# ON THE STRUCTURE OF THE ANTENNULAR ATTACHMENT ORGAN OF THE CYPRIS LARVA OF *BALANUS BALANOIDES* (L.)

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[Plates 38 to 47]

## CONTENTS

The third segment of the antennule of the cypris larva of *Balanus balanoides* is modified as an attachment organ with a disk by which the cyprid attaches to submerged surfaces. The attachment disk is covered with a felt of fine cuticular villi. Opening on to the disk are terminal branches of the cement duct, numerous glands and an array of sensory hairs. The sensory structures are arthropod scolopidia with the dendrites giving rise to cilia which, distally, change to distal sensory processes. It is suggested that the cuticular hairs situated in invaginations of the cuticle around the margin of the disk function as position receptors and that the two setae lying away from the cuticle of the segment are mechanoreceptors. Three of the scolopidia have structures suiting them for chemoreception; the distal sensory processes are exposed to the exterior at the tip of the hair. Two of these hairs are positioned at the margin and one at the centre of the disk. The fourth segment, which arises from the side of the attachment organ, is packed with sensory cell processes which are associated with setae arising at the distal end; its movements are controlled by a single muscle from the third segment.

A suction mechanism of adhesion is precluded as there are no structures which could effect or release suction beneath the disk. The disk could act as an adhesive pad, with the cuticular villi increasing its surface area and the antennular glands possibly secreting a viscous substance. The presence of chemoreceptors on the attachment organ indicates that a chemosensory mechanism could operate during the gregarious behaviour of settling cyprids.

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## INTRODUCTION

The cypris larva is the stage in the life cycle of cirripedes that selects a suitable substrate for settlement and completion of metamorphosis into the adult sessile barnacle. The behaviour of barnacle cyprids has been described by Doochin (1951), Crisp & Barnes (1954), Crisp & Stubbings (1957), Crisp (1955, 1961) and Knight-Jones & Moyse (1961) and it is now well established that the cyprids detect and respond to the chemical and physical nature of substrates. The gregariousness of cirripedes ensures that cyprids will settle in situations where conditions have been favourable for adult life. Gregarious settlement will enable cross-fertilization to occur. Knight-Jones (1953, 1955) and Crisp & Meadows (1962, 1963) have identified the chemical basis of gregariousness, but there has been no description of the structural features by which the cyprid can explore and sense substrates. When cyprids alight on a surface they explore and test it with the antennules. A detailed examination of the antennule should reveal, therefore, the receptor and effector organs controlling much of the behaviour of the cyprids during settlement.

The cypris larva is preceded in development by six nauplius stages. The limbs of the nauplii are the antennules, antennae and mandibles, and these have been described for many species, and a terminology has been devised (Newman 1965). Of the three pairs of nauplius appendages only the antennules are retained as functional limbs by the cyprid, although the latter has also six pairs of biramous thoracic limbs which develop into the characteristic cirri of the adult barnacle. The external morphology of the antennule of *Lepas australis* was described by Darwin (1854), who compared it with that of a number of other species and noted that throughout the cirripedes the cyprid antennule was 'a singularly uniform structure'. In all cases it consists of four segments; the third bears the attachment organ ('adhesive disk', 'prehensile sucker', 'Haftscheibe', etc.), which is either bell-shaped as in *Lepas* and *Balanus* (Darwin 1854), *Conchoderma* (Claus 1869) and *Tetraclita* (Nilsson-Cantell 1921), or hoof-shaped as in *Scalpellum* (Nilsson-Cantell 1921; Kaufmann 1965). This paper describes the cyprid antennule of *Balanus balanoides* (L.), with particular attention to the fine structure of the third segment and attachment organ. The setation of the nauplius antennule has been described by Bassindale (1936) and Crisp (1962). The changes in morphology of this appendage during the moult from the last nauplius to the cypris stage have been followed in order to clarify the homologies in the segmentation and setation of the antennule. The structure of the cypris antennule is discussed with respect to its use in the exploration and selection of surfaces.

## MATERIALS AND METHODS

Larvae of *B. balanoides* were collected from the plankton of the Menai Strait during April and May. The cyprids were narcotized overnight in 0.05 % Nembutal at about 2 °C; this treatment yielded a high proportion relaxed with the antennules extended. The nauplii and cyprids used in the preparation of whole mounts were fixed in a 5 % aqueous solution of glutaraldehyde; the antennules were dissected off and mounted in polyvinyl lactophenol with Evans Blue. For electron microscope study, the cyprids were fixed for 24 h in 5 % glutaraldehyde at pH 7.4 in phosphate buffer to which one drop of 2 % calcium chloride per 20 ml. of buffer had been added. Then they were washed for 3 h in three changes of the same buffer with 0.5 M glucose. Three hours post-fixation in 1 % osmium tetroxide was carried out in the same solution. The cyprids were then dehydrated in alcohol and embedded in Araldite. This preparative procedure was carried out on some specimens in a vacuum oven at a pressure of 400 mm of mercury. The antennules were sectioned on a Cambridge Huxley ultramicrotome using glass knives and examined with an A.E.I. EM 6B electron microscope.

This procedure did not prevent entirely the separation of the tissues from the thick layer of exocuticle, but this is not thought to have disturbed seriously the relative arrangement of structures within the antennule.

## DEVELOPMENT OF THE CYPRIS ANTENNULE

The nauplius antennule is a straight uniramous appendage with four segments and, in the last nauplius stage, bears 13 setae (figure 1*b*). The developing cypris antennule may be observed beneath the cuticle of the late sixth-stage nauplius as shown in figure 1*d* and figure 44, plate 46. The proliferation of cells in the distal postaxial region of the third segment is the rudimentary attachment organ of the cyprid. The fourth segment becomes reduced to about a third of the length of that of the nauplius, drawing the newly formed setae away from the exuvia of the old setae. The homologies of setation between the nauplius and cypris antennule are shown by the numbers of the setae in figure 1. Single rudiments of the terminal setae of the cypris fourth segment can be traced into the terminal setae of the nauplius antennule. Rudiments from the preaxial setae are thin and destined to be vestigial. Whereas there are two postaxial setae on the nauplius fourth segment (8 and 9), the rudiments of four can be traced from a similar position in the cypris fourth segment. The cellular reorganization during metamorphosis takes place around the muscles that extend through the third segment to attach to the base of the fourth segment. In the cypris larva, the antennule is held ventrally as an articulating walking appendage for which a new musculature is formed.

## MORPHOLOGY OF THE CYPRIS ANTENNULE

After the final nauplius moult the distal postaxial region of the third segment assumes a bell-shaped form 50 $\mu$m in length. This is the attachment organ (figure 1*e*) by which the cyprid can attach itself to and walk over surfaces. The adhesion is sufficient to enable the cyprid to cling to vertical and overhead surfaces of smooth glass even in considerable water turbulence.

The structure of the attachment organ has been deduced from electron micrographs of transverse and longitudinal sections. The final interpretation of the morphology is shown diagrammatically in figure 2. Some of these details are included in the diagram of the whole antennule in figure 1*e*, although not all these are visible in this species by light microscopy. The surface and margin of the disk of the attachment organ are covered with cuticular villi (*c.v.*). There are pores (*p.g.*) in the cuticle of the disk which are the outlets of glands that lie within the second and third segments. A complex arrangement of narrow slits amongst the cuticular villi lead into the radial cement ducts (*cm.r*). These are shown in longitudinal section in figure 5*a*, plate 38, and in transverse section in figure 6, plate 39.

A tubular hair (*s.a.*) mounted on a dome of cuticle (*a.d.*) projects from the centre of the disk, and another hair (*s.p.*) protrudes through an eccentrically placed pore towards the postaxial side of the disk. These are the hairs of the axial and postaxial sense organs described later. About eight hairs (*s.r.*) of varying lengths emerge radially from openings in the cuticle between the proximal side of the margin of the disk and a velum (*vm.*). Not all of these radial hairs can be seen with the light microscope. The velum consists of many sheets of solid cuticle which are attached mid-way along the side of the bell and ensheath the distal region of the attachment organ. An annulated postaxial seta (*set.* 2) which will be shown to be sensory, arises mid-way along the third segment. A corresponding but smaller preaxial sensory seta is hidden under the velum.

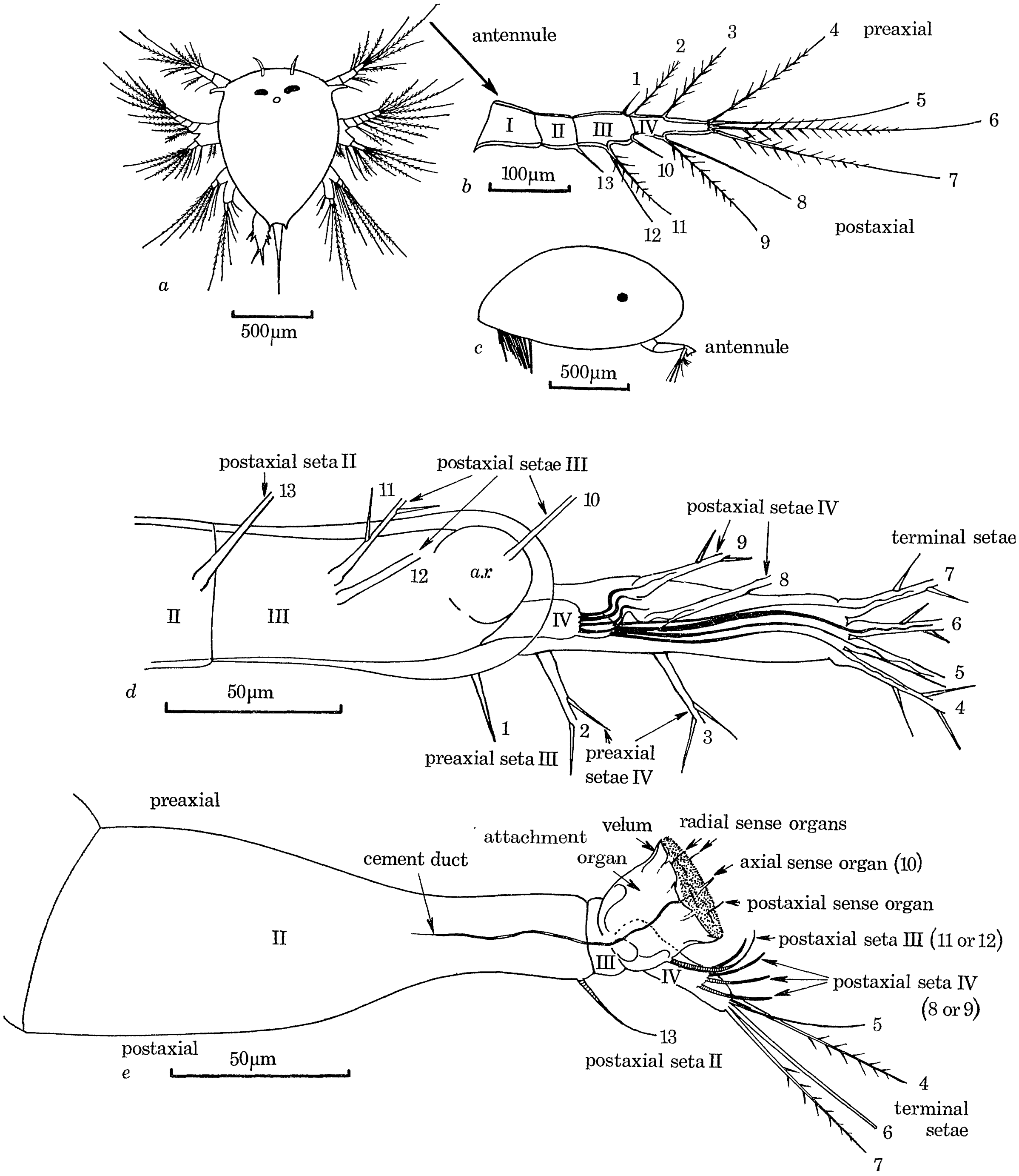


FIGURE 1. The larval antennule of *Balanus balanoides*: (*a*) sixth stage nauplius, dorsal aspect; (*b*) setation of the nauplius antennule; (*c*) cyprid, lateral aspect with one antennule extended; (*d*) late sixth stage nauplius antennule with developing cypris antennule within, postaxial aspect; (*e*) cypris antennule, lateral aspect. Roman numerals refer to the segment number.

The fourth segment arises half-way along the lateral side of the attachment organ. Postaxially on the fourth segment, there is a slight protruberance from which arise four annulated setae which are curved so that they bend towards the attachment disk. The fourth segment has four terminal setae, two of which are similar to each other and bear setules as do their precursors in the nauplius. Of the other two terminal setae, one is short and simple (i.e. without setules), and derived from the simple terminal seta of the nauplius. The other is a long rod-like structure with no apparent setulation, and derived from the long plumose seta of the nauplius. The cypris fourth segment has no preaxial setae corresponding to numbers 2 and 3 of the nauplius in figure 1.

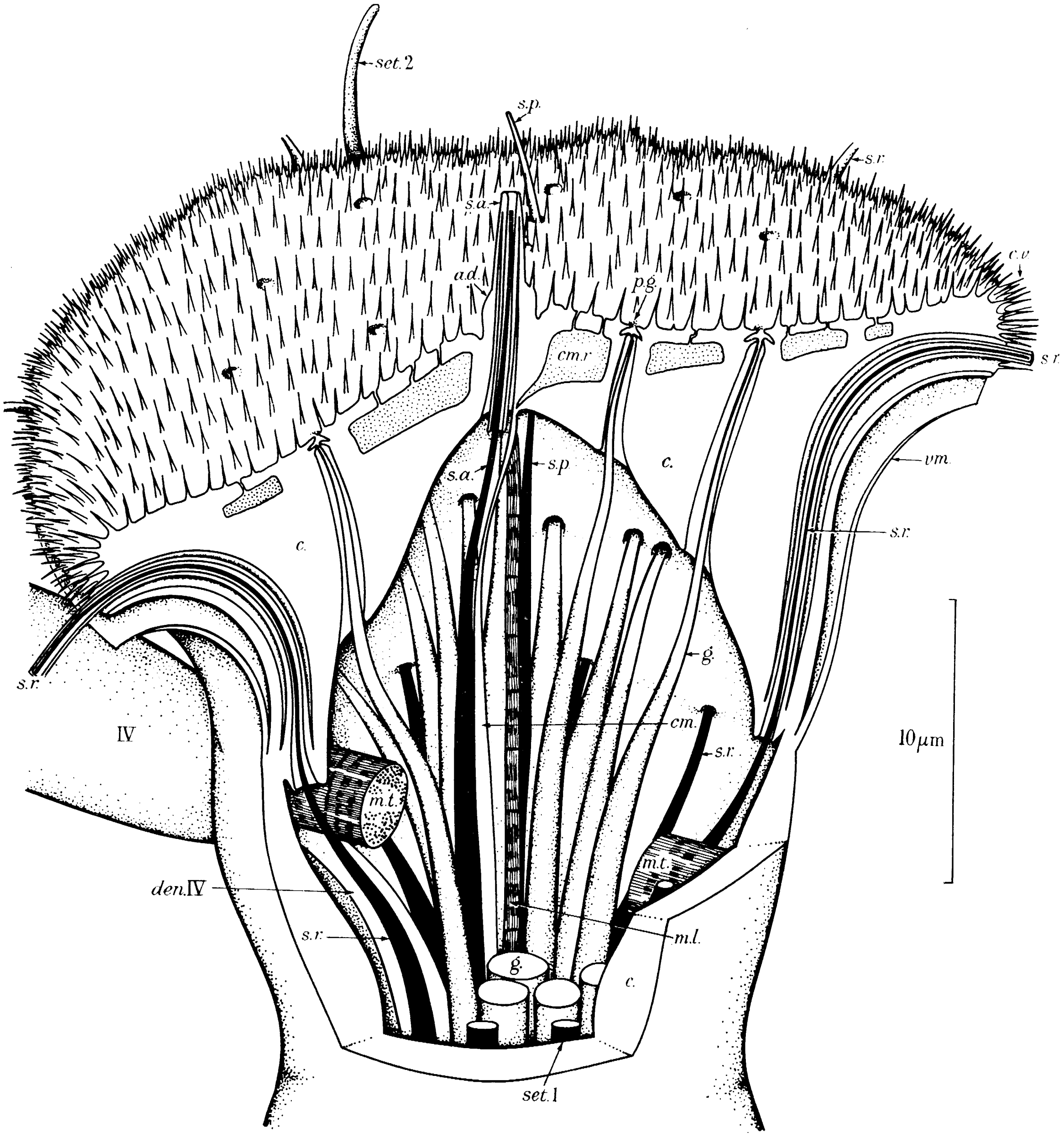


FIGURE 2. The arrangement of the structures within the attachment organ as seen in cutaway section from the preaxial side. The sensory organs drawn in solid black.

The second segment is shorter than that of the nauplius antennule, and is laterally compressed. Its distal postaxial edge bears an annulated seta similar in appearance to that on the third segment. The second segment articulates with the basal segment which is relatively unmodified in shape and inflected proximally for the attachment of the extrinsic limb musculature. Muscles within the basal segment articulate the second segment, and large muscles in the second segment are attached via ligaments on to the proximal cuticle of the third segment where there is apparent cuticular thickening. The cement duct can be traced from the centre of the attachment disk into the axis of the antennule, and thence to the cement glands in the cypris body.

## STRUCTURE OF THE ATTACHMENT ORGAN

A composite electron micrograph of a median longitudinal section of the attachment organ is shown in figure 5, plate 38, and three transverse sections at different levels are shown in figures 6 and 7, plate 39, and figure 8, plate 40. The arrangement of the various structures is shown in figure 2. Internally, the attachment organ has the axially situated cement duct (*cm.*), two muscles (*m.l.* and *m.t.*), and an array of glands (*g.*) and sense organs (*s.a.*, *s.p.* and *s.r.*). The arrangement of the sensory structures at various levels in the attachment organ is depicted in figure 3. Most of the structures originate in regions proximal to the third segment and terminate at the attachment disk. The axial sense organ (*s.a.*), cement duct and longitudinal muscle (*m.l.*) traverse the length of the third segment along the axis of the bell and they are surrounded by the glands and an outer ring of sense organs (*s.r.*).

The wall of the third segment provides anchorage for the transverse muscle (*m.t.*), shown partially cut away in figure 2, which crosses the third segment perpendicular to the axis of the bell and is inserted into the base of the fourth segment. A bundle of dendrites (*den.* IV) passes through the proximal part of the third segment into the fourth segment. The remainder of the lumen of the attachment organ is occupied by cells or cell processes that surround the sense organs and glands.

The orientation of the structures is described with reference to the axis of the attachment organ and not necessarily to the morphological axis of the antennule which is continuous with the longitudinal axis of the fourth segment and bent sharply at the junction between the segments.

### (*a*) *The axial sense organ*

The sensory components of the axial sense organ (*s.a.* in figures 2 and 3) originate from nerve cells situated proximally to the third segment and terminate within an open ended cuticular hair at the centre of the attachment disk. The structure of this organ is shown diagrammatically in figure 4*a*.

In the proximal region of the third segment, the axial sense organ has five dendrites (*den.*) which are enclosed within longitudinal invaginations of a single sheath cell (*sh.c.*). These are shown in figure 15, plate 41. Each dendrite, therefore, is enclosed in a double membrane, where the outer component is either continuous with the plasma membrane of the sheath cell or is the membrane of another dendrite. Thus, in this region there is direct contact between dendrites.

Mid-way along the attachment organ (figure 14, plate 41) ciliary roots and bases (*cil.r.* and *cil.b.*) appear in each dendrite together with supporting rods (*sp.r.*) in the sheath cell. Distal to this region five cilia are freely suspended in an extracellular space surrounded by longitudinal

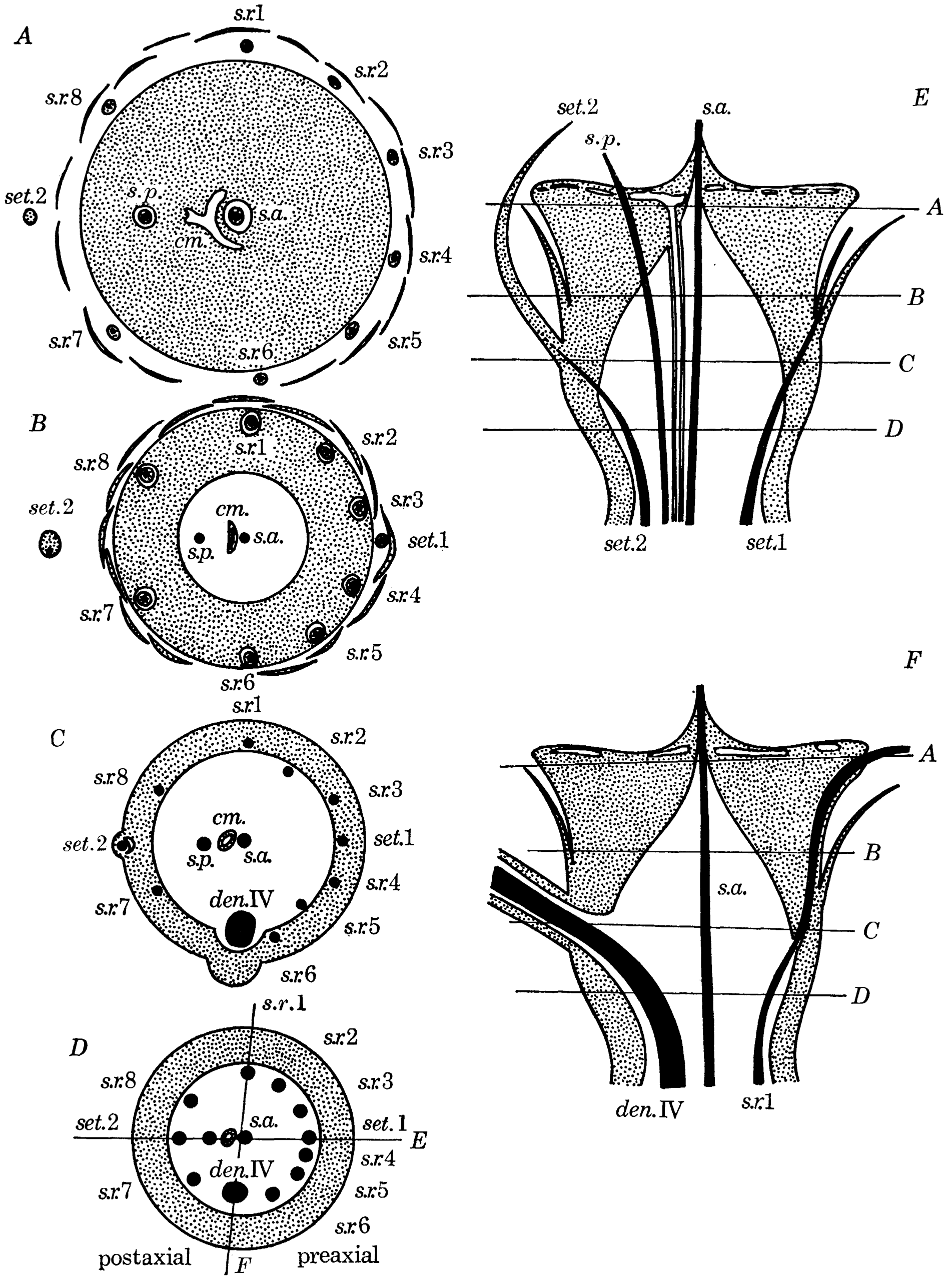
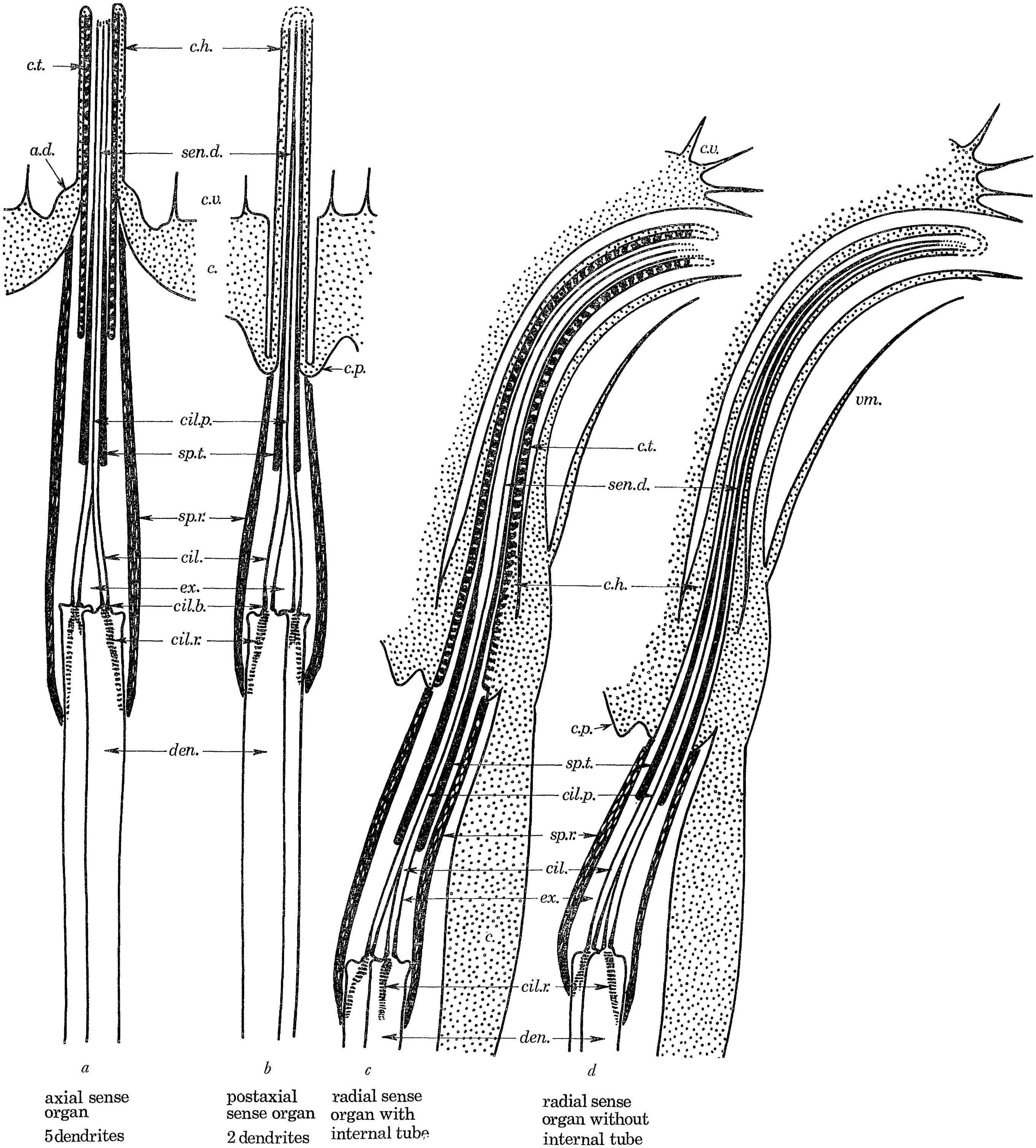


FIGURE 3. Arrangement of the sense organs shown diagrammatically in transverse and longitudinal sections.

folds of the sheath cell. Numerous supporting rods are present in the inner fold and occasional rods in supplementary folds.

Immediately below the cuticle of the adhesion disk (figure 13, plate 41), the cilia become five distal sensory processes (*sen.d.*) which are tightly packed within an electron dense supporting tube (*sp.t.*); they all contain microtubules some of which are paired. Four of the processes have similar transverse dimensions to the cilia, with a diameter of about 250 μm, but the fifth is considerably dilated with an irregularly shaped outline. Folds of the sheath cell with supporting rods continue to ensheath the tube in a longitudinal invagination of the cell. Distally (figure 12,



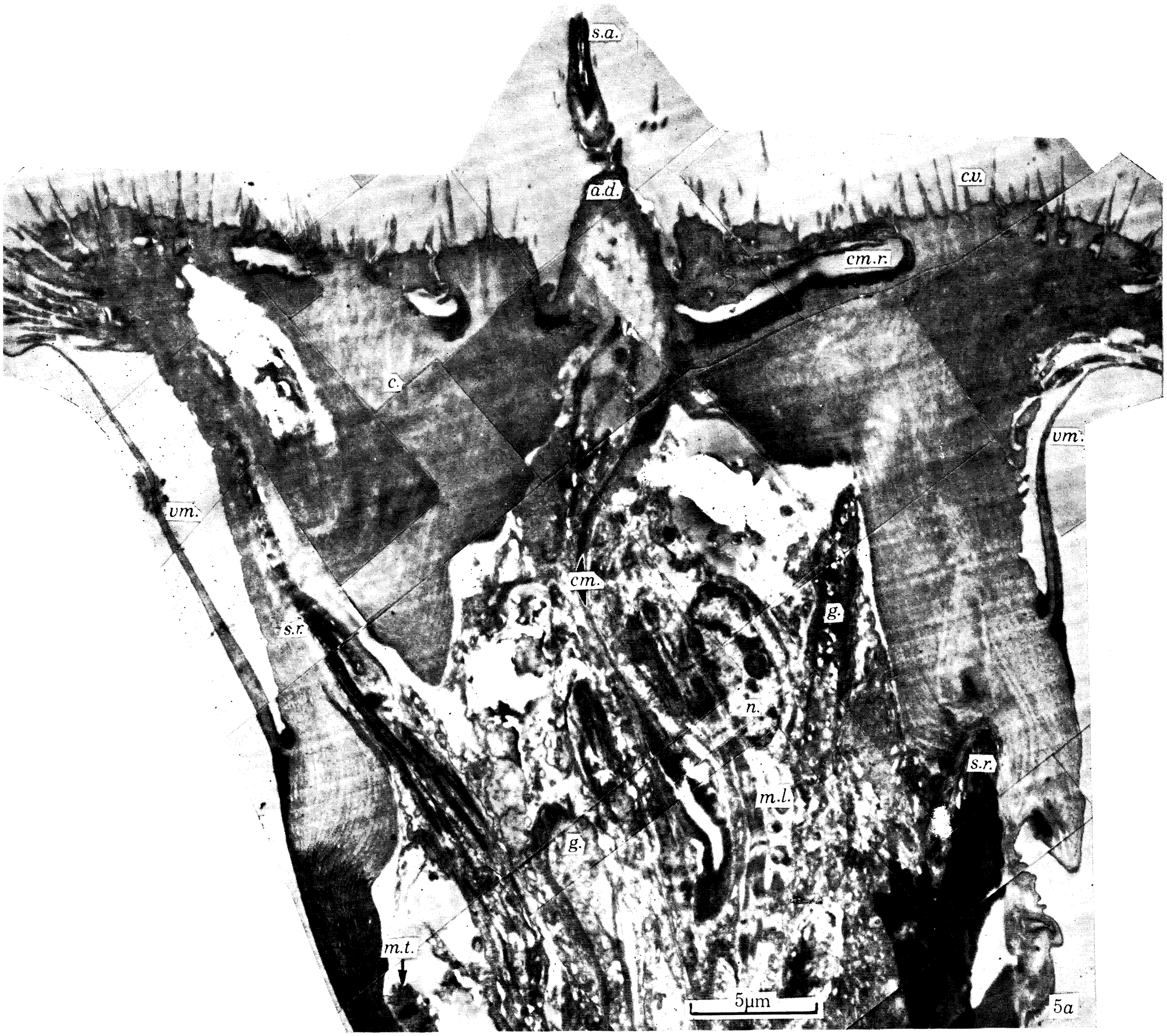
a axial sense organ 5 dendrites

b postaxial sense organ 2 dendrites

c radial sense organ with internal tube 5 dendrites

d radial sense organ without internal tube 2-4 dendrites

FIGURE 4. Schematic diagrams of the attachment disk sense organs in longitudinal section. The number of dendrites is reduced to two in each case and the sheath cells are omitted.



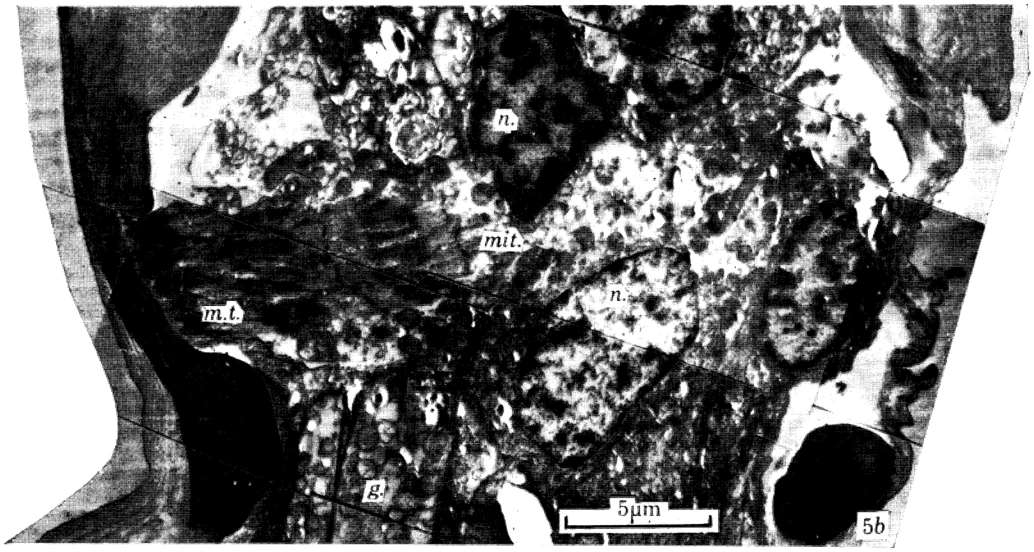
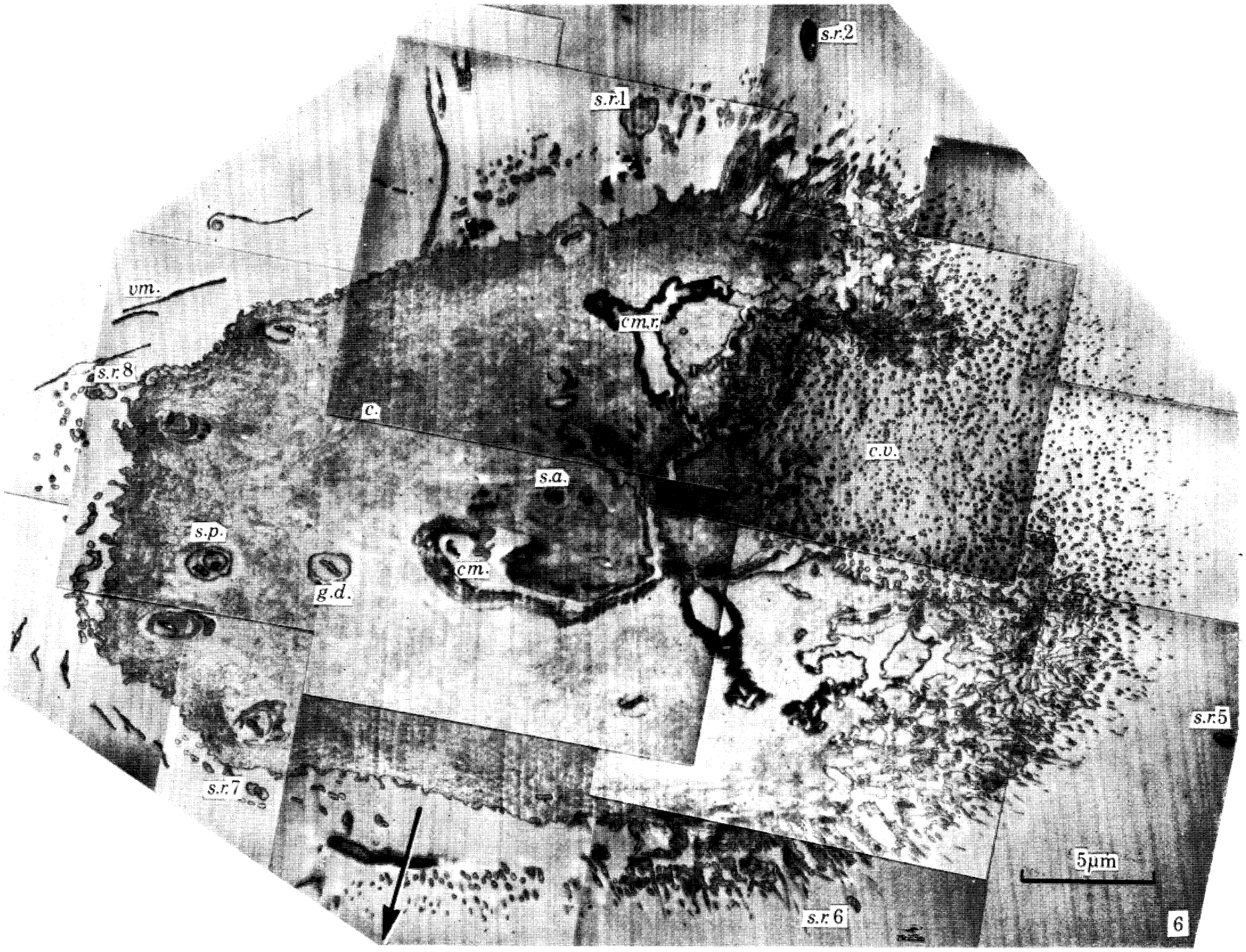


FIGURE 5*a*. Composite electron micrograph of a section through the median, longitudinal axis of the attachment organ of the third antennular segment.

FIGURE 5*b*. Similarly oriented section through the basal region of the third segment, with the transverse muscle crossing to the fourth segment out of the plane of the section to the right.



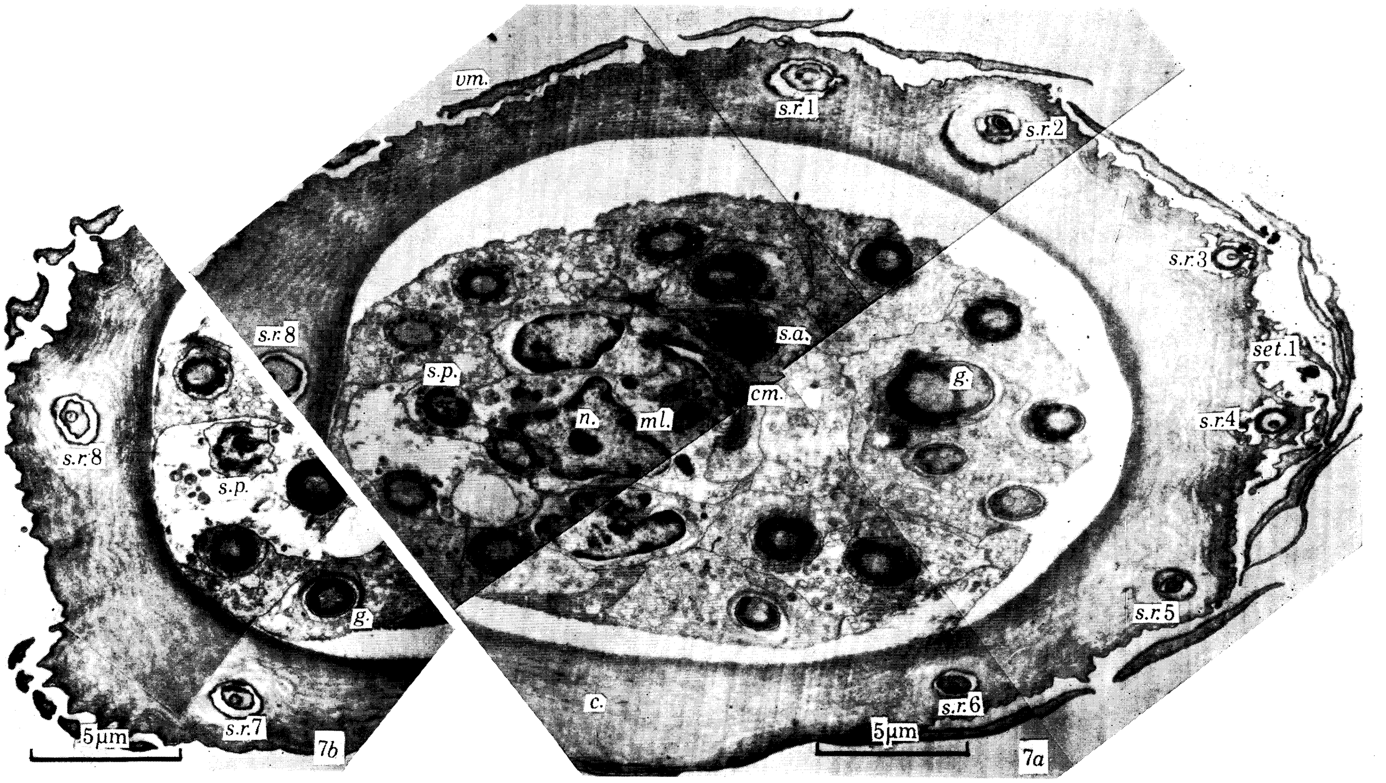
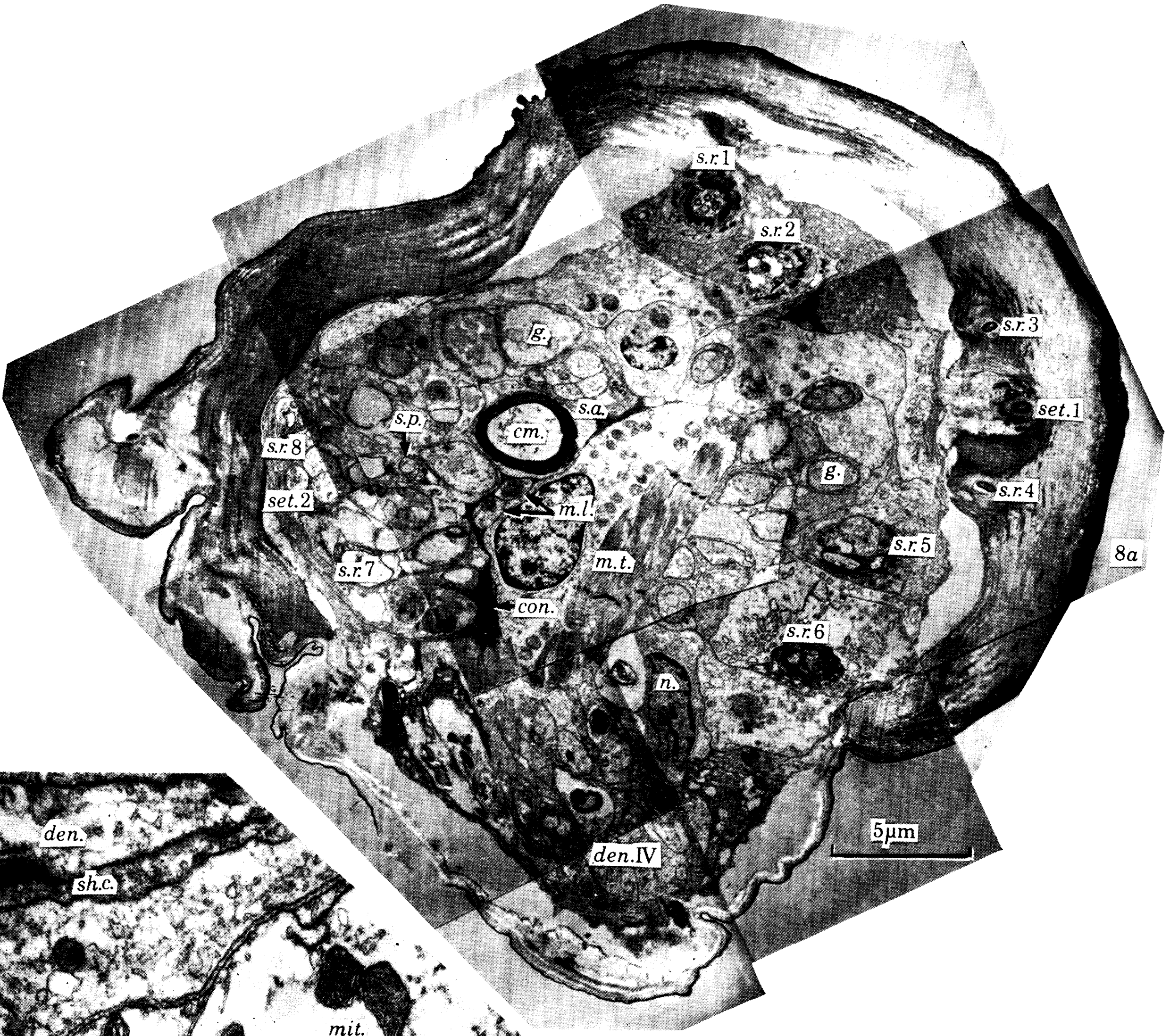
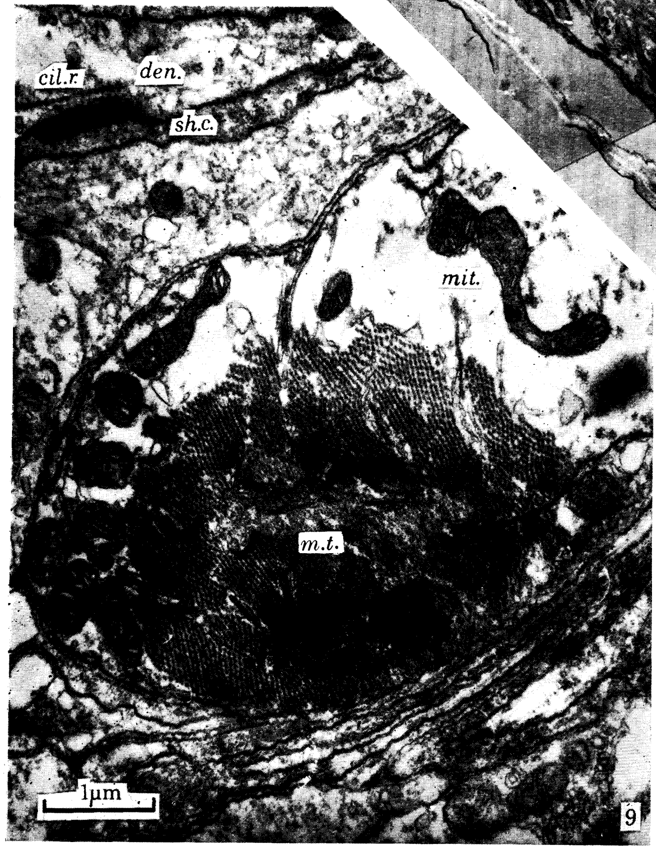


FIGURE 6. Composite electron micrograph of a transverse section (slightly oblique) through the distal region of the attachment organ. The fourth segment lies laterally in the direction indicated by the arrow. The postaxial side of the segment is to the left of the micrograph.

FIGURE 7*a*. Similarly oriented transverse section through the mid-region of the attachment organ.

FIGURE 7*b*. Transverse section taken distally from that of figure 7*a*, to which it is complementary.





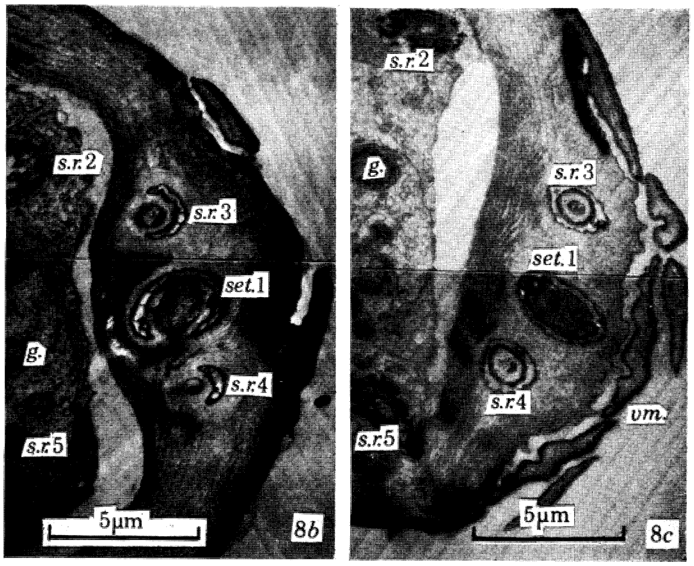
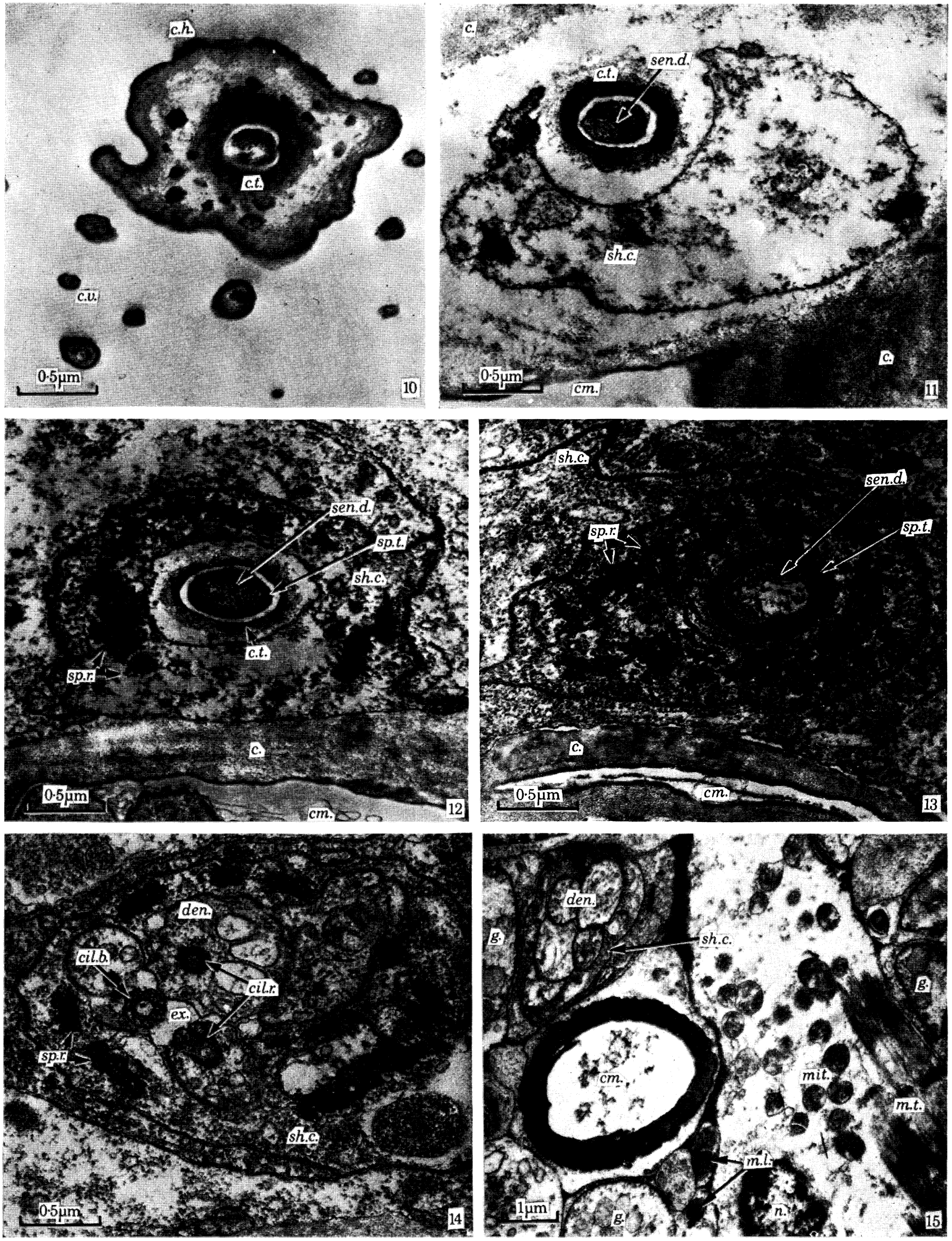


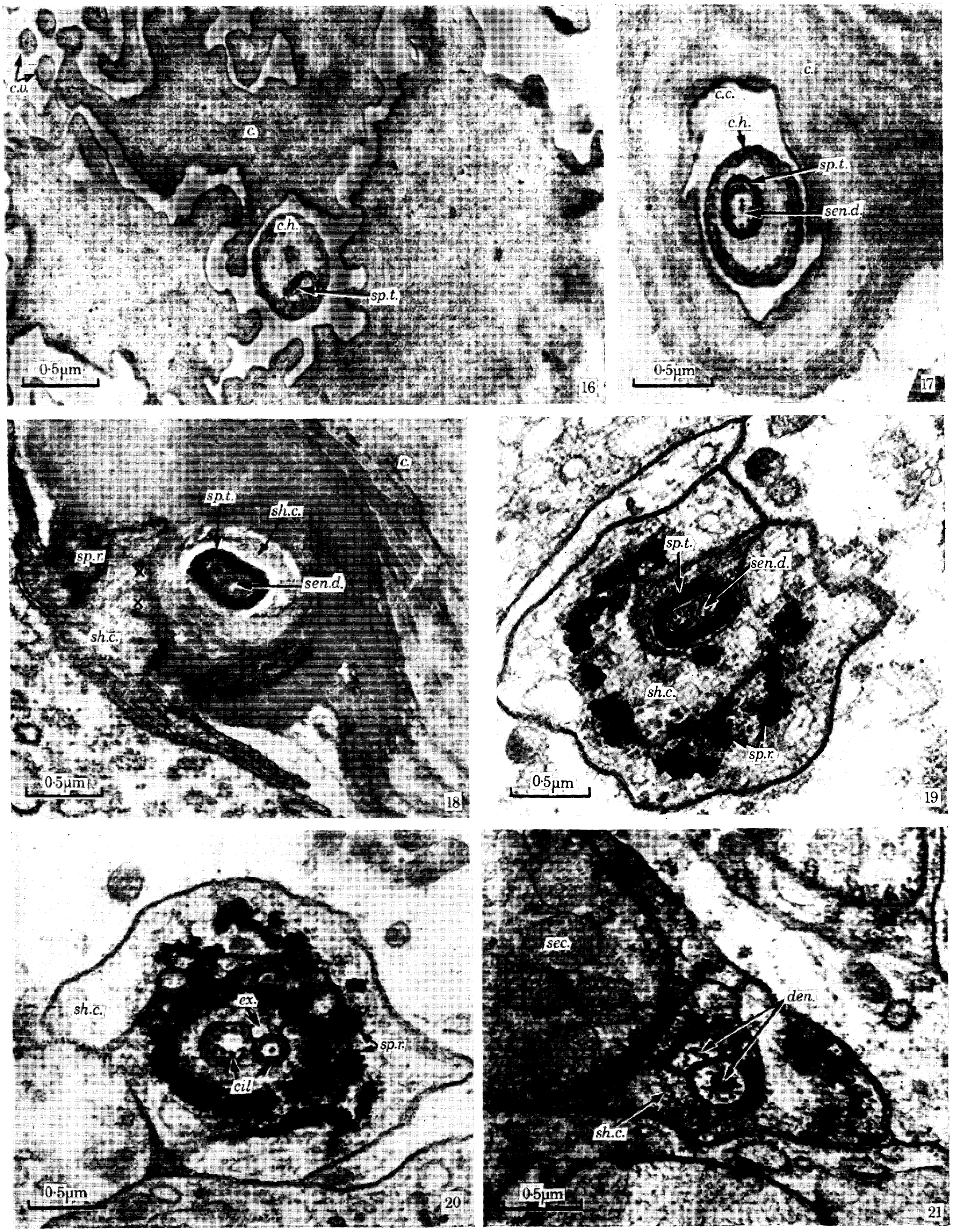
FIGURE 8*a*. A transverse section oriented similarly to those in plate 39, cut at the level of the attachment of the fourth segment (IV).

FIGURES 8*b*, 8*c*. Transverse sections cut distally to figure 8*a* and proximally to figure 7*a* to show the course of the sense organs on the preaxial side.

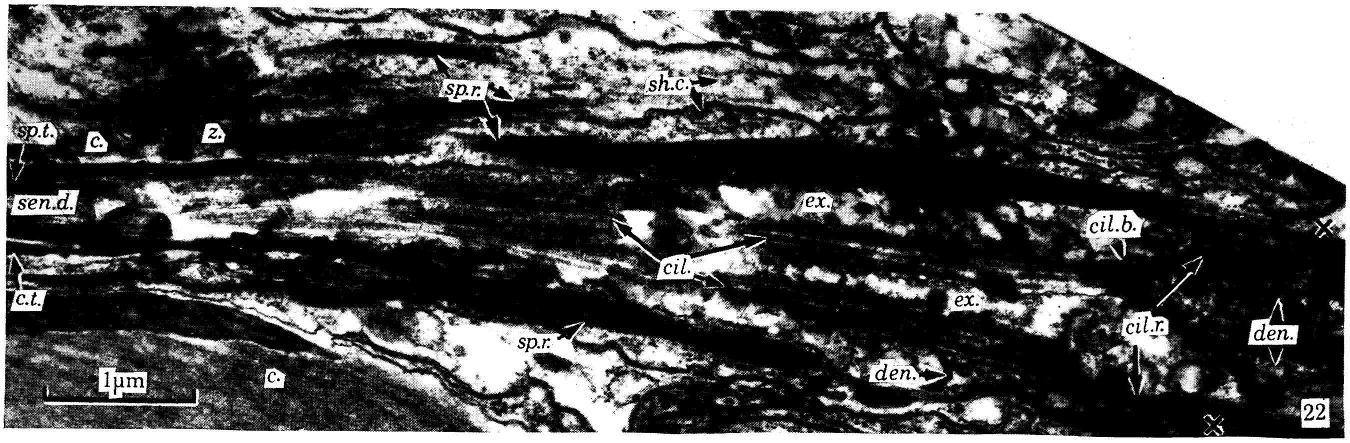
FIGURE 9. A longitudinal section of the third segment showing the transverse muscle in transverse section.



FIGURES 10 to 15. The axial sense organ shown in transverse sections from the cuticular hair in figure 10 to the dendrites in figure 15. Described in the text. Figure 15 at lower magnification to show the arrangement of other structures.



FIGURES 16 to 21. The postaxial sense organ in transverse sections, from the hair emerging from the attachment disk in figure 16 to the dendrites in figure 21. Described in the text.



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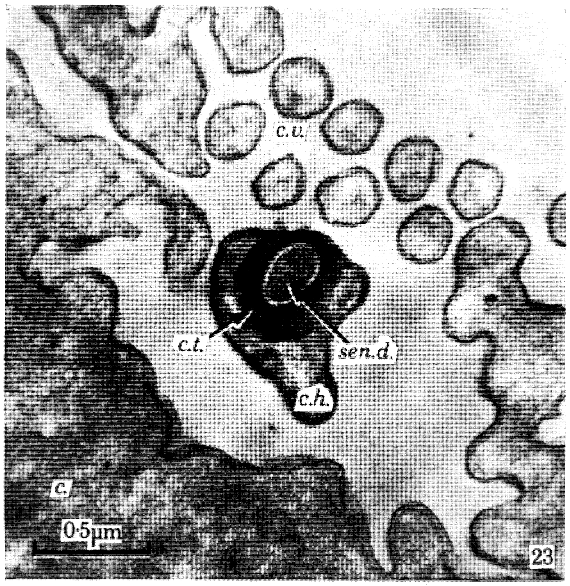

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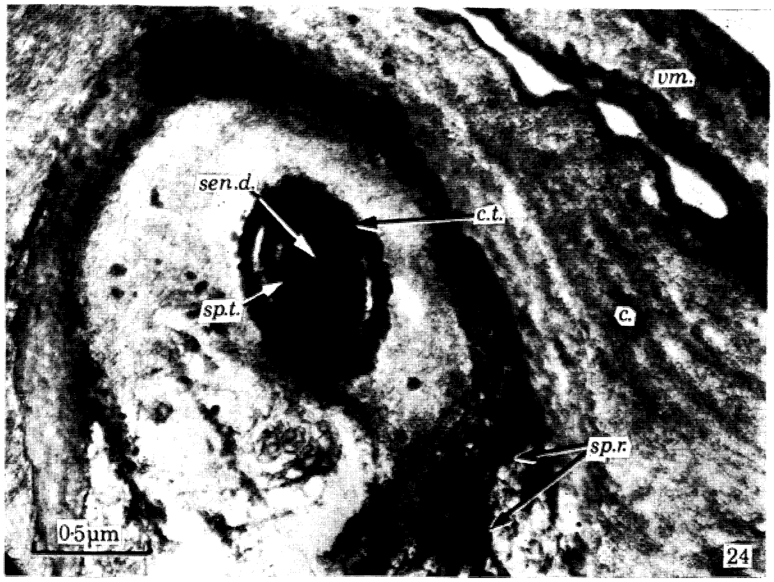

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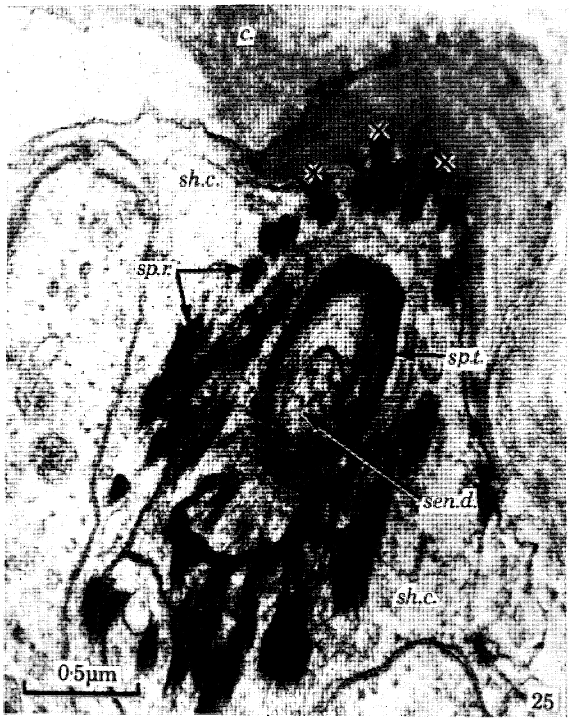

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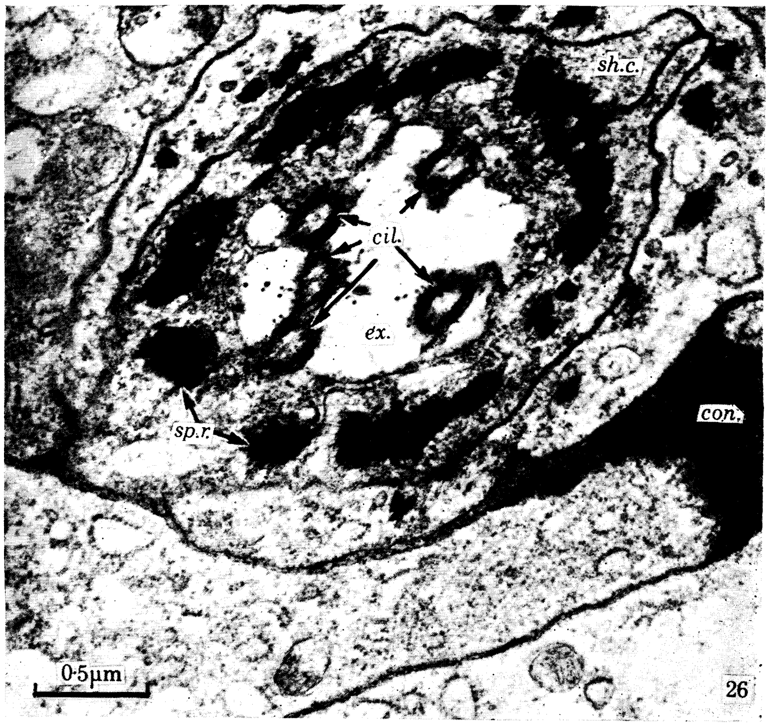

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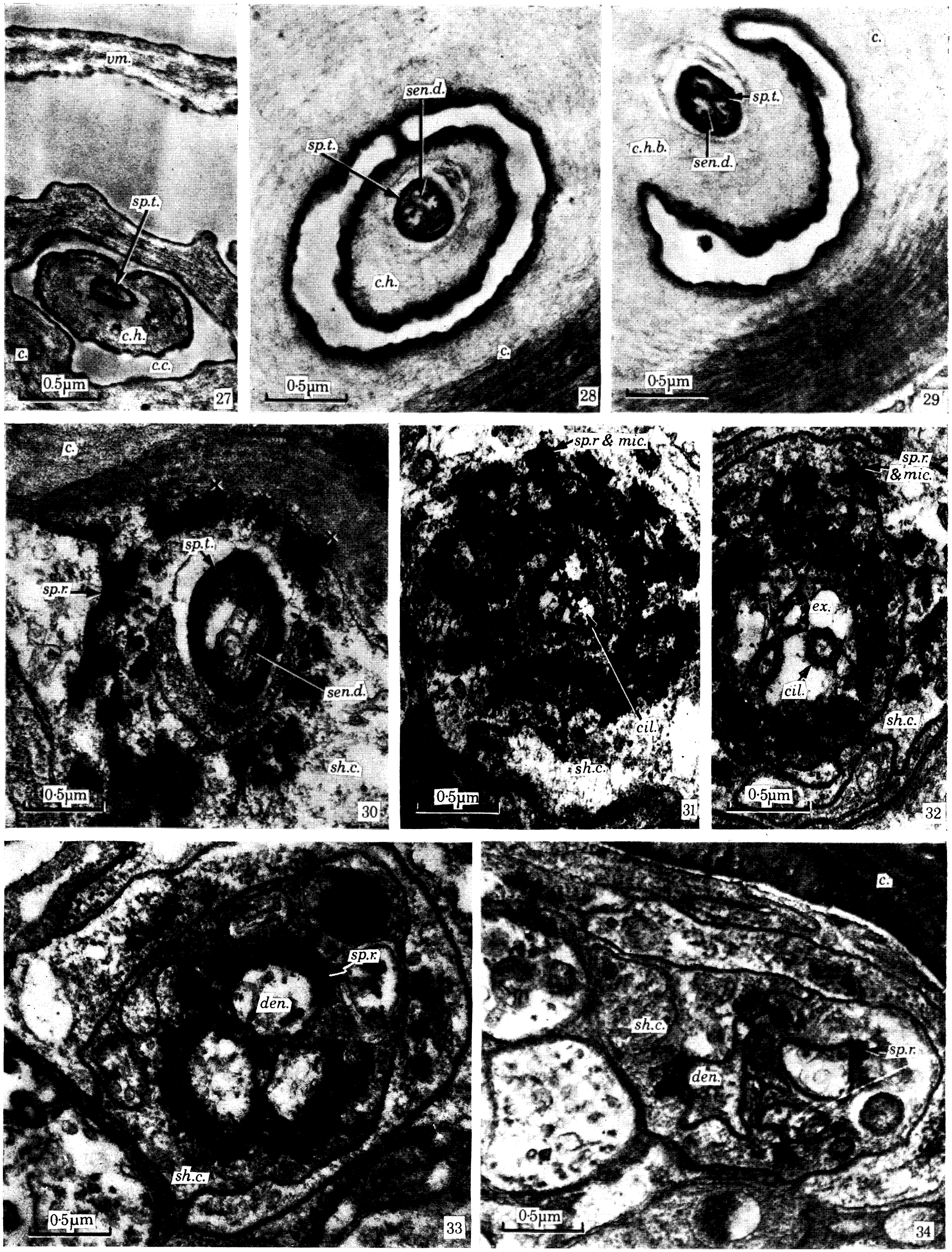
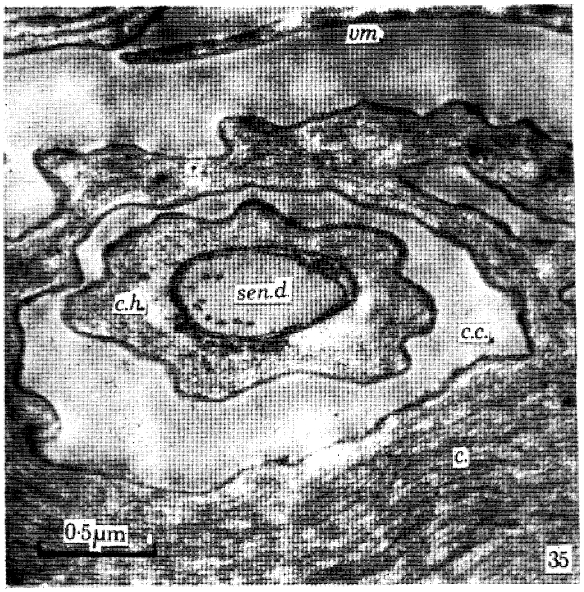
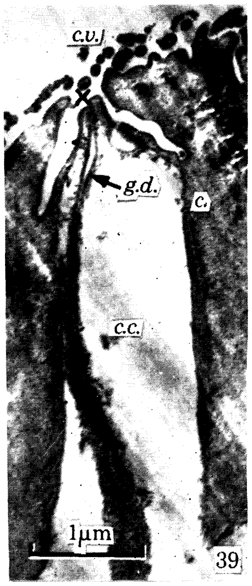
FIGURE 22. Longitudinal section of a radial sense organ showing the transition from dendrites to cilia and to distal sensory processes which enter the internal cuticular tube of the hair. In the proximal region the striated root of each cilium diverges from the axis of the sense organ towards the supporting rods, which approach the dendrites at '*x*'. The dendrites are in direct contact with each other. The supporting rods attach to the cuticle at '*z*'.

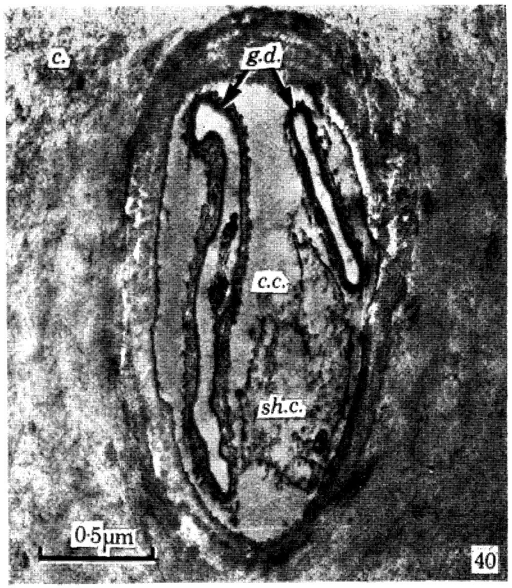
FIGURES 23 to 26. A radial sense organ with internal cuticular tube (*s.r.* 2 of plates 39 and 40) in transverse sections. In figure 25 the attachment points of supporting rods to the cuticle are marked '*x*'. Described in the text.

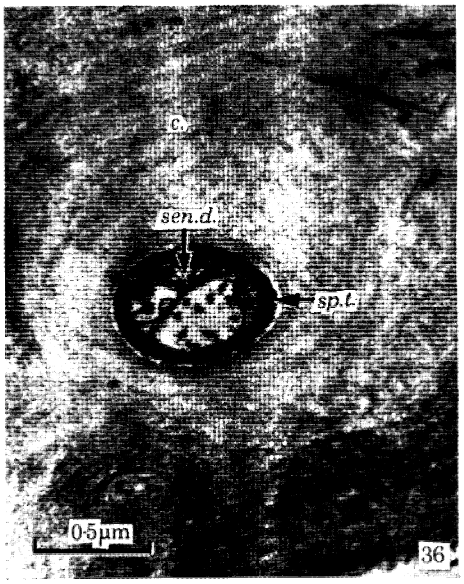


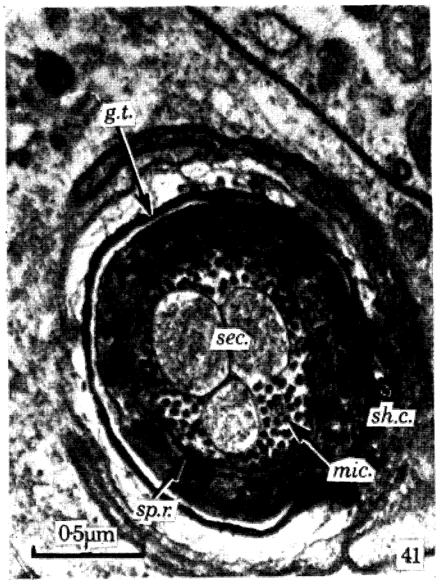
FIGURES 27 to 34. A radial sense organ without internal cuticular tube, in transverse sections from the hair within the cuticular canal in figure 27 to the dendrites in figures 33 and 34. In figure 30 the attachment points of the supporting rods to the cuticle are marked '*x*'. The intimate contact between the supporting rods and the dendrites, together with the electron dense material close to the plasma membrane in the dendrites, adjacent to the supporting rods, is shown in figure 33.

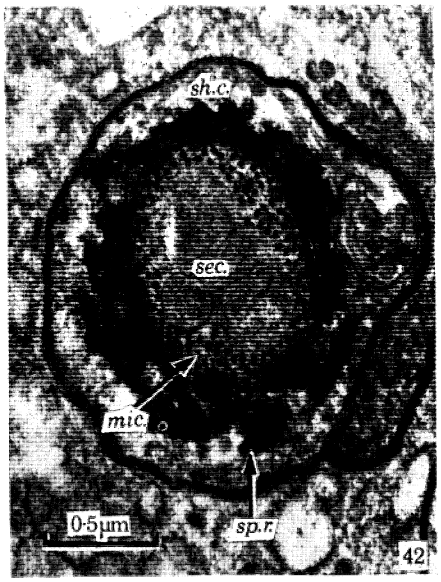


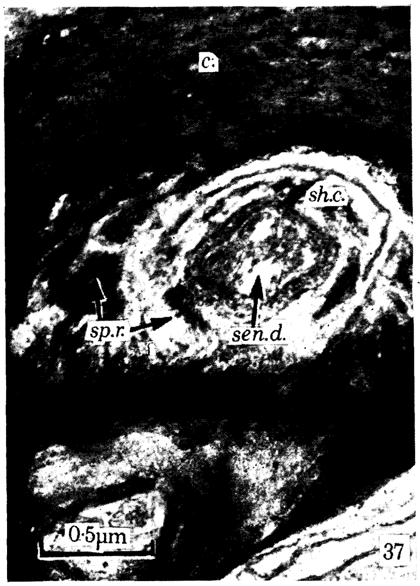


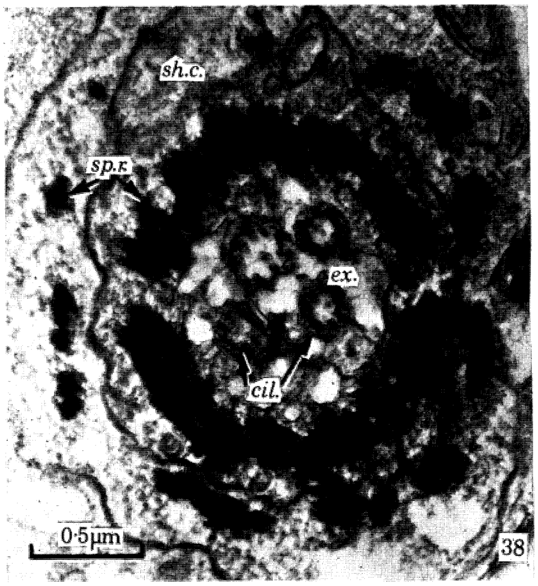


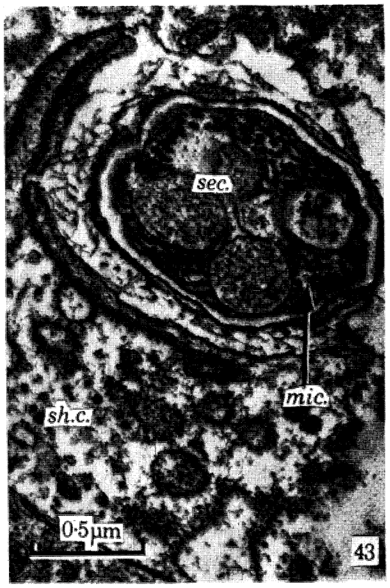










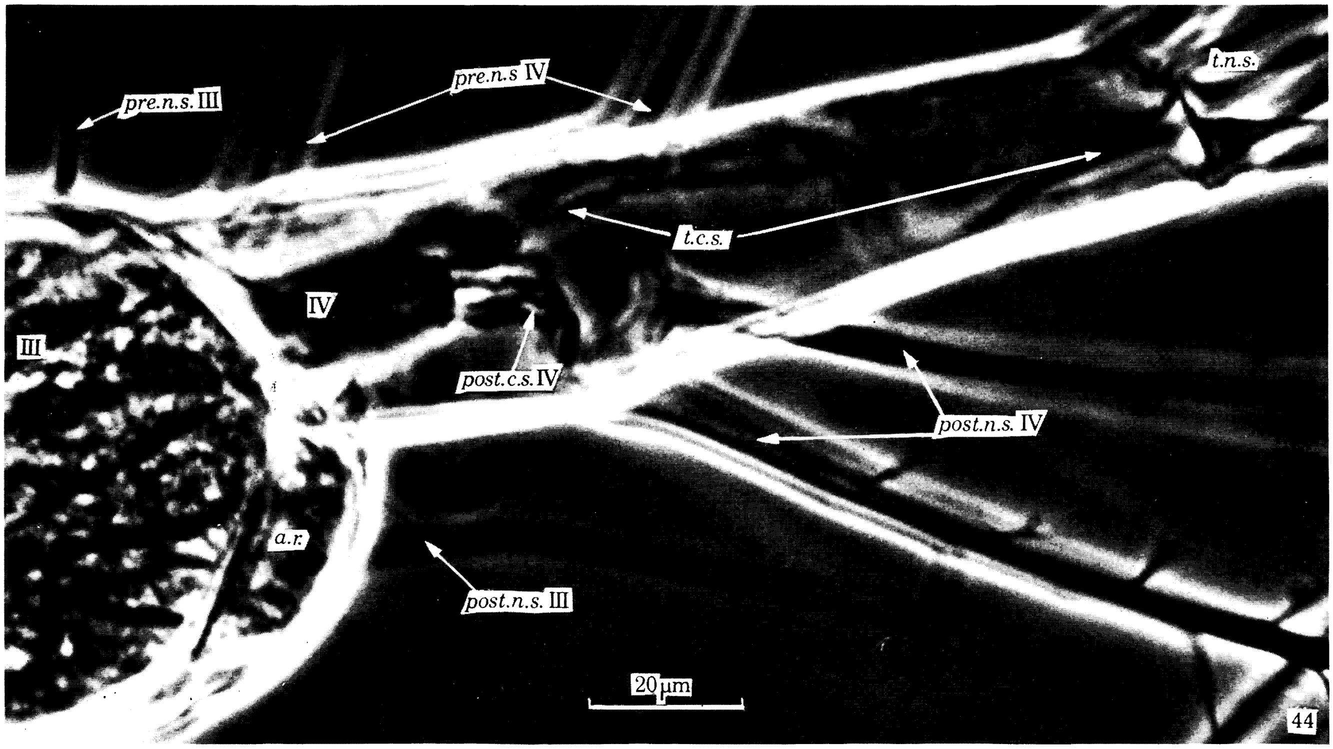


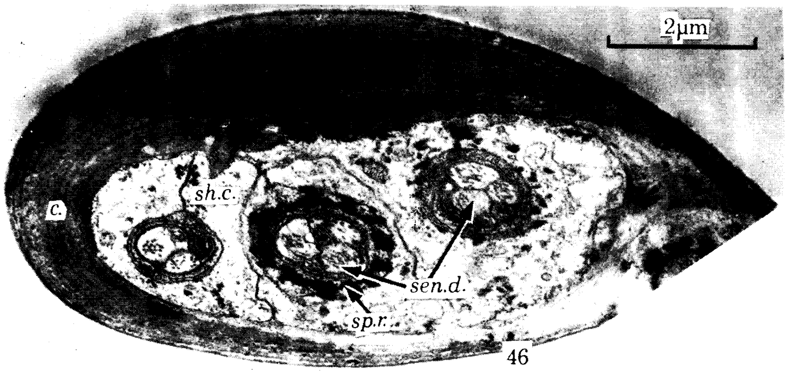
Figures 35 to 38. Transverse sections of the median radial sense organ without internal cuticular tube (*s.r.* 1 in plates 39 and 40). Described in the text.

Figure 39. Longitudinal section through the attachment disk surface showing the opening of the antennular gland duct (marked '*x*').

Figure 40. Transverse section through a cuticular canal in the attachment disk, with the ducts from two antennular glands and a process from a sheath cell.

Figures 41 to 43. Transverse sections through antennular glands taken from regions progressively more proximal from figures 41 to 43. Described in the text.





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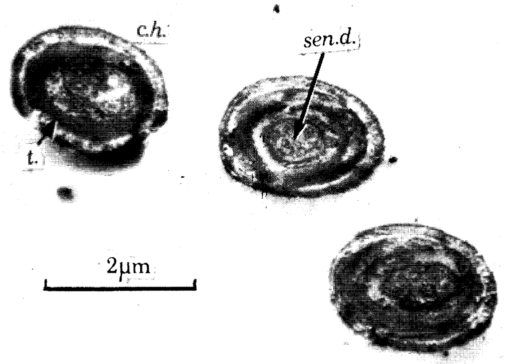

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FIGURE 44. Phase-contrast photomicrograph of the terminal regions of the antennule of the late sixth-stage nauplius, showing the developing cypris antennular structures within.

FIGURE 45. Transverse sections of three of the postaxial setae of the fourth segment of the cypris antennule.

FIGURE 46. Transverse section of the distal region of the fourth segment showing the sensory processes to the terminal setae.

FIGURE 47. A section transverse to the axis of the third segment showing the attachment of the transverse muscle in the base of the fourth segment and the dendrites entering the fourth segment.

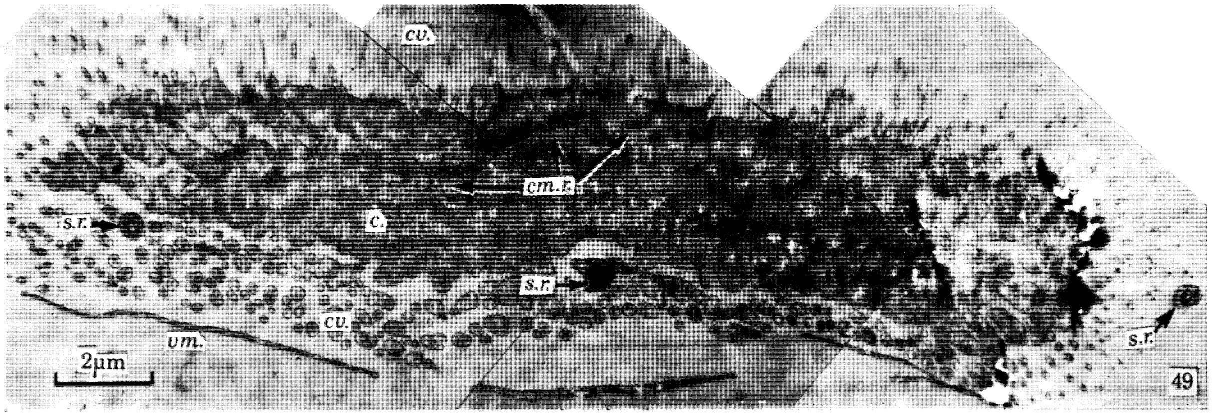

FIGURE 48. A section transverse to the axis of the fourth segment showing the arrangement of the sensory processes into those connected to the terminal setae on the left and those to the postaxial setae on the right. Supporting rods are shown in intimate contact with dendrites at '*x*' with electron dense material within the dendrite, adjacent to the rods.

FIGURE 49. A section longitudinal to the axis of the attachment organ and through the edge of the attachment disk showing three radial sense organs emerging from between the margin of the disk and the velum.

plate 41), at the level of the inner margin of the disk cuticle, the supporting tube enters a cuticular tube (*c.t.*) which extends the length of the axial hair.

After entering the cuticular tube, the wall of the supporting tube becomes progressively thinner and at the level of the disk attachment surface four distal sensory processes are enclosed directly within the cuticular tube. The single sensory process with an irregularly shaped outline terminates within the supporting tube. The four remaining processes have limiting membranes and contain microtubules and continue in this form towards the apex of the axial dome (*a.d.*). The sheath cell continues into the dome, but in this region it only partially ensheaths the cuticular tube which lies in a longitudinal groove on one side of the cell (figure 11, plate 41). Also, the sheath cell at this level is without supporting rods which terminate on the cuticle of the disk.

At the tip of the hair, the cuticular tube folds outwards, being continuous with the exterior cuticle and forming an open end to the sensory hair (figure 5*a*, plate 38). Within the terminal half of the hair, the membranes and microtubules of the distal sensory processes cannot be resolved and the cuticular tube contains only amorphous, electron dense material (figure 10, plate 41). This material continues to the distal end of the tube and as yet there is no evidence suggesting that the tube is sealed by a membrane.

### (*b*) *The postaxial sense organ*

The sensory components of the postaxial sense organ (*s.p.* in figures 2 and 3) terminate within a cuticular hair which passes through a canal in the cuticle of the attachment disk and emerges on the surface towards the postaxial margin. The structure of this organ is shown diagrammatically in figure 4*b*.

In the proximal region of the third segment, the postaxial sense organ consists of two dendrites in contact with each other and enclosed within a longitudinal invagination of a single sheath cell (figure 21, plate 42). Mid-way along the length of the third segment the sensory processes each produce a cilium and these lie in an extracellular space (figure 20, plate 42). This space is bounded by the longitudinal folds of the sheath cell which contain numerous supporting rods. Both cilia have nine peripheral pairs of microtubules and at least one of them has the $9+2$ arrangement for part of its length. Distally, in the region immediately below the cuticle of the adhesion disk, the cilia change in form to distal sensory processes. The distal sensory processes are packed in an electron dense tube with a wall about 0·06 μm thick; they contain microtubules, some of them paired, but the total number is progressively reduced in the distal direction (figure 19, plate 42).

The supporting tube passes through a peg of cuticle projecting into the lumen of the third segment from the adhesion disk and continues into the lumen of a cuticular hair (figure 18, plate 42). The supporting rods of the sheath cell appear to be attached to the projection at '*x*'. Except at its base, the sensory hair is unattached and it traverses the thick disk cuticle through a canal (*c.c.* in figure 17, plate 42). It emerges at the surface of the disk through a simple opening formed by the distal end of the canal (figure 16, plate 42).

At the base of the hair, one of the sensory processes terminates and the other one continues distally in the tube along the hair with a few unpaired microtubules. This process terminates as the hair emerges from the disk and the tube then contains amorphous granular material. This sensory hair does not contain an inner cuticular tube and, therefore, is thought to be closed at the distal end, although its structure has not been observed beyond the cuticle of the disk.

### (*c*) *The radial sense organs*

The radial sense organs (*s.r.* in figures 2 and 3) terminate as cuticular hairs which emerge radially from the margin of the disk. These sense organs show differences in the number of sensory processes and in the structure of the hair but have certain features in common. In the proximal region of the third segment, the sense organs are arranged in a circle located mid-way between the longitudinal axis and the cuticular wall of the segment. Each sense organ consists of a group of dendrites ensheathed within longitudinal invaginations of a sheath cell but they come into intimate contact with each other in the region where the ciliary roots develop. The inner folds of the sheath cell contain supporting rods which terminate in intimate contact with the dendrites proximally (figure 22, plate 43) and attach to the cuticle distally ('*x*' in figure 25, plate 43 and figure 30, plate 44). In transverse section, within the dendrites, there is a layer of electron dense material near the plasma membrane, adjacent to the areas of contact with the supporting rods (figure 33, plate 44). In longitudinal section, the ciliary roots are seen to diverge from the axis of the sense organ to lie against the plasma membrane of the dendrites, adjacent to the supporting rods (figure 22, plate 43 at '*x*'). This structural feature has been observed in longitudinal sections of the axial sense organ.

At the level of the transverse muscle (*m.t.*), mid-way along the segment, the dendrites give rise to cilia which are suspended in an extracellular space bounded by the inner fold of the sheath cell. The supporting rods are very numerous and sometimes occur within two concentric inner cell folds. The dendrite to cilia transition is shown in figure 22, plate 43.

The radial sense organs are oriented at such an angle to the longitudinal axis that they remain in a circle around the axial structures but come to lie adjacent to the cuticular wall of the segment distal to the transverse muscle (figure 8*a*, plate 40). In this region the sensory processes continue obliquely into the lateral wall, entering the cuticular peg projecting into the lumen of the segment, then through a cuticular canal and into a cuticular hair (figure 7, plate 39). The hairs continue obliquely through canals in the wall of the attachment organ and, distally, turn through 90° to emerge exteriorly at the proximal side of the margin of the attachment disk, directed outwards from the axis of the segment (figure 49, plate 47).

#### *Radial sensory hairs with internal tube* (figure 4*c* and plate 43)

There are two hairs of this type (*s.r.* 2 and *s.r.* 5 in plates 39 and 40) and they may correspond to the long hairs (usually two only) seen to emerge from the margin of the disk on whole mounts viewed with the light microscope. They are similar to the axial sense organ in that they have five sensory processes and a cuticular tube within the hair.

The five cilia (figure 26, plate 43) change to distal processes which become ensheathed in an electron dense supporting tube (figure 25, plate 43). The supporting tube enters a cuticular tube and these together penetrate the cuticle of the attachment organ (figure 24, plate 43) and enter the base of the hair. The supporting tube terminates, leaving the distal processes directly enclosed by the cuticular tube. The inner cuticular tube of the hair, when it passes through the cuticle of the third segment and beyond distally, has a wall up to $0{\cdot}15\,\mu$m thick at its widest region (figure 23, plate 43). In transverse section, the tube is always eccentrically placed within the folded outline of the hair, being in close contact with the exterior cuticle of the shaft on the side away from the longitudinal axis of the attachment organ.

Within the base of the hair the number of distal processes is reduced to two or three and these still contain microtubules. Where the hair emerges among the lateral cuticular villi, the thick inner tube contains two or three sensory processes with microtubules. The fine structure of these hairs has not been traced beyond the margin of the disk. By homology with the axial sense organ, the presence of the inner cuticular tube suggests that the cuticle is infolded at the tip of the hair, i.e. it is open-ended.

*Radial sensory hairs without internal tube* (figure 4*d* and plate 44)

These hairs (*s.r.* 3, 4, 6, 7 and 8 in plates 39 and 40) are more numerous than those with an internal tube. They are similar in structure to the postaxial sense organ; there is no cuticular tube within the lumen of the hair and only two or three sensory processes are present.

The dendrites give rise to cilia which are initially within a large extracellular space formed by a longitudinal fold of the sheath cell (figures 34 to 32, plate 44). Distally, the cilia are tightly enclosed by the sheath cell (figure 31, plate 44) and near the point of entry of the sense organ into the cuticle, the cilia change to distal sensory processes within a supporting tube which becomes increasingly electron dense towards the cuticle (figure 30, plate 44). Some of the microtubules in the distal processes are arranged in pairs but distally they are reduced in number and arranged at random.

The supporting tube penetrates the cuticle and enters the base of the hair (figure 29, plate 44), and then continues within the shaft of the hair (figure 28, plate 44). Distally along the hair, the number of sensory processes is reduced and at the region of the margin of the disk the tube is empty and tends to be flattened in transverse section (figure 27, plate 44). The fine structure of the hairs has not been observed beyond the exterior surface of the disk, but by analogy with the postaxial sense organ they are probably not open at the tip.

The radial hair on the median side of the adhesion disk (*s.r.* 1 in plates 39 and 40) shows some structural differences. The area of transverse section of the hair is greater, and this sense organ has four cilia within the lumen of the third segment (figure 38, plate 45). Distally, the cilia become distal sensory processes (figure 37, plate 45) which are tightly packed within the cuticle at the base of the hair (figure 36, plate 45). The processes retain the microtubules which are often paired. The sense organ is enclosed by a sheath cell with supporting rods, until it enters the cuticle. Distally along the hair shaft, the area of transverse section of the supporting tube increases, although the number of distal sensory processes is progressively reduced until one dilated sensory process containing microtubules remains (figure 35, plate 45). The distal end of this hair has not been traced, but the tip is probably closed as an internal cuticular tube has not been observed.

### (*d*) *Setae of the third segment*

The transverse sections of the more proximal regions of the third segment show preaxial and postaxial sensory structures (*set.* 1 and *set.* 2 in figure 8*a*, plate 40) which do not continue distally in the same manner as the radial sense organs. These sensory structures pass more obliquely through the cuticle (figures 8*b* and 8*c*, plate 40). That on the postaxial side (*set.* 2) appears to be associated with the postaxial seta that arises from the third segment below the junction of the velum (figures 2, 3). In its proximal region, the preaxial structure (*set.* 1) is much stouter than the radial hairs (figure 8, plate 40). A supporting tube containing sensory processes penetrates the base of the hair which emerges from the cuticle to lie freely between the velum and the body of the third segment (figure 7*a*, plate 39). It becomes very thin distally and apparently does not extend beyond the edge of the attachment disk.

### (*e*) *Antennular glands*

Numerous glands are arranged around the axis of the second segment of the antennule. Within the proximal region of the third segment, the glands (*g.* in figure 8*a*, plate 40) are arrayed round the axial structures, but towards the disk they diverge towards the wall of the segment (figure 7, plate 39). They enter the attachment disk cuticle in two concentric rings, and pass through canals in the cuticle (*g.d.* in figure 6, plate 39) to open through pores on the surface among the cuticular villi.

Each gland is surrounded by a longitudinal fold of a sheath cell (*sh.c.* in figure 43, plate 45); and in the distal half of the third segment this fold contains numerous electron dense supporting rods (*sp.r.* in figure 42, plate 45). In this region also, each gland contains microtubules (*mic.*) which are oriented longitudinally and packed marginally between the secretion (*sec.*) and the limiting membrane of the gland. The secretion can be seen in sections as electron dense granules packed in vesicles bound by a membrane. The distal region of each gland is enclosed in a tube (*g.t.*), consisting of a heavily 'stained' double membrane, derived from the sheath cell (figure 41, plate 45). An extension of the inner fold of the sheath cell containing supporting rods is also enclosed in the tube. The gland and the sheath cell extension terminate within this tube, which then becomes a flattened duct (*g.d.*) passing through a wide canal (*c.c.*) in the cuticle of the adhesion disk. A process from the sheath cell extends into the cuticular canal with the duct from the gland. Occasionally, the ducts from more than one gland pass through the same canal (figure 40, plate 45).

Distally the canal is closed by a thin layer of cuticle which is folded into a cone sunk beneath the surface of the disk ('*x*' in figure 39, plate 45). There is a pore at the apex of the cone, where the cuticle is folded inwards to become continuous with the duct from the gland. The ducts from the outer circle of glands pass obliquely through the cuticle and the pores occur on the surface of the adhesion disk near to the margin. Ducts from the inner circle of glands pass through the cuticle parallel to the longitudinal axis and the pores occur around the axial sense organ (figure 6, plate 39, and figure 2).

### (*f*) *Cement duct*

The cyprid has two cement glands which are situated laterally in the carapace posterior to the compound eyes and from each gland a cement duct traverses the length of the three proximal segments of the antennule on that side, connecting with the complex arrangement of canals in the attachment disk cuticle.

In the proximal region of the third segment, the cement duct is circular in transverse section and the cuticular wall is very electron dense (*cm.* in figure 8*a*, plate 40). Distal to the region of the transverse muscle, in the terminal half of the segment, the transverse section of the duct becomes crescent-shaped and the cuticle is less electron dense (figure 7*a*, plate 39). As the duct reaches the cuticle of the disk, the crescent shape is lost and the cuticular wall develops folds which eventually extend as radial canals (*cm.r.* in figure 5, plate 38 and figure 6, plate 39) through the cuticle beneath the attachment surface, branching frequently and reaching almost to the margin of the disk. Numerous fine, terminal slits in the disk connect the radial canals to the adhesion surface (figure 5, plate 38).

At the distal end of the crescent-shaped part of the cement duct, the longitudinal muscle (*m.l.*) is attached to the concave side of the crescent. The wall of the duct on the convex side is about

0·26 μm thick and that on the concave side is about 0·13 μm thick, with the lumen reduced to a narrow slit (figure 7*a*, plate 39). This structural arrangement may constitute a valve in the cement duct, which could be opened by the muscle contracting and pulling the thin concave wall away from the thicker convex wall.

### (*g*) *Longitudinal muscle*

From the point of attachment to the cement duct, the longitudinal muscle extends the length of the third segment in close proximity to the duct. The muscle has been traced well into the second segment of the antennule. Transverse sections at all levels of the third segment and the distal region of the second segment show two muscle cells, each containing mitochondria and a myofibril. In the mid-region of the third segment the muscle is supported by amorphous connective tissue (*con.* in figure 8*a*, plate 40).

## FOURTH SEGMENT

Internally, this segment is almost completely filled by the dendritic processes of nerve cells (figure 47, plate 46 and figure 48, plate 47). At particular levels within the fourth segment the sensory processes show differences in their form, and these differences are in accord with sensory structures, namely, dendrite to cilium to distal sensory processes. The more distal parts of the dendrites and their sensory processes are arranged in groups, each surrounded by a fold of a sheath cell. These groups appear to be associated with the setae. The four curved setae arising from the projection on the morphological postaxial side have an annulated appearance due to circular bands of thickening (*t.*) beneath the cuticle of the hair shaft, shown in transverse section in figure 45, plate 46. Sensory processes containing microtubules enter a supporting tube which penetrates the lumen of each curved seta. The distal sensory processes associated with the four terminal setae are shown in figure 46, plate 46. The differences in the fine structure of the sensory apparatus of the setae of the fourth segment have not yet been investigated fully.

The muscle which is inserted into the base of the fourth segment is shown in figure 5*b*, plate 38, figure 9, plate 40 and figure 47, plate 46.

## FUNCTION OF THE SENSE ORGANS

Within the distal regions of the sense organs, the dendrites take the form of ciliary processes lying in an extracellular space and surrounded by folds of sheath cells with supporting rods. The supporting rods are equivalent to the 'scolopale spikes' or 'scolopales' of previous descriptions of the sensory units ('scolopidia') of arthropod sense organs. The rods appear to be attached to the dendrites and closely associated with the ciliary roots of some sensory processes and they are attached distally to the cuticle. The position of the rods around the distal regions of the scolopidia, and also around the distal regions of the antennular glands in the attachment organ, suggests that they are supporting or protective intracellular structures.

Mechanoreceptors have been variously classified according to their situation and the nature of the mechanical stimulation. Hair sensilla and campaniform sensilla that respond to movements of the hair or cap have been described in the honeybee by Thurm (1964, 1965) and in spiders by Görner (1965), sensilla that respond to intersegmental movement are described in the Johnston's organ of *Drosophila* by Uga & Kuwabara (1965) and in the subgenual organ of the termite by Howse (1965), auditory receptors that respond to airborne vibrations have been described in the locust by Gray (1960), and internal proprioceptors from the leg joints of crabs have been described by Whitear (1962, 1965). The features of these scolopidia have been reviewed by Howse (1968). In all cases the ciliary processes are anchored into a cap, which may be solid as in proprioceptors or long and tubular as in the chordotonal receptors of the termite. In the latter, the cap is embedded in the cuticle and movements of the cap by the cuticle provide the stimulus (Laverack 1964; Taylor 1967). The nature of transduction in the dendrite is uncertain, but it seems likely that flexion or stretching of the ciliary processes by the movements of the cap may provide the stimulus. The significance of the connexions between the supporting rods or scolopales and the ciliary roots within the dendrites is not known, although Howse (1968) has suggested that flexions of the scolopales and the resulting compressions of the dendrite tip might provide a stimulus.

The presence of chemoreceptors in Crustacea has been established by Hodgson (1958), Case & Gwilliam (1961) and Laverack (1963). The chemoreceptor sensilla of insects, at least, are hair or peg processes which are probably derived from setae (Dethier 1963). The chemosensory hairs of insects have sensory processes that are connected with the exterior either by numerous pores in the hair shaft as in the honeybee antennae (Slifer & Sekhon 1961) or via a cuticular tube that is an invagination of the cuticle at the tip of the hair, as on the antennae of grasshoppers (Slifer, Prestage & Beams 1957, 1959), flies (Wolbarsht 1965, and Adams cited in Wolbarsht 1965) and on the antennule of the spiny lobster (Laverack & Ardill 1965). Apparently, the sensory processes must be in direct contact with the exterior and stimulation results from the apposition of chemicals to the membranes of the distal sensory processes (Dethier 1962). Davies (1962) has suggested that an impulse is initiated by the penetration and dislocation of a small region of the lipid membrane of the sensory cell by the molecule, the dislocation allowing the passage of ions across the membrane. Amoore (1963) argues that the shape of the molecule allows it to fit an appropriately shaped receptor site on the sensory processes.

In the cyprid antennule there are three structures that are comparable with the described insect chemoreceptors. These are the axial sense organ and two of the radial sense organs (*s.r.* 2 and *s.r.* 5). They have an internal cuticular tube that surrounds the distal sensory processes within the hair which, in the axial sense organ, has been observed to open at the tip. The similarity of the structure of the hair of the axial sense organ and the two radial sense organs suggests that they also open at the tip. The supporting tube does not ensheath the distal sensory processes very far into the hair and some of the processes are directly enclosed in the cuticular tube as they penetrate further into the hair.

The other radial sense organs and the postaxial sense organ on the disk have distal sensory processes that always appear to be ensheathed in a supporting tube within the hair. The absence of an internal cuticular tube at any of the levels sectioned and the absence of pores in the cuticle of the hairs suggests that the sensory processes are not in contact with the exterior.

All the sense organs of the attachment disk are comparable with the chordotonal scolopidia described by Howse (1968), with the supporting tube being homologous to the scolopale cap. The tube is embedded in the hairs which could conform to distortions of the shape of the attachment organ and function as position receptors. Also, there is some evidence that mechanoreceptive processes occur within sense organs which are primarily chemoreceptive and, furthermore, that each chemoreceptive process may respond to different and specific chemicals (Hodgson 1958; Dethier 1963). One of the processes in the axial sense organ and at least two in the radial sense organs *s.r.* 2 and *s.r.* 5, terminate within the supporting tube. It is considered, therefore, that these sense organs could function as mechanoreceptors as well as chemoreceptors.

The postaxial and preaxial setae emerge from the side walls of the attachment organ. They have dendrites and cilia associated with them and these are probably sensitive to movements of the setae. Sections of the preaxial seta have been obtained external to the disk and it is not entirely analogous to insect hair sensilla because the distal processes penetrate the seta within a supporting tube rather than being attached to a more solid cap at the base of the hair as described by Thurm (1964) and Görner (1965). The penetration of the supporting tube or cap into the preaxial seta suggests that flexions along the seta are perceived. The annulated appearance of the postaxial setae of the third and second antennular segments and the four curved postaxial setae on the fourth segment, indicates some function different from that of the straighter and plumose setae or 'feelers' of the nauplius and the terminal setae of the cypris fourth segment. The setae curve towards the surface to which the cyprid is attached and this could indicate a mechanoreceptive mechanism for detecting some physical characteristics of surface texture.

More intensive study of the fine structure of the various sensory structures, complemented by appropriate neurophysiological experiments, is necessary to establish the function of any of these sense organs.

## FUNCTION OF THE ANTENNULAR ATTACHMENT ORGAN

There can be no doubt that the cyprid uses the attachment organ to hold itself on to the substratum while exploring the surface, and as the organ of permanent attachment when the exploratory phase is complete. Movements of the third segment are effected by large muscles in the second segment that are attached by ligaments to the cuticle at the very proximal end of the third segment. These muscles enable the attachment organ and the rest of the third segment to be moved as a palm and wrist move with respect to the arm.

The shape of the attachment organ and the manner in which it is applied to and released from the surface as the animal walks has lead to the assumption that it adheres by suction. The details of its fine structure do not lend support to this idea. First, there is no efficient seal around the edge of the disk to prevent water flowing into the region of low pressure that must be presumed to exist between the disk and the substratum. The velum is unlikely to serve such a function as it is discontinuous at the edge of the disk and the cuticular villi are probably disadvantageous for establishing a seal on to a smooth surface. A means of establishing a seal would be to fill the gaps in the velum and between the marginal villi with viscous material. Although the antennular glands might provide such a material, the fact that they do not discharge at the margins of the disk but rather over the general surface, suggests that the disk is an adhesive pad rather than a suction cup.

Secondly, there is no obvious mechanism to reduce the pressure between the disk and the substratum which would be necessary to produce the apparently strong and continuous adhesion that can be seen when attached cyprids are exposed to strong water currents (Crisp 1955). To apply suction, the centre of the disk must be pulled away from the substratum while the rim must stay in close contact with it. The periphery of the disk cuticle is thick and would give the necessary support, but the inner areas are also fairly thick and appear unlikely to deform readily. Moreover, the only muscle in a position suitable for lifting the centre of the disk is very slender and terminates not on the disk itself but at the cement duct, which it appears to control. In the absence of muscular attachments, the centre of the disk could be lifted from the substratum hydraulically if body fluid were withdrawn from the third segment. Again, there is no apparent mechanism for withdrawing fluid and since the third segment is packed tightly with glands and sense organs and lacks any large haemocoel, there appears to be no excess fluid to be withdrawn. Furthermore, any extensive change in shape of the attachment organ would involve distortion of the sensory and secretory tissues inside.

Structurally, the disk appears more likely to act as a pad with an adhesive surface. There are two sets of glands discharging on to the disk surface which could supply an adhesive secretion; the antennular glands each discharging via a separate pore and the cement glands discharging through a network of canals opening among the villi. The villi would serve to increase the surface area of the disk and promote more efficient adhesion. However, any proposed mechanism of adhesion must take into account the ability of the cyprid to detach the antennules from the substratum. If the adhesion could be broken only by pulling normally to the surface the reaction against the surface would be transmitted to the other antennule, which in turn would also adhere, probably more strongly. In this way the larva would be impaled by its own adhesion. However, an adhesive union can usually be broken more readily by progressive shearing at the margin of the join than by pulling normally to the surface and breaking all the adhesive bonds simultaneously. An analogy is the stripping of adhesive tape from a surface. The circular distribution of the muscles that control the movement of the third segment may be capable of providing such a pull at the margin of the disk on any side.

## PRESETTLEMENT BEHAVIOUR

Before settling, the cyprids display a characteristic change in the exploratory behaviour; they turn sharply, twist around on the antennules and seem to be testing the surface by pulling on the antennules (Crisp 1961). It is not certain whether the attachment is in part involuntary or whether, having recognized by some means the nature of the surface on which the antennule is placed, the cyprid deliberately holds on. The latter seems the more likely since cyprids can be observed to leave the surface if they encounter loose particles, air bubbles or obnoxious stimuli, and they can exercise independent control of the attachment of each antennule during walking. It is only at the final stage of settlement or fixation when cement can be seen issuing round the base of the antennular disk that they are unable to detach the antennules and swim off. Settlement is preceded by close searching and clearing of the settlement site and a well-ordered orientation reaction to a hierarchy of stimuli (Crisp 1961; Knight-Jones & Moyse 1961), and cannot therefore result from an uncontrolled increase in the adhesiveness of the antennules to the substratum. Attachment during exploration right up to the time of fixation is effected, therefore, by a different mechanism from fixation itself. Fixation is generally assumed to result from an outpouring of cement material from the cement glands situated posteriorly to the compound eyes, although there is no proof of this, nor that the cement gland secretion alone is sufficient. The secretion from the antennular glands may serve for temporary attachment, and it may also catalyse the hardening of the cement gland secretion.

### RECOGNITION OF THE MOLECULAR NATURE OF THE SUBSTRATE

Suggestions can now be made, relating the structure of the antennule to the mechanism of the gregarious response of barnacle cyprids. Knight-Jones (1953) showed that adult barnacle shells and bases promoted settling of cyprids and further showed that the chemical properties of the active substance were consistent with those of quinone-tanned proteins which are known to form the epicuticle and attachment cement of barnacles. Knight-Jones suggested two possible mechanisms for the chemical basis for recognition of these substances; first, the secretion of an enzyme capable of attacking the cuticle and releasing recognizable products and, secondly, the recognition by the cyprid of surface patterns on the cuticle.

Crisp & Meadows (1962) have shown that cyprids respond to adsorbed layers of a specific protein extracted from adult barnacles. This protein has not yet been isolated, but Crisp & Meadows identified it with the integumentary proteins or arthropodin, this being the term used by Fraenkel & Rudall (1947). Cyprids prefer to explore and settle on slate panels which have been left for several hours in a weak solution of arthropodin and which have presumably acquired an adsorbed layer, rather than on untreated panels. Crisp (1965) showed that only a few molecular layers are involved and concluded that the structure of the surface layers of molecules must be recognized, as cyprids do not respond to arthropodin when it is in solution. Crisp & Meadows (1963) suggested that the recognition is essentially tactile and that the specificity resides in forces acting between the antennule and the surface; if the surface of the antennular disk is strongly and specifically attracted by the molecular configuration of the surface of physically or chemically bound arthropodin, then the cyprid might be able to detect differences in adhesion. However, this is probably not the operative mechanism, for although these molecular surfaces might be present under experimental conditions, such 'clean' surfaces do not exist in the sea where the gregarious response is operative. Bacterial slimes and other material quickly cover objects exposed to sea water so the exterior cuticle of the adult barnacle would be covered normally with extraneous matter which would interfere with such a 'tactile chemical sense' proposed by Crisp & Meadows (1963). Also, if a sticky secretion is used to enable the disk to attach temporarily to the substratum from which it separates by breaking the cohesive bonds within the secretion, it would not seem possible for the cyprid to form or test other specific, adhesive bonds between the disk and the surface.

In the antennular attachment organ there are sensory structures that appear to be organized to act as chemoreceptors and, furthermore, the axial sense organ is ideally placed for the perception of substances under the attached disk. It is suggested, therefore, that the recognition of specific compounds is dependent, like a normal chemosensory response, on the contact between a stimulating molecule and the distal sensory processes of certain sensory cells. A specific protein molecule as such is probably not recognized as Crisp & Meadows (1963) found that the presence of arthropodin in solution does not lead to settlement behaviour. However, responses by chemoreceptors to amino acids are well documented (in crustacea by Hodgson 1958; Laverack 1963, 1964) and it is possible that the protein of the integument is broken down by enzymatic action and that one or more of the soluble derivatives are responsible for stimulating the chemosensory organs, as suggested by Knight-Jones (1953). The antennular glands discharge on to the surface of the disk and may produce an enzyme. The presence of superficial, extraneous material on the integument of adult barnacles and other surfaces is less limiting to this theory than to one assuming direct contact between receptor and substrate.

This chemosensory mechanism could explain why the cyprid does not respond to protein extracts in solution. When swimming in a dilute solution of arthropodin, the products of any enzyme activity would disperse away from the chemosensory hairs. It can be calculated that, when the cyprid is attached to the substrate, there would be at least 100 times less arthropodin available for breakdown in the volume of fluid beneath the disk, than in an adsorbed film. Electron micrographs of attached cyprids show the volume of fluid beneath unit area of the disk to be not greater than $2.5 \times 10^{-5}$ ml. In an experiment quoted by Crisp & Meadows (1963) the concentration of arthropodin was 0.05 mg/ml, so that the weight of arthropodin beneath the disk would be of the order of $1.25 \times 10^{-6}$ mg/cm$^2$. This contrasts with a coherent protein monolayer having a surface density of about $10^{-4}$ mg/cm$^2$. The concentration of products released from a monomolecular film might be sufficiently greater, therefore, to elicit a response from an adsorbed film but not from a volume of solution.

After recognizing a chemically suitable substrate for settlement the cyprid selects a position for the fixation of the antennules. The manner in which the annulated setae curve towards the substrate when the cyprid is attached, may enable the surface texture and other irregularities of the site to be recognized.

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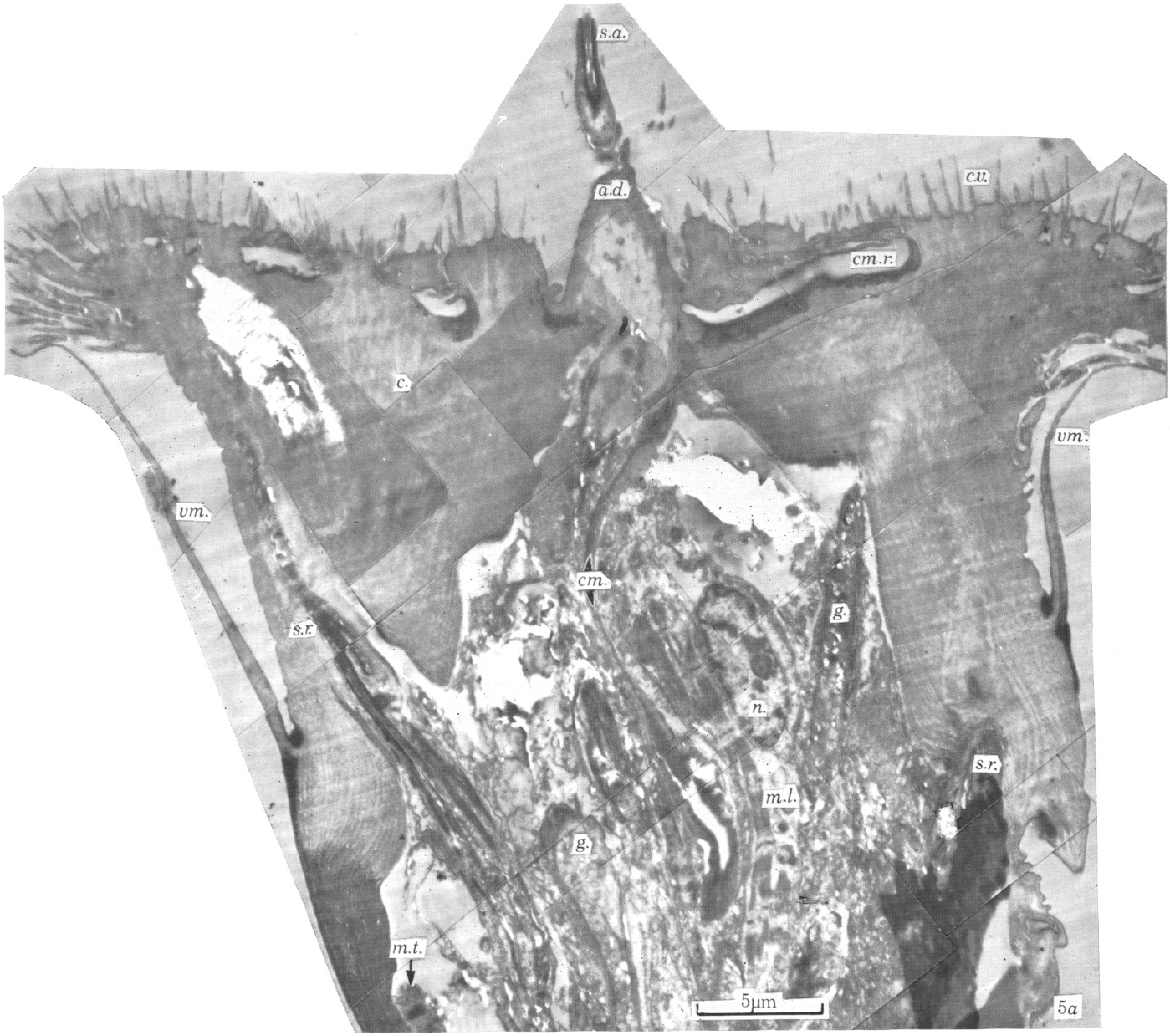
## KEY TO ABBREVIATIONS USED IN THE FIGURES

| | | | |
|---|---|---|---|
| *a.* | attachment disk | *m.t.* | transverse muscle |
| *a.d.* | axial dome | *mic.* | microtubules |
| *a.r.* | rudiment of attachment disk | *mit.* | mitochondrion |
| *c.* | cuticle | *n.* | nucleus |
| *c.c.* | canal in cuticle | *p.g.* | pore of antennular gland |
| *c.h.* | cuticular hair | *post.c.s.* | postaxial setae of cyprid |
| *c.h.b.* | base of cuticular hair | *post.n.s.* | postaxial setae of nauplius |
| *c.p.* | cuticular peg | *pre.c.s.* | preaxial setae of cyprid |
| *c.t.* | cuticular tube | *pre.n.s.* | preaxial setae of nauplius |
| *c.v.* | cuticular villi | *s.a.* | axial sense organ |
| *cil.* | cilium | *s.p.* | postaxial sense organ |
| *cil.b.* | base of cilium | *s.r.* | radial sense organ |
| *cil.p.* | ciliary process | *sec.* | secretion in antennular gland |
| *cil.r.* | ciliary root | *sen.d.* | distal sensory process |
| *cm.* | cement duct | *set.* 1 | preaxial seta |
| *cm.r.* | radial canal of cement duct | *set.* 2 | postaxial seta |
| *con.* | connective tissue | *sh.c.* | sheath cell |
| *den.* | dendrite(s) | *sp.r.* | supporting rods |
| *den.* IV | dendrites to the fourth segment | *sp.t.* | supporting tube |
| *ex.* | extracellular space | *t.* | circular band of thickening |
| *g.* | antennular gland | *t.c.s.* | terminal setae of cyprid |
| *g.d.* | antennular gland duct | *t.n.s.* | terminal setae of nauplius |
| *g.t.* | tube enclosing distal region of antennular gland | *v.* | vesicle |
| *m.l.* | longitudinal muscle | *vm.* | velum |



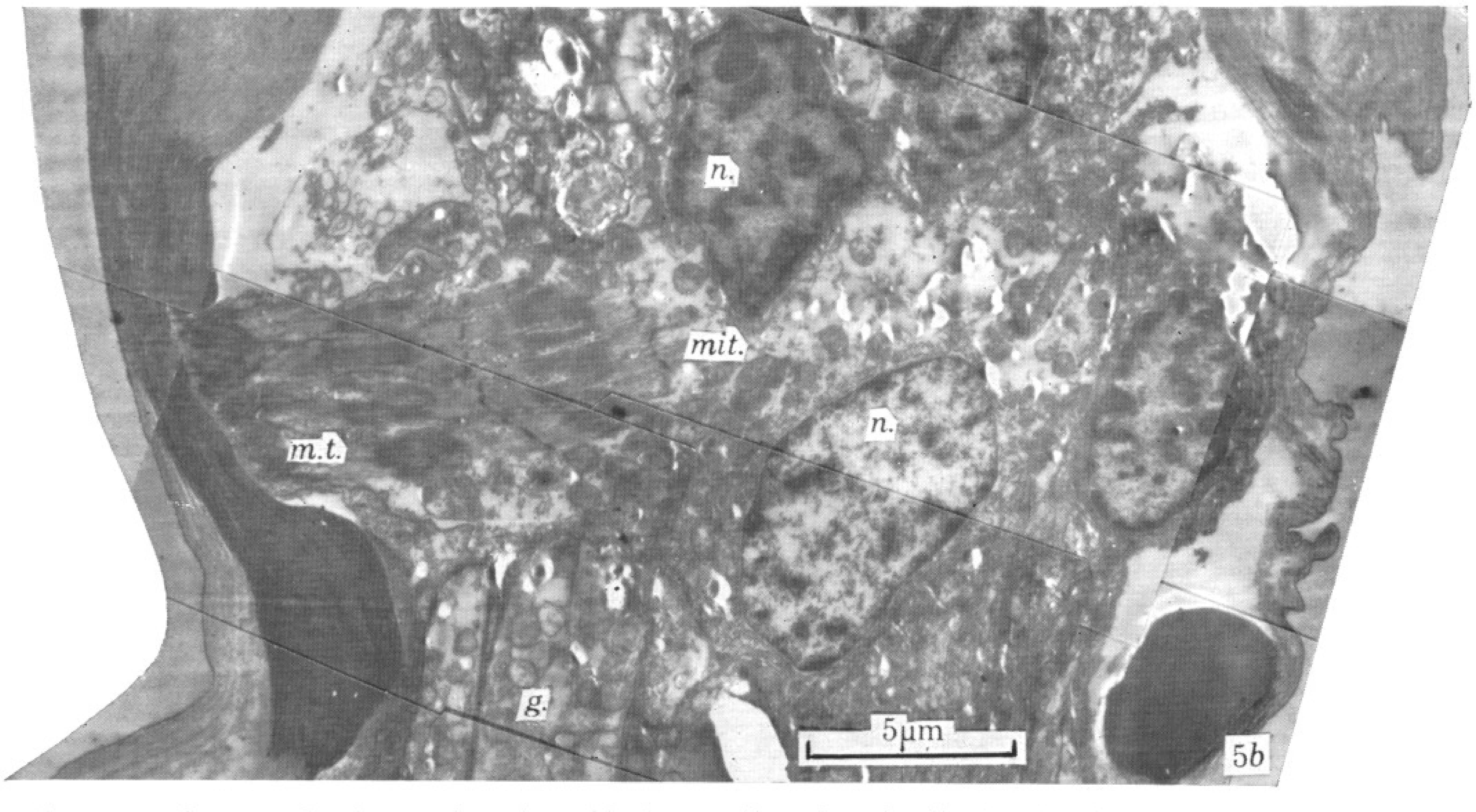
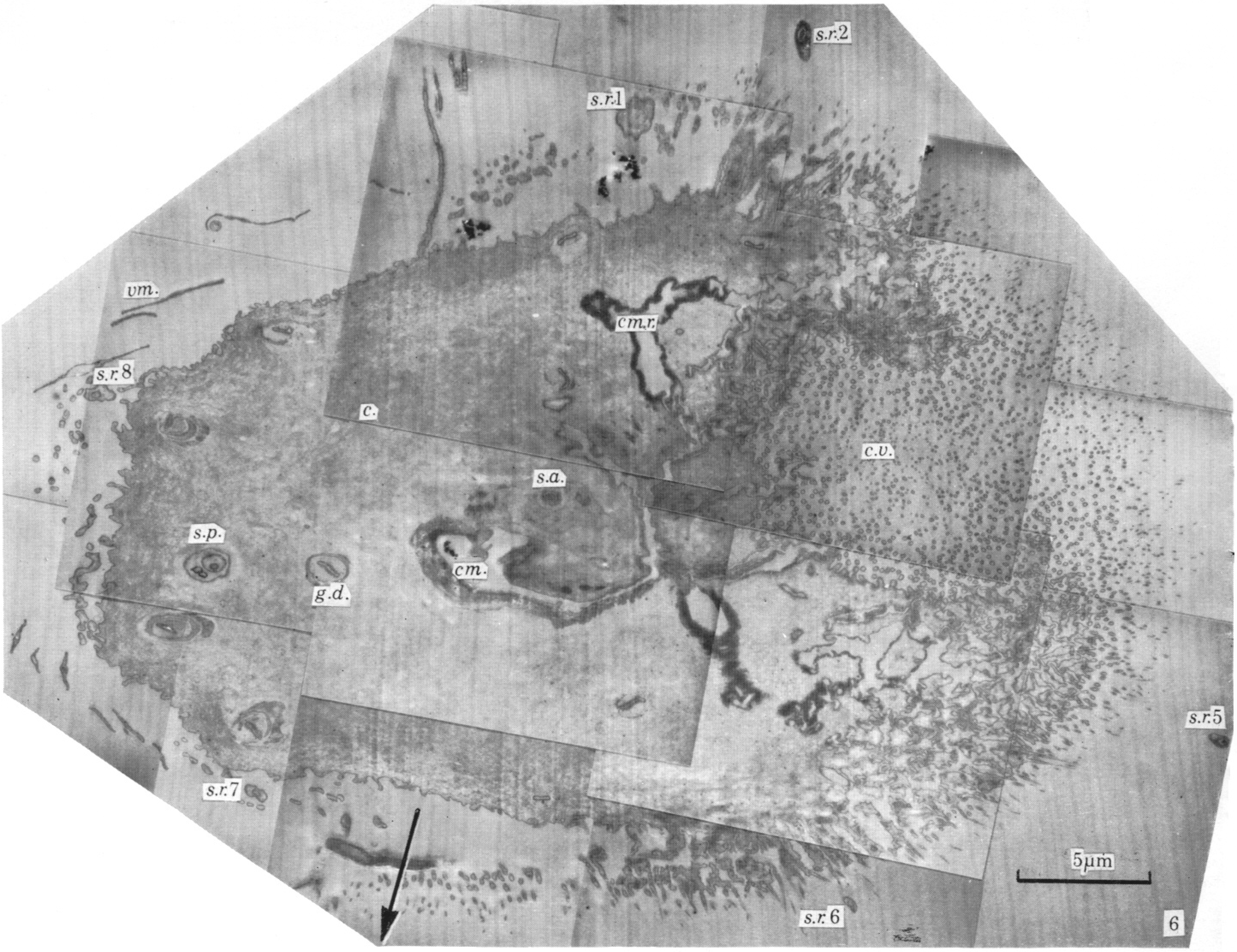


FIGURE 5*a*. Composite electron micrograph of a section through the median, longitudinal axis of the attachment organ of the third antennular segment.

FIGURE 5*b*. Similarly oriented section through the basal region of the third segment, with the transverse muscle crossing to the fourth segment out of the plane of the section to the right.



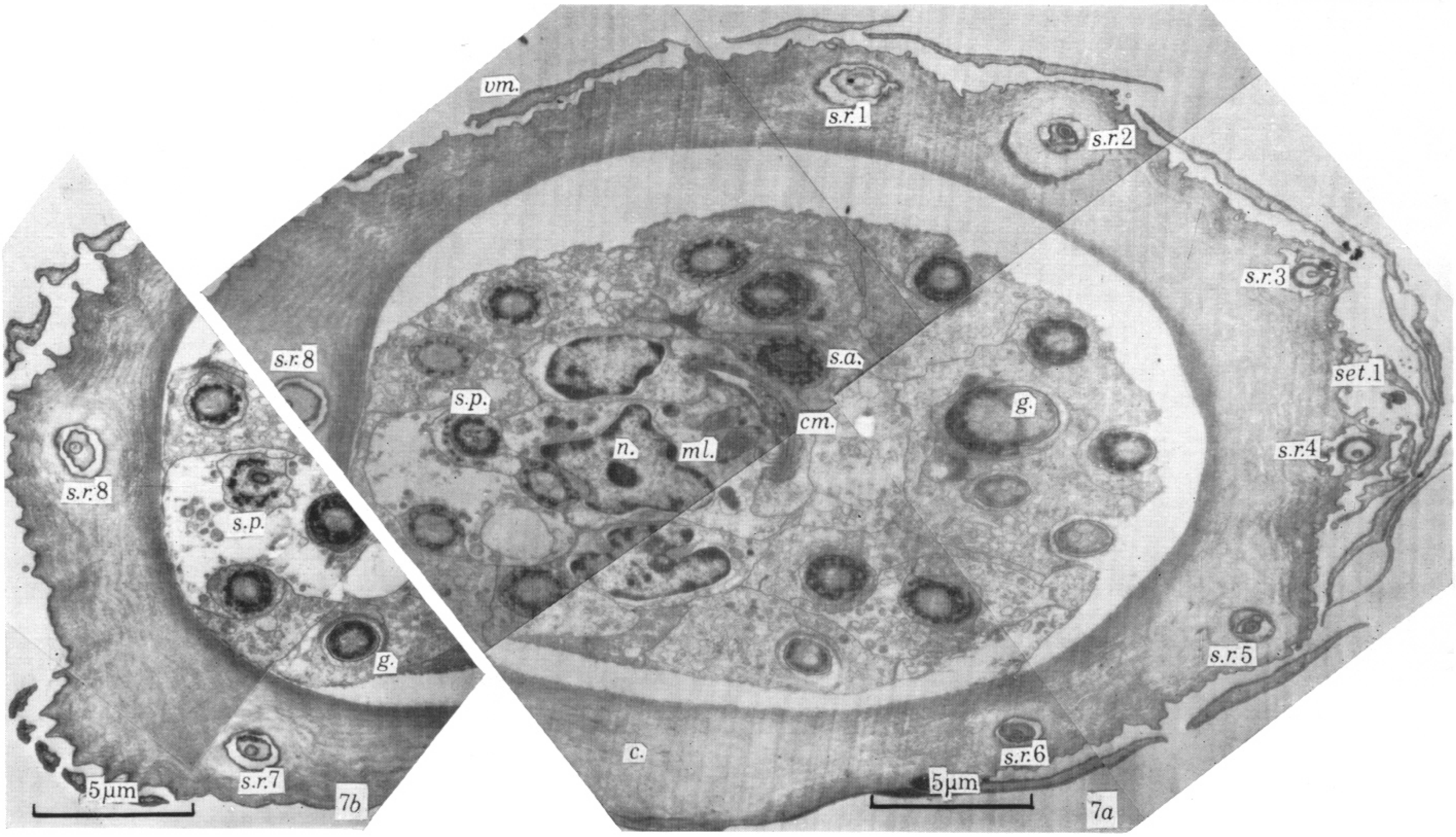
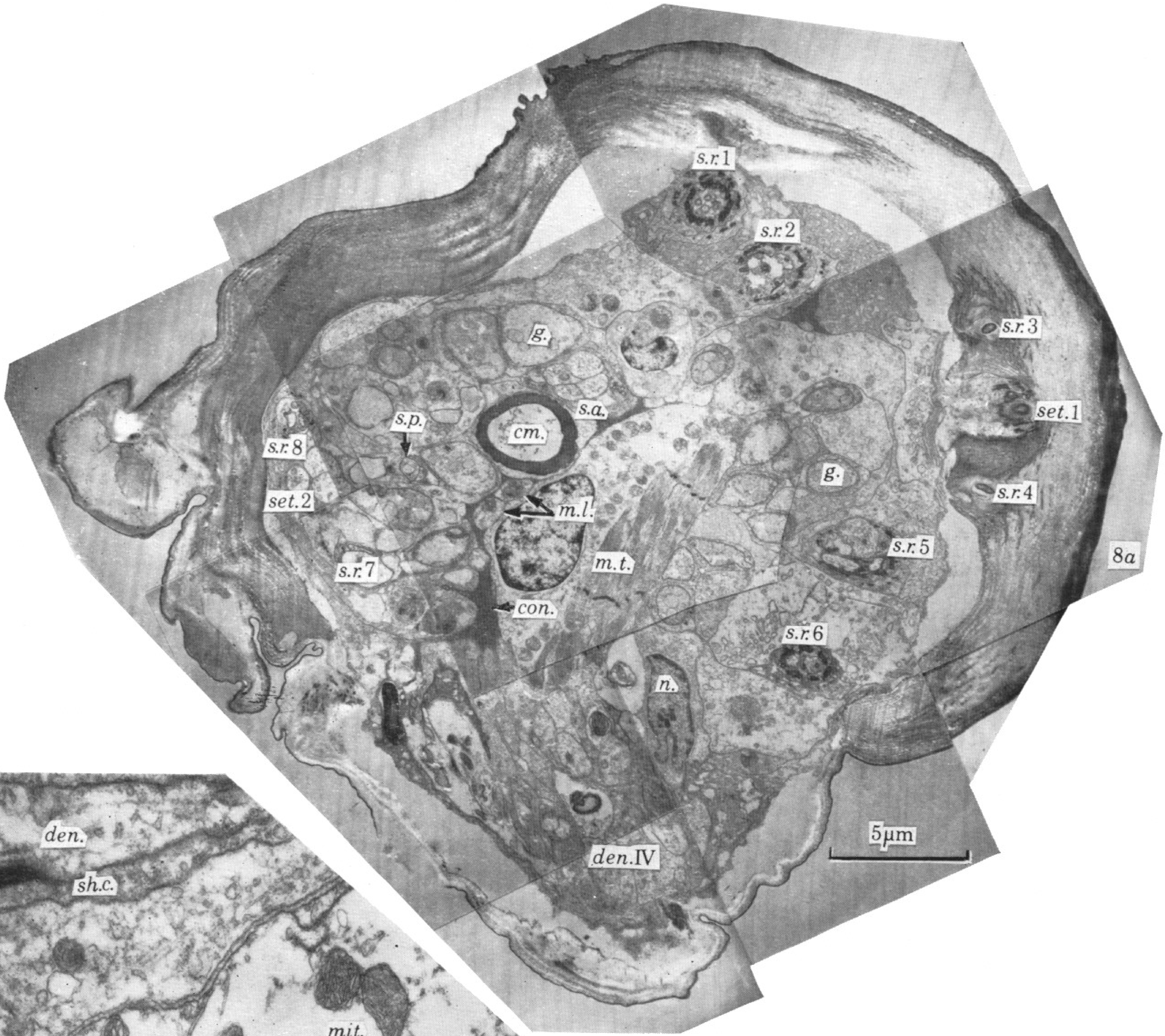
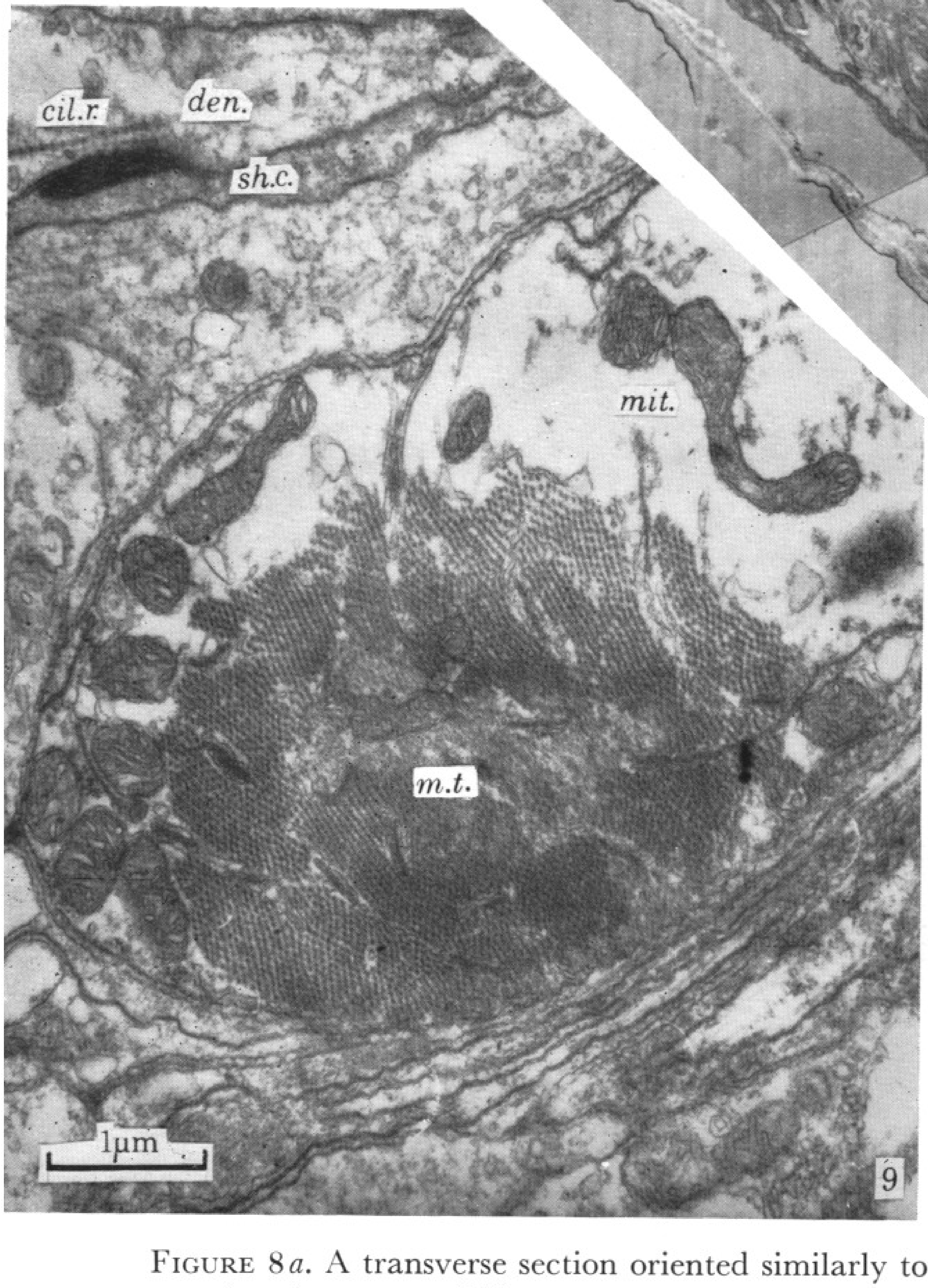


FIGURE 6. Composite electron micrograph of a transverse section (slightly oblique) through the distal region of the attachment organ. The fourth segment lies laterally in the direction indicated by the arrow. The postaxial side of the segment is to the left of the micrograph.

FIGURE 7*a*. Similarly oriented transverse section through the mid-region of the attachment organ.

FIGURE 7*b*. Transverse section taken distally from that of figure 7*a*, to which it is complementary.





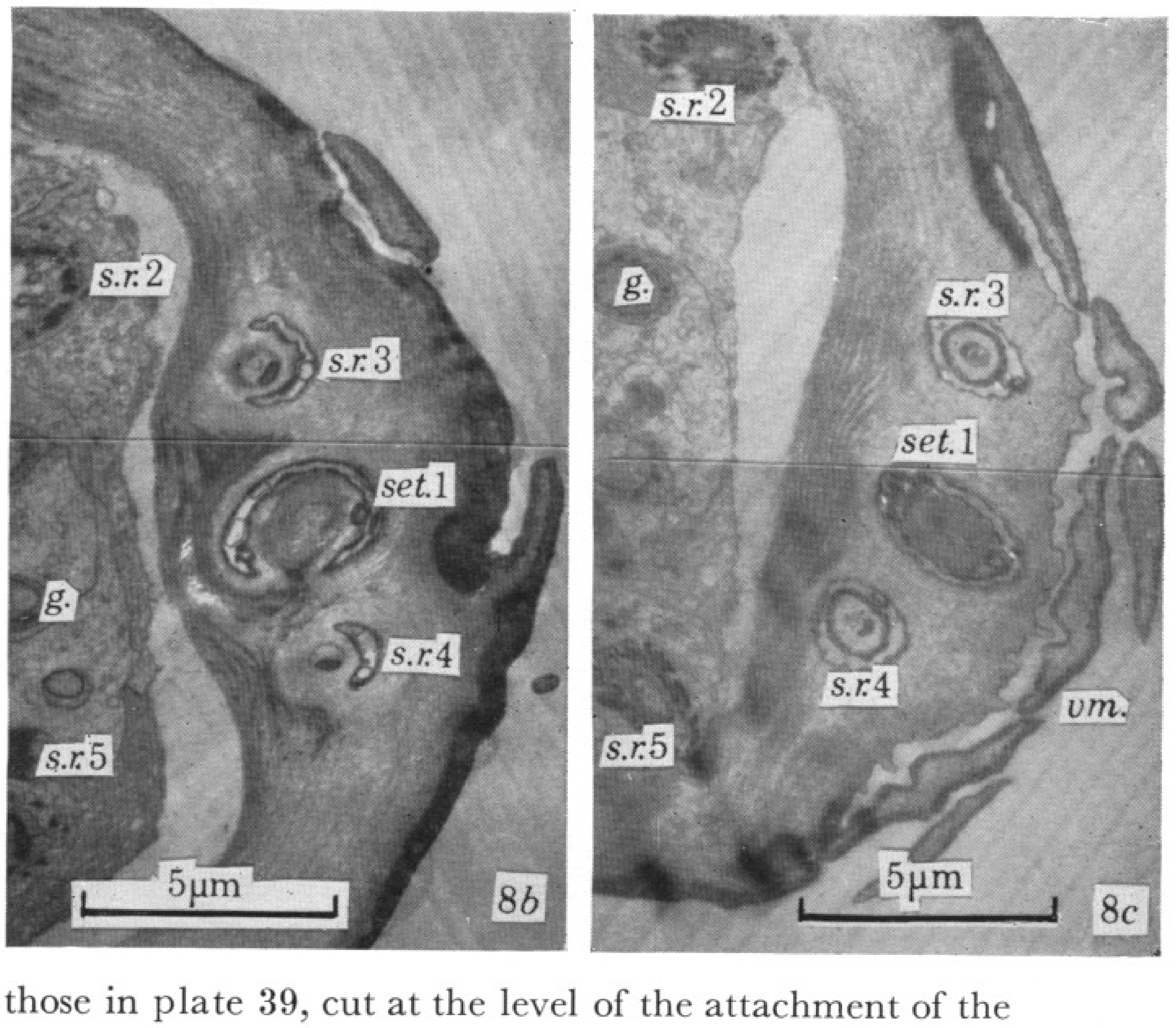
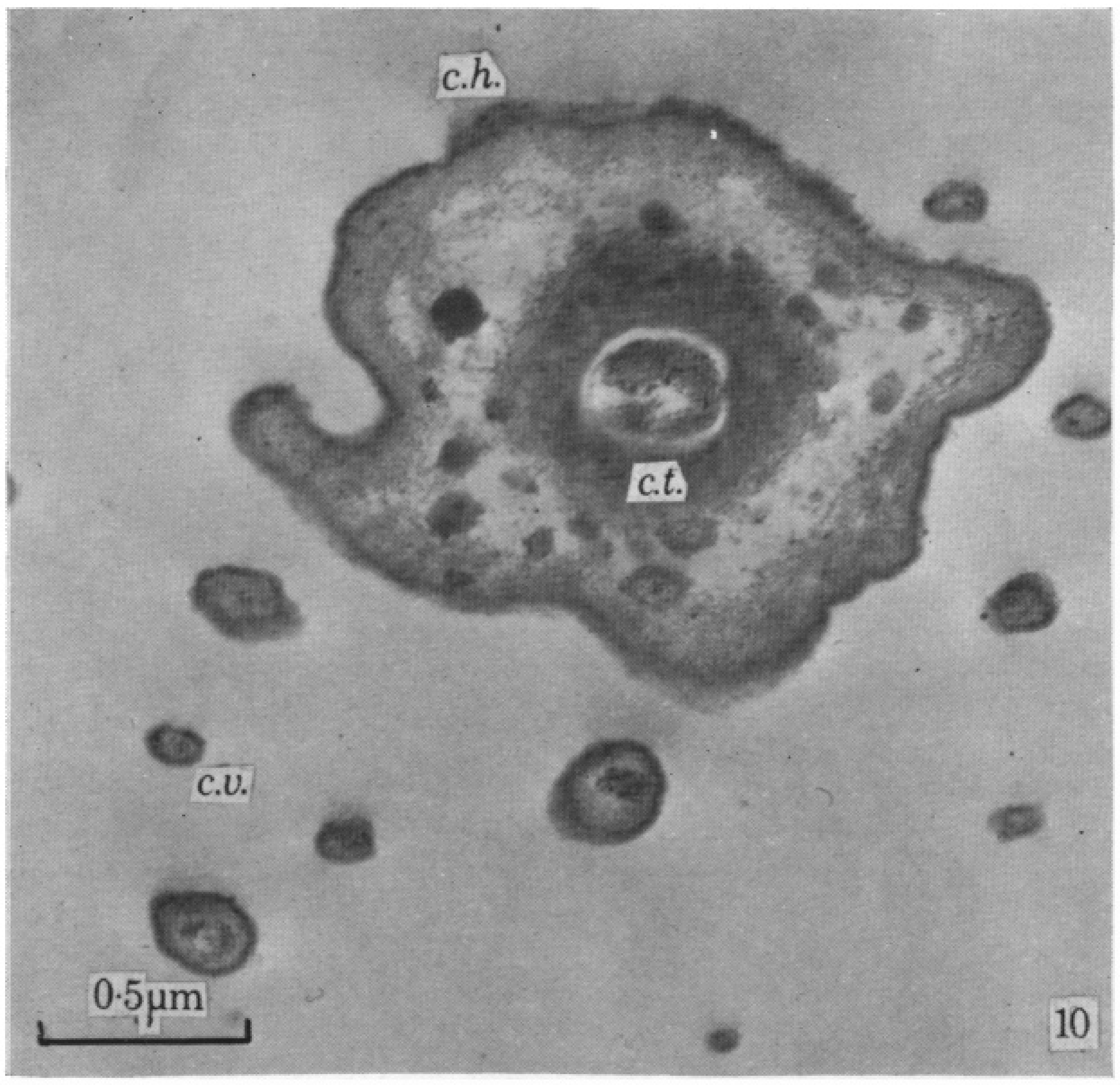
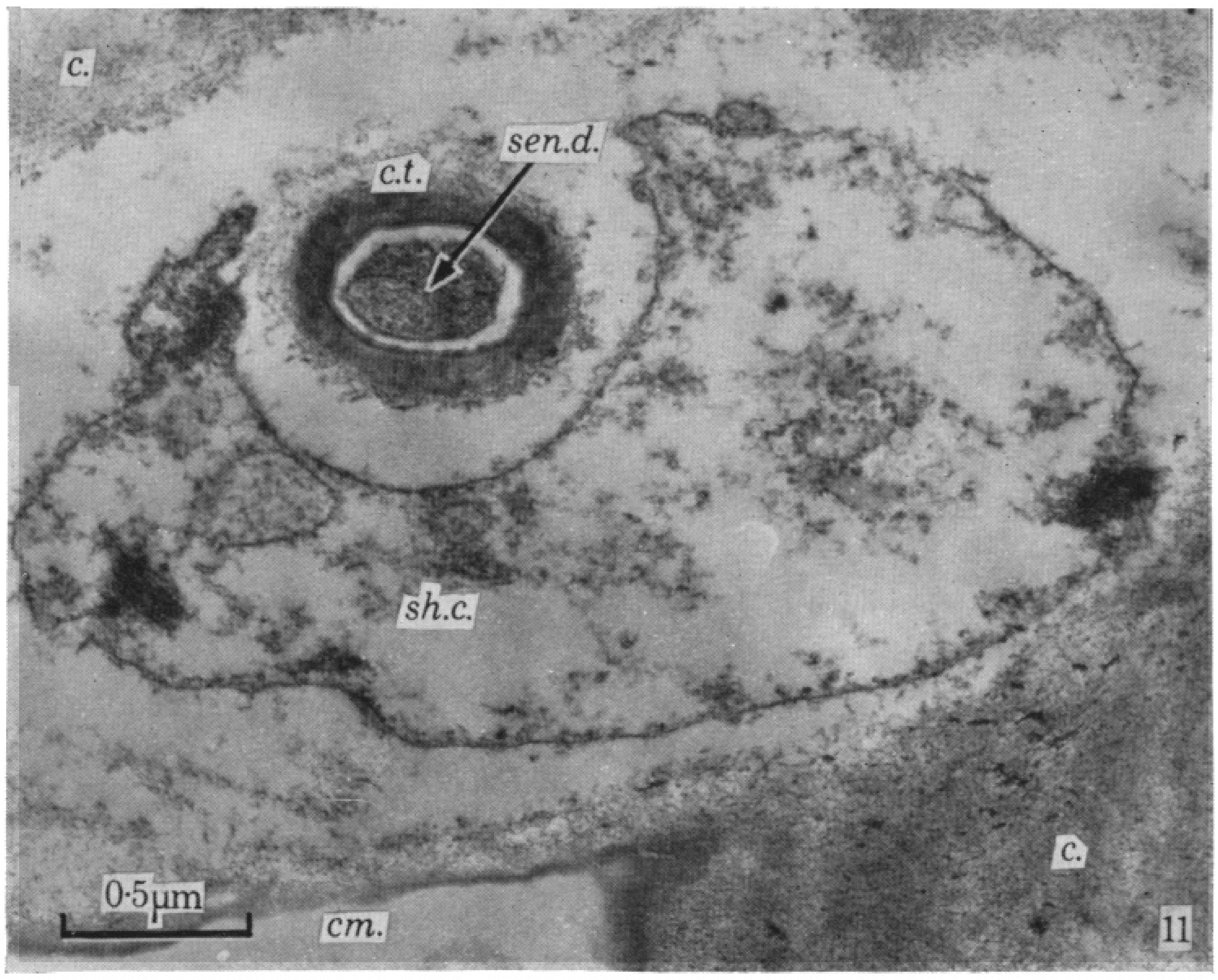


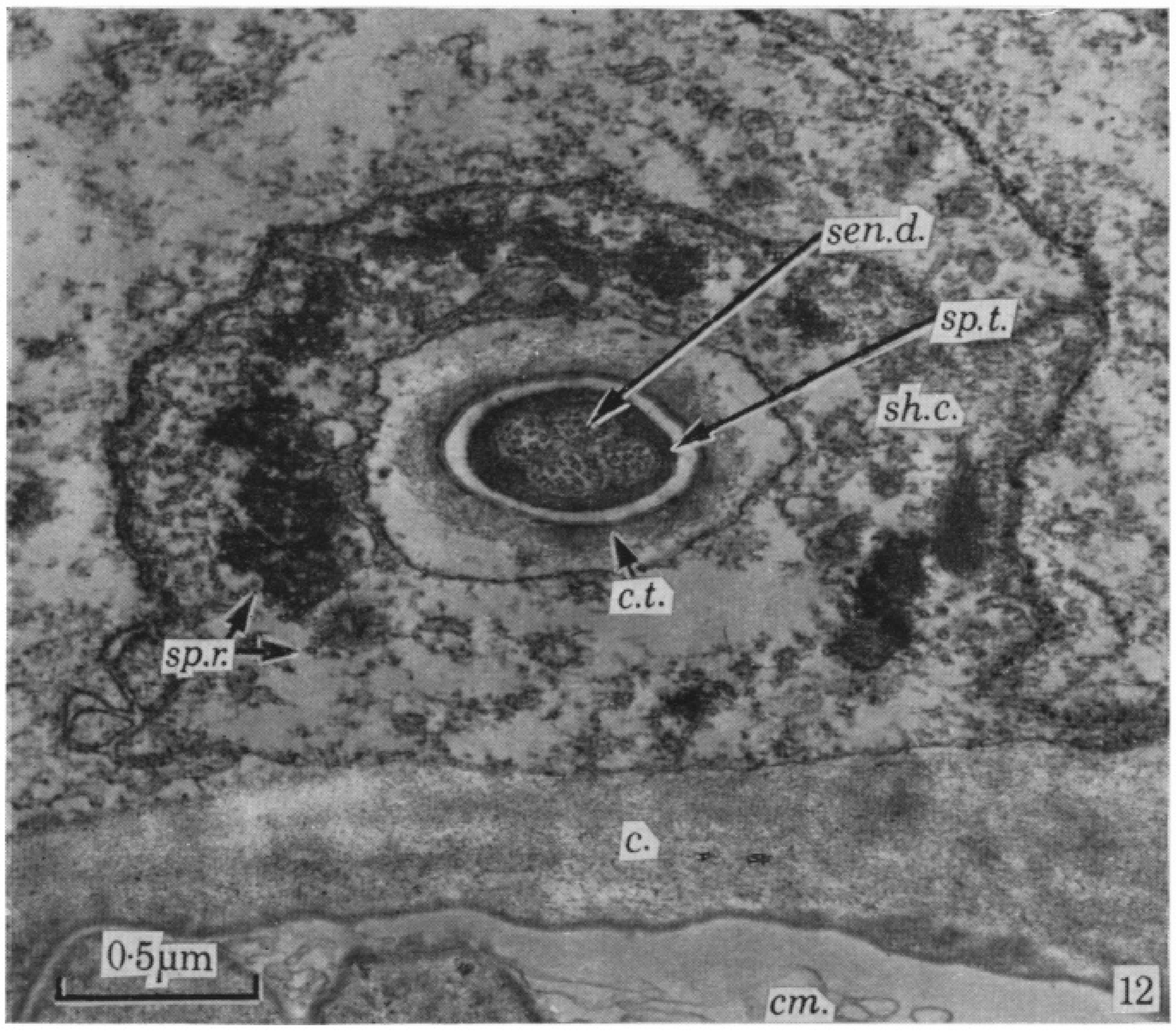
FIGURE 8*a*. A transverse section oriented similarly to those in plate 39, cut at the level of the attachment of the fourth segment (IV).

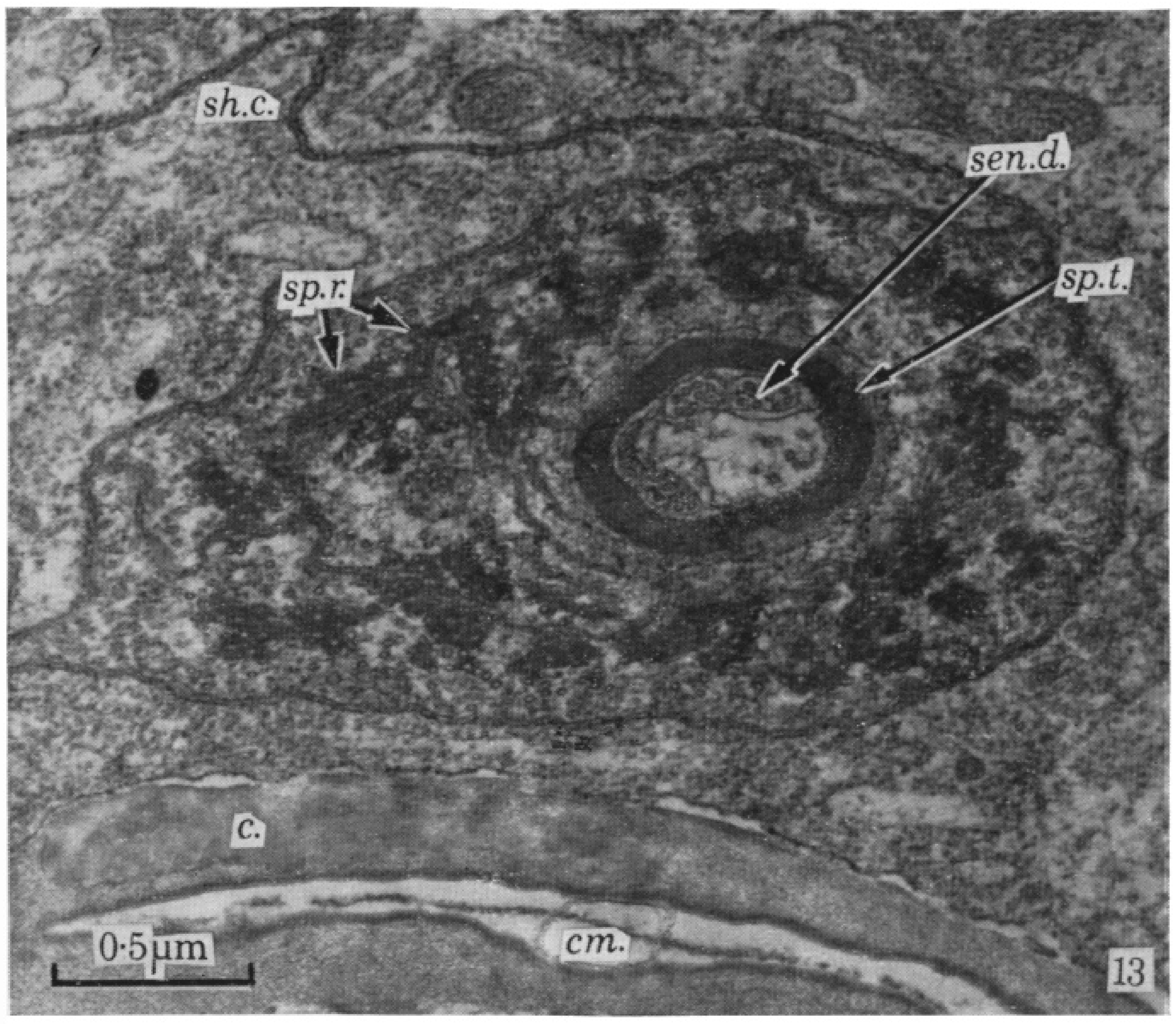
FIGURES 8*b*, 8*c*. Transverse sections cut distally to figure 8*a* and proximally to figure 7*a* to show the course of the sense organs on the preaxial side.

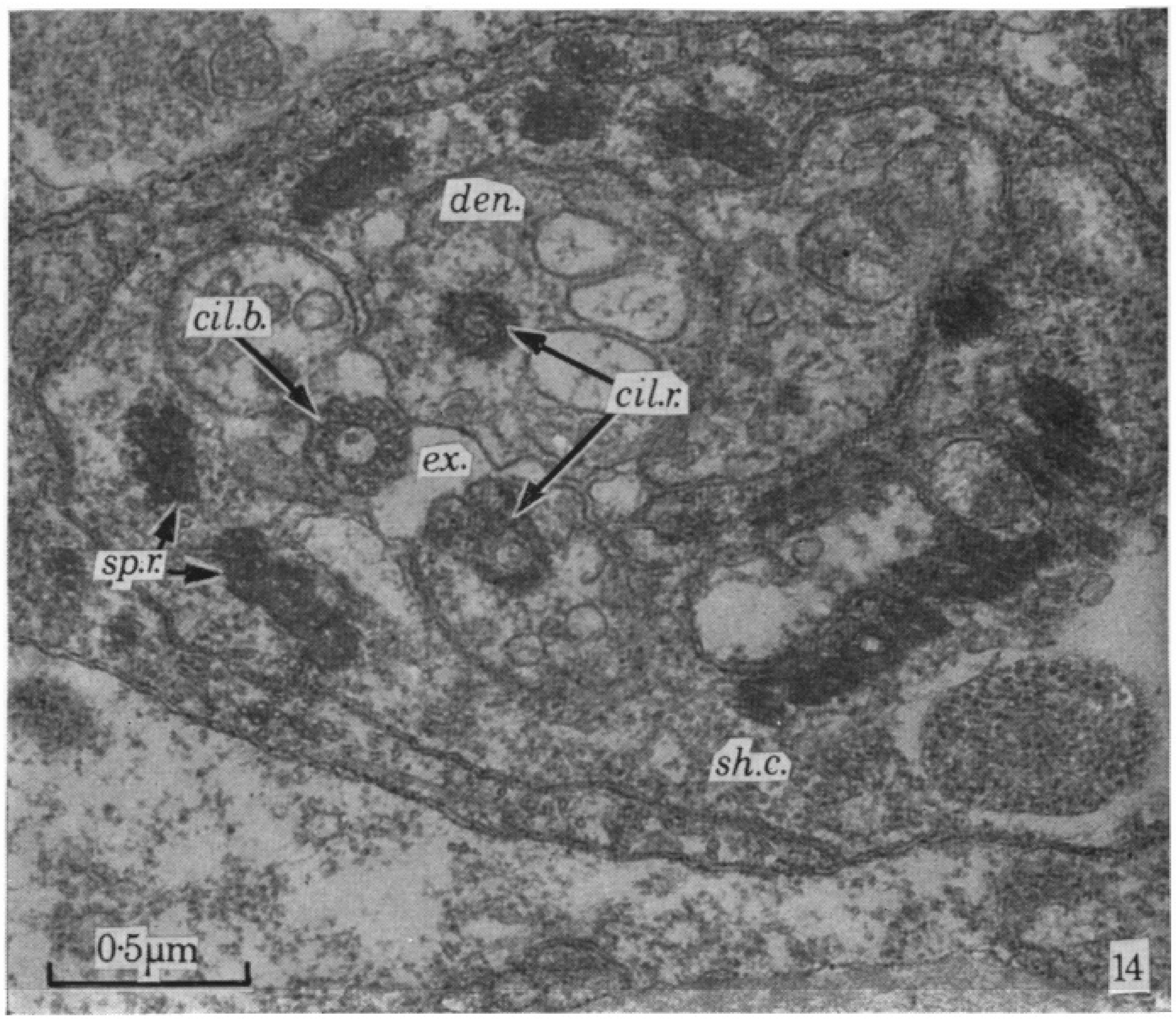
FIGURE 9. A longitudinal section of the third segment showing the transverse muscle in transverse section.

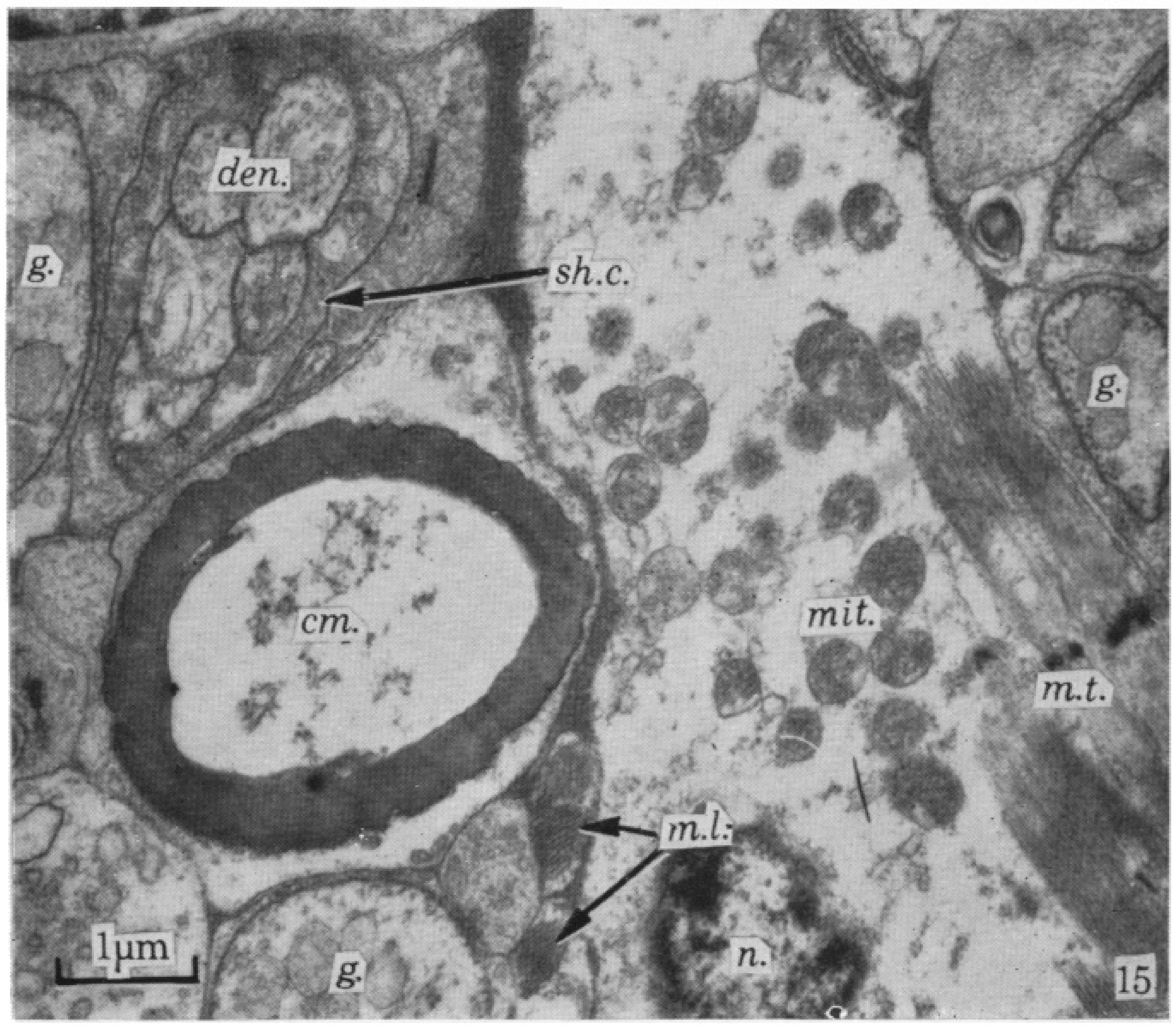




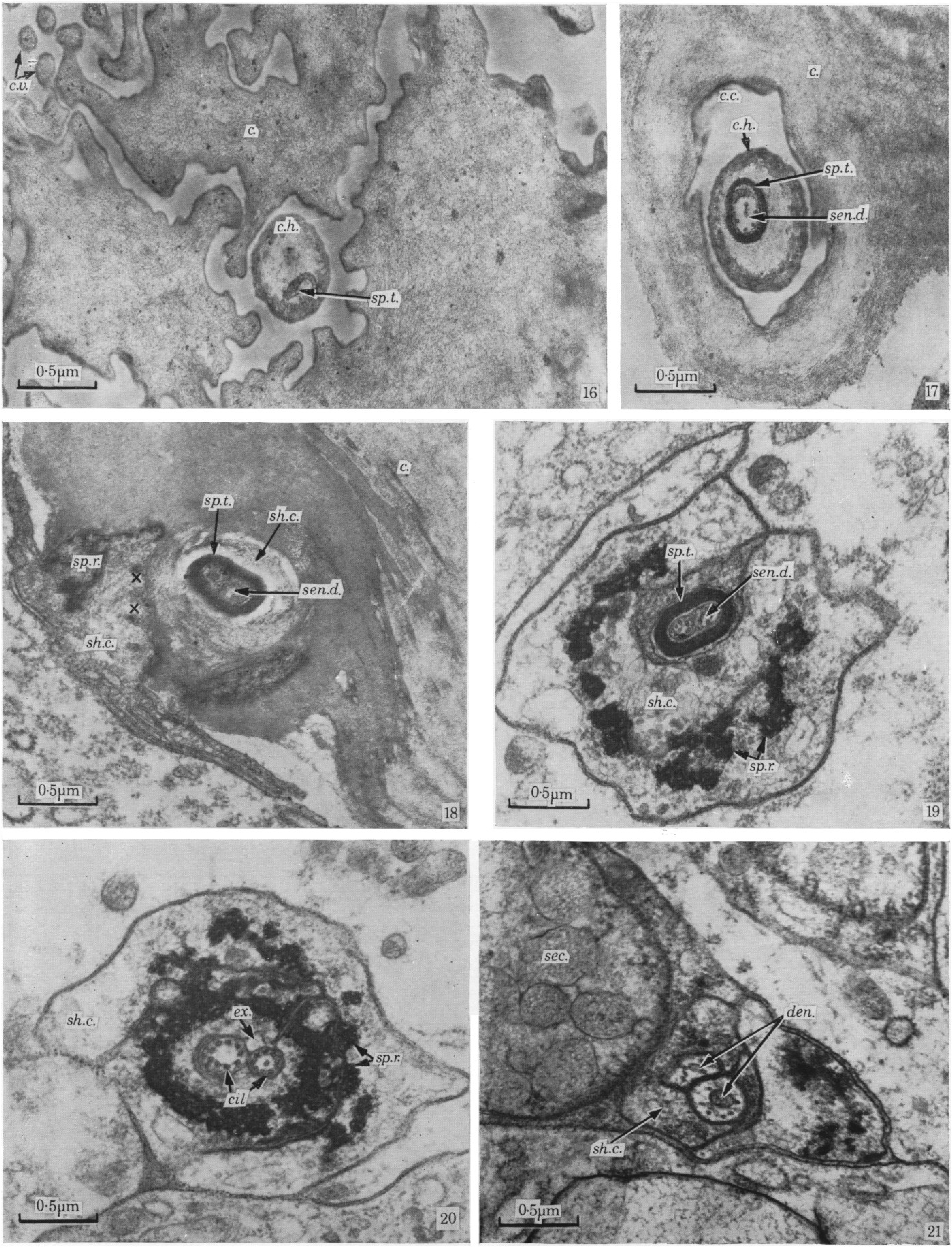




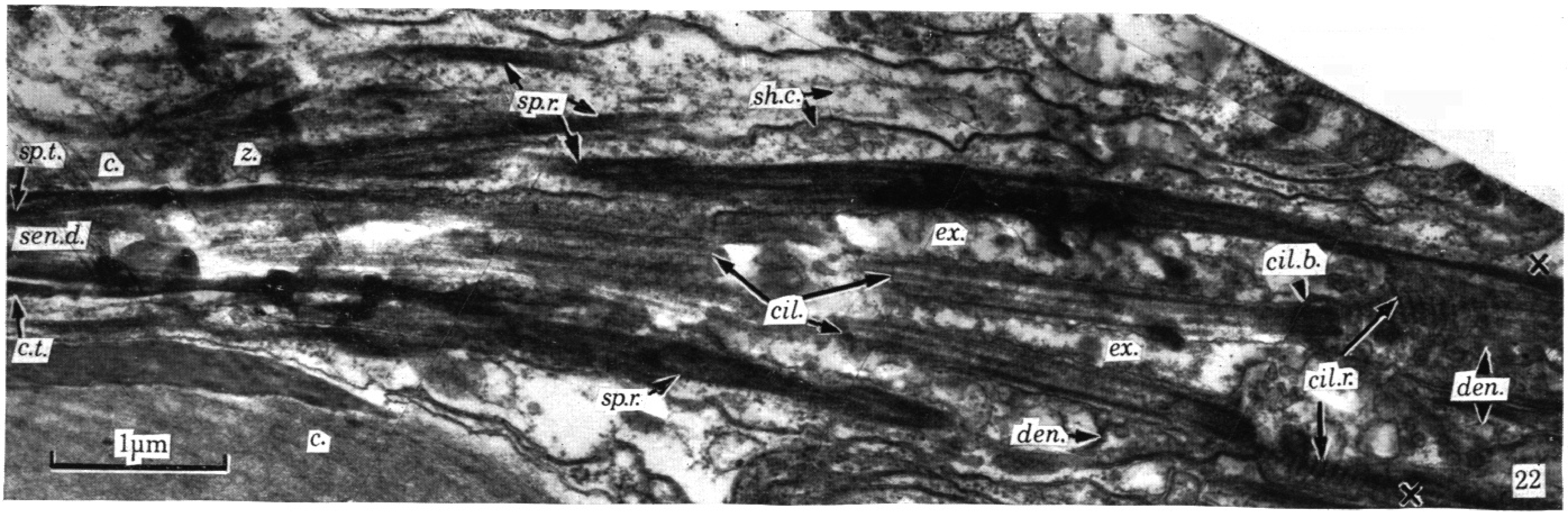


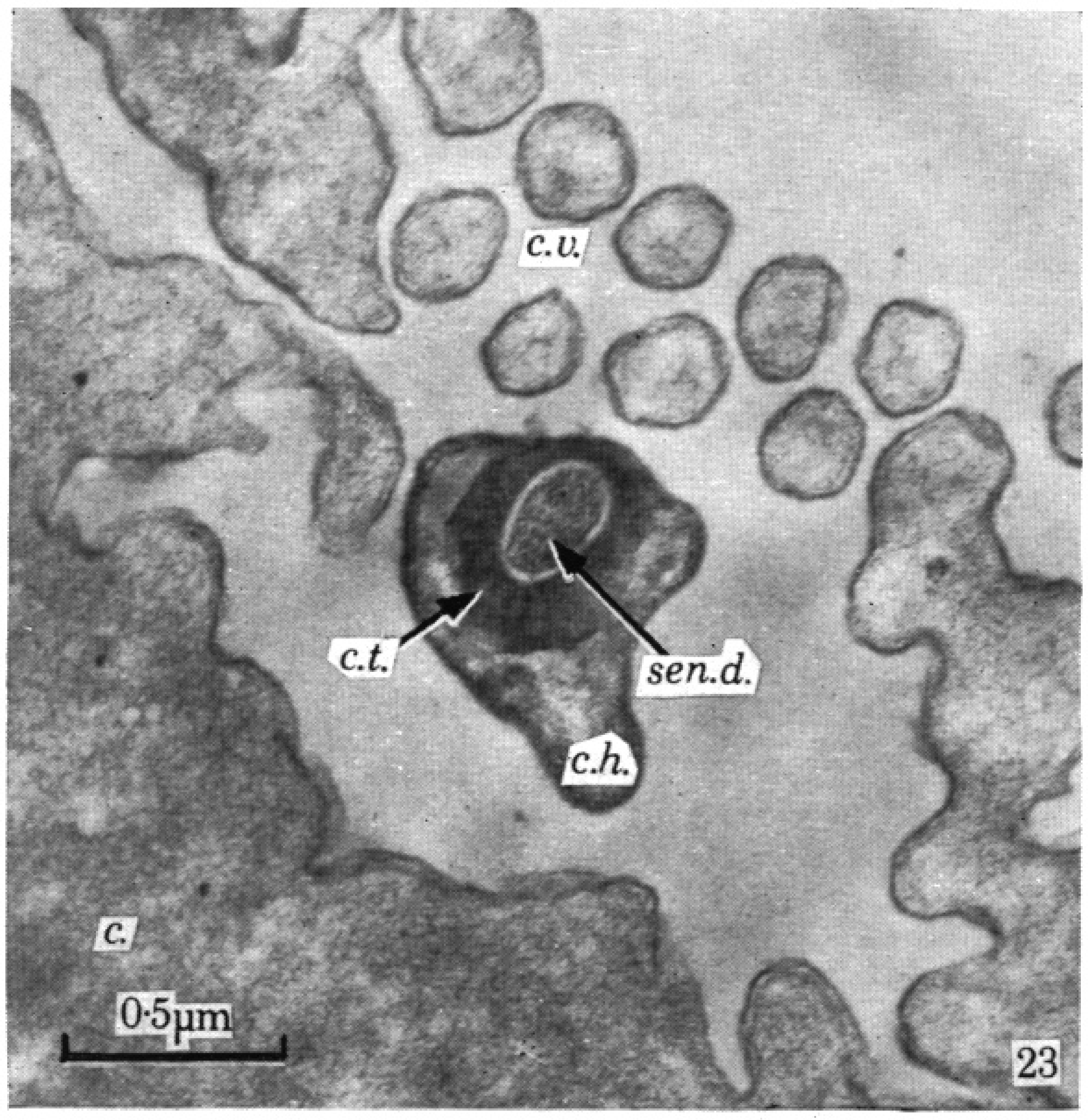


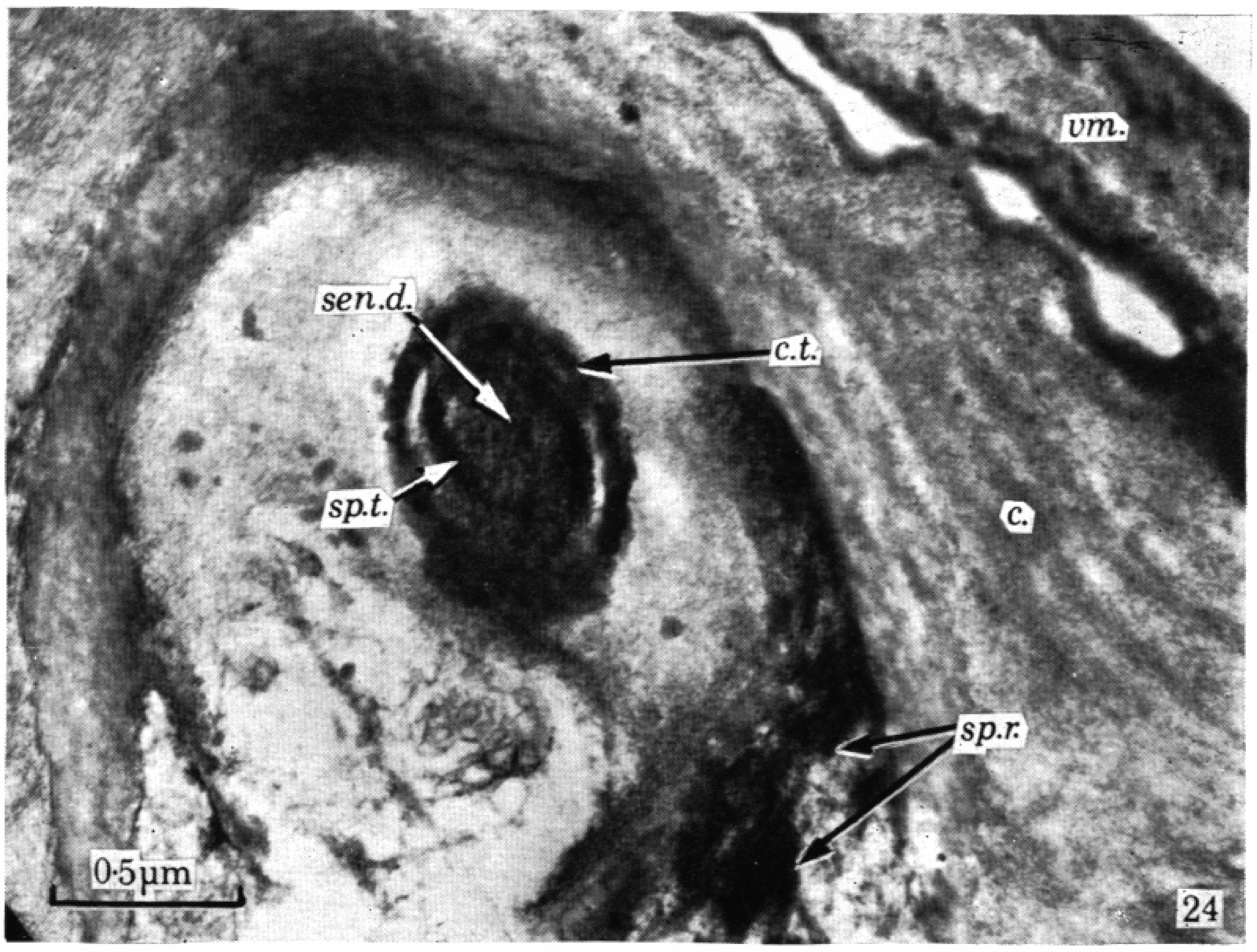
FIGURES 10 to 15. The axial sense organ shown in transverse sections from the cuticular hair in figure 10 to the dendrites in figure 15. Described in the text. Figure 15 at lower magnification to show the arrangement of other structures.

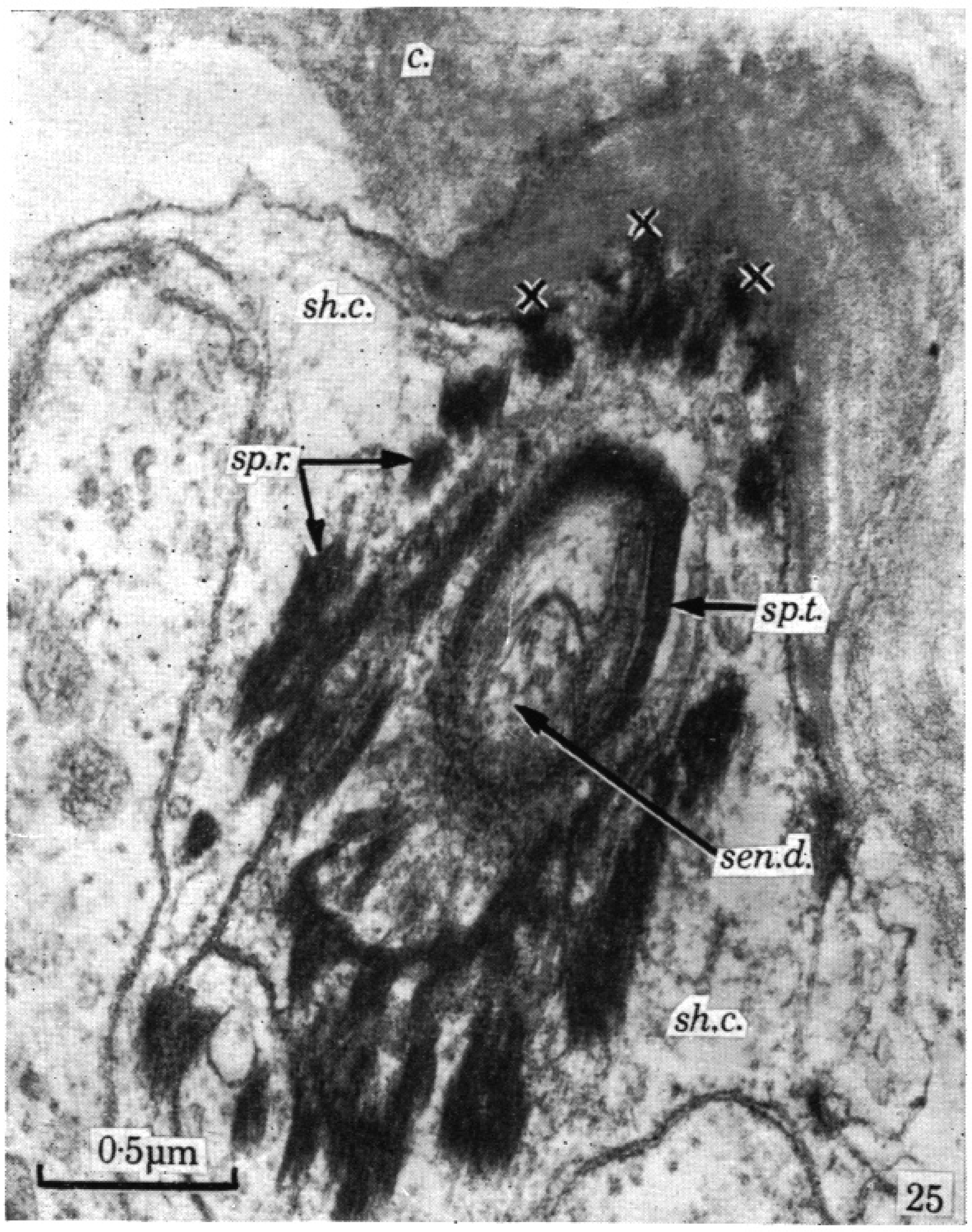


FIGURES 16 to 21. The postaxial sense organ in transverse sections, from the hair emerging from the attachment disk in figure 16 to the dendrites in figure 21. Described in the text.









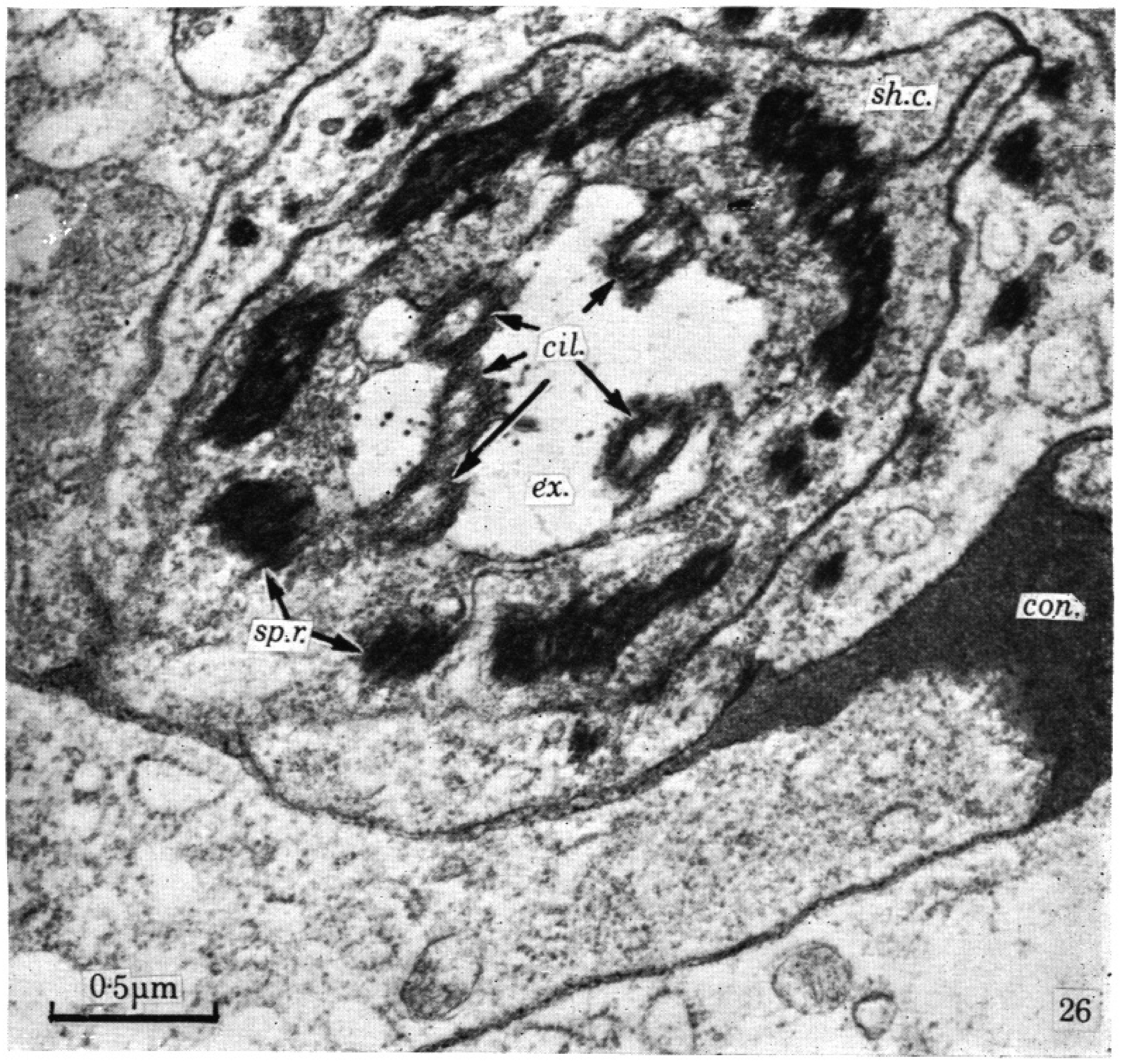
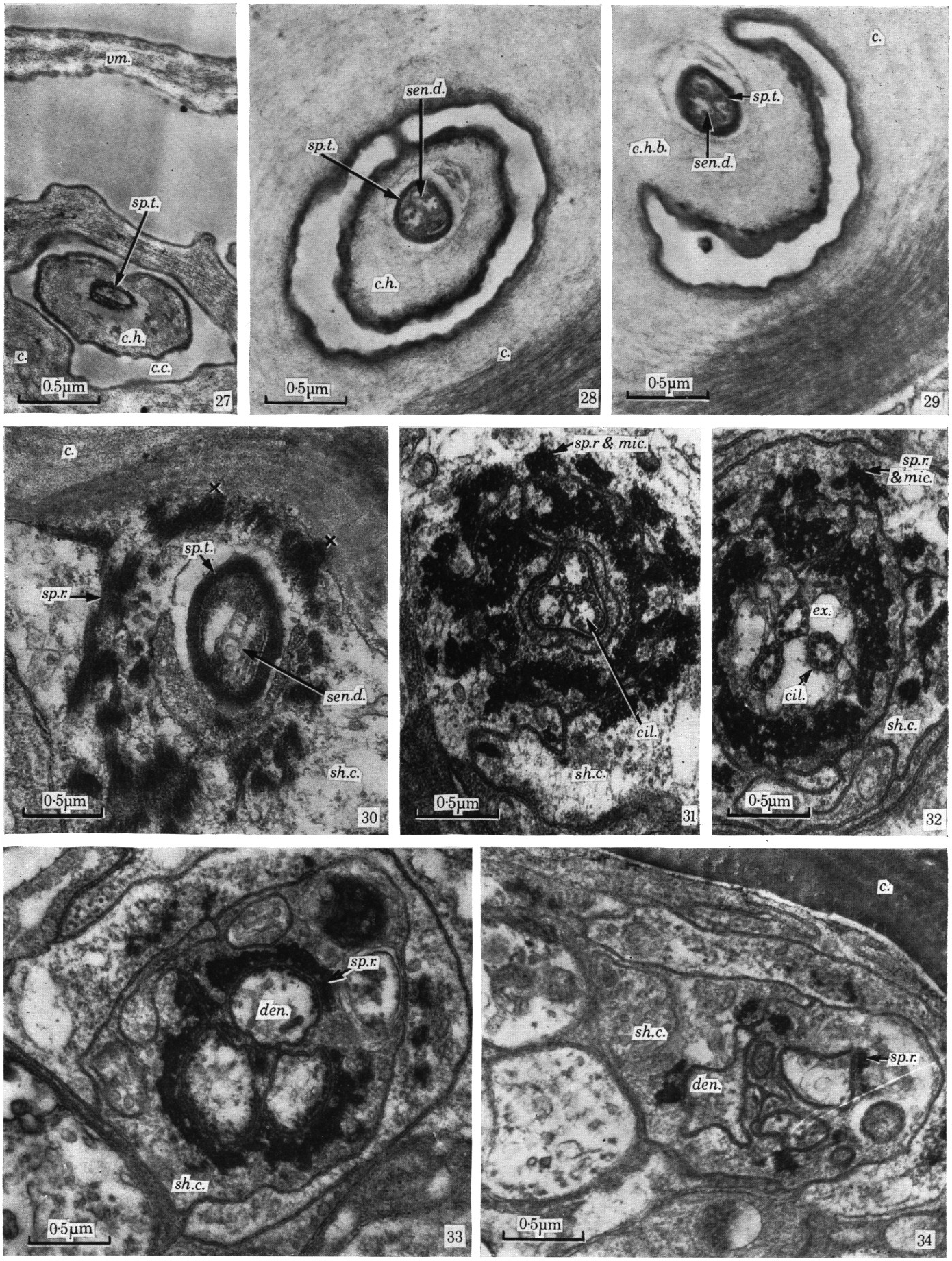
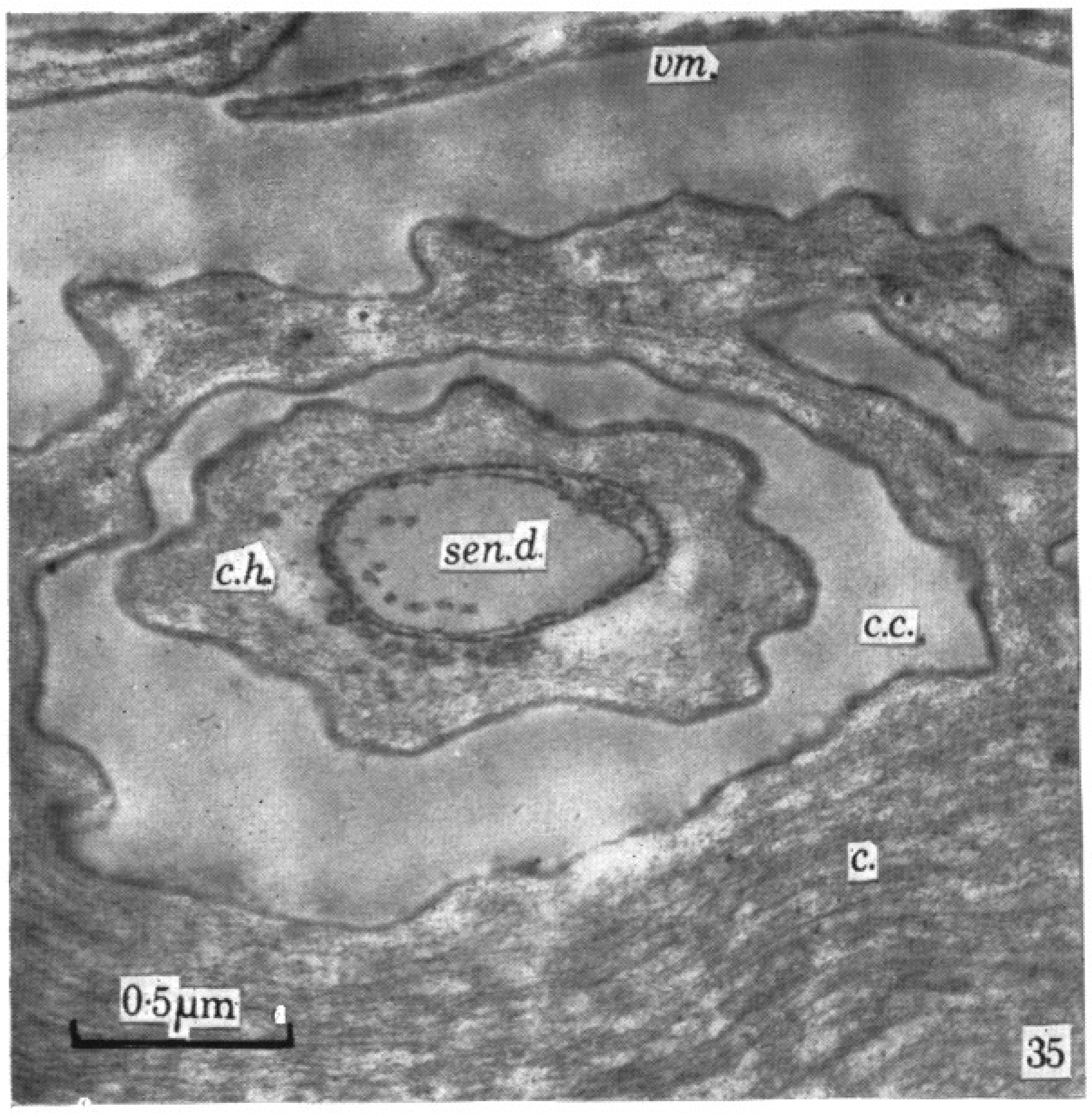


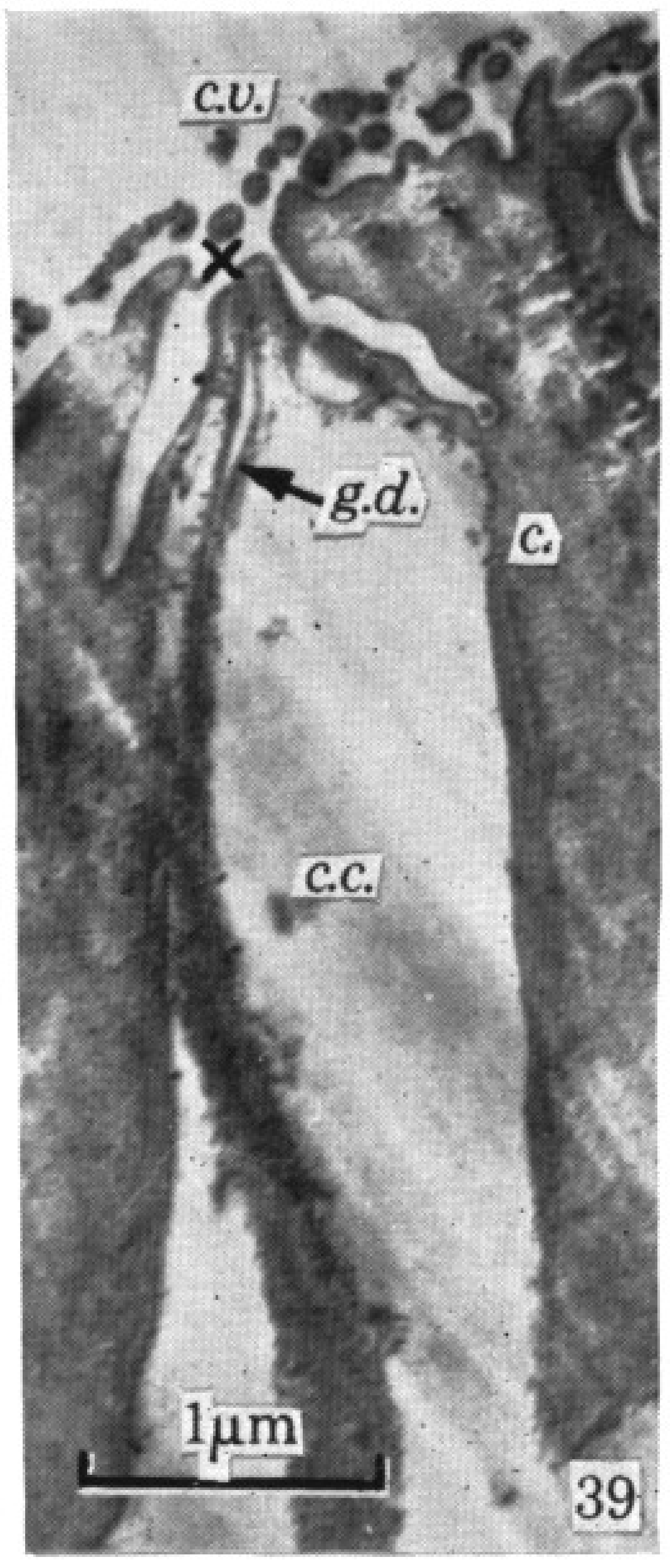
FIGURE 22. Longitudinal section of a radial sense organ showing the transition from dendrites to cilia and to distal sensory processes which enter the internal cuticular tube of the hair. In the proximal region the striated root of each cilium diverges from the axis of the sense organ towards the supporting rods, which approach the dendrites at '*x*'. The dendrites are in direct contact with each other. The supporting rods attach to the cuticle at '*z*'.

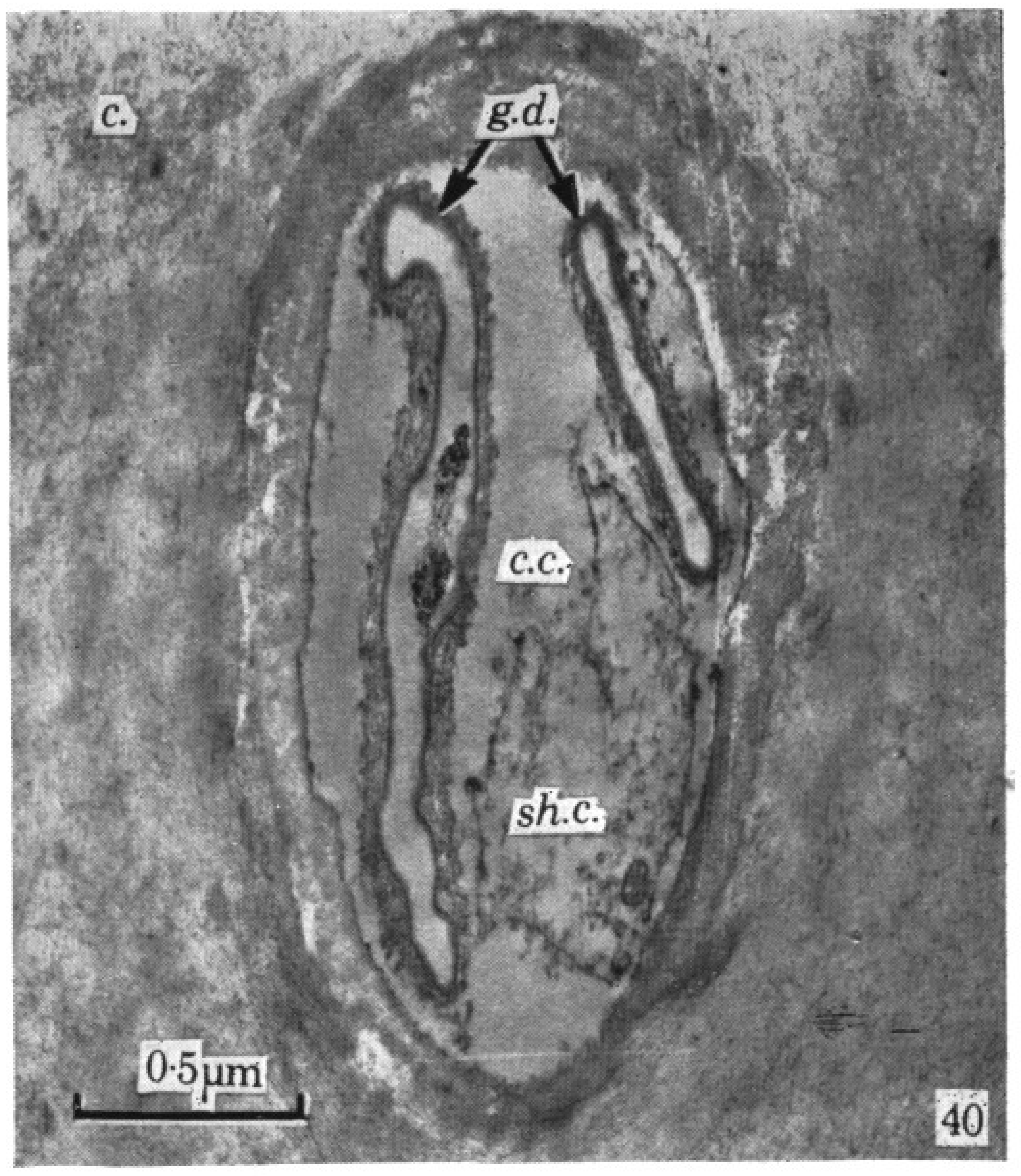
FIGURES 23 to 26. A radial sense organ with internal cuticular tube (*s.r.* 2 of plates 39 and 40) in transverse sections. In figure 25 the attachment points of supporting rods to the cuticle are marked '*x*'. Described in the text.

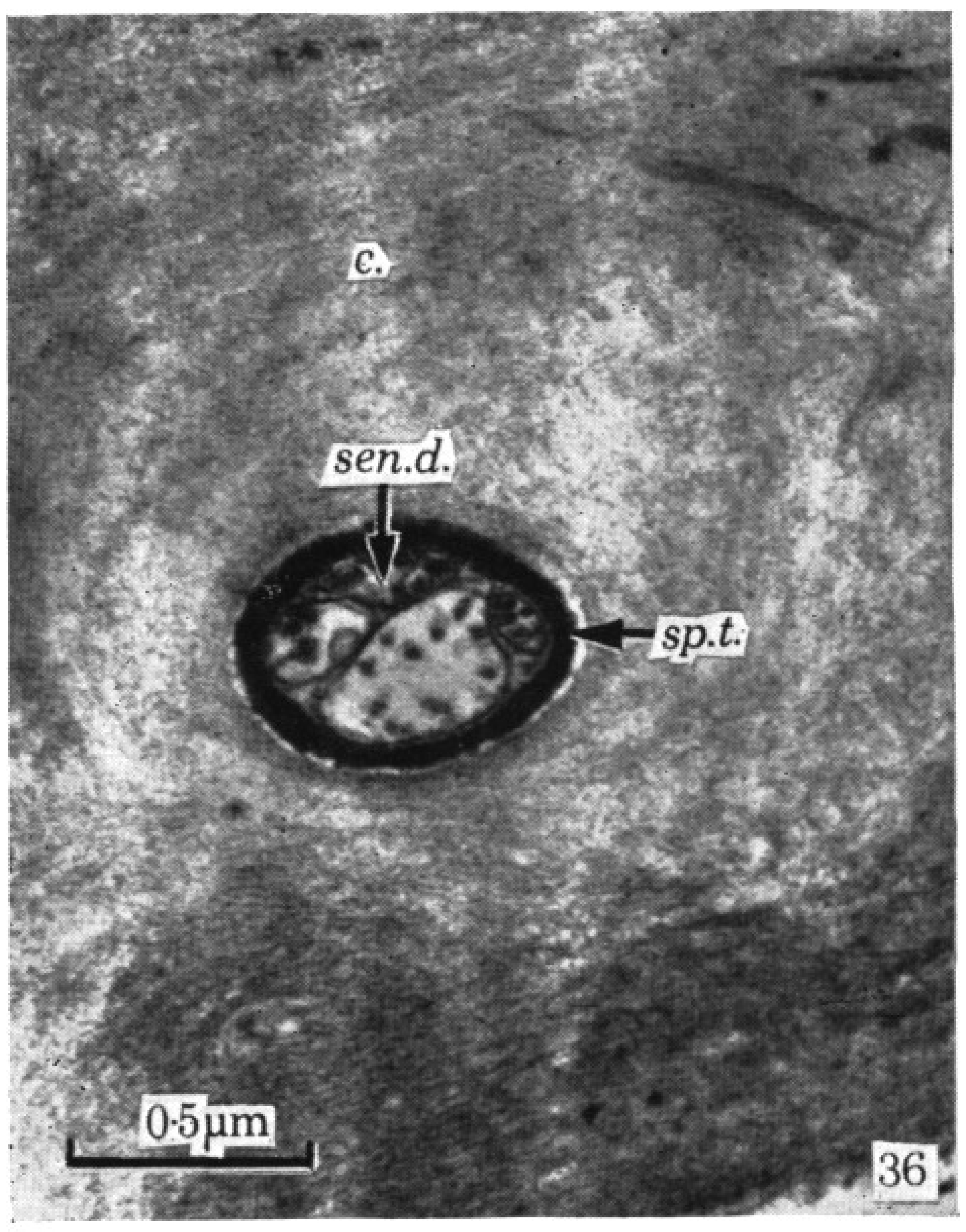


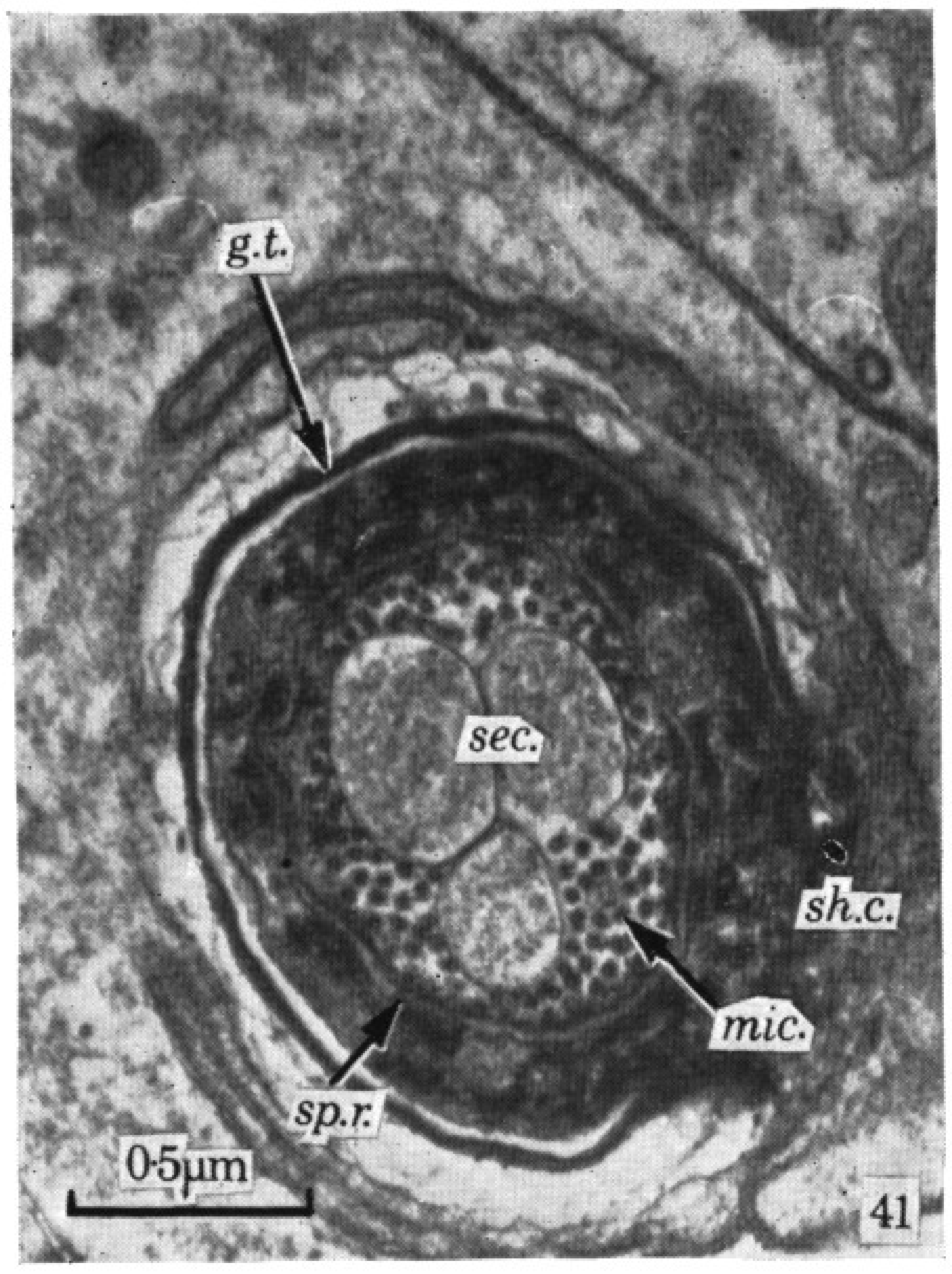
FIGURES 27 to 34. A radial sense organ without internal cuticular tube, in transverse sections from the hair within the cuticular canal in figure 27 to the dendrites in figures 33 and 34. In figure 30 the attachment points of the supporting rods to the cuticle are marked '*x*'. The intimate contact between the supporting rods and the dendrites, together with the electron dense material close to the plasma membrane in the dendrites, adjacent to the supporting rods, is shown in figure 33.

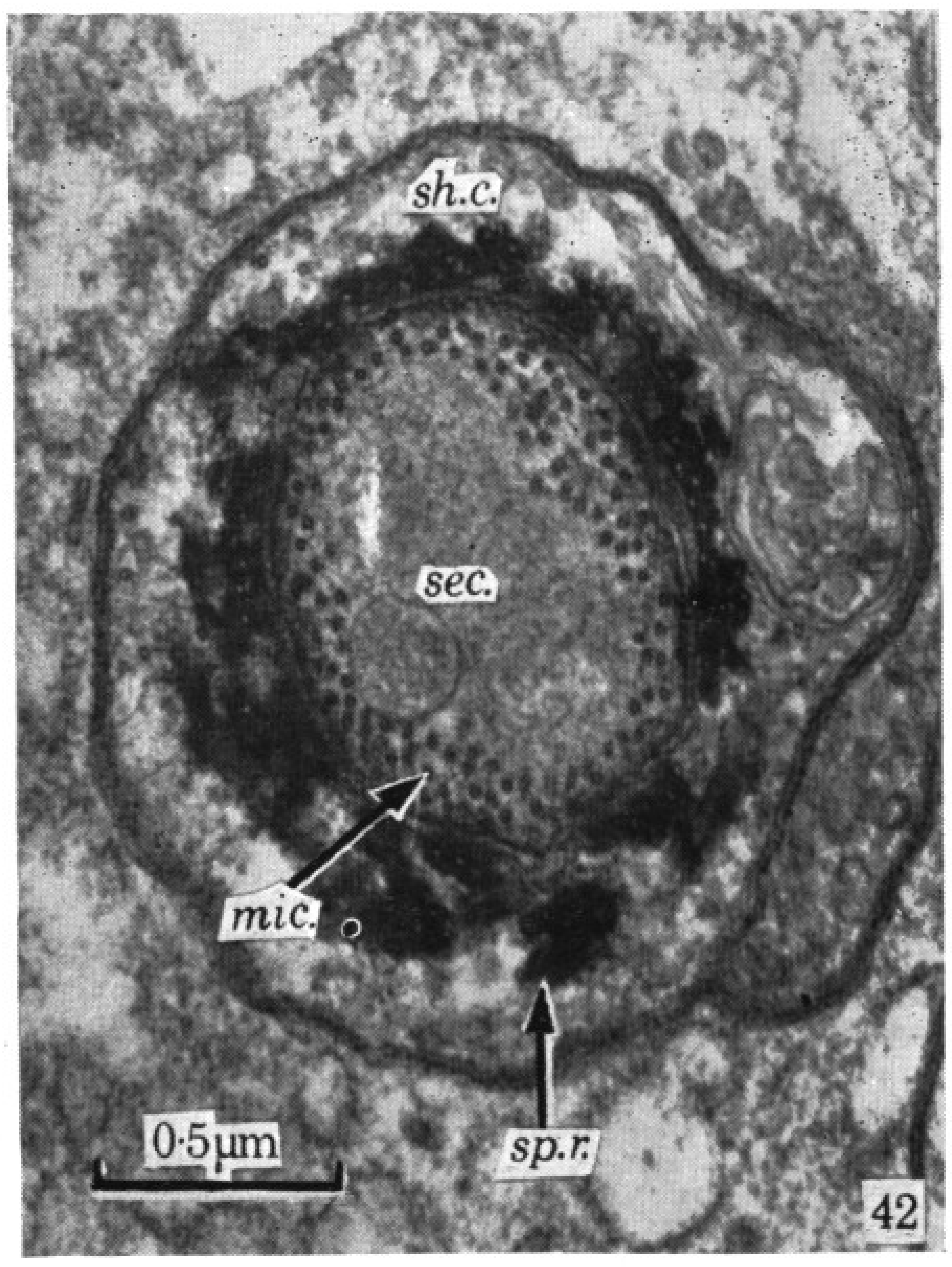


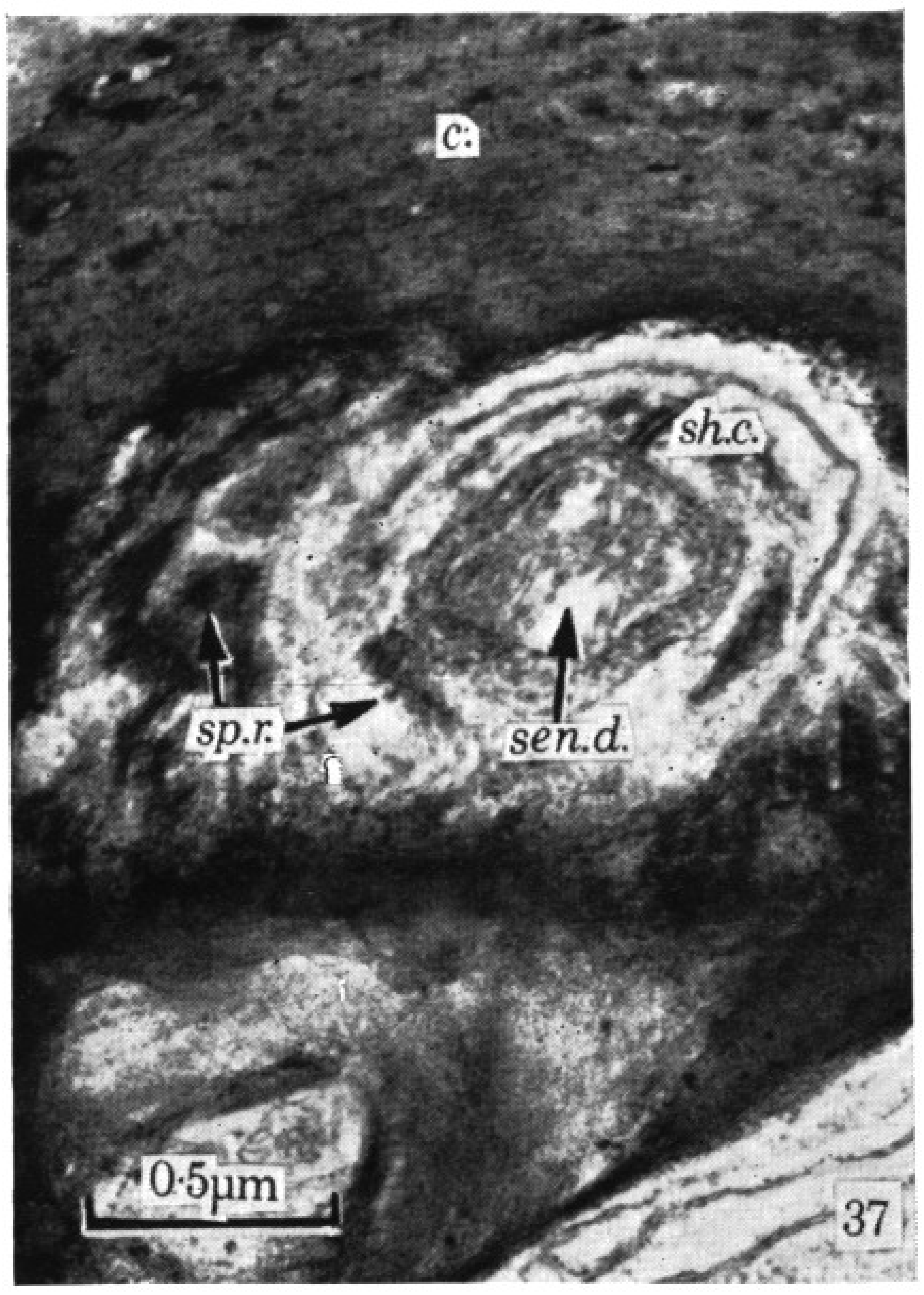


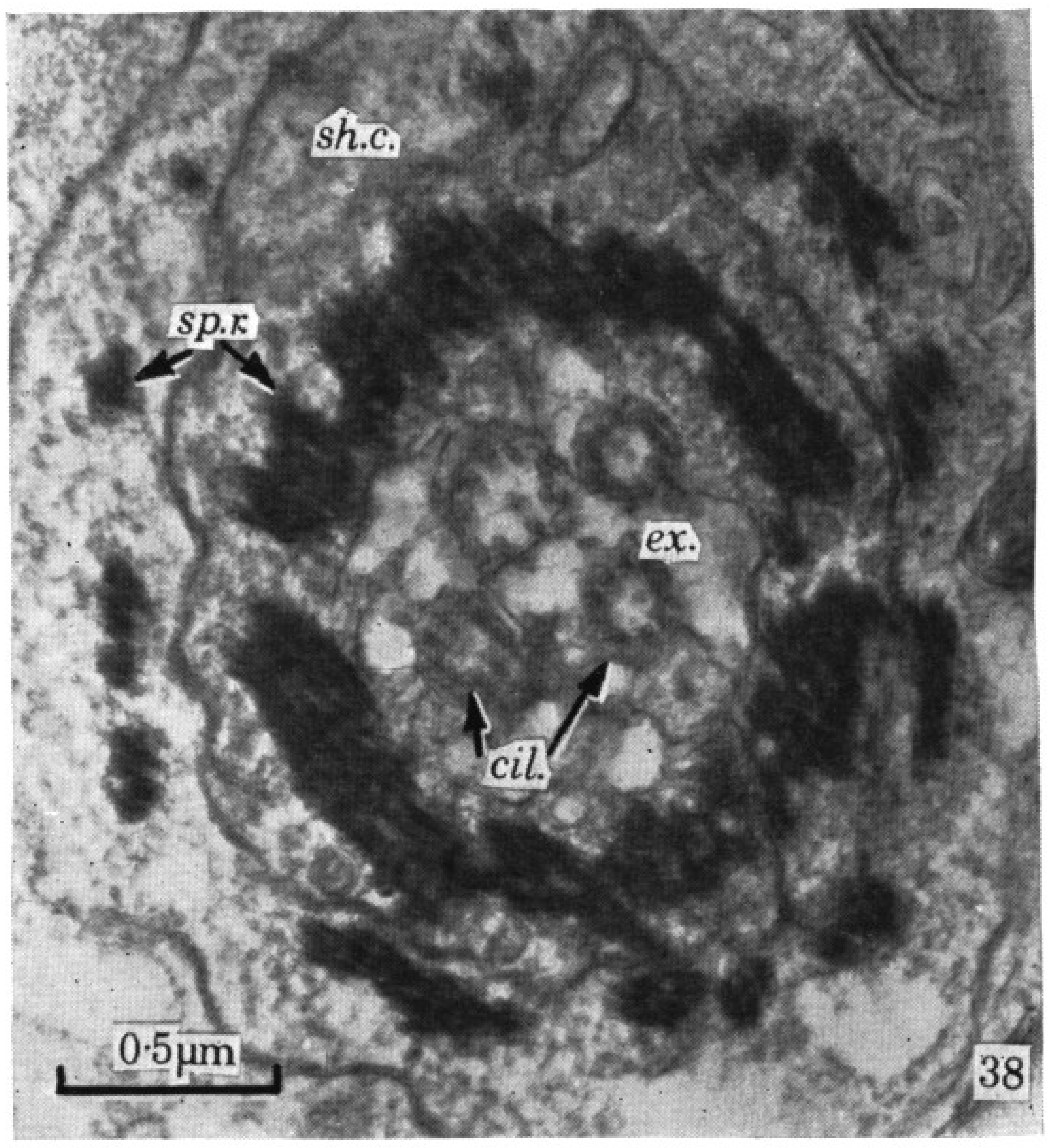


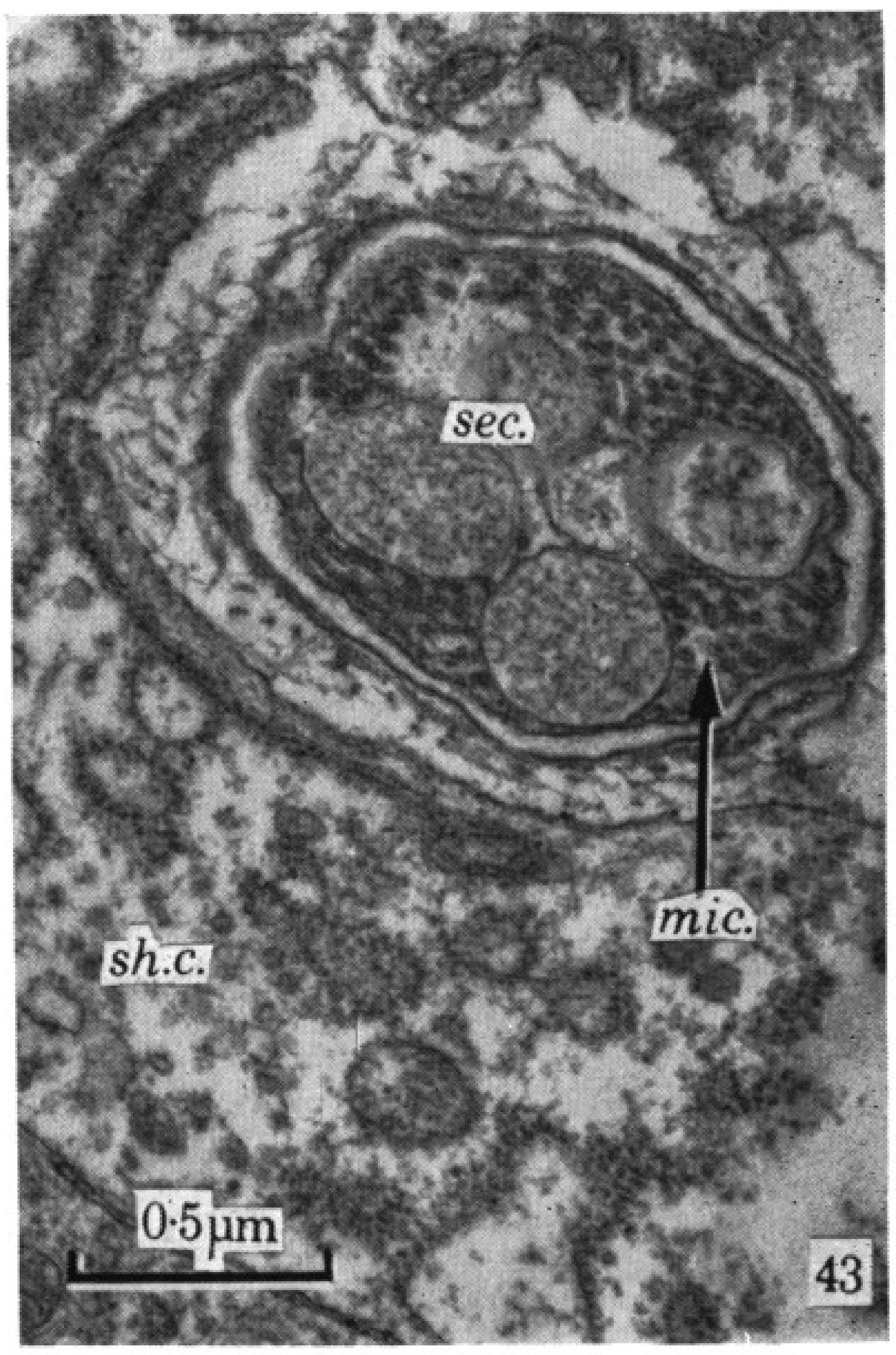










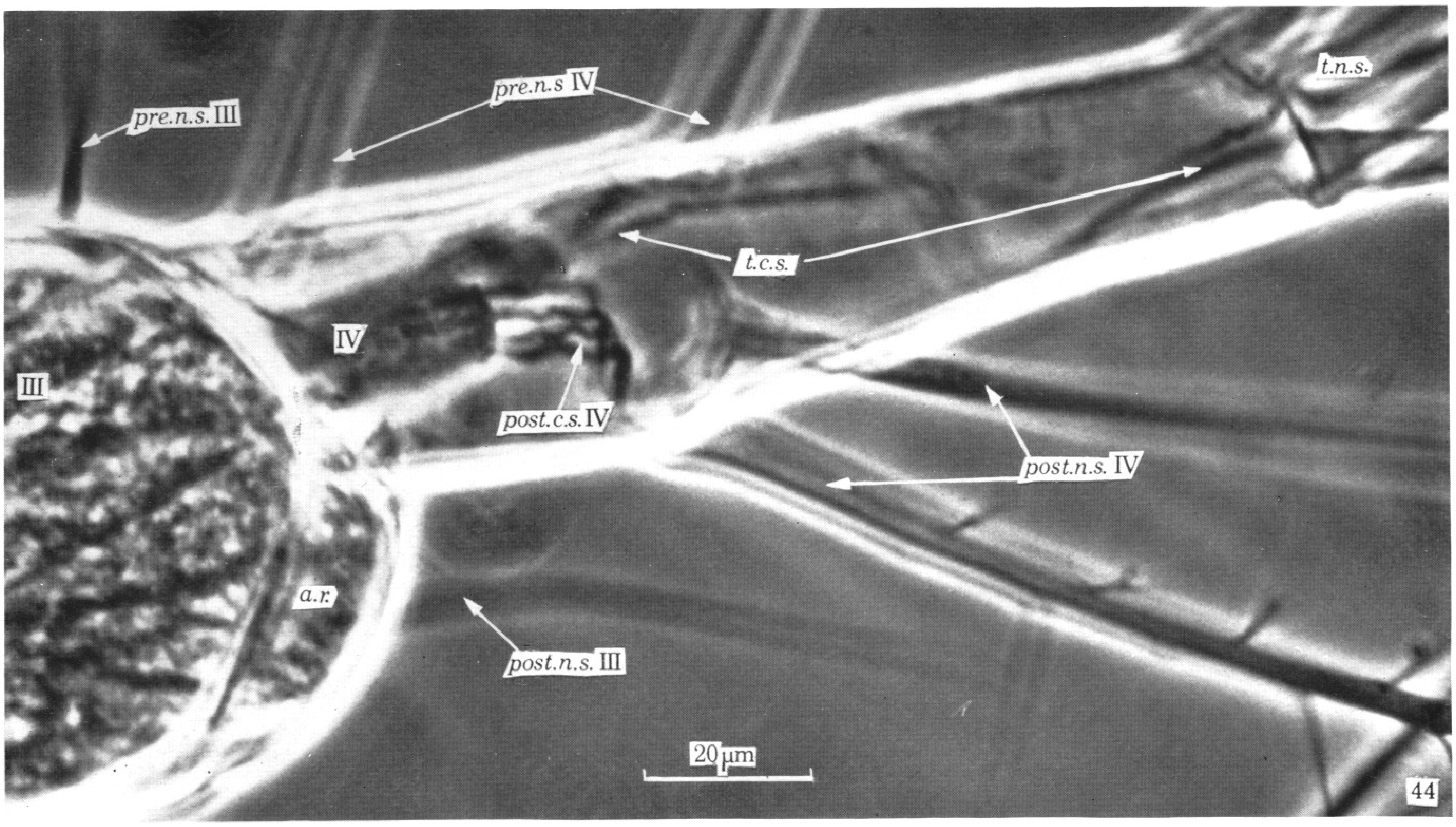


FIGURES 35 to 38. Transverse sections of the median radial sense organ without internal cuticular tube (*s.r.* 1 in plates 39 and 40). Described in the text.

FIGURE 39. Longitudinal section through the attachment disk surface showing the opening of the antennular gland duct (marked '*x*').

FIGURE 40. Transverse section through a cuticular canal in the attachment disk, with the ducts from two antennular glands and a process from a sheath cell.

FIGURES 41 to 43. Transverse sections through antennular glands taken from regions progressively more proximal from figures 41 to 43. Described in the text.



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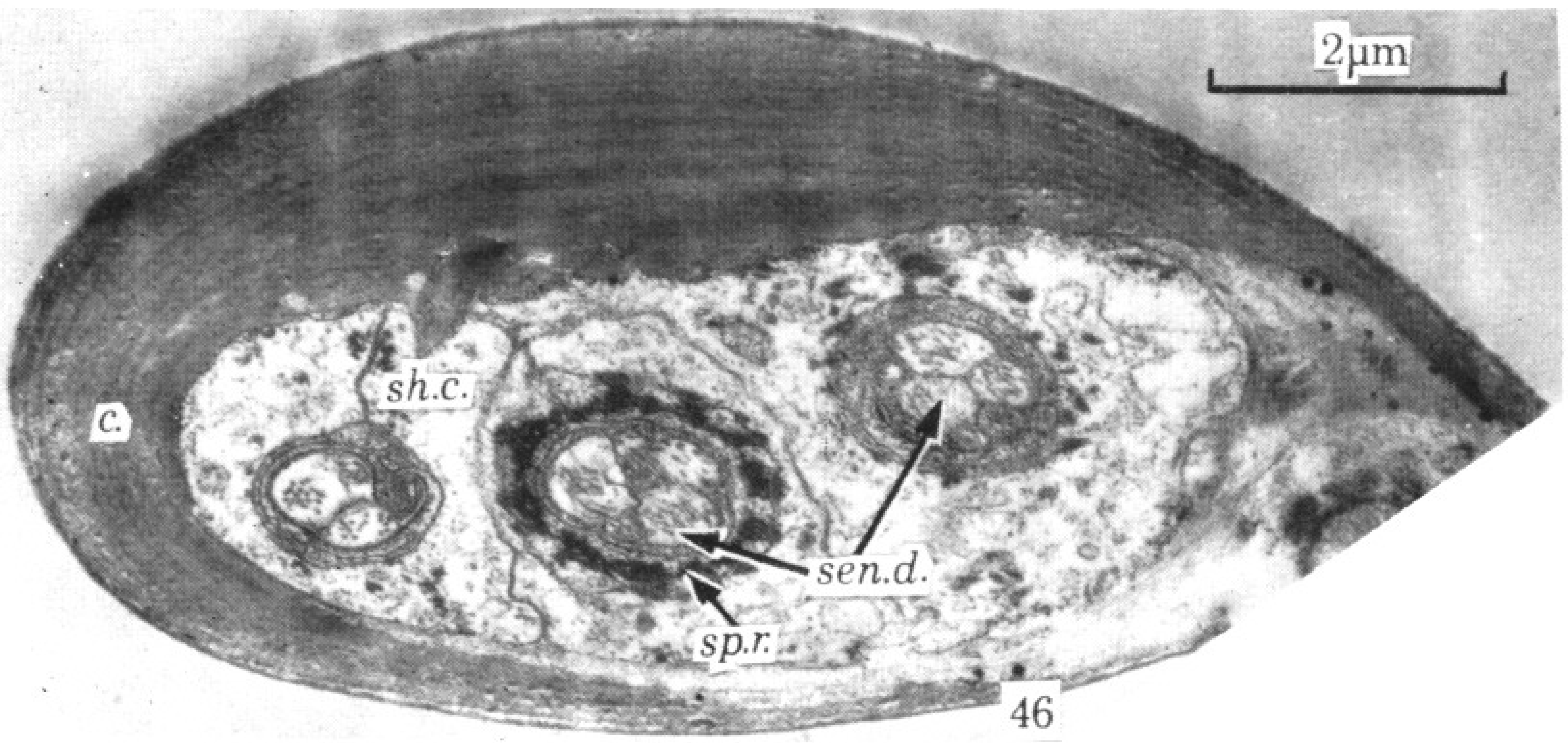

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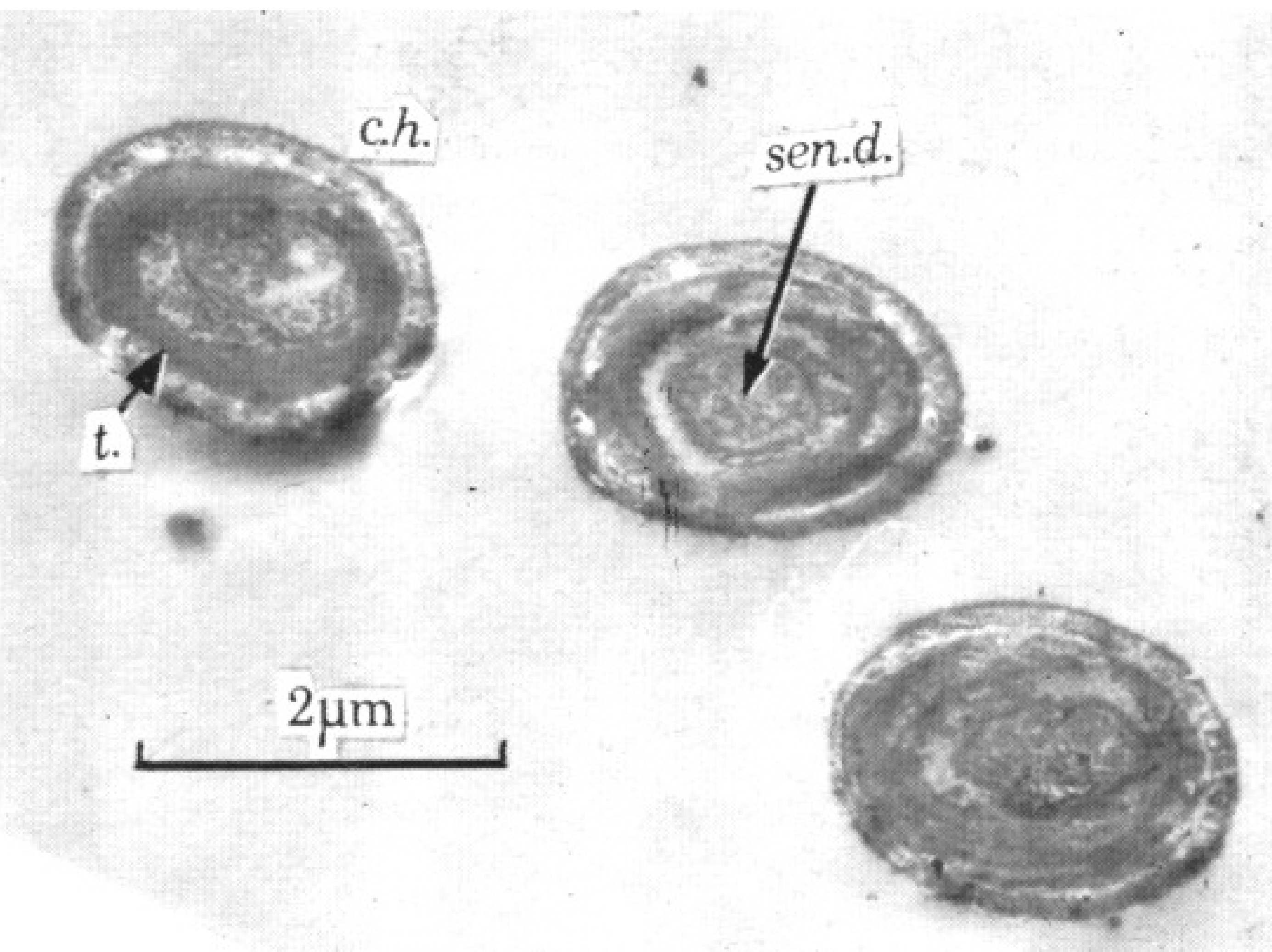

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FIGURE 44. Phase-contrast photomicrograph of the terminal regions of the antennule of the late sixth-stage nauplius, showing the developing cypris antennular structures within.

FIGURE 45. Transverse sections of three of the postaxial setae of the fourth segment of the cypris antennule.

FIGURE 46. Transverse section of the distal region of the fourth segment showing the sensory processes to the terminal setae.

FIGURE 47. A section transverse to the axis of the third segment showing the attachment of the transverse muscle in the base of the fourth segment and the dendrites entering the fourth segment

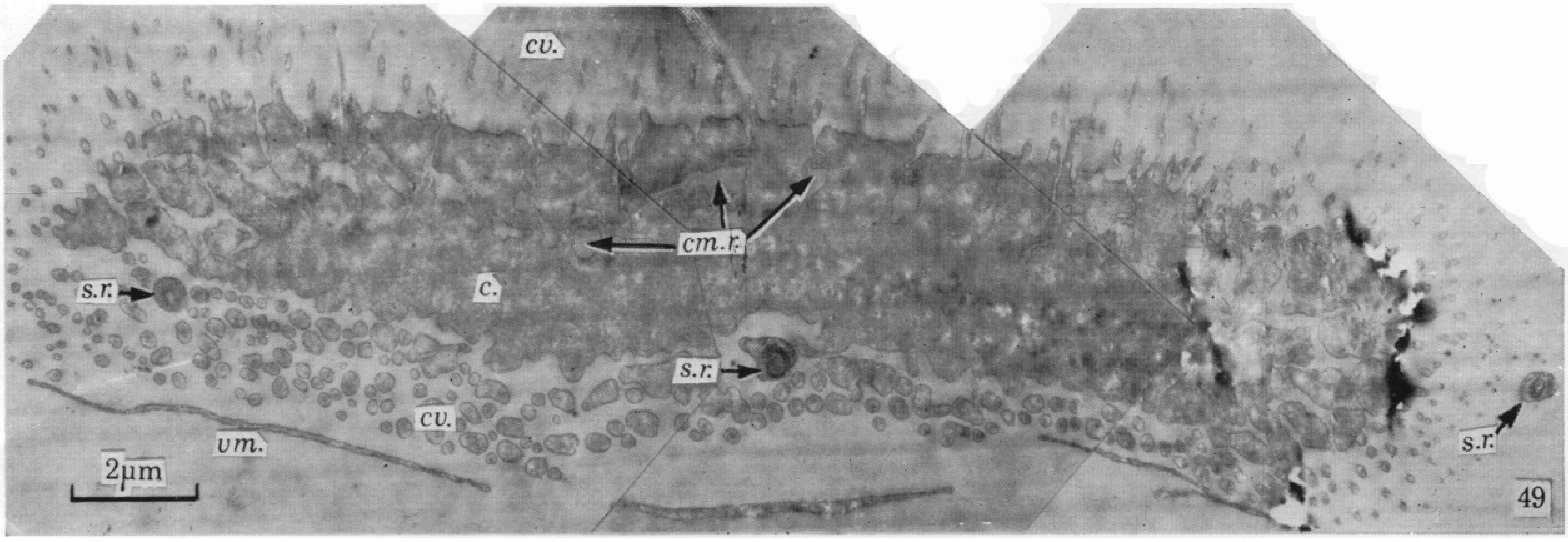

FIGURE 48. A section transverse to the axis of the fourth segment showing the arrangement of the sensory processes into those connected to the terminal setae on the left and those to the postaxial setae on the right. Supporting rods are shown in intimate contact with dendrites at '*x*' with electron dense material within the dendrite, adjacent to the rods.

FIGURE 49. A section longitudinal to the axis of the attachment organ and through the edge of the attachment disk showing three radial sense organs emerging from between the margin of the disk and the velum.