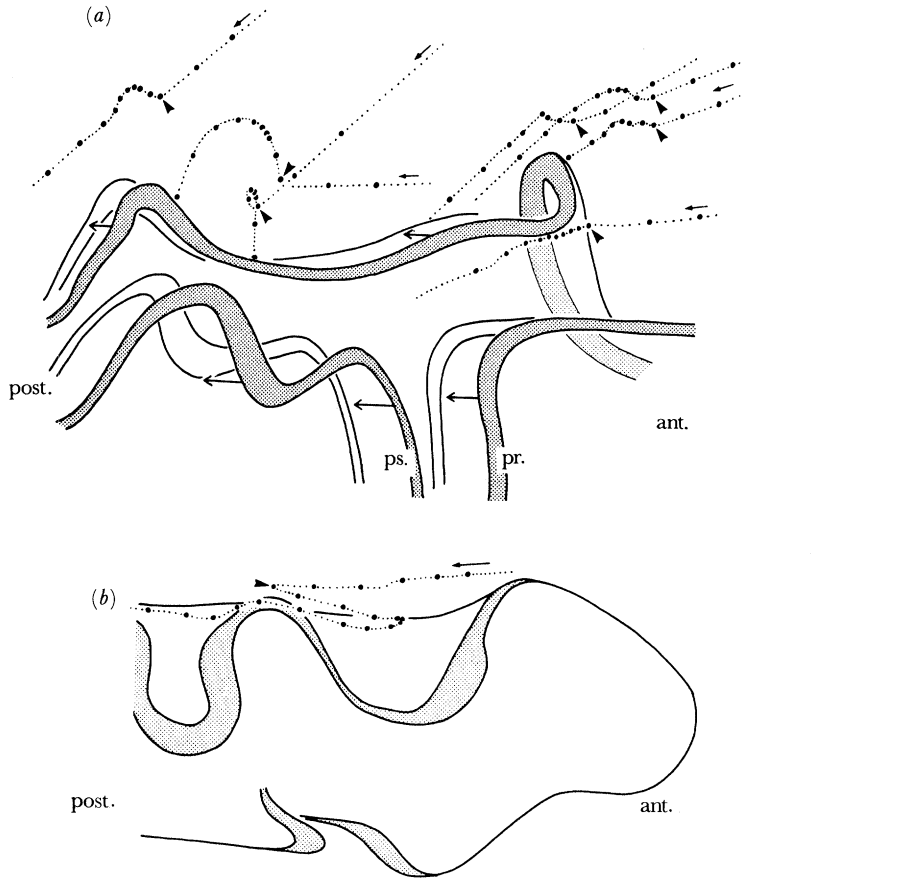


Figure 29. Particle traces from videotapes. There is no external flow in this example, so particle movement is entirely the result of ciliary currents. Each shows a single contact event. Dotted lines show particle paths with the position of each particle shown at regular intervals. Arrows parallel to these show the direction of movement. Arrowheads indicate the position of each particle at the onset of the event. Anterior is to the right in both instances, and the ciliary band is shaded. (*a*) A *Pisaster* larva, *ca.* 18 days, during a typical contact event. Particle position is shown at 0.1 s intervals from 0.2 s before the event. There is a shift in body position during the first 0.2 s after contact, shown by arrows. Note that the gap between the preoral and postoral bands does not change, i.e. there is no flexure in this case. (*b*) A *Parastichopus californicus* auricularia, lateral view with the dorsal surface facing up, from work in progress on this species. The sequence shows a single reversal of the lateral band, causing a reversal and recovery in the particle path. Particle position is shown at intervals of $\frac{1}{3}$ s, i.e. the particle travels more slowly than those in (*a*). This accords with the slower swimming speed of this larva than the bipinnaria. Other reversals involving particles elsewhere around the body showed the same effect.

to observe and quantify. Instead, using the results from the pluteus as a guide, we have tested various transmitters and their agonists for their overall effects.

Three cholinergic agents were tested. All induce hood flexure and impair ciliary beat, but with varying efficacy. With all the drugs, flexing fatigues after the first prolonged flexure, which typically lasts 5–10 s, but sometimes longer. The larvae then become unresponsive, producing at most small twitches in response to either touch or increased dosage. The ciliary effect of cholinergic drugs, at sufficient concentration, was usually a sustained one. The aminergic effect on cilia, in contrast (see below), was transitory. Acetylcholine itself produced pronounced flexures at 20–30 μM, but to block swimming completely required concentrations an order of magnitude higher. There were effects below these thresholds on swimming rate and cilium beat, but they were variable and erratic. High thresholds for acetylcholine are not unusual, based on our experience with other larvae. The larvae are simply being bathed in the drug, and rates of penetration may be limiting if cholinesterase is present in quantity. The pluteus has cholinesterase distributed throughout the band (Ryberg 1973), and the bipinnaria may too. Carbachol was slightly more effective than acetylcholine. It induced flexure at *ca.* 10 μM and impaired ciliary beat at micromolar concentrations, so that larvae typically stopped swimming or slowed after several minutes. Much higher concentrations (e.g. 20 times higher) were needed to block ciliary beat immediately, and the cilia looked damaged at such concentrations. Nicotine was the most effective agonist tested. At *ca.* 1 μM, it produced flexing and some signs of ciliary impairment. Swimming was blocked and the cilia became essentially immobile at concentrations of 2–3 μM, and all these effects were reversible on removal of the drug. The thresholds for both carbachol and nicotine are about an order of magnitude higher than those for ciliary reversal in the pluteus.

Two catecholamines were tested, adrenaline and dopamine, and both produced very similar effects. Both induce hood flexure, and swimming slows or

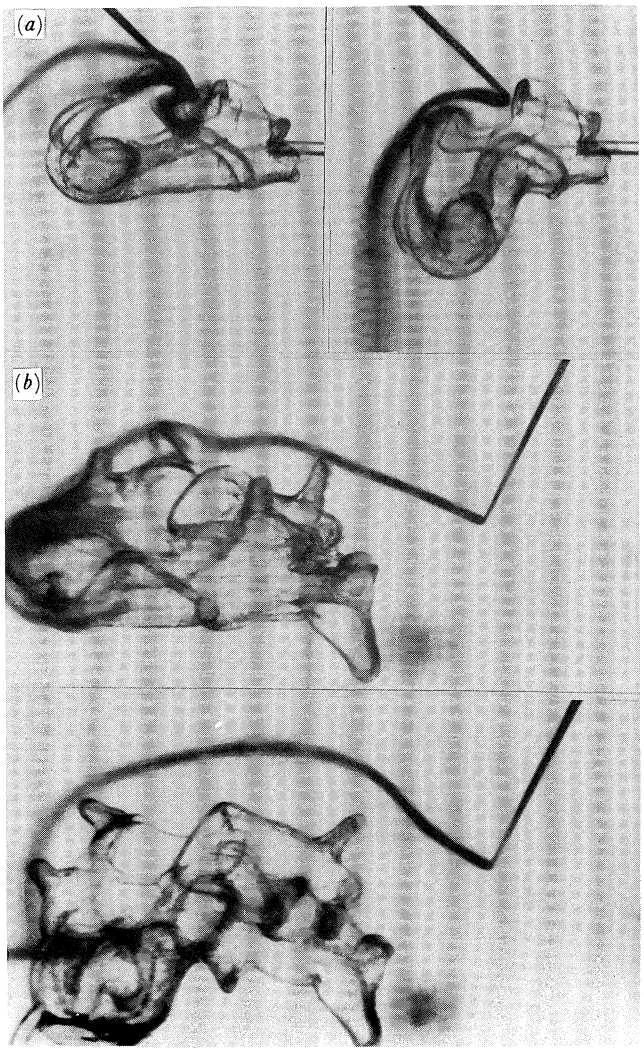


Figure 30. Flow-pattern changes before and during flexure, shown by dyestreams. (*a*) An 8-day larva. The dyestream is drawn into the mouth from one side and discharged medially during normal feeding. During flexure, it is diverted laterally away from the oral region. (Magn. × 70.) (*b*) An early brachiolaria, *ca.* 28 days. The dyestream passes over the ventral surface of the larva and slightly to one side of the midline. During flexure, it also is pushed away. The pipette tip and the preoral region of the larva do not shift position in this instance; the dyestream does. (Magn. × 35.)

stops, but the effects are transitory. The larvae resume normal swimming, usually within 10–20 s, and are refractory to further doses of either drug. The threshold for adrenaline was *ca.* 4 μM, and for dopamine 10–15 μM.

Serotonin has an entirely different effect, blocking both normal and drug-induced flexure. The threshold is on the order of 6–8 μM. The larvae swim at normal speed, but in a rigid posture, and are refractory to contact or touch. Ten times the threshold concentration completely blocks nicotine-induced flexure at the highest concentration we have tested, *ca.* 25 μM. Ciliary beat is severely impaired at these concentrations, however, and we cannot clearly show any protection by serotonin against the nicotine effect on ciliary beat.

In summary, the ciliary response to drugs in *Pisaster* larvae is one of modulation and progressive impairment of beat. The cilia cease beating under some conditions, notably in response to nicotine, but do not show coordinated arrests. Hood flexure is induced by both aminergic and cholinergic agents, and is blocked by serotonin. The response undergoes fatigue, which suggests that some element in the system is being depleted, either at the level of the nerves involved or the muscle itself. The nicotinic effect on cilium beat does not undergo fatigue, evidence that ciliary modulation may be under direct cholinergic control.

5. SUMMARY AND DISCUSSION

(*a*) *Nerve cell types and patterns of innervation*

Section 3 provides more comprehensive information on cell types and patterns of innervation in the band than is available from previous EM studies, and on this basis some of the inconsistencies in the literature can be resolved. Burke (1983) identified two catecholamine-containing cell types in *Pisaster* larvae, which he designated n1 and n2 (figure 31). The n1 cells lie within the band and are bipolar with individual fibres that run some distance along it. The n2 cells lie along one margin of the band and produce a diffuse plexus of slender fibres. The intensity of staining appears to differ between preparations, but none show large nerve tracts, and only the n2 cells occur in the preoral transverse band. We find an extensive plexus of fibres under the band, with slender aminergic fibres running through it. Because of their dense-core vesicles, these probably correspond with Burke's fibres. They belong to the cells we have called sensory and bipolar cells. From their positions within the band and distribution, they evidently correspond with Burke's n2 and n1 cells, respectively.

We also find a single large ciliary nerve running along the band. Nakajima (1987) found large single nerves in both pluteus and bipinnaria bands by using the same methods as Burke. From their size, these must be the ciliary nerve we have found, so the fibres in that nerve are presumably also aminergic. If so, they must stain too faintly in *Pisaster* larvae to be easily visible, because Burke's figures do not show an obvious ciliary nerve.

Nakajima (1988) has also found serotonin-containing cells in the ciliary band, but in most preparations such cells occur predominantly in the apical region of the larva, in association with the apical plate (Bisgrove & Burke 1986, Nakajima 1988). Nothing comparable with the multipolar cell described here has yet been seen in preparations stained or immunolabelled for either catecholamines or serotonin.

We interpret previous ultrastructural reports on larval nerve cell types as follows: the cells identified as nerve cells in figures 13, 17, and 21/22 in Burke (1983) are examples of bipolar cells, mucus cells and multipolar cells, respectively. Despite different fixation, there is a qualitative similarity between Burke's results and ours: the bipolar cell in his figure 13 shows some shrinkage, but is otherwise similar to our bipolar cells, and also resembles bipolar cells found along the ciliary nerve in plutues larvae (Burke 1978). The multipolar

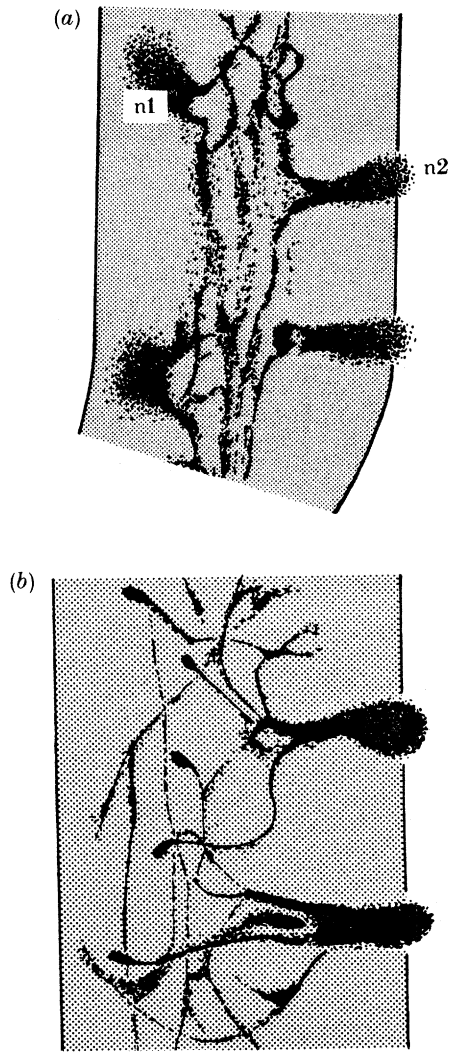


Figure 31. Nerve cells in the *Pisaster* ciliary band: two examples of the type of results obtained by Burke (1983) with the glyoxylic acid method, redrawn from his figures 33 and 37/38. (*a*) A segment of the lateral band showing two cell types, bipolar n1 cells within the band and n2 cells along its margin. (*b*) A segment of the preoral transverse band, where only n2 cells occur.

cell in Burke's figures 21 and 22 is badly extracted, but this is consistent with our conclusion that the very dense appearance of these cells in our preparations is due to sensitivity to fixation, and results from clumping of the cytoplasmic components and chromatin, followed by extraction. Burke's figures clearly show the multipolar nature of this cell type in the bipinnaria, and similar cells occur in the pluteus (see below).

In functional terms, judging by the spatial extent of the various fibre types, type II fibres are good candidates for coordinating responses involving the band as a whole. They are the main component of the ciliary nerve, and probably run most of the length of the band. The type I fibres evidently do not travel as far. We found at most four or five fibres in our reconstructions, and based on Burke's work, these come from cells spaced *ca.* 30 μm apart. To account for the numbers in this example, their fibres can travel no more than 150 μm on average, which makes them poor candidates for coordinating activities in band segments of more than moderate length.

The behavioural observations suggest that both aminergic and cholinergic transmitters are involved in controlling ciliary beat. Our interpretation of data on pluteus reversals (Lacalli & Gilmour 1990) is that sensory input is aminergic, and the effector neurons are cholinergic, specifically nicotinic. Ciliary modulation in the bipinnaria is more difficult to study, but we have evidence for both aminergic and cholinergic effects, of which the latter are clearly the most direct. This suggests that the multipolar cells may be cholinergic and responsible for ciliary modulation. They contain the right vesicle type, i.e. clear vesicles, and their processes ramify extensively through the plexus. They are also postsynaptic to the cells we identify as being sensory, and though the junctions in question are not particularly well defined or synapse-like, conventional synapses are generally absent in echinoderm tissue (Cobb & Pentreath 1977; see also Cobb (1987) for examples). An apparently similar type of cell, with similar apical processes and basal neurites, has been described from the pluteus (Nakajima 1986), and we find microtubule-filled processes running along the apical surface of the band between cilia in the pluteus of *Lytechinus pictus* (unpublished data). Nakajima showed that the processes have a ciliary rootlet at their bases, i.e. they are evidently modified cilia. They could function in sensory reception, but an effector function seems more likely, i.e. they could act at the apex of the ciliary cell or at the cilium directly. There is precedent for an effector function by such processes in salps. Bone *et al.* (1980) describe branched cilium-derived processes that are responsible for signal transmission between zooids. These form characteristic specific junctions with the apical surfaces of their target cells. We find similar junctions between apical processes and ciliary band cell apices in the pluteus. These are structurally quite specialized with, for example, post-junctional vesicle arrays, which we have not found in the bipinnaria. Differences in the number and distribution of and degree of differentiation processes in the pluteus and bipinnaria could explain the behavioural differences between them. The comparatively narrow pluteus band has ciliary nerves, but lacks the extensive basal plexus of the bipinnaria. It is nevertheless well supplied with apical processes, such that most cells have direct access to them, and the ciliary response is coordinated and well controlled. The broader bipinnaria band is more sparsely supplied with processes, and access to them probably varies between cells. The less dramatic modulatory effect that we observe in bipinnaria bands, and its variability along the band, could be a consequence of this. The bipinnaria may also rely more on transmission via basal processes and less on apical processes then the pluteus.

(*b*) *Comparative aspects and phylogenetic significance*

Marine invertebrates with distinct ciliary bands probably evolved from a uniformly ciliated ancestor much like the coelenterate planula, and this appears to

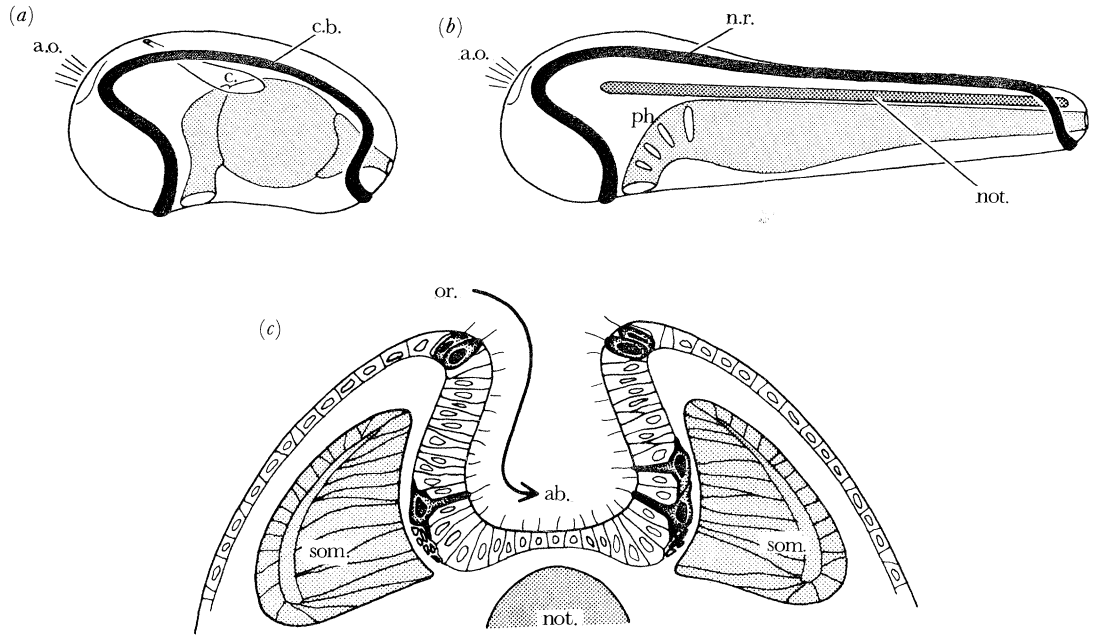


Figure 32. Garstang's scheme for deriving the chordate body plan from a dipleurula larva. (*a*) Schematic dipleurula-type larva. Shows the apical organ (a.o.), circumoral ciliary band (c.b.), digestive tract (light shading), and one coelomic pouch (c.), with hydrophore. (*b*) Schematic chordate derived from (*a*) by elongation of the body, addition of a notochord (not.), conversion of the anterior portion of the original gut into a pharynx (ph.) with gill slits, and with developing neural ridges (n.r.) in place of the ciliary band. (*c*) The consequences of neurulation for the chordate in (*b*) showing the neural tube in transverse section, somites (som.) and notochord. The oral–aboral axis (arrow) of any band that gets incorporated into the neural tube will be reoriented and become dorso-ventral. If most of the neurogenic tissue of the neural tube is derived from the ciliary band, then the cell types we have identified would be positioned as shown (darkened cells): sensory cells would always be dorsal (e.g. at the neural ridges) with respect to effector neurons, i.e. the multipolar cells and their processes, which are shown deeper within the neural folds. This is the basic pattern seen in chordate spinal cord, and there may be significant similarities in the types of transmitters released by cells occupying comparable positions in the two systems. See text for further discussion.

have been accompanied by the evolution of a simple intratrochal form of ciliary band innervation. Typically, in primitive spiralian larvae, this involves uniciliate sensory cells located in the band, whose basal neurites form nerve tracts that run beneath the band, for example as seen in Müller's larva and the pilidium (Lacalli 1982; Lacalli & West 1985). It is not clear which neurotransmitters are involved. Staining trials show that both serotonin and catecholamines are present (A. Hay-Schmidt (1990*b*), for the pilidium), but the presence of cholinesterase (Kotikova (1981), for Müller's larva) means acetylcholine may also have a role. The absence in these larvae of complex behavioural responses makes the function of the sensory cells and ciliary nerves problematic. In more advanced protostome larvae, e.g. trochophores and veligers, intratrochal innervation tends to be reduced in favour of innervation by nerves from the apical organ or precociously differentiated elements of the adult CNS. This is one aspect of a general trend recognized in such larvae towards precocious development of a variety of adult structures, producing larvae that are both structurally and behaviourally more complex than primitive forms, e.g. the veliger (Mackie *et al.* 1976) in comparison with the trochophore (Lacalli 1984, 1986). The implication is that there are disadvantages to retaining intratrochal innervation in its primitive form.

The dipleurula-type larvae of echinoderms, in contrast, have evolved diverse ways of using very simple ciliary bands simultaneously for both food capture and locomotion. Echinoderm larvae are capable of a wider range of behavioural responses than primitive protostome larvae, and a greater variety of cell types seems to be involved. The principal ciliary band is clearly not homologous with that in protostomes (i.e. with the prototroch and its homologues). Indeed, the origin of the former as a circumoral system that displaces or replaces the downstream feeding system of protostome larvae may be the key to understanding how an improved form of band innervation evolved. Of the cells involved, the uniciliate sensory and bipolar cells described here are morphologically closer to the nerve cells found in primitive protostome larvae than is the multipolar cell, which has no obvious counterpart in more primitive larvae. Its appearance, as a new cell type directly involved in ciliary control, could account for the improved behavioural capabilities of echinoderm larvae and their consequent evolutionary success. It is difficult to envisage how so bizarre a range of morphological types could evolve, all operating with such efficiency as particle collecting devices, without the means to control cilium beat with some precision, and differentially, in differentially, in different parts of the system.

Too little information is available on the other main dipleurula-type larva, the hemichordate tornaria, for a useful comparison with the results reported here on the bipinnaria, but we cannot ignore the possible sig-

nificance of our observations in relation to the origin and basic organization of the CNS (i.e. spinal cord) in chordates. The origin of the whole of the axial complex in chordates, i.e. the notochord, neural tube and axial muscles, is a phylogenetic puzzle of the first order. Only Garstang's explanation for the origin of spinal cord by dorsal convergence of the ciliary bands in an ancestral dipleurula larva (Garstang 1894, 1928) enjoys some degree of acceptance. The essence of his idea, illustrated in figure 32, is that the larval ciliary bands define the margin of the developing neural tube, i.e. they are in some way involved with the origin of the neural ridges and, to a degree as yet undetermined, contribute to the neural tube itself. The external body surface is thus derived by expansion of the oral field, and what remains of the aboral ectoderm, along with the apical organ, is rolled into the neural tube. Oral–aboral differences in the ciliary band become, on transformation into neural tube, dorso-ventral ones, as shown in figure 32*c*. From our work, the cell types and functional differences associated with the two margins of the band correspond reasonably well with the pattern seen in spinal cord. The oral margin in our larvae is associated predominantly with aminergic cells and sensory input. Moving across the band towards its aboral margin, we find several cell types, including effector neurons that are probably cholinergic, and nerve tracts. The aboral margin is then a point of entry for nerves from the aboral epithelium, mainly, in the case of the bipinnaria, diffuse fibres from serotonin-containing cells in the dorsal and apical epithelium (Nakajima 1988). Serotonergic cells are found in the apical plate of both echinoderm and lophophorate larvae (Bisgrove & Burke 1987; Hay-Schmidt 1990*a*), suggesting that the association between this transmitter and the apical organ is an ancient one.

Spinal cord shows a comparable organization in several respects. The point of entry of sensory nerves is dorsal, effector cells and nerve tracts are lateral and ventral, and catecholamines are mainly associated with cells derived from the dorsal margin, i.e. the neural crest. We are suggesting here that the oral margin sensory cells in the bipinnaria may in some way be related to the neural crest lineage in vertebrates. Also of interest is the tendency for serotonergic neurons in chordates to be ventral and medial in position. In lower vertebrates and vertebrate larvae, serotonin-containing cells occur principally in the ventral midline of the spinal cord (van Dongen *et al.* (1985), for lamprey) and midbrain/hindbrain area (Brodin *et al.* 1986; van Mier 1986), the latter including the raphespinal plexus, which lies approximately where the apical organ might be expected to lie in vertebrates if Garstang is correct i.e. at the level of the mid brain or anterior hindbrain.

The evidence from the bipinnaria, in terms of cell types, their relative position and transmitters is thus at least consistent with Garstang's proposal. It should be pointed out that there are other schemes that would produce quite different predictions that are not supported by the data. For example, suppose the chordate body were derived by elaboration of a muscular feeding appendage, perhaps a tentacle, of a filter-feeding ancestral form. Such a structure would be expected to have a ciliated margin and a median gutter continuous with the oral field which, if folded into a neural tube, would have an orientation opposite to that in Garstang's scheme, i.e. oral would be ventral, and aboral would be dorsal. Ultimately it should be possible to establish homologies more convincingly on the basis of specific molecular probes, but the above provides at least provisional support for Garstang's scheme.

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ABBREVIATIONS USED IN THE FIGURES

ab.	aboral margin of ciliary band
an.	anus
ant.	anterior
a.o.	apical organ
bp.	bipolar cell
c.b.	ciliary band
c.n.	ciliary nerve
ep.	epaulette
m.	multipolar cell
m1–m6	specific multipolar cells
mo.	mouth
mu.	mucus cell
n.	nerve, e.g. the ciliary nerve
n1, n2	nerve cell types, from fluorescence data
n.r.	neural ridge
not.	notochord
or.	oral margin of ciliary band
p.	ciliary band plexus
p.a.	posterior larval arm
ph.	pharynx
post.	posterior
pr.	preoral transverse band
ps.	postoral transverse band
s.	sensory cell
som.	somite
st.	stomach
*	specified in figure description

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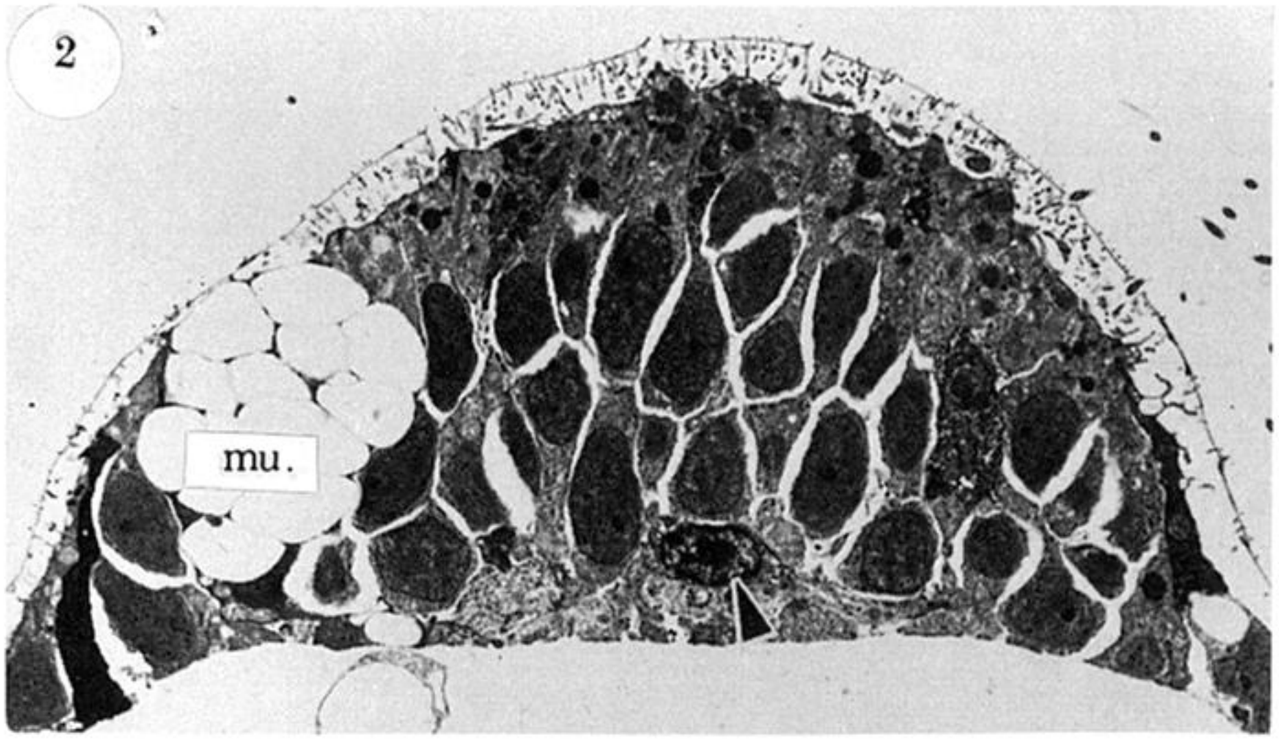
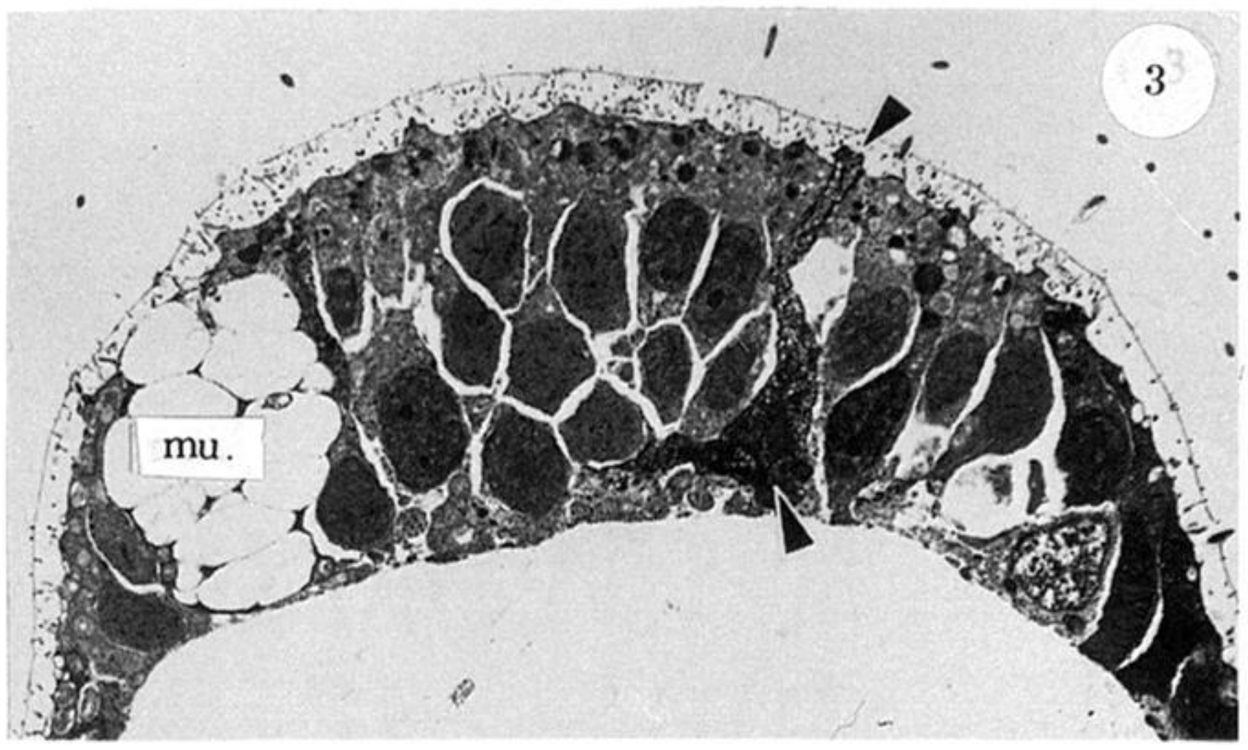
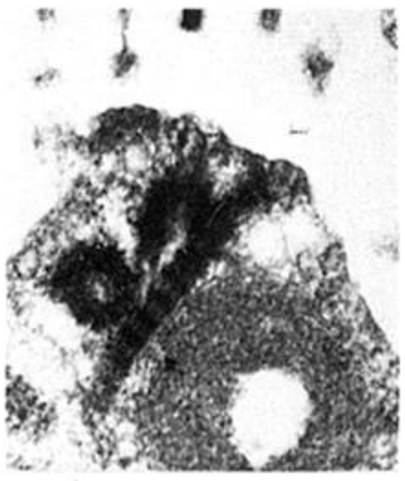
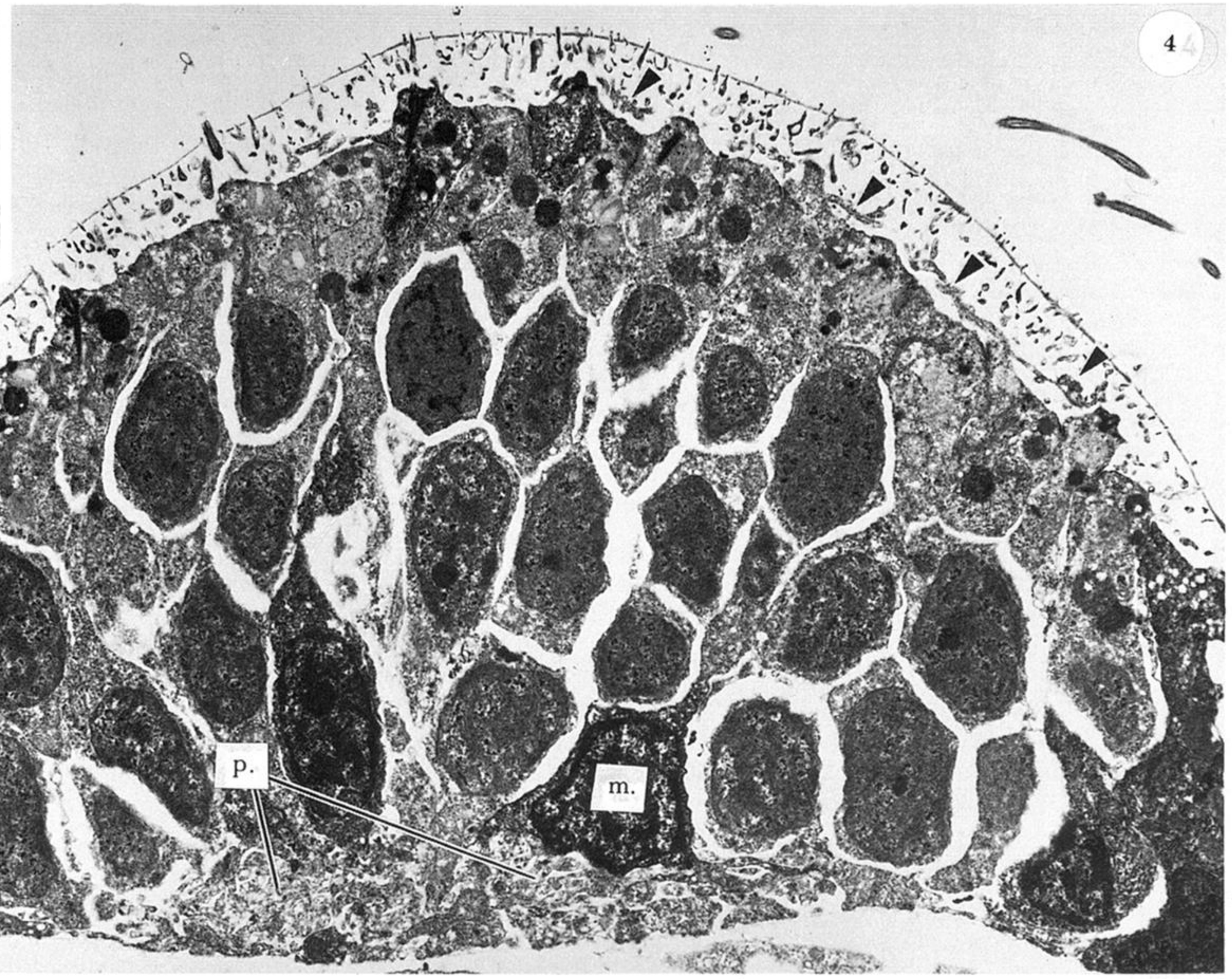
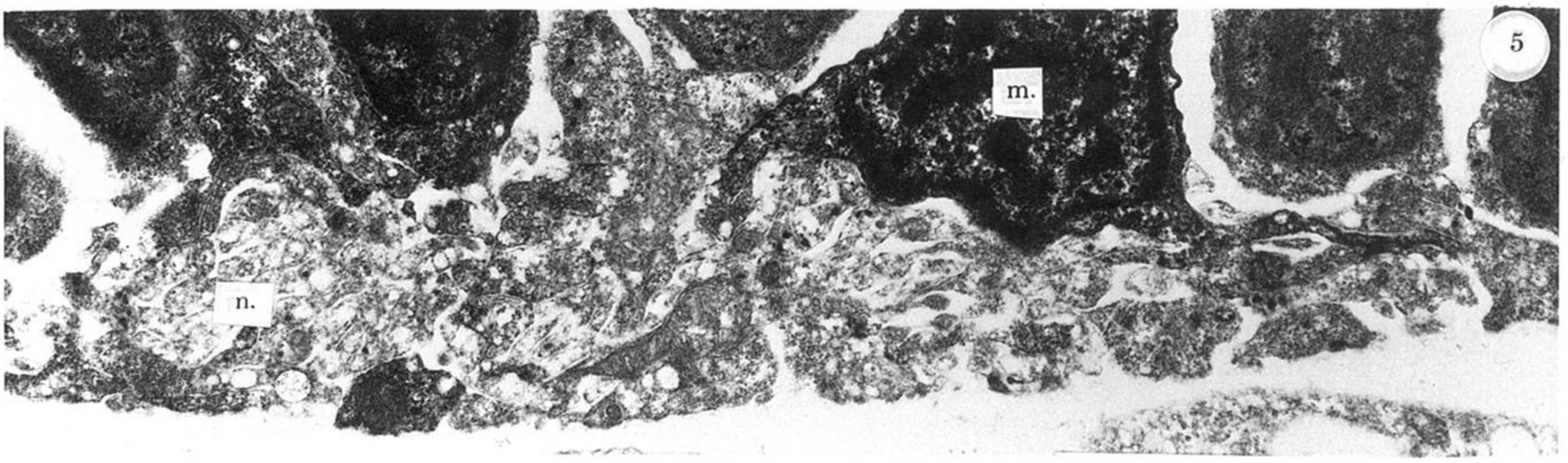










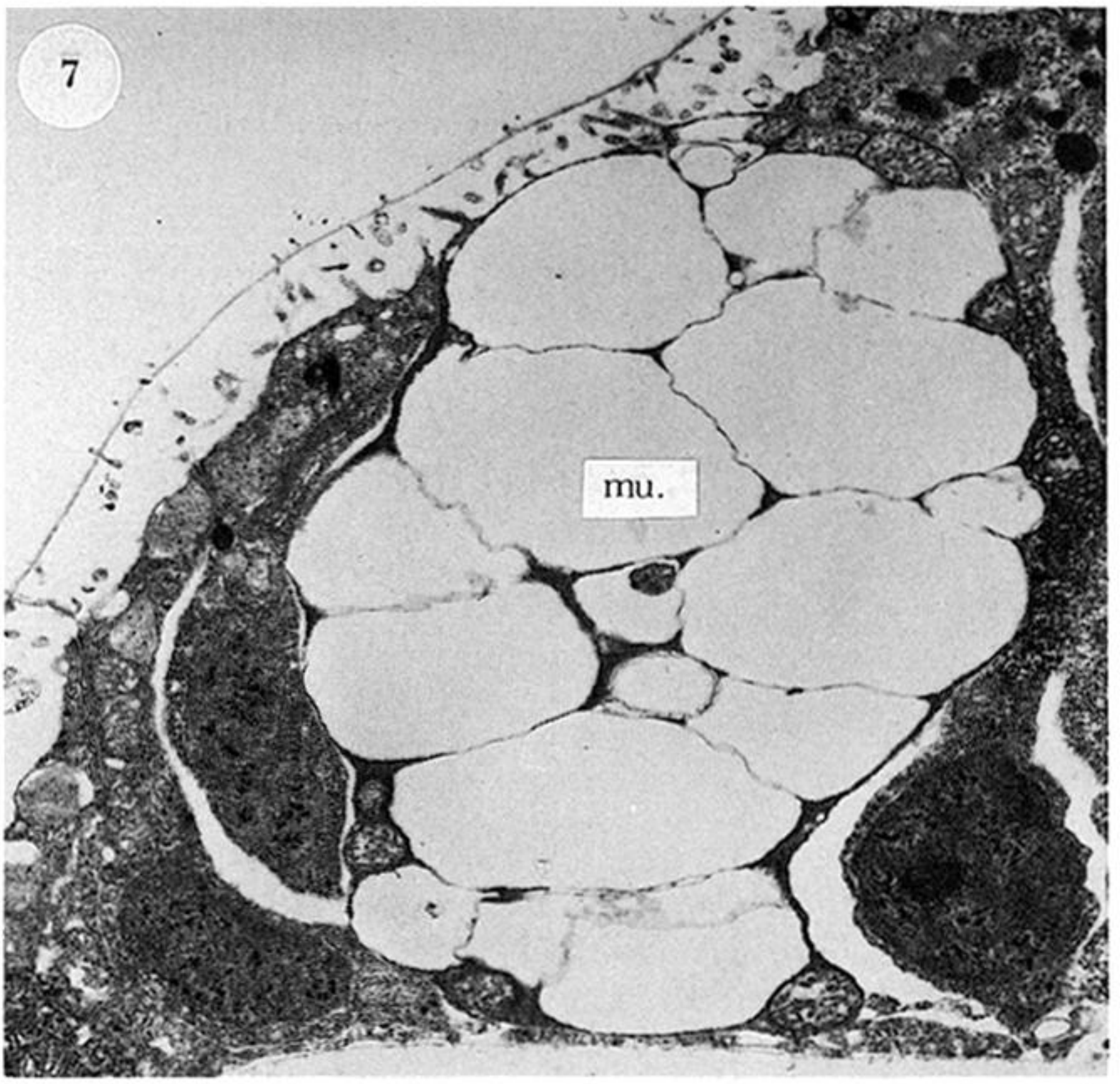
Figures 2–5. Transverse sections through the ciliary bands, all oriented with the oral field to the right so the direction of ciliary beat is from right to left.

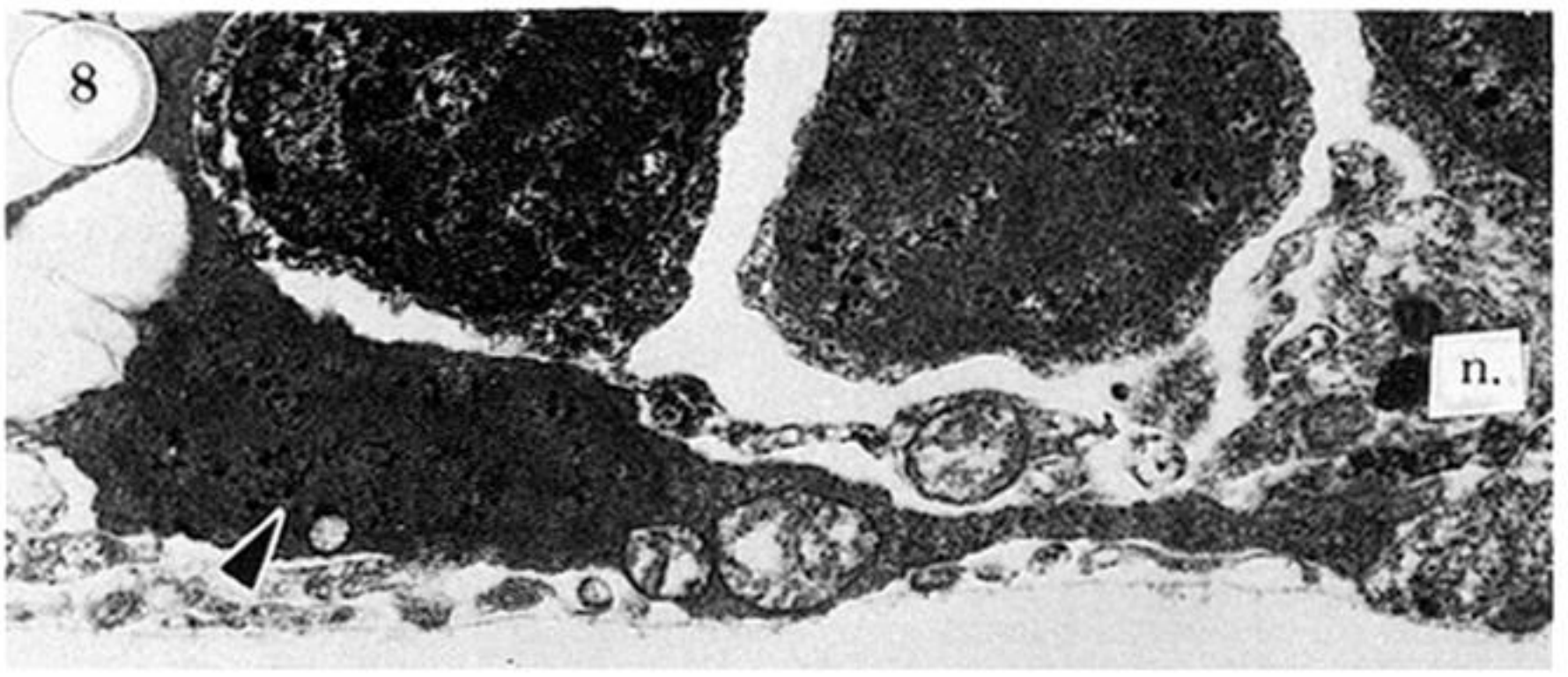
Figure 2. Survey view of the preoral transverse band near the midline. There is a mucus cell (mu.) at the aboral margin of the band. The arrow indicates the cell body of a multipolar cell. (Magn. × 3400.)

Figure 3. Survey view of the postoral transverse band, as in figure 2 with a mucus cell and the basal cell body (lower arrow) and apical projection (upper arrow) of a multipolar cell. (Magn. × 3200.)

Figure 4. Enlarged view of the preoral band. Inset shows the rootlet complex of a typical cell in correct orientation, i.e. with the accessory centriole on the downstream side. The section also contains the cell body of one multipolar cell (m.) lying just above the plexus of nerve cell processes (p.). This particular cell is m2 in the reconstructions (figures 21–23). Arrows show multipolar cell processes traversing the surface of the ciliary band surface. (Magn. × 8250, inset × 32 000.)

Figure 5. An enlargement of figure 4 to show the plexus, including the ciliary nerve (n.), which lies at its left-hand (aboral) side. (Magn. × 17 050.)





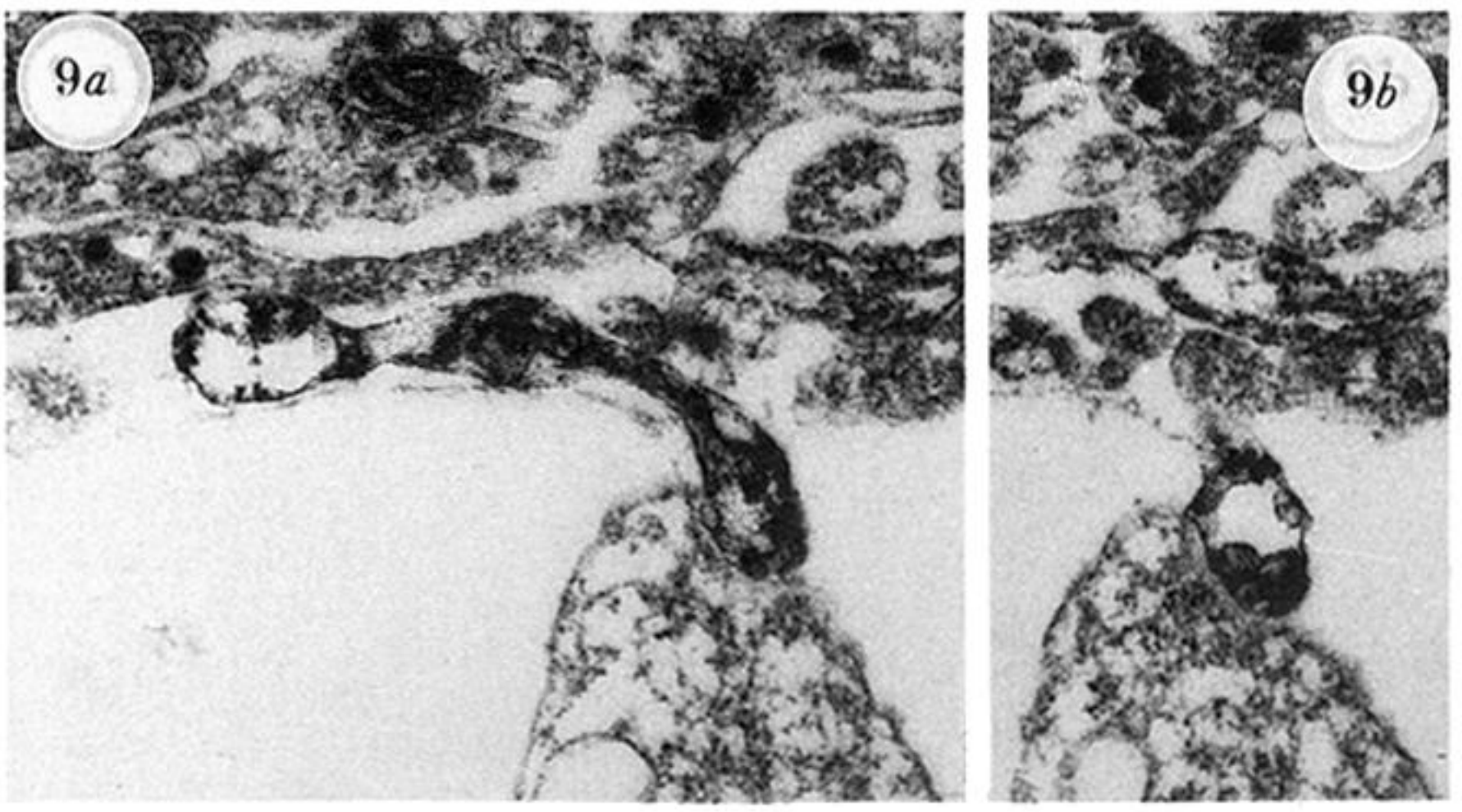


Figure 7. A mucus cell. (Magn. × 9120.)

Figure 8. Basal nucleus (arrow) of a mucus cell showing a typical flattened basal process extending into the plexus. (Magn. × 15 700.)

Figure 9*a*, *b*. Two sections through possible neuromuscular junction between a fibre from the ciliary nerve and one of the mesenchyme cells lying just under the band. (Magn. × 10 890.)

Figures 10–16. Nerve cells and fibres containing dense-cored vesicles.

Figure 10. A sensory cell from the oral margin of the postoral transverse band. Dense-cored vesicles typically occur in the apical region of these cells in association with the golgi, as well as the basal region. (Magn. × 16 200.)

Figure 11. Bipolar cell from the lateral band showing its association with the ciliary nerve (n.). (Magn. × 22 900.)

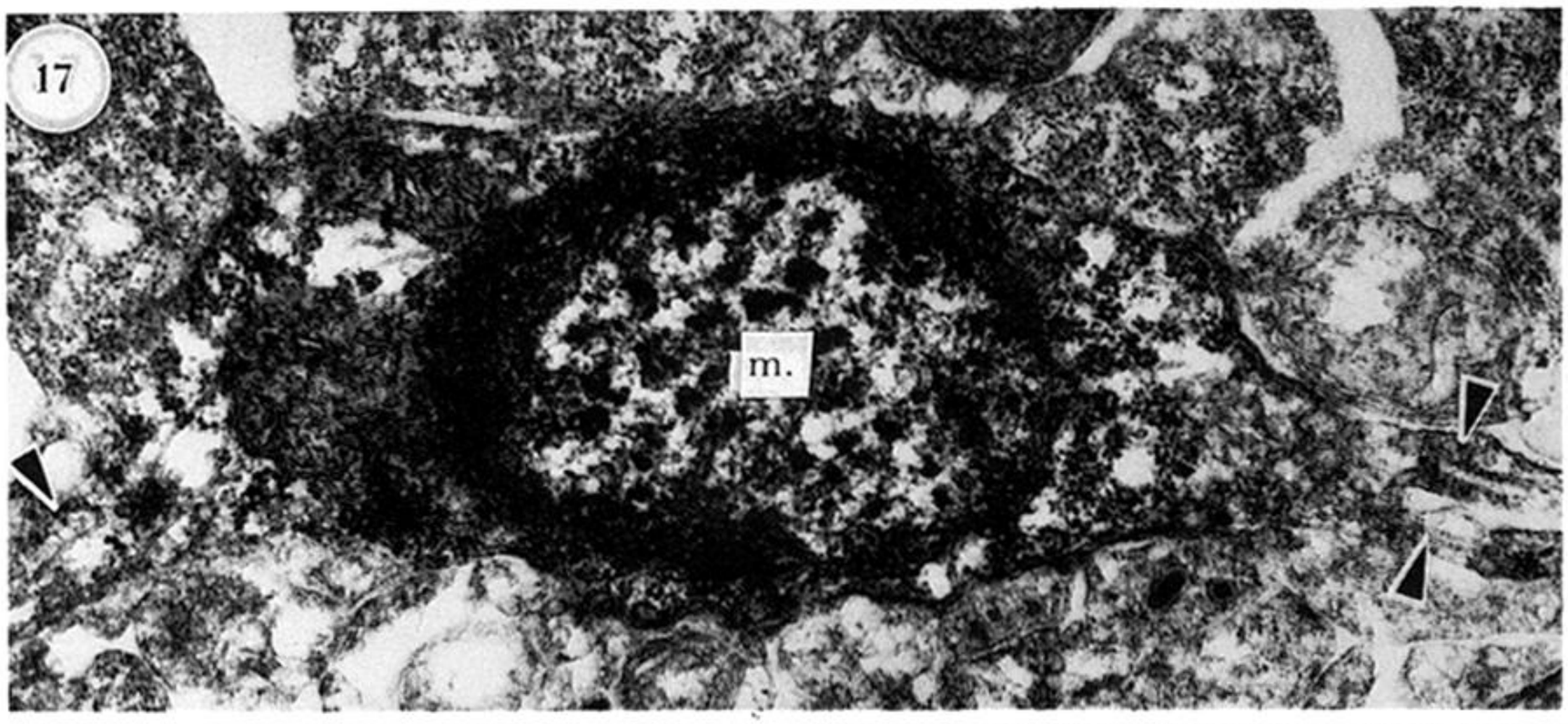
Figure 12. Detail of fibres in the plexus. Shows vesicle-containing varicosiites of two type I aminergic fibres adjacent to the underside of a multipolar cell. (Magn. × 47 550.)

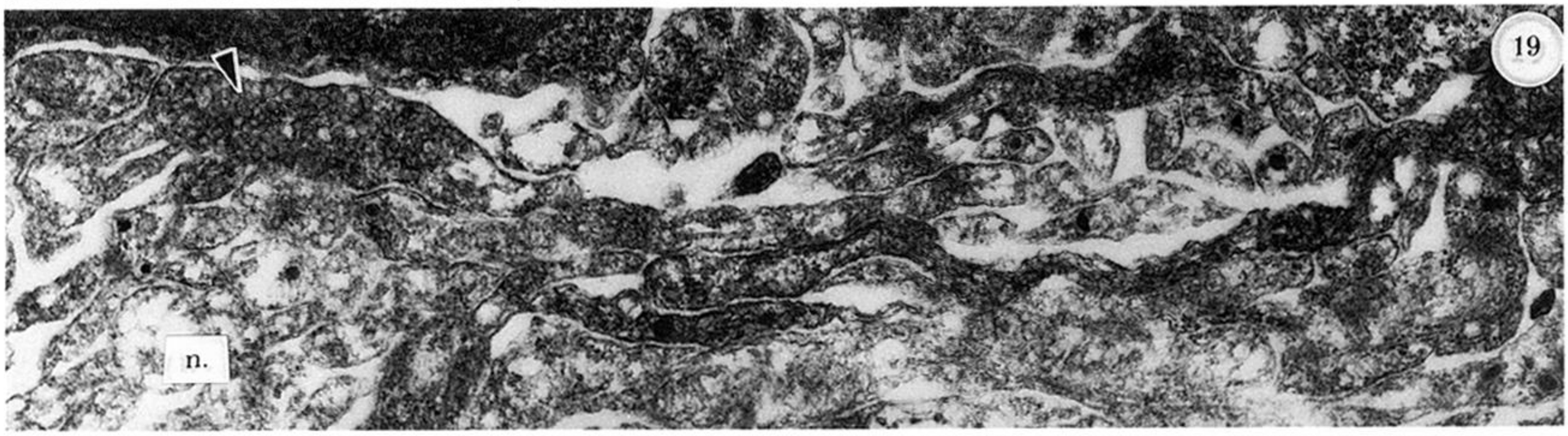
Figure 13. Detail of fibres in the plexus showing examples of type II aminergic fibres (arrows). (Magn. × 44 650.)

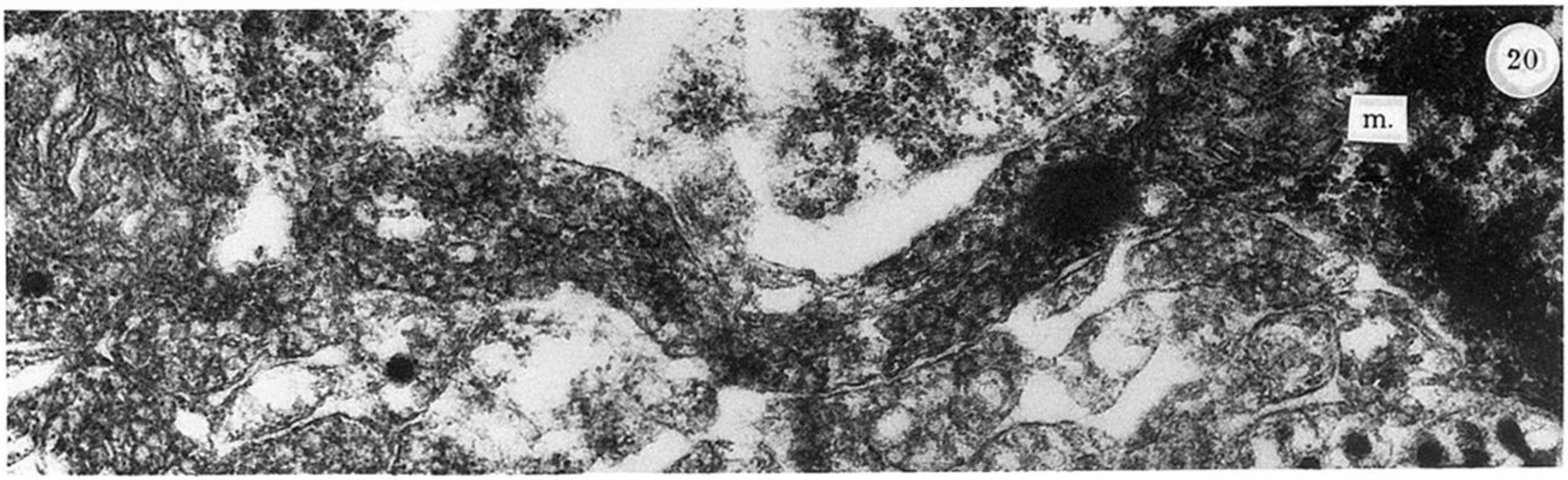
Figure 14. A sensory cell from the oral field just adjacent to the band. This is the cell shown in figure 22*b*. (Magn. × 17 680.)

Figure 15. Basal body and accessory centriole of the cell in figure 14, for comparison with that in figure 10. (Magn. × 61 550.)

Figure 16. One of several nerves entering the preoral transverse band across the oral field. Shows a mixture of fibre types. (Magn. × 29 060.)







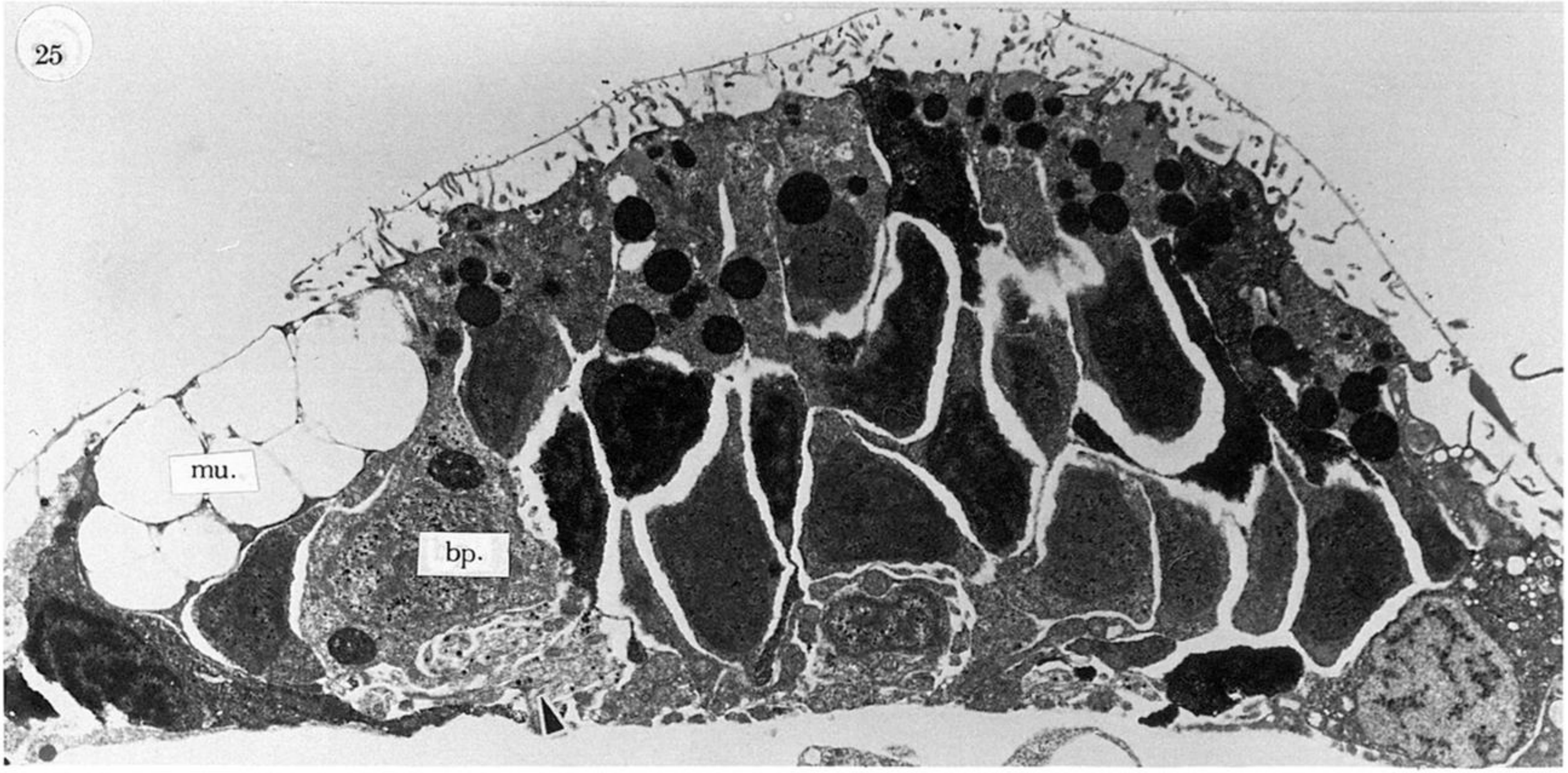
Figures 17–20. Multipolar cells and their processes.

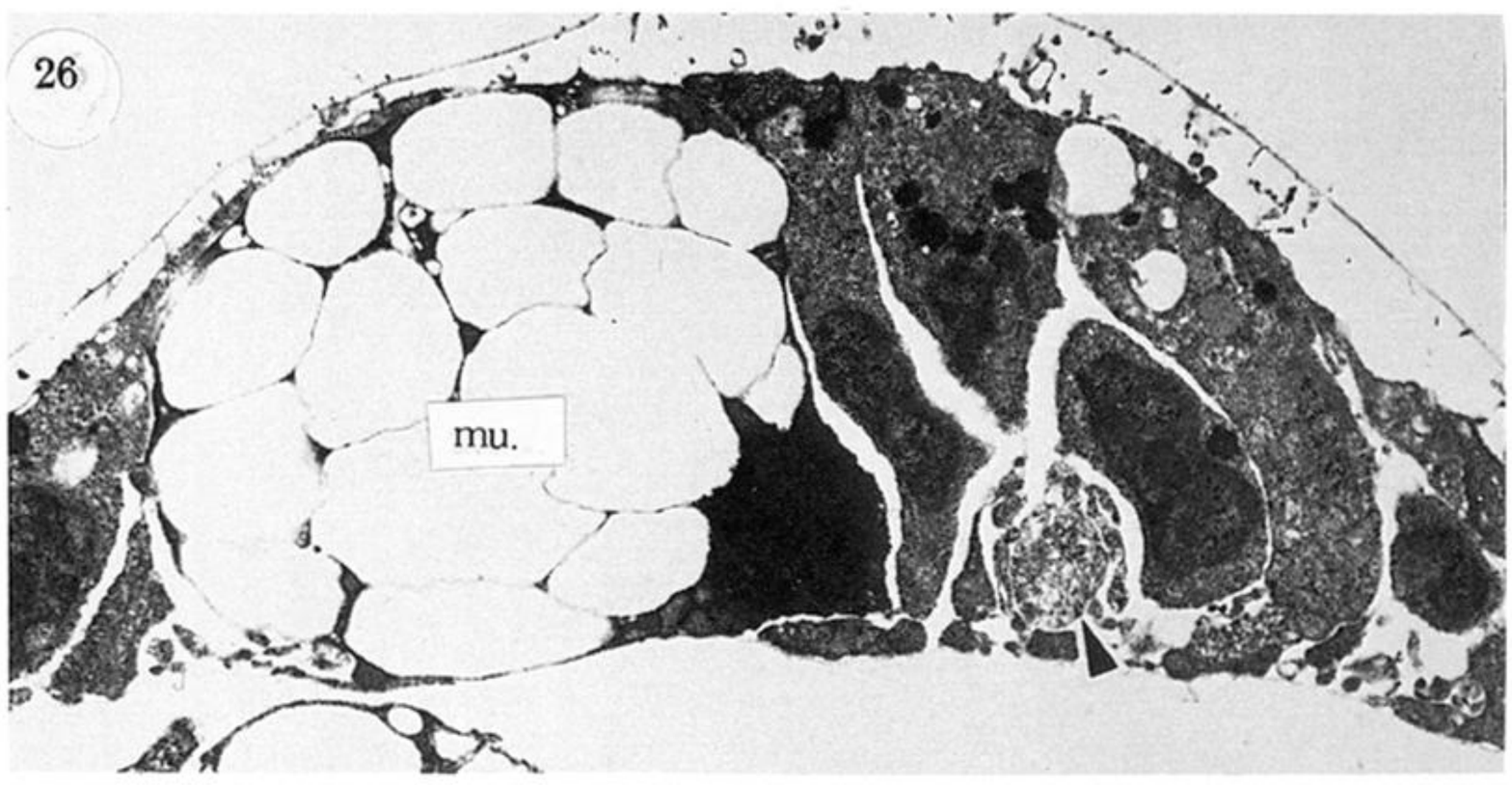
Figure 17. Cell body of a multipolar cell showing its characteristic dense cytoplasm and points of origin of several basal processes (arrows). (Magn. × 32 120.)

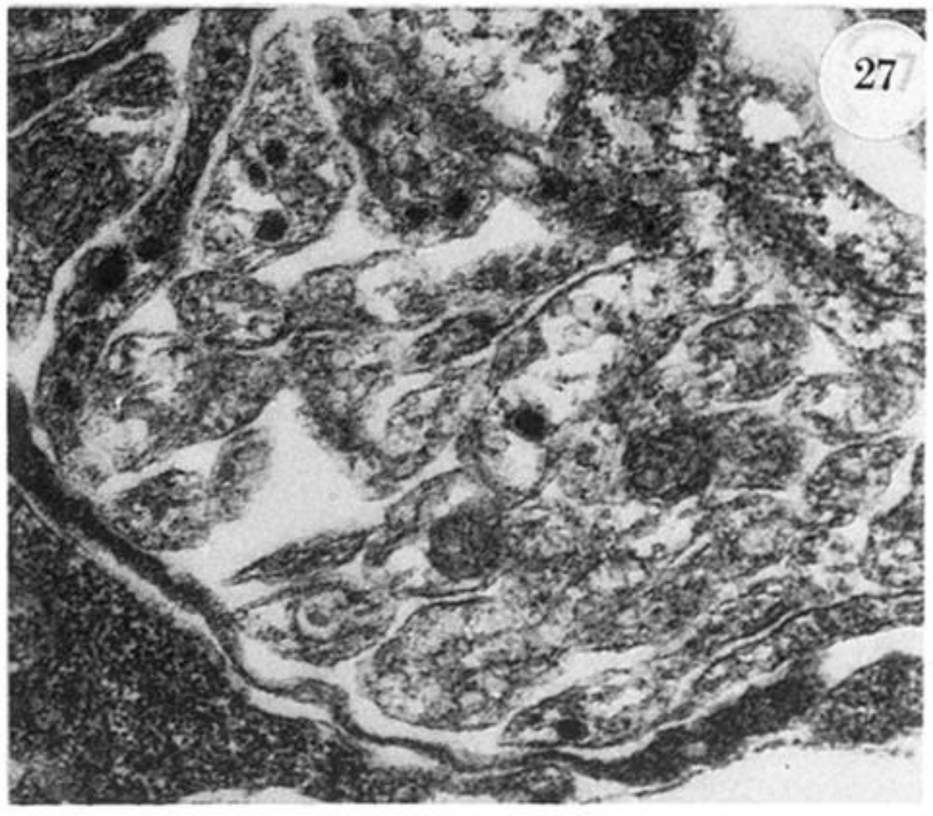
Figure 18. Apical surface process of a multipolar cell showing the dense body from which the microtubules radiate. (Magn. × 29 850.)

Figure 19. Vesicle-filled processes belonging to multipolar cells showing an example (arrow) of contacts with fibres in the ciliary nerve (n.). (Magn. × 33 290.)

Figure 20. As in figure 19, a further detail. (Magn. × 53 820.)







Figures 25 and 26. Typical sections through lateral parts of the band showing its variable size, the relative prominence of the mucus cells, and the ciliary nerve (arrow). (Magns: figure 25, ×6710; figure 26, ×6200.)

Figure 27. Detail of fibres in the ciliary nerve in a lateral part of the band. (Magn. ×32460.)

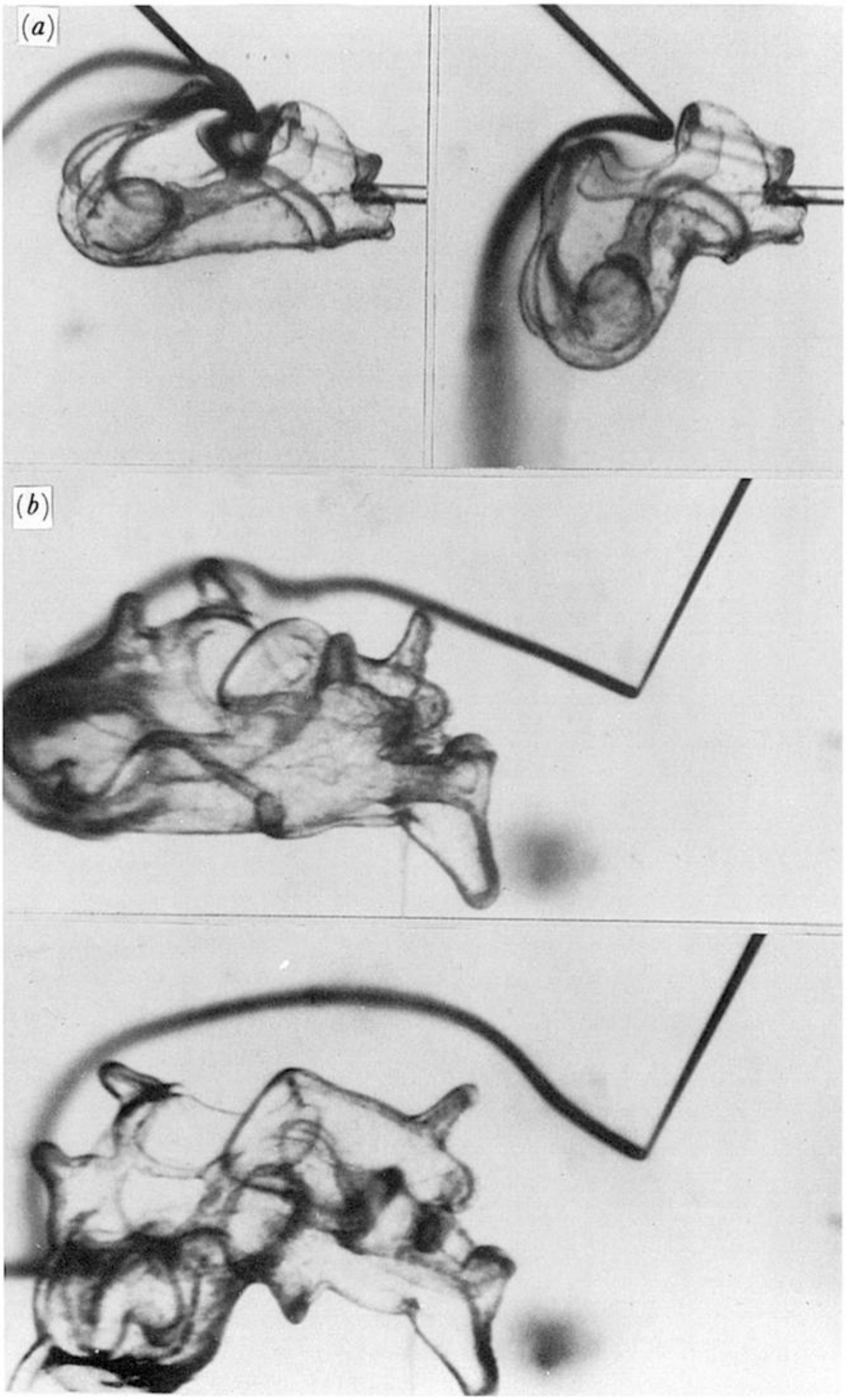


Figure 30. Flow-pattern changes before and during flexure, shown by dyestreams. (*a*) An 8-day larva. The dyestream is drawn into the mouth from one side and discharged medially during normal feeding. During flexure, it is diverted laterally away from the oral region. (Magn. × 70.) (*b*) An early brachiolaria, *ca*. 28 days. The dyestream passes over the ventral surface of the larva and slightly to one side of the midline. During flexure, it also is pushed away. The pipette tip and the preoral region of the larva do not shift position in this instance; the dyestream does. (Magn. × 35.)