THE STRUCTURE AND FUNCTION OF A CONNECTIVE CHORDOTONAL ORGAN IN THE COCKROACH LEG

BY D. YOUNG*

Department of Zoology, University College, Cathays Park, Cardiff

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[Plates 77 to 88]

CONTENTS

The micromorphology of the tibio-tarsal joint of *Periplaneta* is described, based on the mesothoracic limb. There are two muscles acting on the joint, numbered 144 and 145. There is a connective chordotonal organ, which branches off from the trunk of N5 proximally and inserts on the intersegmental membrane distally. In addition, a previously undescribed group of campaniform sensilla is reported from the tibio-tarsal joint.

The tibio-tarsal chordotonal organ subdivides into a main branch and two side branches. It contains 26 bipolar sensory neurons, whose dendrites are associated distally with 14 scolopales. These scolopidia are arranged in three groups which differ in position, fine structure and number of cells per scolopale. There is one group 1 scolopidium in each of the side branches and two proximally in the main branch. Group 2 scolopidia are spread out along the main branch and group 3 scolopidia occur distally in the main branch.

Group 1 scolopidia consist of two bipolar neurons, 15 to 20 μm in diameter, whose dendrites, heavily sheathed, insert in a single scolopale. Group 2 scolopidia consist of two bipolar neurons, 8 to 15 μm in diameter, whose dendrites are less well sheathed and insert in a single scolopale. Group 3 scolopidia consist of a single bipolar neuron, about 10 μm in diameter, with a short, poorly sheathed dendrite inserting in a single scolopale.

The scolopales of groups 1 and 2 are identical, consisting of the usual pattern of a ring of scolopale rods inserting into a distal cap and enclosing the cilia on the dendrite terminations. In this case, the cap is particularly long and pointed and both the cilia and the scolopale rods penetrate a long way into it.

* Present address: Research School of Biological Sciences, P.O. Box 475, Canberra City, A.C.T. 2601.

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The cilia of the two members of a pair of dendrites are identical but the ciliary roots differ between the two members of a pair.

Electrophysiological recordings show that the organ responds to downward and backward deflexion of the tarsus. The response comprises at least two classes of sensory fibre distinguishable both by their size and by their behaviour. The larger fibres show a unidirectional phasic and tonic response to extreme deflexion of the tarsus and are identified with the group 1 scolopidia. The smaller fibres show a unidirectional tonic response to the full range of deflexion of the tarsus and are identified with the group 2 scolopidia. On structural evidence, it is suggested that the differences in adaptation between these two groups of scolopidia is not likely to be caused by differences in mechanical attachment.

The combination of electrophysiological and fine structural evidence indicates that in this chordotonal organ the adequate stimulus of the scolopidia is an increase in their longitudinal tension but it is not possible to say which fine structural component responds to strain.

1. Introduction

The structure of insect chordotonal organs has been investigated in detail by the anatomists of an earlier generation and widespread agreement has been reached concerning their general structure (see, for example, Debauche 1935; Snodgrass 1926). They are composed of numbers of sensilla, the scolopidia, each of which characteristically contains scolopales associated with the dendrites of one or more bipolar sensory neurons. More recently, the electron microscope has thrown light on the fine structure of the scolopidium. Chordotonal organs were studied first in the electron microscope by Gray (1960) for insects and by Whitear (1962) for Crustacea. Electron microscope studies have been extended and reviewed by Howse (1968). Fortunately the terminology used by these authors is fairly uniform and has been followed here as far as possible. The adjective 'connective' was introduced by Howse (1968) to designate those integumental chordotonal organs, like the present one, which span, or connect, two structures as opposed to those which form tympanal organs.

Most of what is known about the physiology of connective chordotonal organs is based upon those in the decapod Crustacea, which are easier to study on account of their larger size. The chordotonal organ at the propodite–dactylopodite (PD) joint in crabs is one of the best known. Burke (1954) showed that the isolated organ contains small sensory fibres which respond tonically to the length of the organ and large sensory fibres which respond phasically to a change in the organ's length. These properties enable the organ *in situ* to signal the position and movement of the joint. Our understanding of the organization of this organ has been extended by the analysis of single PD organ neurons by Wiersma & Boettiger (1959) and Hartman & Boetigger (1967). Chordotonal organs having similar properties span the other leg joints in the decapods (Wiersma 1959; Bush 1965*a*, *b*).

Less is known about the chordotonal joint receptors in insects. In an incomplete study, Becht (1958) recorded from the coxa–trochanteral organs in the cockroach which are sensitive to extension of the trochanter. He found small tonic fibres and large phasic fibres but it is not clear which of the organs these came from. Recently Usherwood, Runion & Campbell (1968) have studied the femoral chordotonal organ in locusts. They found tonic and phasic responses but have not, as yet, related them to the cell groups which they have described. Thus the study of the individual structure and function of insect connective chordotonal organs has been hardly touched and so a particular chordotonal organ in the leg of the cockroach, *Periplaneta americana* (L.) has been chosen for study here, namely the connective chordotonal organ at the tibio–tarsal joint.

In general, the chordotonal organs in Crustacea and insects serve to signal the position and movement at the limb joints. But the crustacean work in particular makes it clear that the

precise character of their sensitivity results from a subtle combination of the mechanical properties of the organ and the neural properties of its sensilla. Hence, for a full understanding of chordotonal organ function, it is desirable to know both the detailed structure of the organ and the precise mode of response of its sensilla to the parameters of the stimulus. The tibio–tarsal chordotonal organ lends itself well to this kind of study. The small size of the organ makes it possible to obtain a complete description of its fine structure and to obtain a good qualitative picture of its behaviour with the simplest of electrophysiological methods. The combination of these approaches enables useful conclusions to be drawn about the functioning of individual cells.

The occurrence of chordotonal organs in the legs of insects was surveyed for several orders of insects by Debaiseaux (1936, 1938). In the majority of orders he found femoral, proximal tibial (subgenual), distal tibial and tarsal chordotonal organs. There are also coxal organs which Debaiseaux did not describe. Because of the close association of the distal tibial organ with the joint in *Periplaneta* it is referred to here as the tibio-tarsal chordotonal organ. Debaiseaux did not examine *Periplaneta* but his description of this organ in *Phyllodromia* (= *Blatella*) corresponds fairly closely with what we have found in *Periplaneta*.

Nijenhuis & Dresden (1952) made a preliminary study of the sense organs of the cockroach leg but they did not study the leg beyond the subgenual organ. As a result their paper contains no information on the tibio–tarsal joint. Hence, what follows is the first description of the tibio–tarsal chordotonal organ in *Periplaneta*.

2. Material and methods

(*a*) *Methods for light and electron microscopy*

The study was based on the mesothoracic legs of adult male cockroaches, *Periplaneta americana*. These were selected from the laboratory colony while their cuticle was still soft and transparent from the final moult and were used at once.

Preliminary studies were carried out by injecting dilute solutions of methylene blue into the freshly moulted leg. For detailed study, serial sectioning was found to be the only satisfactory method. So as to study the sense organ in as natural a position as possible, it was fixed *in situ* by cutting off a short length of leg and immersing this in the fixative. The fixative was alcoholic Bouin's fluid (Pantin 1946). After 2 h fixation at 60 °C the lengths of leg were dehydrated in graded ethanols and embedded in Gurr's histological paraffin wax, m.p. 54 °C. The whole process of fixation and embedding was completed within 10 h. By this method it was possible to obtain good fixation with minimal hardening of the specimen.

Longitudinal and transverse serial sections, 15 μm thick, were cut on a Bausch and Lomb rotary microtome. These sections were stained with Baker's modification of Masson's triple stain (Pantin 1946). Six such sets of serial sections were studied. In particular, two sets of longitudinal sections were used to prepare reconstructions of the sense organ using the technique of Pusey (1939 and personal communication). One of these reconstructions is shown in figure 4.

For electron microscopy the chordotonal organ was also fixed *in situ* by immersing short lengths of freshly moulted leg in the fixative. In this way information was obtained about the natural geometry and fine structure of the sense organ which was directly comparable with the results of the histological methods. It should be emphasized that this was the purpose of the electron microscope study since this method gave fixation which was poor for ultrastructure

but which was perfectly adequate for studying the fine structure and orientation of the sensory cells.

Fixation and dehydration were carried out in a refrigerator at 4 °C. The fixative was 3 % glutaraldehyde in phosphate buffer, mol l^{-1}, pH 7.4. Eight hours fixation was followed by a rinse in the buffer overnight and post-fixation for 1 h in 1 % osmium tetroxide in phosphate buffer, pH 7.4 (Glauert 1965). The lengths of leg were transferred from the osmium tetroxide directly to 50 % ethanol. They were dehydrated in graded ethanols, then passed through propylene oxide and embedded in Araldite (Glauert & Glauert 1958; Glauert 1965).

Four specimens were sectioned on a Cambridge 'Huxley' ultramicrotome for a preliminary study in the electron microscope. Then a further three specimens were subjected to a combined study with the light and electron microscopes, two using transverse sections and one using longitudinal sections. For this, the Araldite block was orientated so that the leg would be cut either transversely or longitudinally and it was trimmed until the most proximal part of the sense organ was reached. Then 1 μm sections were taken at regular, known intervals using an L.K.B. semi-automatic ultra-microtome. These thick sections were stained with 0.5 % toluidine blue in 0.5 % borax for examination in the light microscope. At points of particular interest thin sections were cut for examination in the electron microscope. Thin sections were 'stained' with 1 % potassium permanganate (Lawn 1960) for 10 min, followed by lead citrate (Reynolds 1963) for 1.5 min. They were examined in an A.E.I. E.M.6 electron microscope.

The block was worked through systematically until the entire chordotonal organ had been sectioned. By comparative study of the series of thick and thin Araldite sections in the light and electron microscopes respectively it was possible to reconstruct details of the neurons and their satellite cells and to check these against the results obtained with wax sections. This method yielded accurate reconstructions at different levels of magnification, some of which are reproduced as text figures.

(*b*) *Physiological methods*

All the experiments were performed on isolated mesothoracic legs of adult cockroaches. For most experiments the leg was cut off at the trochanter and otherwise left intact, but it was possible to obtain a satisfactory preparation consisting of the distal half of the tibia and the proximal segment of the tarsus only.

Fine tungsten needles were used as recording electrodes and these were inserted into the tibia through two small holes made in the cuticle, close to the chordotonal organ. The electrodes were connected, via a Cossor 1440 preamplifier, to a Cossor 2000 dual beam oscilloscope. Recordings were taken with a Cossor 1431 oscillograph camera.

The chordotonal organ was left *in situ* and stimulated by passive movement of the tarsus, so as to provide a mode of stimulation approximating to the natural condition. Controlled movement of the tarsus was provided with the apparatus shown in figure 1. The isolated leg was held down on the stage with the tibio–tarsal joint centred over the shaft of a d.c. potentiometer (a wire-wound volume control). Two small pins, soldered to the end of the shaft moved the tarsus through the same angle without strain on the joint. The shaft was rotated by hand by means of a rod which moved against a protractor scale enabling known angular displacements to be applied. A record of the movement was obtained by incorporating the potentiometer in the circuit shown in figure 1 whose output is connected across the second channel of the oscilloscope.

The time course of adaptation and the tonic responses were studied by moving the tarsus through a known angle and leaving it in the deflected position. Phasic responses were studied by simple constant velocity displacements. These were effected by hand by moving a ruler at steady speed so as first to strike the rod and then come to rest against a stop. With practice this method produced quite passable ramp functions (figure 35).

With these methods, the impulse frequency in response to a given stimulus was found to vary with such factors as the exact placing of the electrodes and the time after setting up the preparation. Accordingly, the results are presented as graphs, each of which is plotted from a single preparation, judged to be representative in respect of the variable in question.

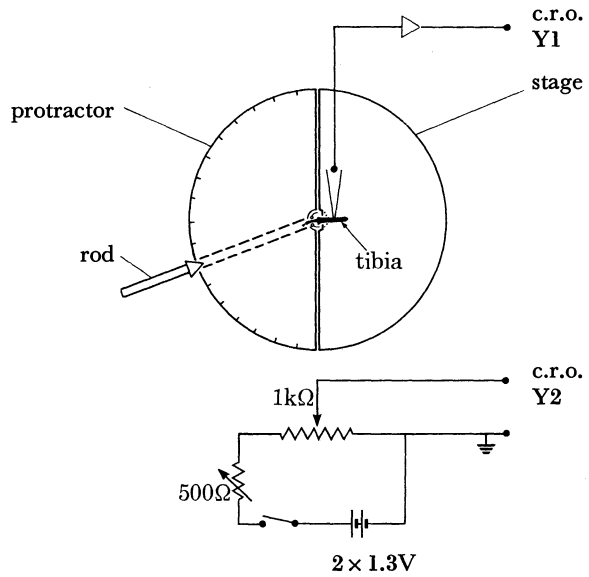


FIGURE 1. The apparatus used to provide controlled movement of the tibio–tarsal joint. The isolated leg is held on the stage with the joint centred over the shaft of a d.c. potentiometer, which is arranged to move the tarsus. The shaft is turned by a rod, which moves against a protractor scale. Electrodes inserted in the leg are connected to the first channel of a cathode ray oscilloscope (c.r.o.). The potentiometer is incorporated in the circuit shown below, which is connected across the second channel of the c.r.o.

3. THE MUSCLES AND SENSE ORGANS AT THE JOINT

The main features of the cockroach leg at the tibio–tarsal joint are shown in figures 2 and 3. Dorsally, the cuticle is folded in upon itself so as to form a true extrinsic condyle. This provides what is effectively a ball and socket joint, permitting movement of the tarsus in many planes. Anteriorly, the joint is covered by a flange of cuticle which projects from the tibia. Except for the condylar region the tibia and tarsus are joined by an extensive intersegmental membrane, which is flexible and transparent.

The most conspicuous structure inside the tibia is the tracheal trunk, which is expanded into a large tracheal sac in the region of the joint. At the joint it narrows right down and continues on into the tarsus as a small trachea.

The main leg nerve runs along the anterior surface of the tracheal sac. This nerve derives from the fifth nerve trunk to leave the mesothoracic ganglion and so is called nerve 5, following Nijenhuis & Dresden (1955) after Pringle (1939). Nijenhuis & Dresden have shown that nerve 5 is the only nerve to supply the leg beyond the coxa. A small ramus of this nerve runs along

the posterior surface of the tracheal sac. Both the ramus and the nerve trunk pass on into the tarsus. Shortly before the joint, a minute branch of nerve 5 supplies the chordotonal organ which inserts on the intersegmental membrane under the flange of cuticle projecting from the tibia.

There are two muscles in this region of the tibia which insert on the base of the tarsus, below and on either side of the condyle. These muscles have been designated by numbers rather than attempting to devise suitable names and the term tendon has been used for the skeletal ingrowth which provides their distal attachment (some authors use the term apodeme). The numbers follow the system of Carbonell (1949). He did not study the muscles all the way along the leg but left six numbers (141–146) for the muscles distal to the coxa in the mesothoracic leg. Dresden & Nijenhuis (1953) studied the femoral muscles but not the tibial ones. They gave numbers 141 to 143 to the main femoral muscles and 146 to the tarsal claw muscle, which occurs in the femur and is attached by a long tendon which passes right through the tibia. They suggested numbers 144 and 145 for the two tibial muscles and these are the numbers adopted here.

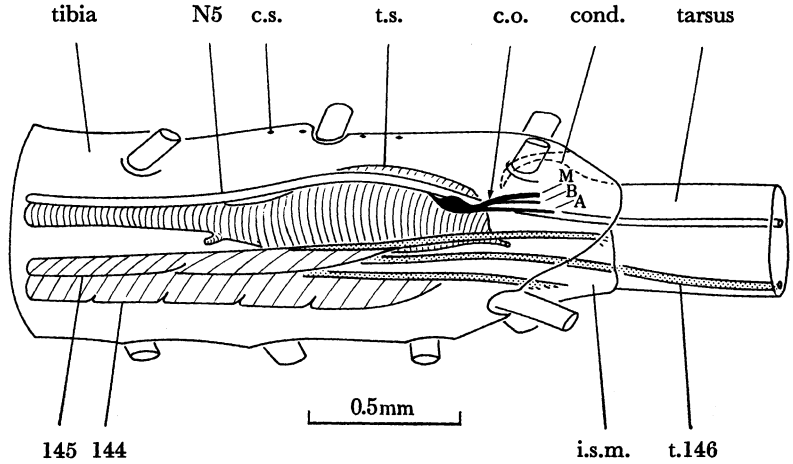


FIGURE 2. Anterior view of the tibio–tarsal joint of the left mesothoracic leg of *Periplaneta* showing the connective chordotonal organ (c.o.) at this joint. The main branch (M) and two side branches (A) and (B) of the chordotonal organ are shown. The chordotonal organ branches off the trunk of the main leg nerve (N5) which is closely apposed to the surface of the large tracheal sac (t.s.) present near this joint. The chordotonal organ is also close to the tendon of one of the two muscles (144 and 145) which move the tarsus. Dorsally there is a line of four campaniform sensilla, the most proximal of which is labelled (c.s.). i.s.m., intersegmental membrane; t.146, tendon of muscle 146.

The direction in which each muscle pulls the tarsus was determined by cutting the tendon of one and then electrically stimulating the other, in a number of preparations. When both are stimulated together they act as depressors in relation to the animal as a whole (figure 3), thus holding the tarsus down on the substrate. Both muscles respond with single twitches to low frequency stimulation.

The chordotonal organ leaves N5 and inserts on the intersegmental membrane as described below. There is a campaniform sensillum at the base of each of the large tibial spines near the joint, as described by Chapman (1965). In addition, there is a group of campaniform sensilla associated with the joint, which has not been described by previous workers (Pringle 1938; Nijenhuis & Dresden 1952; Guthrie & Tindall 1968). There are four campaniform sensilla longitudinally orientated and situated in a line on the dorsal surface of the tibia, about 0.5 mm

from the joint (figures 2 and 3). This corresponds to the location of those on the tarsal segments and is the logical position for them to respond to the compression component in the cuticle when the animal is standing (J. W. S. Pringle, personal communication). Guthrie (1967) has described multipolar sense cells at the femoro–tibial joint but none were found at the tibio–tarsal joint in the present study.

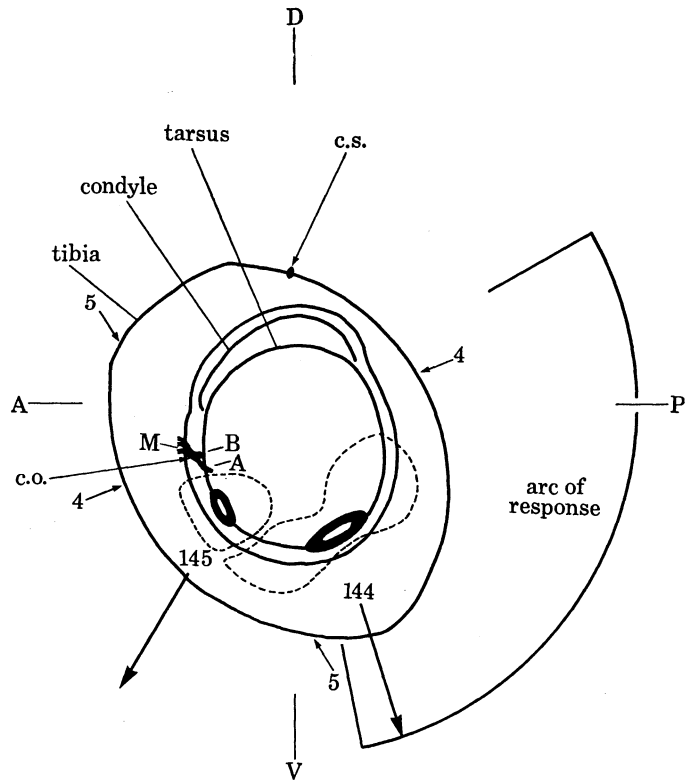


FIGURE 3. The tibio–tarsal joint seen end on, at its natural angle in relation to the anterior–posterior (A–P) and dorso–ventral (D–V) axes of the animal as a whole. The location of muscles 144 and 145 in the tibia is shown by the broken lines and their insertion on the tarsus by heavy lines. The direction in which each (acting separately) moves the tarsus is shown by a corresponding arrow. The location of the chordotonal organ (c.o.) is shown, together with the arc of movement of the tarsus to which it responds. The orientation of the chordotonal organ's main branch (M) and side branches (A) and (B) is also shown. The paired arrows, numbered 4 and 5, indicate the exact plane of sections used for the reconstructions reproduced in figures 4 and 5 respectively. c.s., campaniform sensilla.

4. THE STRUCTURE OF THE CHORDOTONAL ORGAN

(*a*) *The histology of the chordotonal organ*

A reconstruction of the tibio–tarsal chordotonal organ from longitudinal wax sections is shown in figure 4. It consists of a thin strand of tissue suspended between nerve 5 proximally and the tibio–tarsal intersegmental membrane distally. It is closely accompanied by a tracheole which branches off the tracheal sac (figure 11, plate 78). The chordotonal organ travels near the tendon of muscle 145 but it is not attached to it in any way. Scrutiny of serial sections revealed no proximal anchorage apart from that provided by the sensory axons entering the trunk of nerve 5. Nerve 5 itself is closely apposed to the surface of the tracheal sac.

The chordotonal organ consistently shows the fan-wise arrangement of branches which is illustrated in figures 2, 4 and 5. There is a main branch, M, which subtends the largest angle

with respect to the nerve trunk, and two side branches, A and B, which are more nearly parallel to the nerve trunk. Side branches A and B diverge both downward and inward with respect to the main branch, A more so than B. Distally, these branches all split into finer strands which pass through the hypodermis and insert on the cuticle of the intersegmental membrane. The constancy of geometry often extends to remarkably fine details of structure. For instance, in both light and electron microscope reconstructions, side branch A always gives off a sub-branch which diverges at a considerable angle to insert close to the insertion of side branch B, as illustrated in figures 4 and 5.

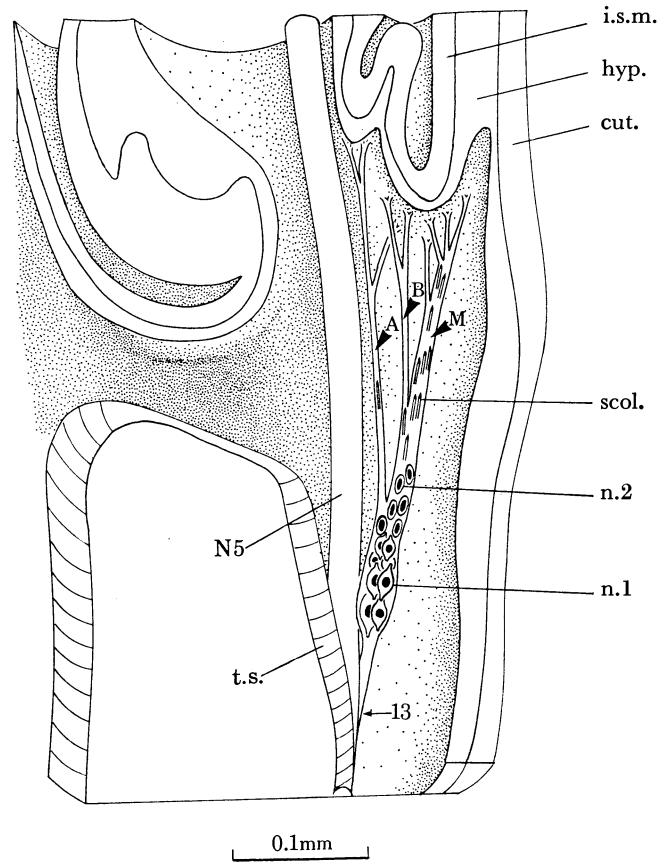
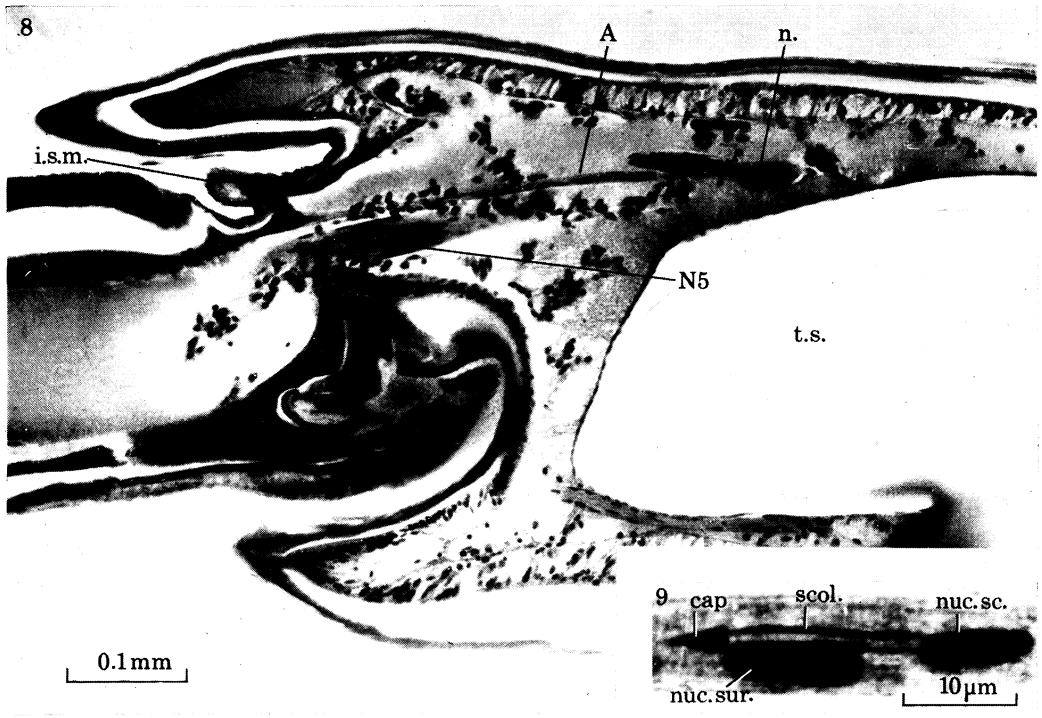


FIGURE 4. Reconstruction from longitudinal wax sections of the tibio–tarsal chordotonal organ, seen in ventral view. The exact plane of section is indicated in figure 3 by a pair of numbered arrows. The chordotonal organ is anchored only by its axons entering the fifth nerve trunk (N5) and its branches insert on the intersegmental membrane (i.s.m.). There are two side branches (A and B) each with one scolopale and the main branch (M) with groups of scolopales (scol.) scattered along its length. Proximally there are corresponding groups of bipolar neurons (n.1, n.2). The arrow, numbered 13, shows the level of section of figure 13, plate 79. cut., cuticle; hyp., hypodermis; t.s., tracheal sac.

In the light microscope sections both neurons and scolopales can be made out clearly. In thick, longitudinal wax sections, scolopales appear as a tube, 15 μm long, closed off distally by a pointed cap (figure 9, plate 77) which stains red with Masson's stain, similarly to exocuticle. When cut transversely the scolopale tubes are seen to be oval in section and to enclose two cilia one each from two sensory neurons. There are several scolopales loosely grouped along the length of the main branch but side branches A and B each contain one scolopale only. The total number of scolopales which could be distinguished clearly in the two light microscope



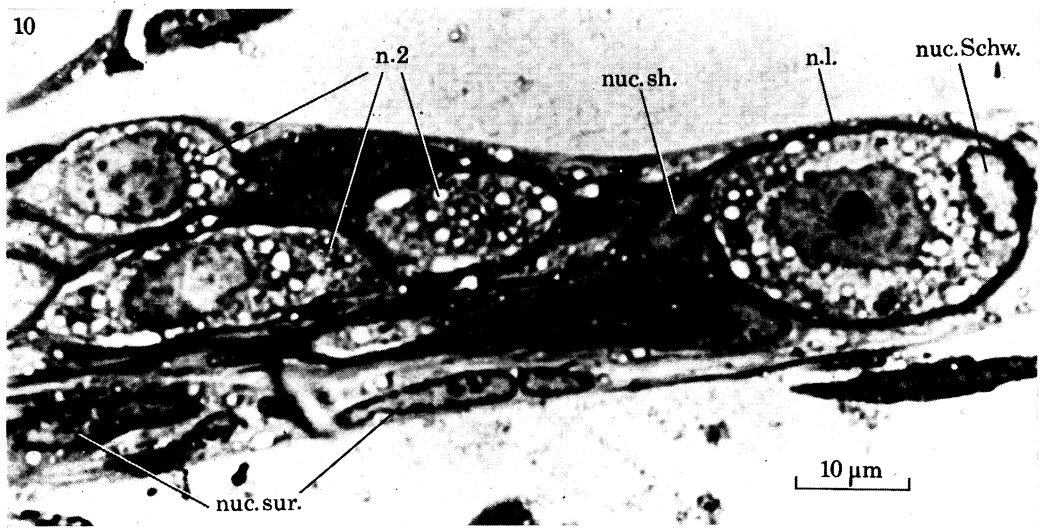


FIGURE 8. One of the longitudinal wax sections, 15 μm thick, on which the reconstruction in figure 4 is based. Some of the bipolar neurons (n.) are seen near the tracheal sac (t.s.). Side branch A can be seen along its whole length from the neurons to its insertion on the intersegmental membrane (i.s.m.). N5, the fifth nerve trunk.

FIGURE 9. Scolopale of side branch A enlarged from figure 8, showing the scolopale rods (scol.) and the pointed cap. nuc. sur., surrounding cell nucleus; nuc. sc., scolopale cell nucleus.

FIGURE 10. A longitudinal Araldite section, 1 μm thick, through the sensory neurons. This section passes through the nucleus of a group 1 neuron (n.1.) and through the nuclei of its Schwann cell (nuc. Schw.) and its sheath cell (nuc. sh.). On the left are three group 2 neurons (n.2.). nuc. sur., surrounding cell nucleus.

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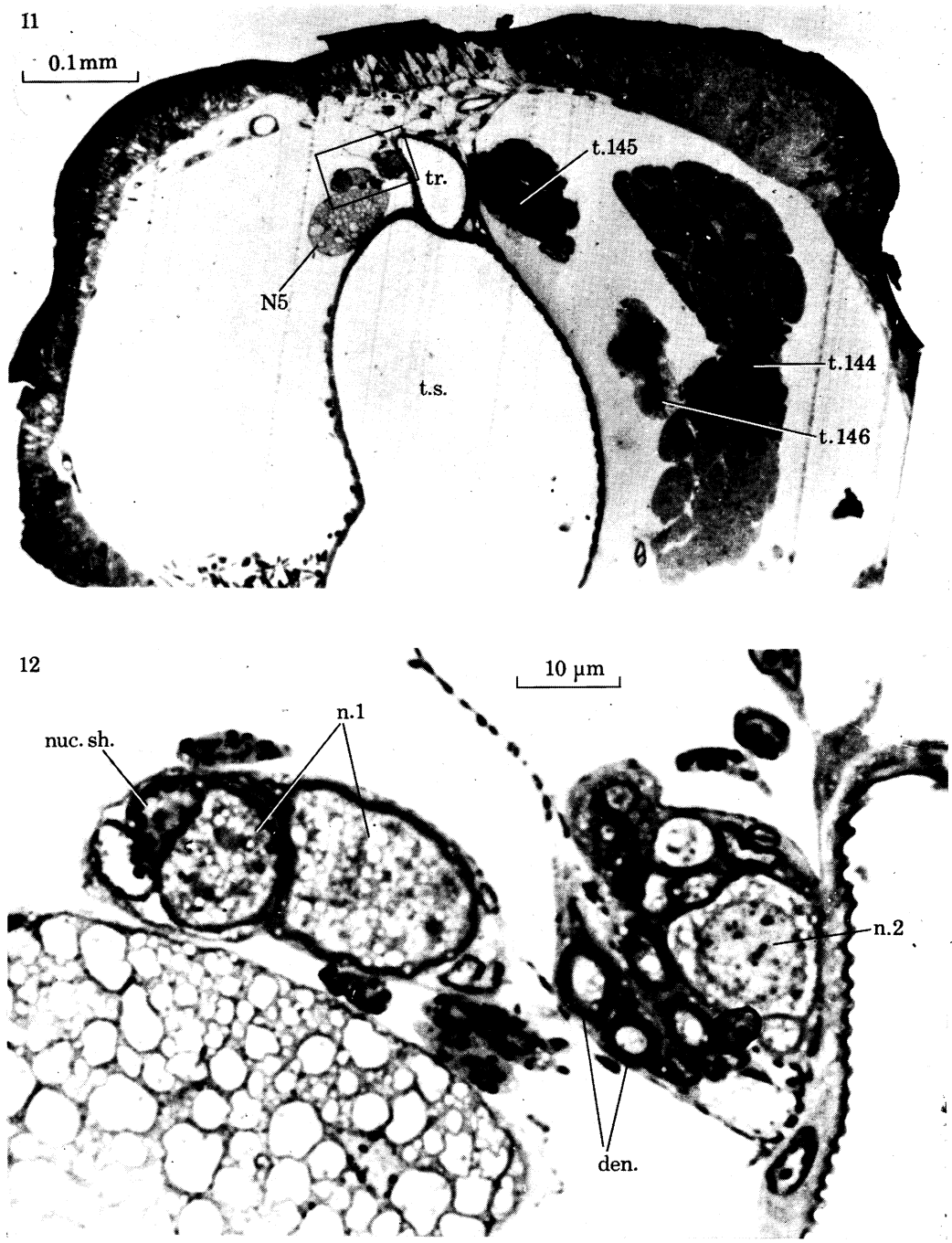


FIGURE 11. Transverse Araldite section through the tibia of the right mesothoracic leg of *Periplaneta*. The chordotonal organ (within the rectangle) is shown at higher magnification in figure 12. Note that the chordotonal organ is closely accompanied by a tracheole (tr.) and the tendon of muscle 145 (t.145). N5, the fifth nerve trunk; t.s., tracheal sac; t.144, tendon of muscle 144; t.146, tendon of muscle 146.

FIGURE 12. The tibio-tarsal chordotonal organ enlarged from figure 11. The level at which this section is cut is shown in figure 5 by a pair of numbered arrows. On the left are shown the two group 1 neurons (n.1.) which supply the single scolopale of side branch B. On the right is the main branch showing a group 2 neuron (n.2.) cut through its nucleus and pairs of group 1 dendrites (den.), the labelled pair cut through the region of heaviest sheathing. nuc.sh., nucleus of sheath cell.

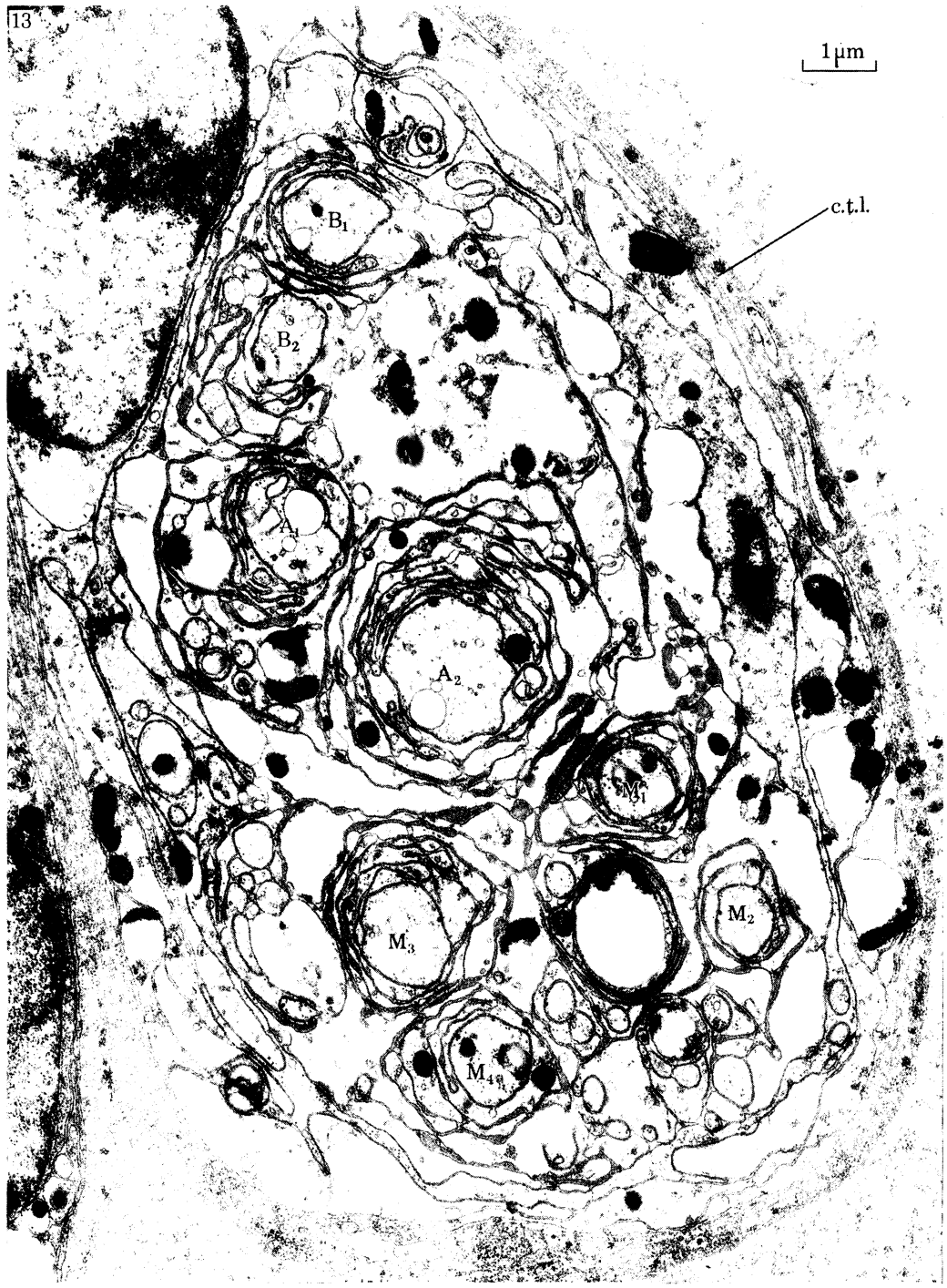


FIGURE 13. An electronmicrograph of a transverse section of the chordotonal nerve at the point where it leaves the main nerve trunk, as shown by the numbered arrow in figure 4. There are about two dozen axons, varying from 0.2 to 1.5 μm in diameter, intermingled with Schwann cell cytoplasm. The nerve is surrounded on the outside by a connective tissue layer (c.t.l.). The axons of group 1 (A_1A_2,B_1B_2,M_1–M_4) can be individually identified and are labelled here according to which neurons they derive from. This labelling is the same as that in figure 5.

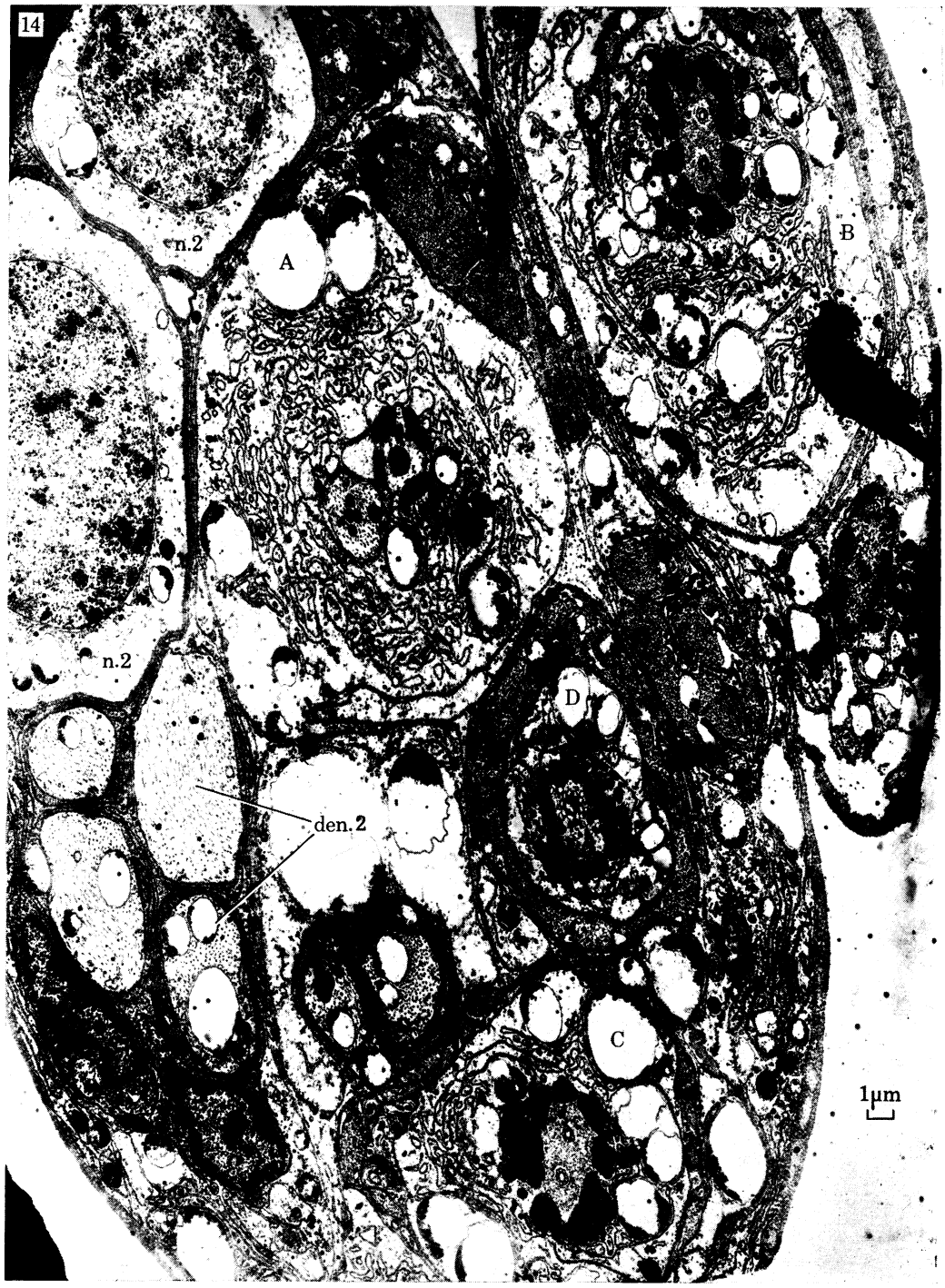


FIGURE 14. An electronmicrograph surveying a transverse section of the chordotonal organ at the point where side branch B leaves the main branch. The approximate level of section is shown by a pair of numbered arrows in figure 5 but this specimen shows a greater degree of overlap between the groups of scolopidia in the proximo–distal axis than does that in figure 5. The section shows four scolopale cells (A, B, C, D) cut at different levels: A, a group 2 scolopale cut through the base of the scolopale; B, the group 1 scolopale of side branch B cut at the level of the ciliary base: C, a group 2 scolopale cut through the mid region; D, a group 1 scolopale cut at the level of the ciliary dilatation. den. 2, group 2 dendrites; n.2., group 2 neurons.

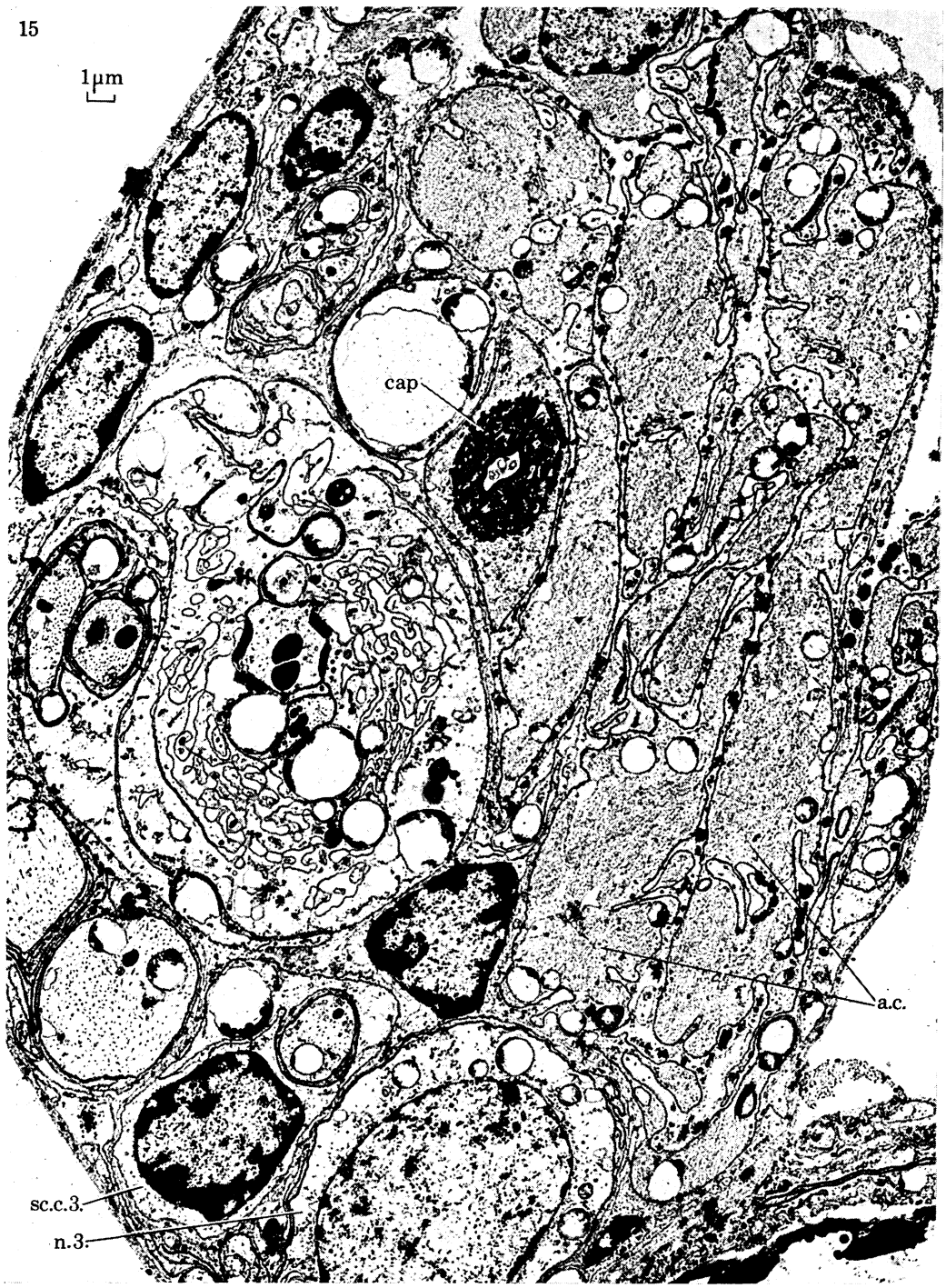
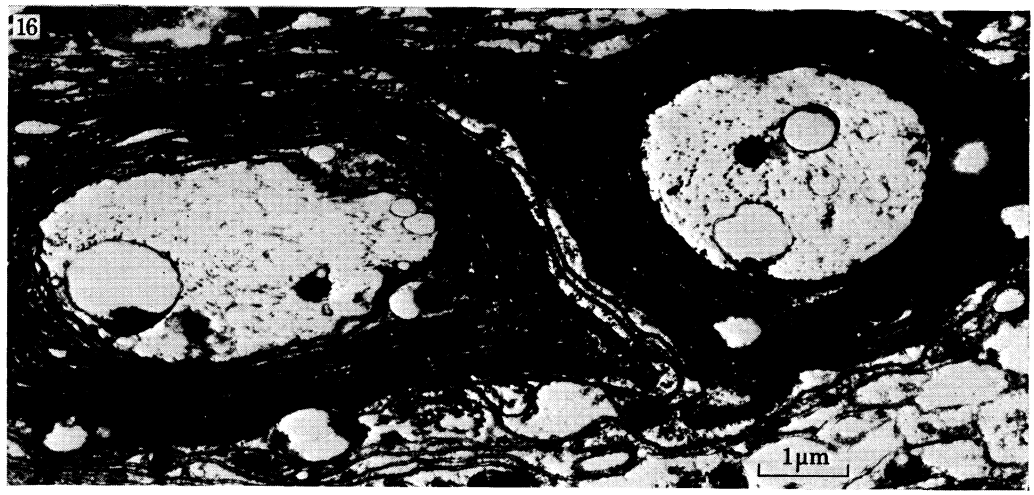


FIGURE 15. An electronmicrograph surveying the chordotonal organ at a level indicated in figure 5 by a pair of numbered arrows. This spciemen shown a greater degree of overlap between the groups of scolopidia in the proximo–distal axis than does that in figure 5. At this level the chordotonal organ main branch consists mostly of attachment cells (a.c.). There are a few group 2 dendrites and scolopales, especially one through the cap. Also at this level can be seen a group 3 neuron (n.3.) and a group 3 scolopale cell, (sc.c.3), cut through its nucleus and enclosing a single dendrite.



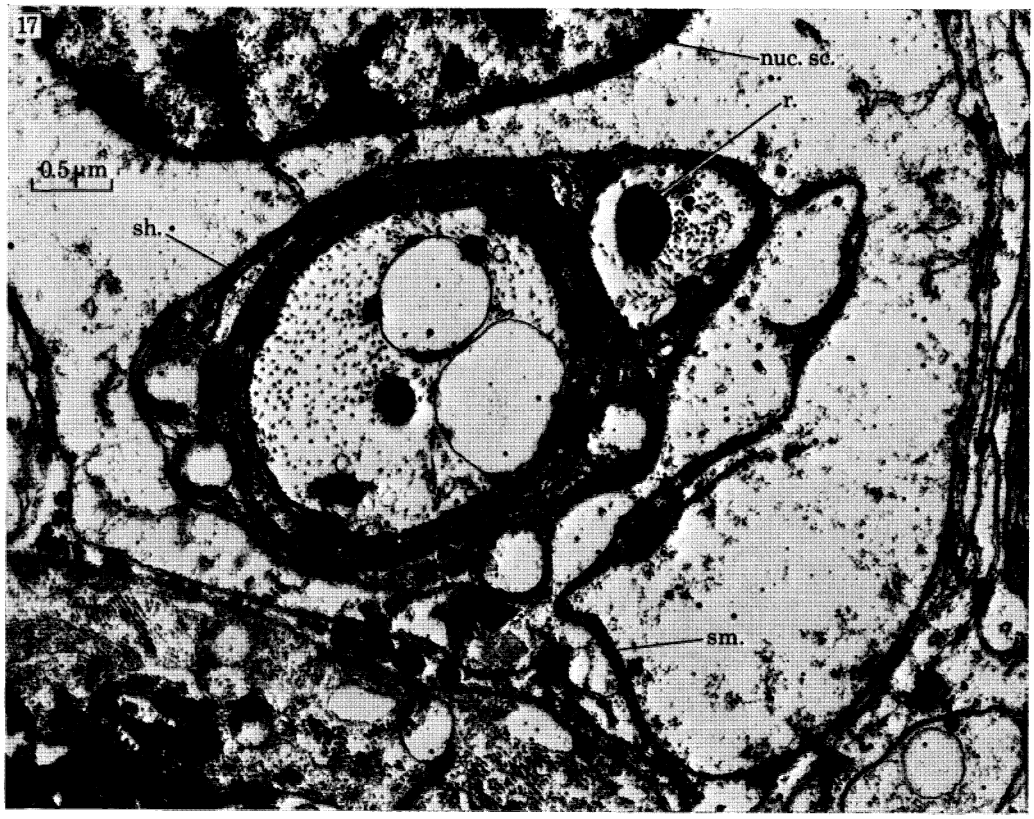


FIGURE 16. Electronmicrograph of a thin section from the same specimen as the thick section shown in figure 12. This section, cut at a short distance distally to that in figure 12, shows the same pair of dendrites which are labelled (den.) in figure 12. The thick sheath can be seen to consist of ten to twelve densely wound folds of the double surface membrane of the sheath cell.

FIGURE 17. Transverse section through a group 1 scolopidium at the level of the scolopale cell nucleus (nuc.sc.) as shown by the numbered arrow in figure 6. An infolding of the surface membrane (s.m.) of the scolopale cell encloses the two dendrites. At this level the sheathing (sh.) of the dendrites has dwindled, more so on the right hand dendrite than on the left hand one. The right hand dendrite contains the large root (r.) of its cilium. Both dendrites contain neurotubules.

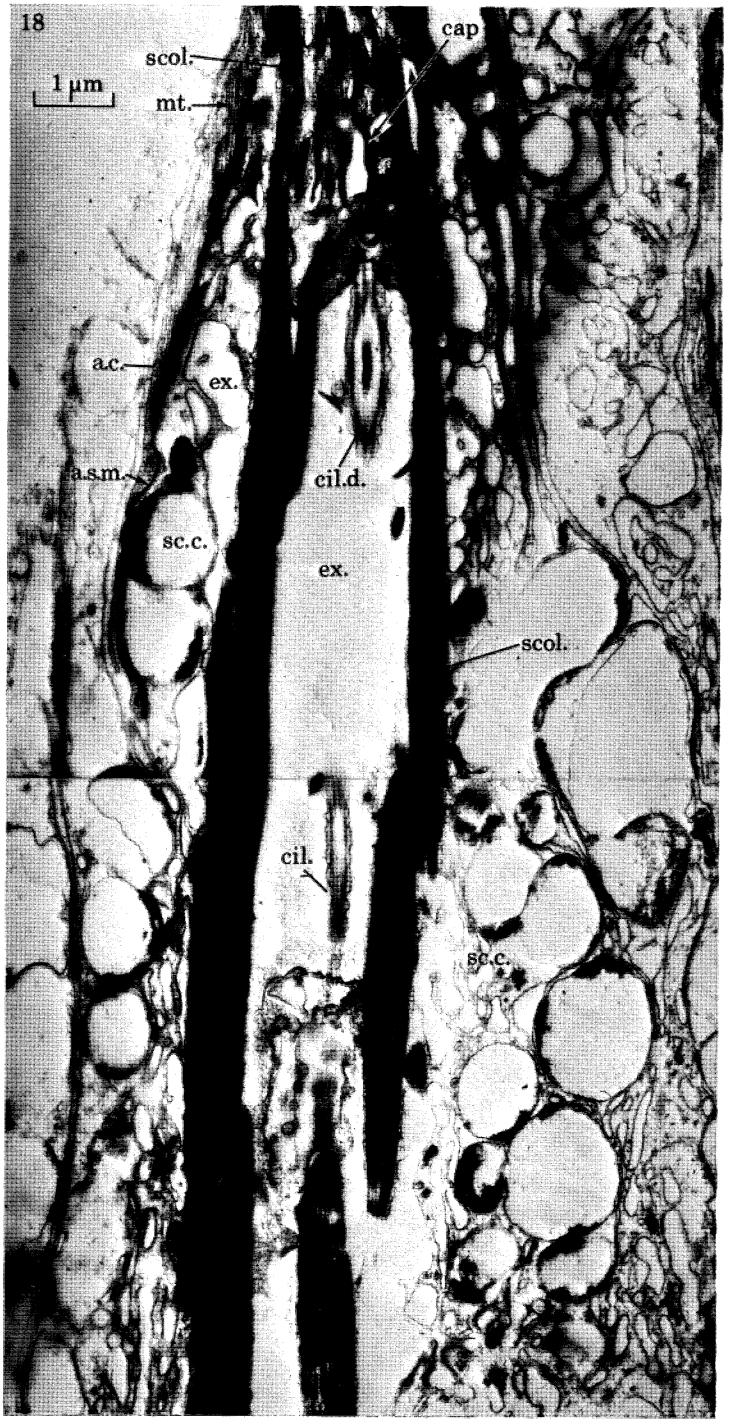
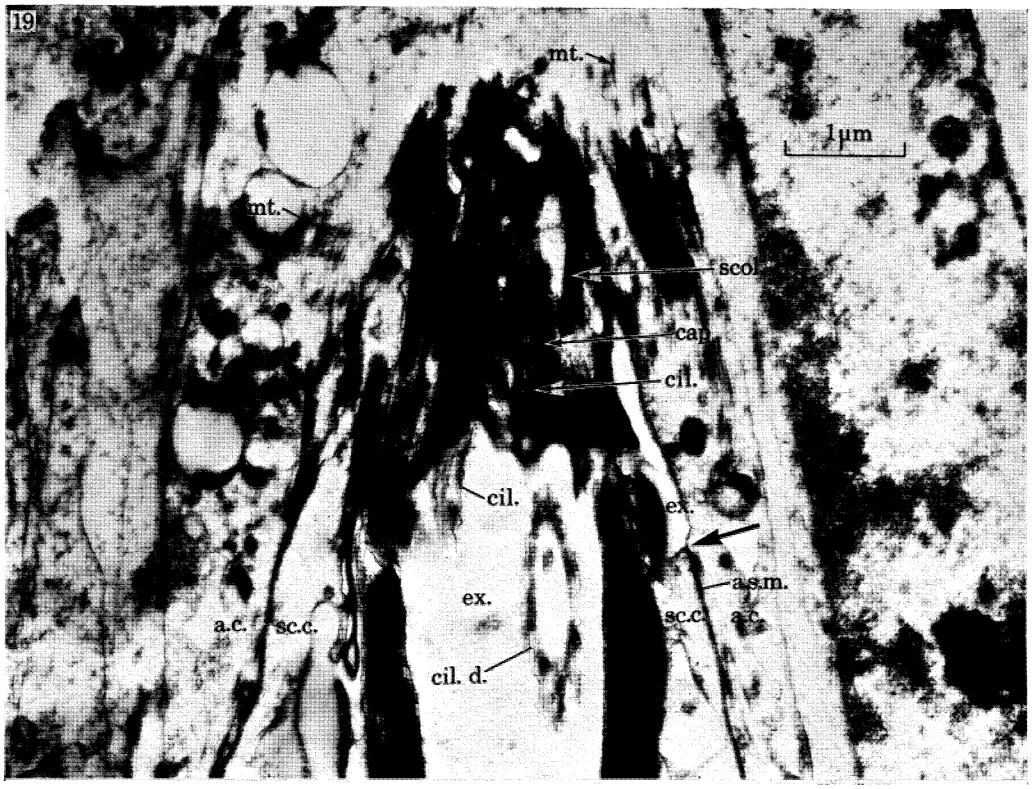


FIGURE 18. Longitudinal section of the scolopale unit, passing through the base of one cilium (cil.) and through the dilatation (cil. d.) of the other cilium. The scolopale rods (scol.) are surrounded by the scolopale cell (sc.c.), whose surface membrane is closely apposed (a.s.m.) to that of the attachment cell (a.c.), which contains many microtubules (mt.). den., dendrite; ex., extracellular space.



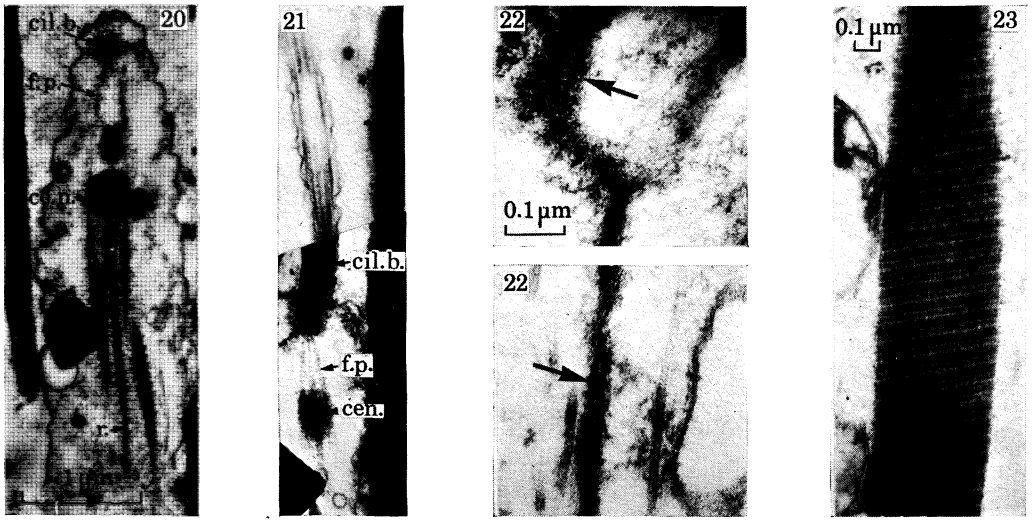


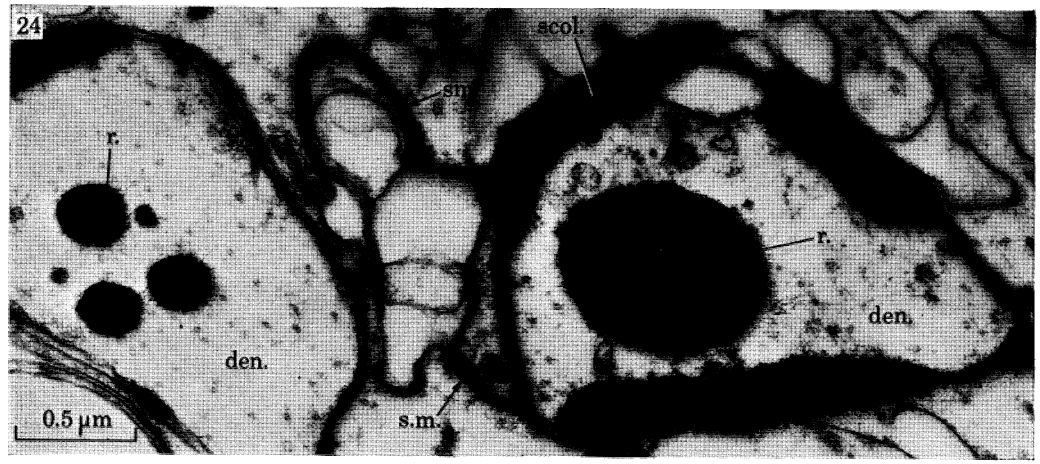
FIGURE 19. Longitudinal section of the distal part of the scolopale unit. The arrow on the right indicates the point where the apposed surface membranes (a.s.m.) of the scolopale cell (sc. c.) and attachment cell (a.c.) separate to pass around the cap material, which is situated in the extracellular space (ex.). The scolopale rods (scol.) penetrate a long way into the cap. The two cilia (cil.) also penetrate well into the cap, fitting tightly into clefts in the cap material. Compare with figures 30 and 31. cil. d., ciliary dilatation; mt., microtubules.

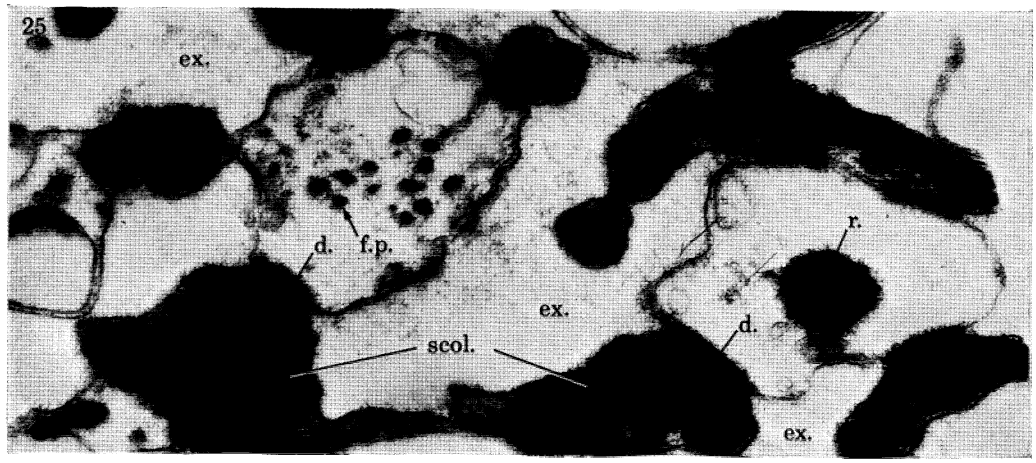
FIGURE 20. Longitudinal section of the centriolar region of the dendrite with rootlets. ce.n., centriole; cil. b. ciliary base; den., dendrite; f.p., finger processes; r., rootlet.

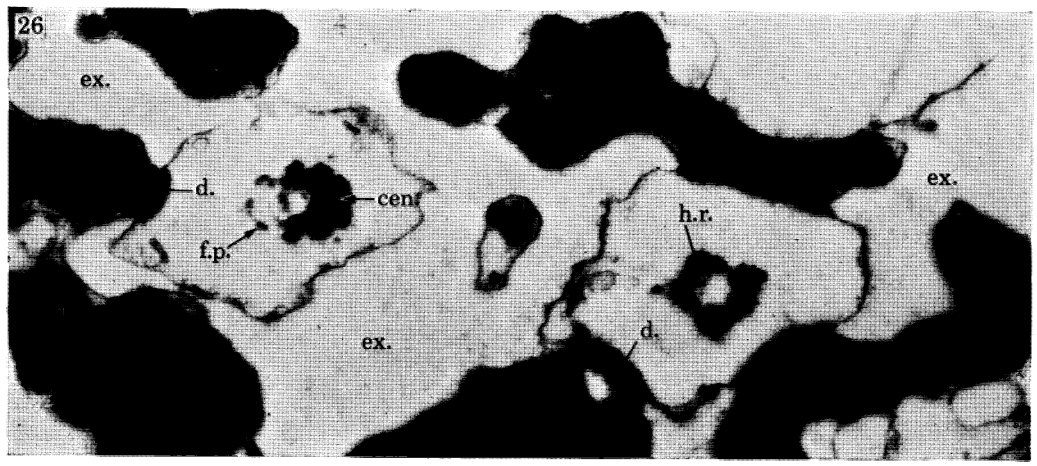
FIGURE 21. Montage of two consecutive longitudinal sections of the centriolar region of the dendrite with the single, large root. Magnification and labelling as in figure 20.

FIGURE 22. Section showing septate desmosomes between the apposed membranes of the dendrite and scolopale cells. The lower part of the picture shows the typical appearance of cross-bridges (arrow) between the apposed membranes. In the upper part of the picture this same pair of membranes have been cut tangentially, showing the cross-bridges to be composed of an hexagonal array (arrow).

FIGURE 23. Longitudinal section through the single, large root, showing the pattern of cross striation.







FIGURES 24, 25 and 26 are electronmicrographs of transverse sections taken at short intervals along the same scolopidium, at the base of the scolopale unit. The level at which each is cut is shown by a corresponding numbered arrow in figure 7.

FIGURE 24. The two dendrites (den.) are surrounded only by the scolopale cell, whose surface membrane fits closely round them and meets itself between them (arrow, s.m.). The dendrite on the left contains three rootlets (r.) and that on the right a single large root (r.). A few scolopale rods (scol.) make their appearance in the scolopale cell.

FIGURE 25. The two dendrites have shrunk away from the scolopale cell leaving an extra-cellular space (ex.) between them. Contact is maintained where scolopale rods (scol.) occur in the scolopale cell and at these points desmosomes (d.) can be clearly seen in the dendrites. On the left the three rootlets have split into bundles of finger processes (f.p.) and on the right the single root (r.) has narrowed considerably. More scolopale rods are now present.

FIGURE 26. The finger processes (f.p.) of the left hand dendrite have become arranged as a ring of nine around the centriole (cen.). This section glances across the top of the centriole so that only some of the finger processes are seen connected to the central tube of electron-dense material. On the right, the single root has become hollow (h.r.) and is just dividing into finger processes.

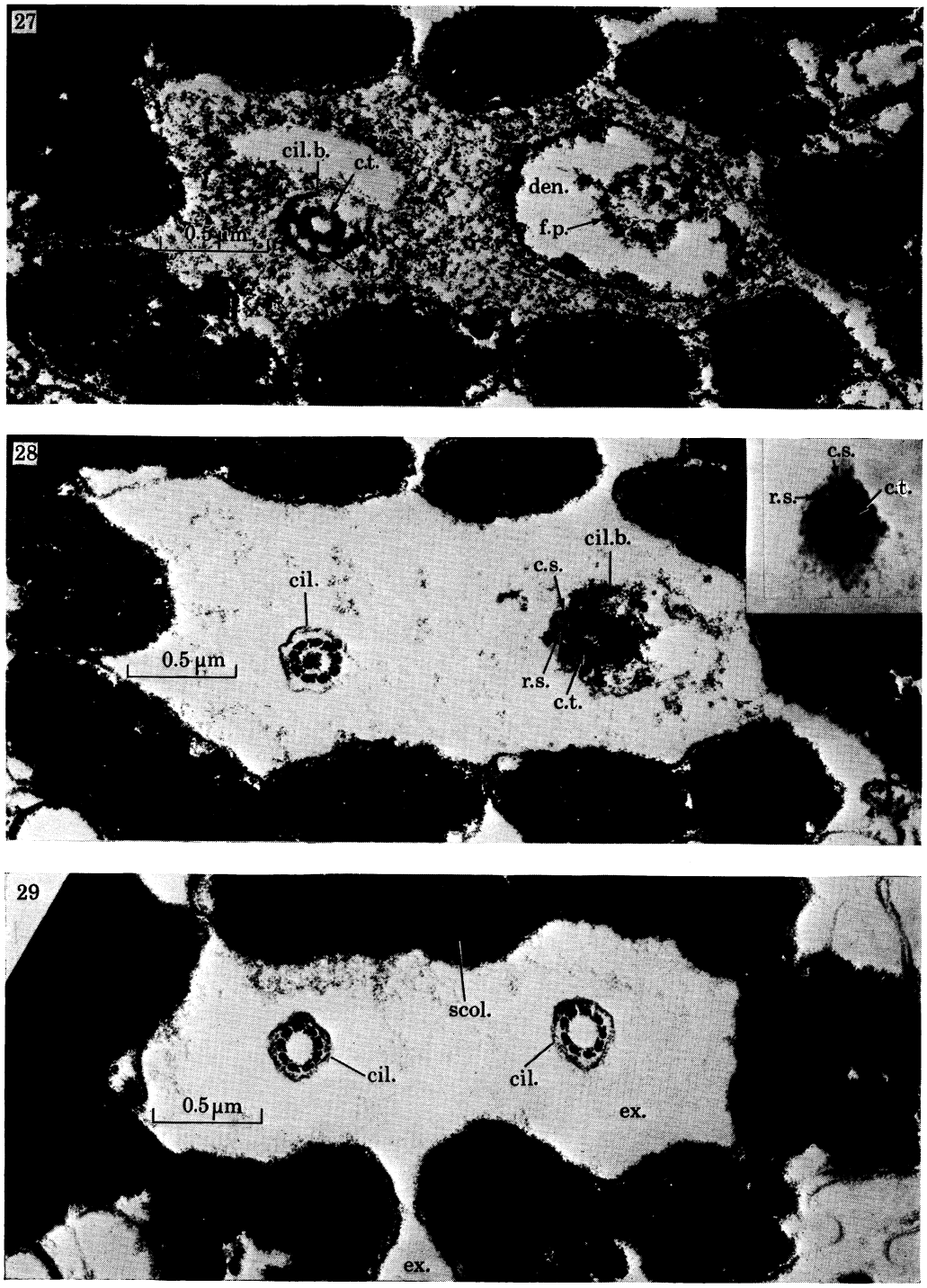
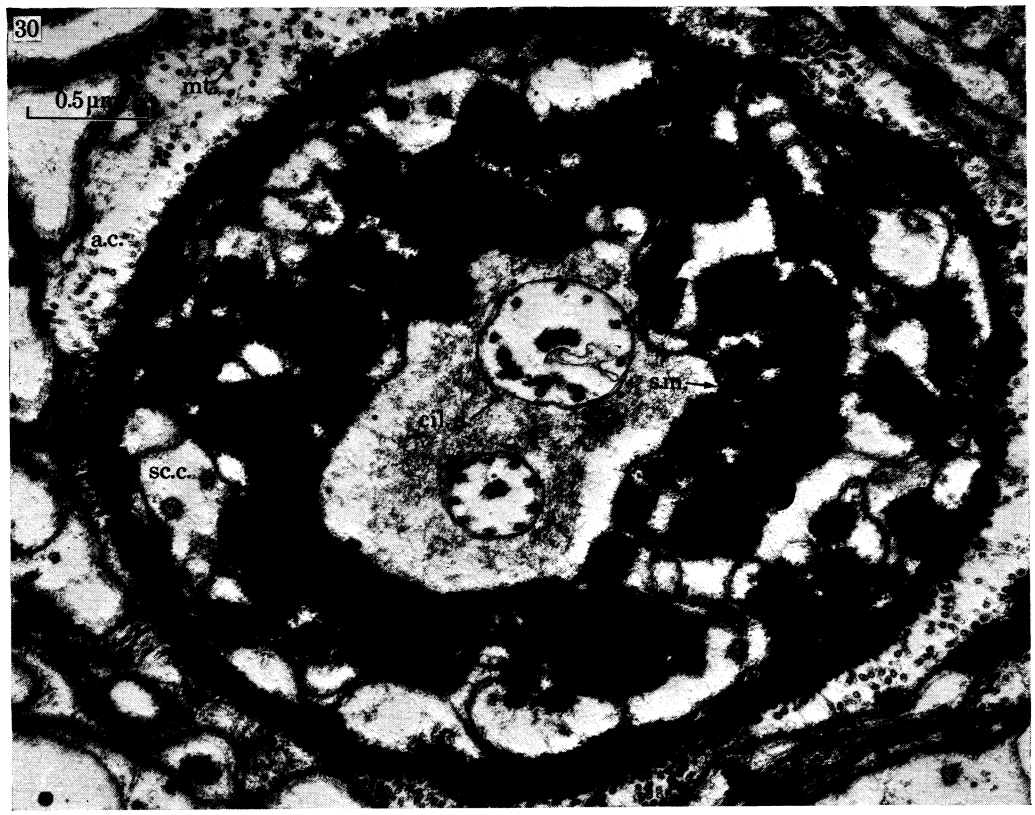


FIGURE 27. Transverse section of the scolopale unit at the level indicated by the numbered arrow in figure 7. On the left, the section passes through the ciliary base (cil. b.) just distal to the point where the ciliary shaft emerges from the surface of the dendrite. At this level, the central tube (c.t.) of the ciliary base is joined to the outer membrane by radial filaments (r.f.). On the right, the dendrite (den.) is cut through the ring of finger processes (f.p.) just below the centriole.

FIGURE 28. The same specimen as shown in figure 27 cut at the level indicated by the numbered arrow in figure 7. On the left, the section passes through the ciliary shaft (cil.). On the right, the section passes through the ciliary base (cil. b.) at the point where the ciliary shaft emerges from the surface of the dendrite. At this point, the central tube (c.t.) bears nine radiating spokes (r.s.), which are cross-connected by oblique strands (c.s.). *Inset.* Another section through the ciliary base at the point where the ciliary shaft emerges from the surface of the dendrite, showing the radiating spokes and their cross-connecting strands. Magnification and labelling as in the main figure.

FIGURE 29. Transverse section through the mid region of the scolopale unit, as indicated by the numbered arrow in figure 7. The two cilia (cil.) are alike, each containing a ring of nine ciliary fibrils. The cilia are surrounded by the extracellular space (ex.) which extends out between the scolopale rods (scol.) in a few places.



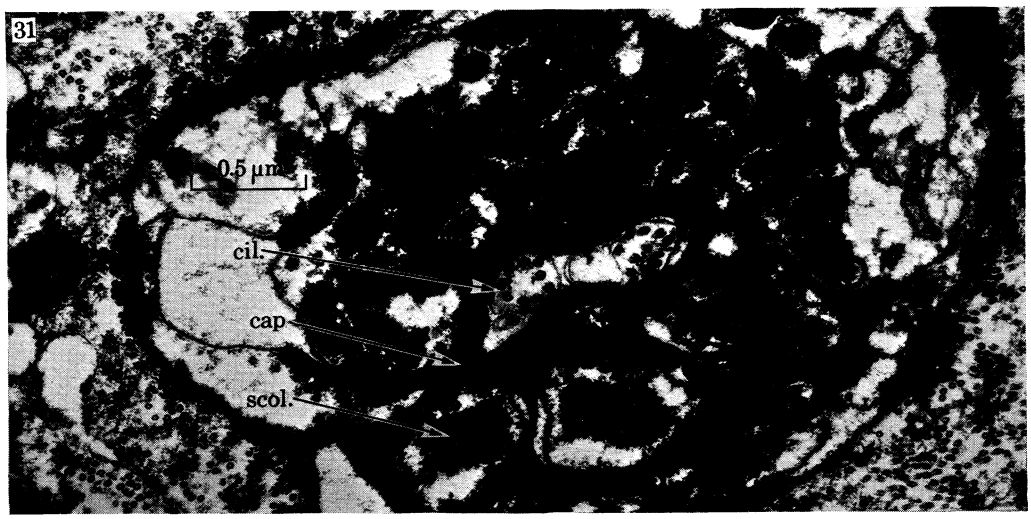
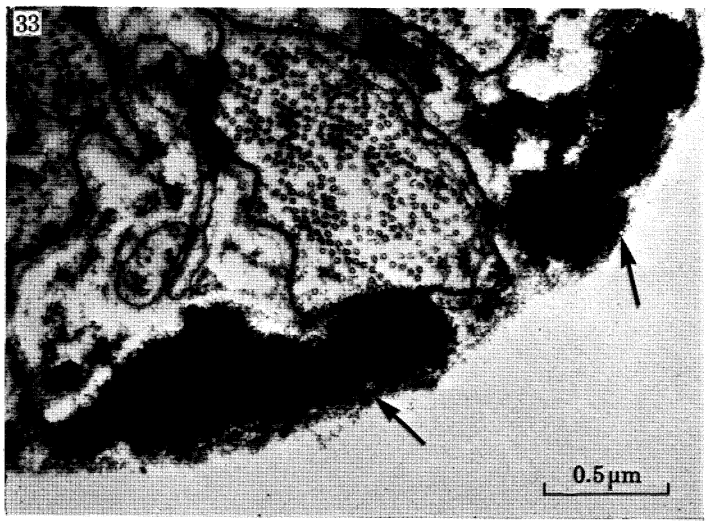


FIGURE 30. Transverse section through the ciliary dilatation (cil. d.) at a level indicated by the numbered arrow in figure 7. At this level the ring of scolopale rods is largely fused except where the surface membrane (s.m.) of the scolopale cell passes through. The scolopale cell (sc. c.) is greatly narrowed and is surrounded by the attachment cell (a.c.) which contains numerous microtubules (mt.).

FIGURE 31. Transverse section through the scolopale cap at a level indicated by the numbered arrow in figure 7. The tips of the two cilia (cil.), each bounded by the nerve cell membrane, are wedged in a cleft in the cap. The tips of the scolopale rods (scol.), each surrounded by the scolopale cell membrane, are inserted into the cap. The cap material itself is extra-cellular. A similar section through a cap can be seen at lower magnification in figure 15.



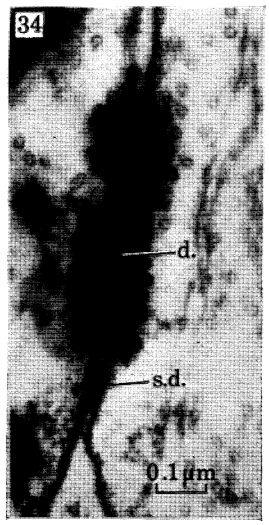


FIGURE 32. Transverse section through the attachment cell some distance beyond the cap. Many of the microtubules are arranged rather regularly in rows along infoldings of the surface membrane.

FIGURE 33. Transverse section through the neural lamella in the distal region of the chordotonal organ, showing the electron dense material (arrows) embedded in the neural lamella.

FIGURE 34. Transverse section through a region where infoldings of the attachment cell surface membrane are closely apposed to each other. Desmosomes (d.) and a small septate desmosome (s.d.) can be seen.

reconstructions were nine in one and eleven in the other. The neurons can be distinguished by their large nuclei and extensive mass of cytoplasm. Most of the sensory neurons are clustered proximally in two groups (figures 4 and 5). The more proximal of these, group 1, contains the largest neurons found in this sense organ, while group 2, which extends along the main branch, contains a somewhat larger number of smaller neurons. The distinction between these two groups of cells is clearly seen with the light microscope. Some way along the main branch is a third group of still smaller neurons but these can hardly be distinguished by light microscopy. Around the neurons there are many satellite cells which have small, round or elongate nuclei and lack a clearly delineated mass of cytoplasm. The relations of some of these can be determined with the electron microscope.

(*b*) *The number and arrangement of the scolopidia*

By reconstruction from light and electron microscope sections of Araldite-embedded material it was possible to locate every neuron and scolopale in the chordotonal organ (figure 5). The neurons associated with a given scolopale could not be identified in every case and so counts of scolopidia are based on counts of scolopales and neurons. The number of scolopidia belonging to each of the groups is shown for the three Araldite reconstructions in table 1.

TABLE 1. THE NUMBER OF SCOLOPALES AND NEURONS BELONGING TO EACH OF THE GROUPS

specimen number	scolopale total	group 1	group 2	group 3	neuron total
EM2	15	4	7	3	25
EM5	14	4	8	2	26
EM7	15	4	8	3	27

The group 1 neurons and scolopales can all be seen in wax sections but the more distal groups are more difficult to detect and cannot all be seen in wax sections. This accounts for the lower total number obtained in the light microscope reconstructions from wax sections.

If there is no axonal fusion in the chordotonal nerve, then the number of axons in the chordotonal nerve should be the same as the number of neurons recorded on the right of table 1. The reconstructions yield neuron counts of 25 to 27 and good agreement is found when a section through the chordotonal nerve is examined (figure 13, plate 79). The total number of axons is about two dozen. It is not possible to give a more exact figure because the smallest axons cannot always be distinguished from infoldings of Schwann cell membranes, even in serial sections. An exact result can be obtained for group 1 neurons. As these are the largest and most proximal it is possible to identify particular neurons with particular axons. There are eight group 1 neurons and eight group 1 axons in the chordotonal nerve; in figure 13 these are labelled according to which particular scolopidium they derive from. Thus there is no axonal fusion before the chordotonal nerve enters the trunk of N5.

Especially with the group 1 neurons, individual neurons can be identified from specimen to specimen by virtue of their characteristic size, location and orientation. The eight group 1 neurons are always arranged in a cluster, stacked dorso-ventrally (figures 4 and 5). This dorso-ventral arrangement of the group 1 neurons is also reflected in the arrangement of their axons in the chordotonal nerve (figure 13, plate 79). The eight group 1 neurons comprise four scolopidia whose specific arrangement is shown in figure 5 and this arrangement remains remarkably constant

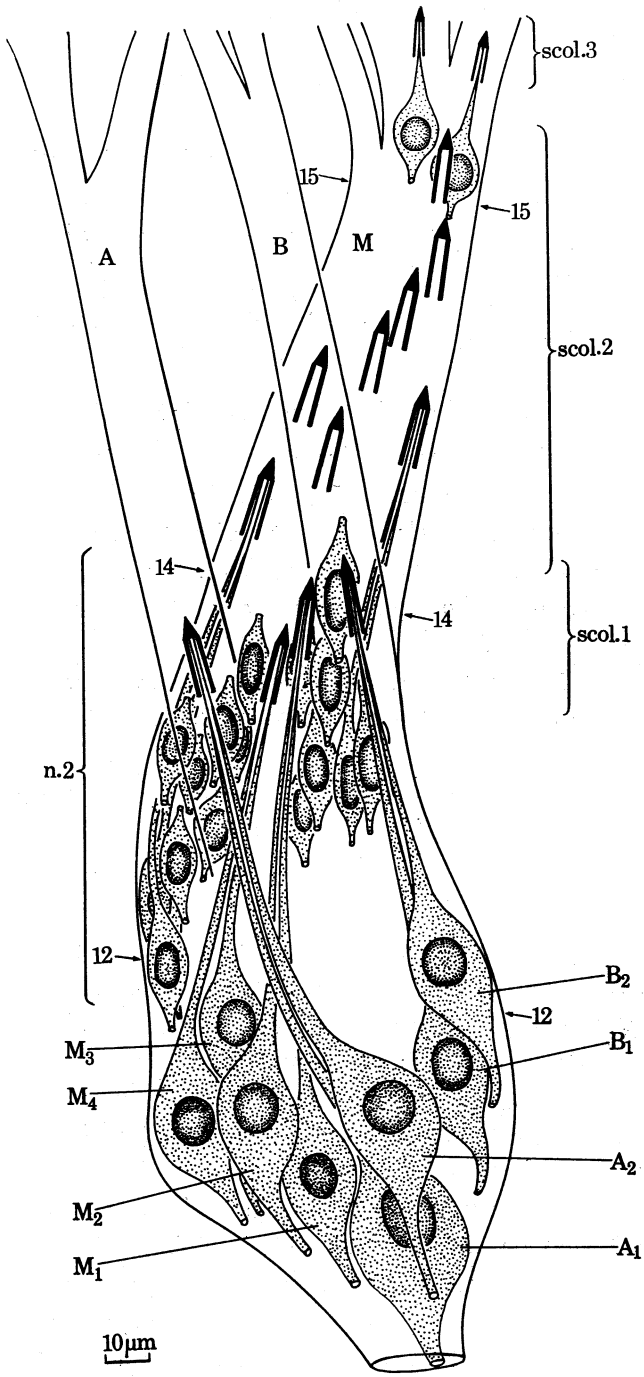


FIGURE 5. Reconstruction of the tibio–tarsal chordotonal organ from light and electron microscope sections of Araldite-embedded material. The exact plane of section is indicated in figure 3 by a pair of numbered arrows. Seen in posterior view, this reconstruction shows the location of every scolopale and neuron and their arrangement in three groups of scolopidia. The group 1 neurons are labelled individually according to which of the group 1 scolopales (scol. 1) they supply: A_1 and A_2 supply the scolopale of side branch A; B_1 and B_2 supply side branch B; M_1 and M_2 supply the first, and M_3 and M_4 the second, of the scolopales of the main branch (M). To save confusion, the dendrites of the group 2 neurons (n.2) have not all been connected to their corresponding scolopales (scol. 2). Also for clarity, this figure is taken from the specimen which shows least overlap between the groups of scolopidia in the proximo–distal axis. The paired arrows, numbered 12, 14 and 15, show the level of section of the corresponding plate figures. scol. 3: group 3 scolopales.

in all the material examined. The two neurons supplying the scolopidium of side branch B (B_1 and B_2) are situated most dorsally and are orientated downwards and inwards with respect to the main branch. The two neurons supplying the scolopidium of side branch A (A_1 and A_2) are situated next most dorsally and are always the two largest neurons. Their dendrites also curve downward and inward with respect to the main branch. The four neurons of group 1 which supply the most proximal two scolopidia of the main branch (M_1 to M_4) are situated ventrally and their dendrites curve upwards and outwards. Thus, within group 1, the two main branch scolopidia and the two side branch scolopidia pass across each other like a pair of scissors. In the proximo–distal axis the location of the neurons is more flexible. The neurons, A_1, A_2, B_1 and B_2, always occur close together but may shift as a group relative to the neurons, M_1 to M_4, which also occur close together.

The arrangement of the group 2 scolopidia is also remarkably constant (figures 4 and 5). The neurons of the most proximal of the group 2 scolopidia usually overlap in the proximo–distal axis with the most distal group 1 neurons and their corresponding scolopales also overlap. The remaining group 2 neurons spread upwards and outwards along the main branch, with their corresponding scolopales similarly spread out along the main branch. They all have a similar orientation. The small group 3 scolopidia are always found in the distal extremities of the main branch and usually overlap with the most distal of the group 2 dendrites and scolopales.

This spatial distribution of the scolopidia is reflected in the organization of the chordotonal organ when seen in transverse sections. Thus at the level shown in figure 12, plate 78, there are mostly group 1 neurons and dendrites with the first of the group 2 neurons. At the level shown in figure 14, plate 80, there are group 1 scolopales alongside group 2 neurons, dendrites and scolopales. While at the level shown in figure 15, plate 81, the chordotonal organ consists of groups 1 and 2 attachment cells, alongside the last of the group 2 scolopales and a group 3 neuron and dendrite.

(*c*) *The sensory neurons and their satellite cells*

The arrangement and fine structure of the sensory neurons and their satellite cells are described first for the group 1 scolopidia. The other groups will then be compared with this. Most detail was obtained for group 1 by taking advantage of the fact that side branches A and B each contain only one scolopale. In the case of these side branches it can be seen clearly that two sensory neurons occur together, rather distinct from the rest (figure 12, n.1), and that these have sheathed dendrites which pair off (as in figure 12, den.) and supply a single scolopale (figure 14, B). This made it easier to reach reliable conclusions about the relations between the neurons and their satellite cells. A detailed reconstruction of the scolopidium of side branch B is shown in figure 6*a*.

The two neurons of a scolopidium occur side by side. They are both alike, being about 15 to 20 μm in diameter except for A_1 and A_2 which are about 25 μm, with a nucleus of 8 to 12 μm. Each neuron has a proximal process or axon, which is about 1 to 1.5 μm in diameter at the point where it enters nerve 5, and a distal process or dendrite, 60 to 100 μm long, which tapers gradually until it reaches the scolopale.

Each cell body and axon are surrounded by folds of Schwann cell cytoplasm (figure 13, plate 79) The nucleus of the Schwann cell is situated near the cell body of the neuron with which it is associated (figure 10, plate 77). The Schwann cell sheath conforms to the description given by Smith & Treherne (1963). It consists of a layer of cytoplasm, bounded by the cell membrane, which is loosely spiralled round the axon two or three times, leaving an extracellular space between the

folds. Homogenous extracellular material often occurs in dilatations of the extracellular space giving the whole a beaded appearance. In the region of the axon hillock the Schwann cell sheath becomes more elaborately folded and thicker in extent but retains its loose arrangement so that around the cell body the sheath closely resembles that of a motor neuron.

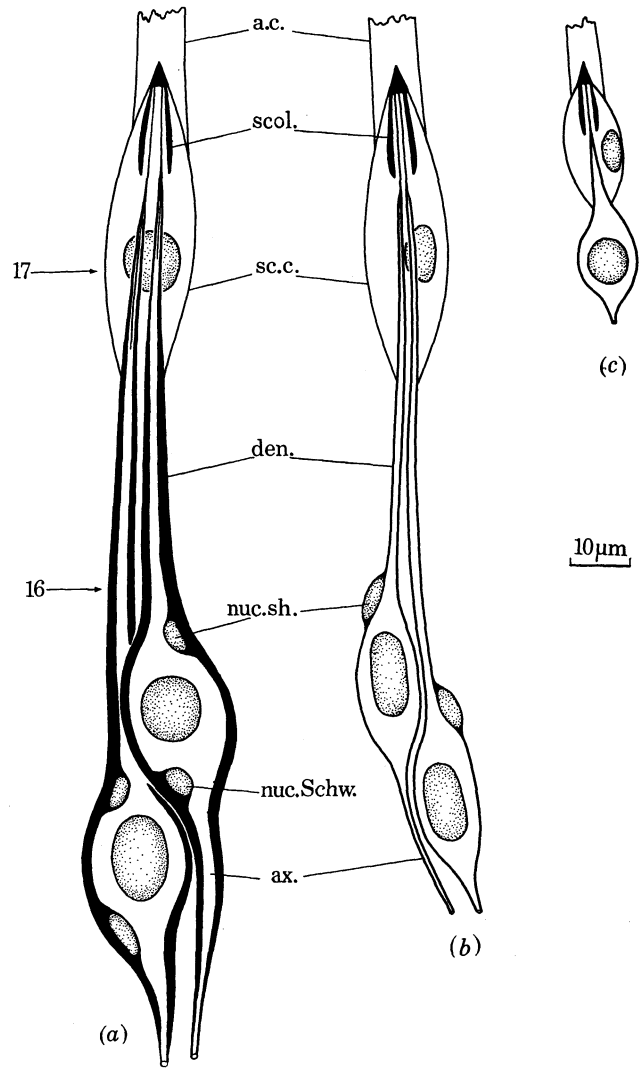


FIGURE 6. Details of the three groups of scolopidia found in the tibio–tarsal chordotonal organ: (*a*) group 1 consisting of two bipolar neurons, 15 to 20 μm in diameter, whose dendrites, heavily sheathed, insert in a single scolopale. (*b*) group 2, consisting of two bipolar neurons 8 to 15 μm in diameter, whose dendrites, less well sheathed, insert in a single scolopale. (*c*) group 3, consisting of a single bipolar neuron, about 10 μm in diameter, with a short, poorly sheated dendrite inserting in a single scolopale. Reconstructed from light and electron microscope sections of Araldite-embedded material. The arrows numbered 16 and 17 show the level of section of the corresponding plate figures. a.c., attachment cell; ax., axon; den., dendrite; nuc.sh., sheath cell nucleus; nuc.Schw., Schwann cell nucleus; scol., scolopale; sc.c., scolopale cell.

The dendrite is surrounded by a thick sheath formed from a cell whose nucleus is situated at the base of the dendrite, close to the cell body (figures 10 and 12, plates 77 and 78). The sheath is thickest at the base of the dendrite and dwindles towards the tip of the dendrite. Electron micrographs show that the sheath consists of concentric, tightly wound folds of double

surface membrane of the sheath cell, up to twelve in number at the thickest part (figure 16, plate 82). The nucleus of the cell is enclosed between the folds of membrane and the neuron cell body. The overall appearance is similar to that of vertebrate myelin except that the membranes are not quite as tightly wound. These sheaths also appear similar to the glial sheaths of the lateral motor axons of *Rhodnius* described by Wigglesworth (1959*b*).

Distally the sheathing round the dendrites dwindles and a pair of dendrites become surrounded instead by a single scolopale cell (figure 17, plate 82). The details of the scolopale cell and its associated structures are described in a following paragraph.

(*d*) *The groups of scolopidia compared*

Group 2 scolopidia are generally similar to those of group 1, having two neurons inserting into a single scolopale (figure 6*b*), but the neurons are smaller, being about 8 to 15 μm in diameter, with nuclei of 5 to 10 μm. They are also more elongate cells than group 1, and are flattened so that their maximum diameter may be almost twice their minimum diameter. In wax sections their cytoplasm stains less heavily with Masson's stain than group 1 neurons. Their axons are distinctly narrower, being 0.5 μm or less in diameter, and they do not have individual Schwann cells. It was difficult to determine whether the neuron cell bodies had individual Schwann cells or not and so Schwann cell nuclei have been left out of the reconstruction in figure 6*b*. The dendrites are 60 to 90 μm long and are surrounded by individual sheath cells. However, the sheath is less substantial, having only three or four layers of double surface membrane (figure 14, den. 2, plate 80). The scolopale cells and scolopales appear to be identical with those of group 1.

Group 3 scolopidia were detected only in the electron microscope sections. They are rather different from the other groups, having only one neuron per scolopale (figure 6*c*). The neuron is quite small, 8 to 10 μm in diameter with a large nucleus-to-cytoplasm ratio (figure 15, n.3, plate 81). The cell body has two or three layers of Schwann cell wrapping. It was not possible to trace their individual axons but they are assumed to be the smallest ones found in the chordotonal nerve, which are 0.2 to 0.3 μm in diameter (figure 13, plate 79). The dendrite is quite short, sometimes as little as 10 μm, and it is surrounded only by the scolopale cell (figure 15, sc.c. 3, plate 81). Group 3 scolopales are generally similar to those of group 1 apart from the obvious distinction of containing only one cilium.

(*e*) *The surrounding cells and the perineurium*

In addition to the specialized cells surrounding the neurons there are many similar cells which contribute to the general surrounding tissue and are not specifically associated with any one neuron. These may be termed the surrounding cells. The surrounding cells also form the outer connective tissue layer which consists of an outer, homogenous, non-cellular layer, the neural lamella, and beneath this the sheath cells or perineurium, following the terminology of Ashhurst (1959, 1968). Especially in the more distal regions, there are rods of fibrous material embedded in the neural lamella (figure 33, arrows, plate 88). Hess (1958) showed that in the peripheral nerves of *Periplaneta* the same class of cells make up the perineurium as sheath the axons and so he called them all Schwann cells. Wigglesworth (1959*a*, *b*) found that the same was true of *Rhodnius* and he considered that this same class of cells became specialized in the central ganglia to form the four types of glial cells. He called all these cells glial cells, as do Smith & Treherne (1963). Similarly, it would seem legitimate to regard the specialized satellite

cells of the sensory neurons as derivations of this same class of cells. In electron microscope sections they all have similar nuclei which differ characteristically from neuron nuclei (figures 10, 14 and 15, plates 77, 80 and 81).

(*f*) *The scolopale unit*

The fine structure of the scolopale cell and its associated structures is shown in figure 7 and is illustrated by electron micrographs in figures 18 to 34, plates 83 to 88.

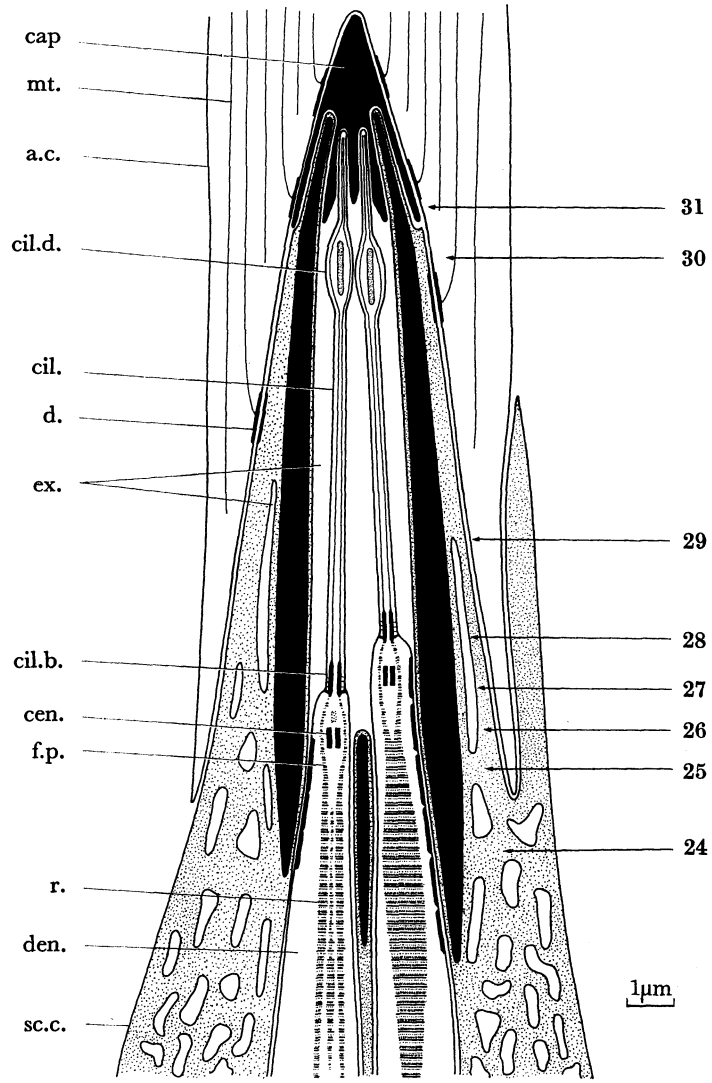


FIGURE 7. Reconstruction of the scolopale unit found in the tibio–tarsal chordotonal organ. A detailed description is given in the text. Some transverse sections of this unit are shown in figures 24 to 31 and the levels at which they are cut are shown by corresponding numbered arrows in this figure. a.c., attachment cell; cen., centriole; cil., cilium; cil.b., ciliary base; cil.d., ciliary dilatation; d., desmosome; den., dendrite; ex., extracellular space; f.p., finger processes; mt., microtubule; r., root; sc.c., scolopale cell.

At their tips, the paired dendrites of the sensory neurons each bear a cilium which inserts into the scolopale. Each cilium has a root, which projects back along the dendrite. In longitudinal section the root appears cross-striated, in a manner identical with that described by Gray (1960). The structure of this root differs consistently between the two members of a pair

of dendrites. In one member of the pair there is a single, large root which extends back for up to 30 μm before splitting into rootlets and terminating. At its widest part, just proximal to the scolopale, it is almost 1 μm in diameter (figure 24, plate 85) but it narrows down as it enters the scolopale (figure 25, plate 85). Then the root becomes hollow (figure 26, plate 85) and divides into nine finger processes, which form a ring enclosing a centriole before inserting on the ciliary base (figure 21, plate 84). In the other member of the pair there are three or four rootlets, each less than 0.5 μm in diameter (figure 24), which extend back for a short distance only. As they enter the scolopale these rootlets do not fuse but split into groups of finger processes (figure 25) which form a ring enclosing a centriole before inserting on the ciliary base (figures 20 and 26, plates 84 and 85). Usually, but not invariably, the dendrite containing this latter form of root does not penetrate quite as far into the scolopale as the other (figure 7). Also, the structures in the centriolar region are more drawn out longitudinally in the dendrite with the rootlets than in that with the single, large root (compare figures 20 and 21, plate 84). However, the structure of the centriole itself is similar in both cases. It comprises a central tube of electron-dense material, about 0.2 μm in outer diameter and 0.35 μm long, connected by nine radial flanges to the nine finger processes, which form an outer ring about 0.4 μm in diameter. The whole is embedded in a matrix of moderately electron-dense material (figure 26, plate 85, c.f. figures 20 and 21, plate 84).

The cilia are identical in both members of a pair. The ciliary base consists of a centriole-like structure of slightly different design from the one associated with the finger processes. There is a central tube of electron-dense material, about 0.2 μm in outer diameter and up to 1 μm long, into which the finger processes insert (figures 20 and 21). Embedded in this central tube are nine groups of microtubules which are continuous with the nine ciliary fibrils (figure 21). At the base of the ciliary shaft, each of these groups of microtubules is connected by a radial filament of electron-dense material to a thickening on the surface membrane of the cilium, at one or two points along their length (figure 27, plate 86). At the point where the shaft of the cilium emerges from the surface of the dendrite, the central tube bears nine radiating spokes of electron-dense material, one arising from each of the nine groups of microtubules. These spokes pass tangentially outwards and are cross-connected to each other by oblique strands of electron-dense material, forming an outer ring about 0.4 μm in diameter (figure 28 and inset). The ciliary shaft contains a ring of nine ciliary fibrils but there are no central fibrils. Each of these fibrils has the typical appearance in section of an electron-dense rod in the middle with a tube attached on one side and a pair of flanges (or 'arms') on the other (figure 29, plate 86). The cilia are about 0.3 μm in diameter but near their tips each cilium has a dilatation where it reaches 0.7 μm in diameter (figures 18, 19 and 30, plates 83, 84 and 87). In the dilatation, there is fibrous material in the centre of the cilium which is not found elsewhere along the length of the cilium. This fibrous material is usually confined to the centre but sometimes it almost fills the dilatation (figure 14, D). Also in the dilatation the ciliary fibrils take on the appearance of two tubes, a form which they retain for the rest of their length. Shortly beyond the dilatation the two cilia insert into the cap, where they are wedged tightly in a cleft in the cap material (figures 19 and 31, plates 84 and 87).

This arrangement of cilia and roots is similar to that reported from other insect chordotonal organs (Gray 1960; Howse 1968). These authors do not describe the radiating spokes associated with the ciliary base but these structures could be missed easily. Similar radiating spokes have been described in a similar location for crustacean chordotonal organs (Whitear 1962) and for

the kinocilia of the vertebrate acoustico-lateralis system (Flock & Duvall 1965; Wersäll, Flock & Lundquist 1965). The description of the vertebrate kinocilium basal body given by these authors closely resembles the detailed design of the ciliary base described here. The basal region of the cilium may be thought of as having two centriole-like structures in series, an arrangement found in several other insect sense organs such as chordotonal organs (Howse 1968), other mechanoreceptors (Thurm 1964) and chemoreceptors (Slifer 1967). It is rather remarkable that the arrangement of centriolar structures in this sense organ is so similar to that of the chemoreceptors described by Slifer & Sekhon (1964). Also of particular interest is the association in one scolopidium of identical cilia with unlike ciliary roots, which has not been reported before in the literature.

At its proximal end, the scolopale can be seen to consist of about eight rods of electron-dense tubular material (figures 24 and 25, plate 85). These rods come together to form a ring of material which is oval in transverse section (figures 27 and 28, plate 86). The individual rods may partly subdivide in the mid region (figure 29, plate 86) but in the region of the ciliary dilatation they become partly fused (figure 30, plate 87). They insert into the cap as distinct rods (figure 31, plate 87). The scolopale is narrower at the cap than at the base. The scolopale rods are entirely enclosed within the scolopale cell.

Proximally to the scolopale, the scolopale cell encloses the paired dendrites within an infolding of the surface membrane. Its nucleus is situated in this proximal region of the cell (figure 17, plate 82). Where the dendrite sheath cells dwindle away, the scolopale cell fits tightly around the dendrites and separates them (figure 24, plate 85). In this region, where the surface membranes of the dendrite and the scolopale cell are apposed to each other, scattered septate desomosomes (Locke 1965; Wood 1959) can be seen between the apposed membranes. In strictly longitudinal or transverse sections they appear as simple cross-bridges (figure 22, lower part, plate 84) but when cut tangentially they can be seen to consist of an hexagonal array (figure 22, upper part). Where a scolopale rod occurs in the scolopale cell, desmosomes (*maculae adhaerens*, Farquhar & Palade 1963) are found at the apposed membranes of dendrite and scolopale cell (figures 25 and 26, plate 85). Within the scolopale there is a large extracellular space between the cilia, which are bounded by nerve cell membrane, and the scolopale rods, which are bounded by scolopale cell membrane. This extracellular space extends out between the scolopale rods and forms complex channels through the body of the scolopale cell so that membrane-bounded cytoplasm and extracellular spaces are intermingled. Thus the scolopale rods are contained within columns of membrane-bounded cytoplasm, surrounded by irregular extracellular spaces. These features can be seen especially in figures 14, 25 and 26, (plates 80 and 85).

As the scolopale narrows towards the cap, the scolopale cell narrows considerably as well and becomes surrounded by the attachment cell (figures 14 and 30, plates 80 and 87). The scolopale cell terminates where the scolopale rods insert into the cap (figure 19, plate 84). The attachment cell is firmly apposed to the scolopale cell but it passes round the cap, which is an extracellular structure. The cap is cone shaped (figure 9, plate 77). It has no filament or tube projecting distally to the point of attachment as in some other kinds of scolopidium (Howse 1968). The attachment cell fits closely round the cap and extends distally to the intersegmental membrane, its length varying according to the position of the scolopale. It contains many microtubules orientated longitudinally and these are particularly dense where contact occurs with the scolopale cell (figure 30, plate 87). Where the attachment cell and scolopale cell

membranes are apposed to each other both desmosomes and septate desmosomes are found and sometimes the microtubules arise from the desmosomes. Distally the microtubules are often arranged rather systematically along infoldings of the surface membrane (figure 32, plate 88) and both desmosomes and septate desmosomes are found in places where these infoldings are closely apposed to each other (figure 34, plate 88).

5. The physiology of the chordotonal organ

The recording method employed would pick up impulses from both campaniform sensilla and the chordotonal organ when they were stimulated. However, the chordotonal spikes are readily distinguished from those of campaniform sensilla in routine preparations. Though about the same order of size as the larger chordotonal spikes, the pattern from the campaniform sensilla is more regular and of higher frequency than that from the chordotonal organ. The possibility of confusing this organ with others nearby (subgenual organ and tarsal organs) may be eliminated by employing a short length of leg from which they have been cut off. It is also possible to cut a small hole in the cuticle over the chordotonal organ and, using a micromanipulator to tug the chordotonal organ, to obtain its characteristic discharge in this way. However, this is not a practical method for routine work.

(*a*) *The response of the chordotonal organ*

The chordotonal organ is found to respond to movements of the tarsus in certain directions. Since the tarsus is free to move in many directions it is not helpful to describe this in terms of flexion and extension. Instead it was possible to sample many planes of movement by fixing the leg on the stage of the apparatus at different angles and so to determine what may be termed the arc of response (figure 3). A response, that is to say, an increase in impulse frequency, is obtained from the chordotonal organ whenever the tarsus is moved within the arc shown but not otherwise. In relation to the sense organ this is effectively a unidirectional response. In relation to the animal as a whole, the arc may be described as downward and backward.

Figure 35 shows a typical response of the chordotonal organ to deflexion of the tarsus within the arc of response. The response comprises at least two distinct classes of fibre which differ considerably in spike size and so will be termed the large and small fibres. The small fibres, which are initially firing fairly regularly, increase their impulse frequency to a new steady level in response to displacement of the tarsus. Their response ceases temporarily during the return movement. The large fibres, hitherto silent, respond to the displacement with an irregular discharge, which ceases immediately the return movement is begun.

The chordotonal organ is also moderately sensitive to vibration. A rap on the bench or a tap on the apparatus elicits a burst of impulses but ordinary movements about the room do not. This response was not studied systematically.

(*b*) *The tonic response*

The time course of adaptation for the large fibres when the tarsus is moved through successive displacements is shown in figure 36*b*. When the tarsus is moved to each new position the large fibres produce a fairly high frequency response which adapts rapidly at first and then more slowly. This may be contrasted with the corresponding behaviour of the small fibres (figure 36*a*) which show no initial high frequency and adapt slowly from the start.

The relation between the unadapted and adapted impulse frequencies and the angle at which the tarsus is held is shown for both classes of fibre in figure 37 from which it can be seen that the small and large fibres differ clearly in their tonic responses. The small fibres respond over the full range of deflexion within the arc of response with an impulse frequency which is very nearly linearly related to the angle of the tarsus. The large fibres respond only in the more extreme positions and then show a non-linear relation, the impulse frequency rising sharply at first and then falling off in the most extreme position.

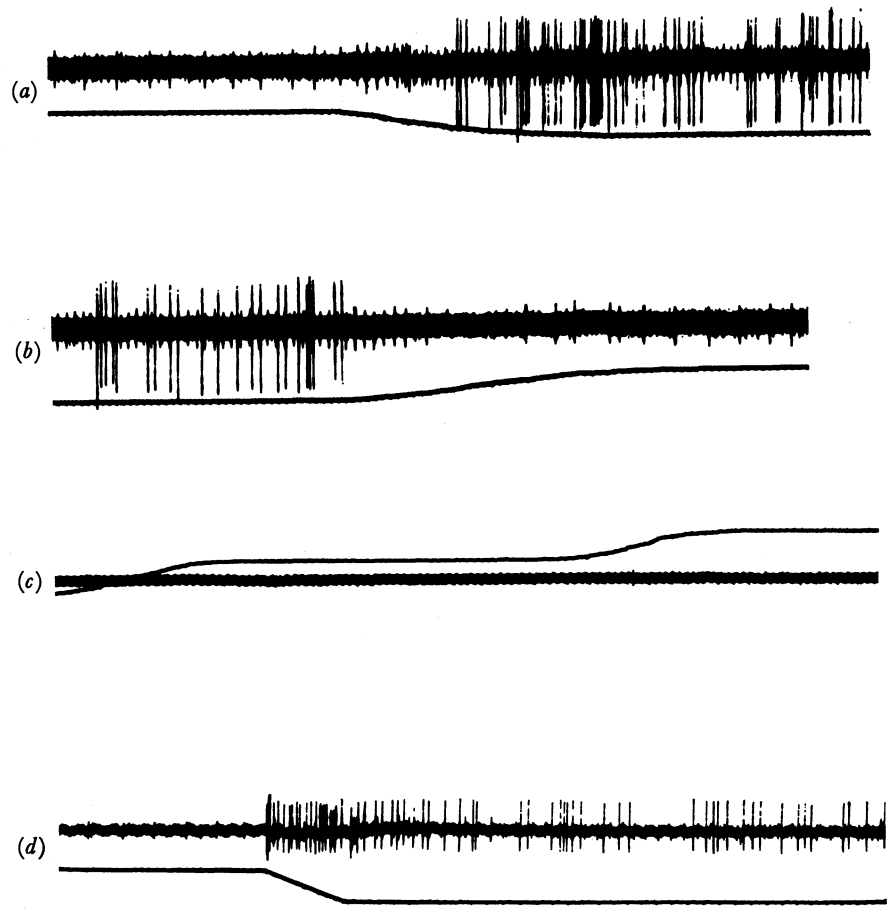


FIGURE 35. Oscilloscope records of the response of the tibio–tarsal chordotonal organ. The spikes are recorded on the upper trace and the movement on the lower. A downward deflexion of the lower trace indicates a downward movement of the tarsus and vice versa. (*a*) and (*b*) are at maximum gain to show both small and large spikes clearly. (*a*) Response of the chordotonal organ to movement of the tarsus from 15 to 30° downward and (*b*) the return movement about 10 s later. (*c*) Two upward movements of the tarsus, showing the lack of responses by the chordotonal organ. The first movement is from 5° downward to 15° upward and the second from 15 to 35° upward. (*d*) The response of the chordotonal organ to movement of the tarsus from 25 to 40° downward at a constant velocity of 150° s^{-1}. The phasic response of the large fibres can be seen clearly; the tonic response of the small fibres can just be made out.

(*c*) *The phasic response*

The response of the large fibres to ramp function displacements of the tarsus at different (constant) velocities is shown in figure 38*b*. Plotting the results on a common time scale makes it easy to see that in each case there is an increase in the impulse frequency during the period of movement. At low velocities this increment is marginal but at high velocities it is considerable.

Figure 39 shows how the increment due to the movement is related to the velocity of movement; the relation does not seem to be constant but is substantially linear in mid-range. The small fibres differ clearly from the large in this respect also, for even at very high velocities they show only the slightest increment in impulse frequency during the period of movement (figure 38*a*).

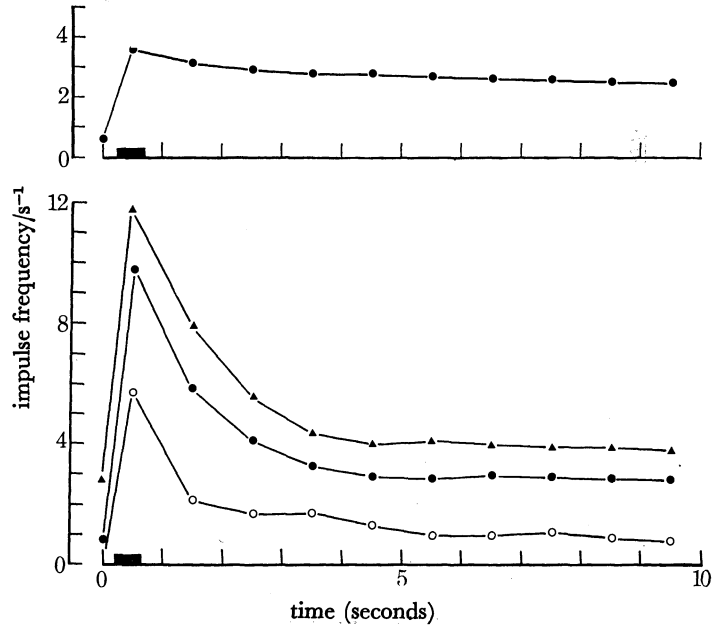


FIGURE 36. The adaptation rate of the small and large fibres. For each point, the impulse frequency is averaged over the whole second and plotted in the centre of the second. The heavy line over the abscissa indicates the duration of the movement. (*a*) The adaptation of the small fibres following movement of the tarsus from 5° to 20° deflected. (*b*) The adaptation of the large fibres following movement of the tarsus to three successive positions. ○, 20 to 30° deflected; ●, 30 to 40° deflected; ▲, 40 to 50° deflected.

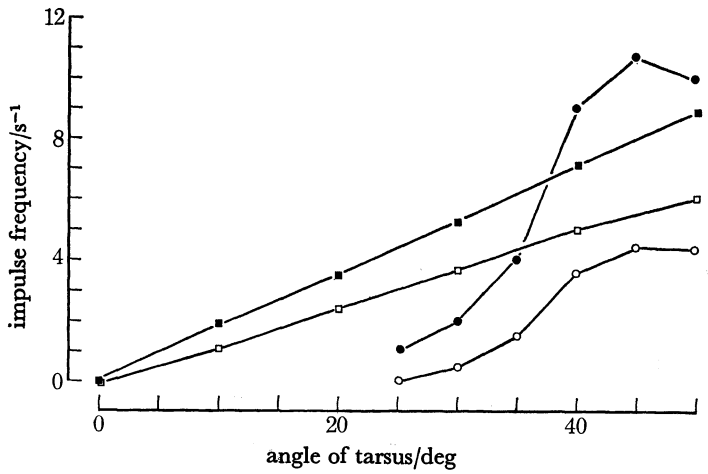


FIGURE 37. The response of both small and large fibres to different extents of deflexion of the tarsus. For both classes, the unadapted impulse frequency and the frequency after 10 s adaptation are plotted against the angle at which the tarsus is held with respect to the tibia. ■, small fibres, unadapted; □, small fibres, adapted; ●, large fibres, unadapted; ○, large fibres, adapted.

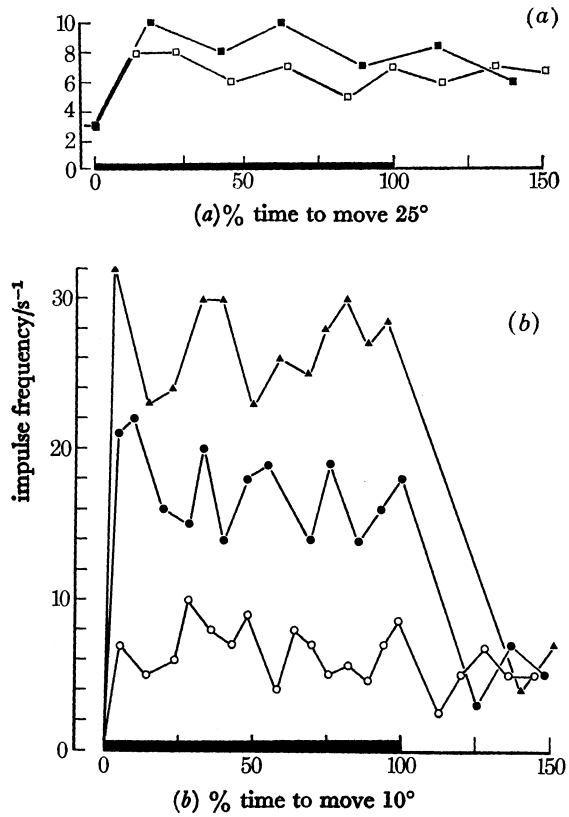


FIGURE 38. The response of the small and the large fibres to movement of the tarsus at different, constant angular velocities. The time scale is expressed as a percentage of the duration of movement, indicated by a heavy line above the abscissa. The impulse frequency is the reciprocal of the time between successive spikes. (*a*) The response of the small fibres to movement of the tarsus through 25° at two different angular velocities. □, 200°/s^{-1}; ■, 400°/s^{-1}. (*b*) The response of large fibres to movement of the tarsus through 10° at three different angular velocities. ○, 30°/s^{-1}; ●, 150°/s^{-1}; ▲, 300°/s^{-1}.

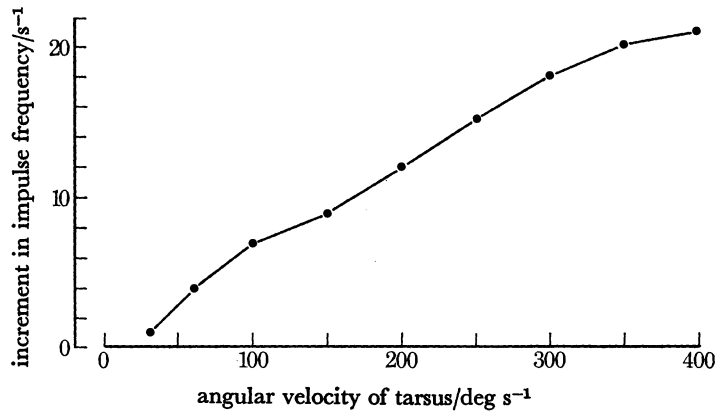


FIGURE 39. The phasic response of the large fibres to movement of the tarsus through 10°. The average increase in sensory impulse frequency during the period of movement, due to the movement, is plotted against the slope of the ramp function, expressed as angular velocity.

(d) Correlation with cell groups

It is interesting to see whether the large and small fibres described here can be correlated with any of the groups of scolopidia described above. Since intracellular recording is impracticable in this case, the following indirect method of correlation was employed. It is assumed that for all these fibres the ion flow per unit area of axonal membrane during an impulse will be the same, so that the external potential change recorded between the electrodes should be linearly related to the diameter of the axons. Hence, it should be possible to obtain a correlation between the size distribution of impulses on an oscillograph record taken from the chordotonal nerve and that of axon diameters in microscopic sections of the chordotonal nerve. Such a comparison is shown in figure 40.

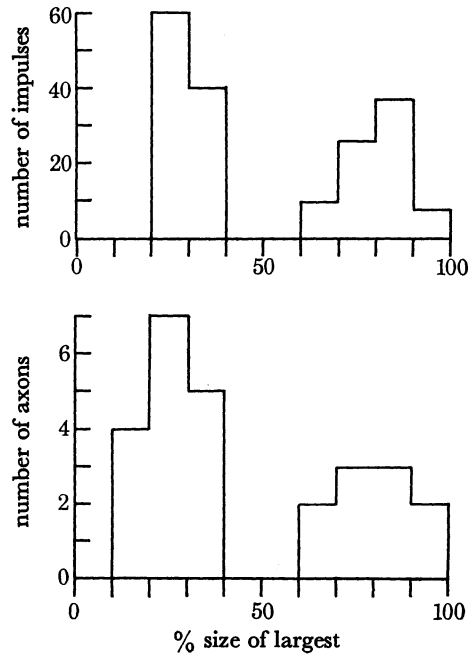


FIGURE 40. Comparison of the size distribution of impulses and axons in the chordotonal nerve. For ease of comparison, the size classes in each case are expressed as a percentage of the largest. The impulse frequency is the figure obtained from an arbitrary length of oscillograph record from one preparation. The axon frequency is a count from a single representative electronmicrograph of a section across the chordotonal nerve.

The impulses fall into two distinct groups corresponding to the large (60–100 % of largest) and small (20–40 %) fibres. The axons between 60–100 % of the largest are those of group 1 scolopidia, those between 20–40 % are group 2 and those between 10–20 % are group 3. From the similarity of the two size distributions, it would seem that the large fibres correspond with group 1 scolopidia and the small fibres with group 2 scolopidia. On this interpretation, failure to record impulses in the 10–20 % range corresponding to group 3 is understandable on account of their minute size.

6. DISCUSSION

The tibio–tarsal chordotonal organ described here conforms closely to the typical pattern found by Debaiseaux (1936, 1938). In most genera, this organ branches off the anterior tibial nerve and inserts on or near the tibio–tarsal intersegmental membrane. In some cases, such as

Forficula, it inserts on the tendon of the tarsal extensor muscle. Debaiseaux found between five and fifteen scolopidia typically in this organ; an exceptional case is *Apis*, where he found sixty. Thus the present example seems to be representative in respect of these general features.

(*a*) *The specificity of neurons*

One of the more remarkable features which emerges from this study is the complexity of cell types and the specificity of their layout in what is at first sight a simple chordotonal organ. Detailed study shows that individual neurons can be recognized on the basis of their size, shape and position. Increasingly this is found to be the case in other preparations known in sufficient detail (e.g. Baylor & Nicholls 1968). This implies that at the level of individual cells their morphological features are precisely determined in relation to their functional requirements. The adequate stimulus is the same for all these neurons and the specific differences probably relate to receptive fields and thresholds of response.

For instance, the constant difference in orientation between the group 1 neurons of the main branch and those of the two side branches is clearly correlated with the insertion of their attachment cells on to different regions of the intersegmental membrane. Again, the specific arrangement of group 2 scolopidia along the main branch may confer a steady increment in the mechanical threshold of the neurons. The attachment cells probably possess some degree of elasticity on account of the many microtubules which they contain. If this were so, the longer the attachment cell the greater would be the degree of extension required to reach a constant neural threshold. Consequently the most distal scolopidia, that is those with the shortest attachment cells, would respond to initial deflexion of the tarsus and the more proximal scolopidia would respond only to more extreme deflexion of the tarsus. This is consistent with the group 1 scolopidia being most proximal of all since they respond only to extreme deflexion of the tarsus.

(*b*) *The functional significance of the cell types*

The organization of this chordotonal organ makes interesting comparison with that in Crustacea. The small tonic fibres, with their lack of phasic response, closely resemble the pure position fibres of the crustacean chordotonal organs. Bush (1965*a*) provides a graph of the position fibre responses, which shows the increase in impulse frequency to be nearly linearly related to the angle of deflexion, as in the present case. No pure movement fibres were found in the tibio-tarsal chordotonal organ but the large fibres show a relation between impulse frequency and velocity of displacement which is similar to that of the crustacean movement fibres (graphs in Bush 1965*a*, *b*).

In the case of the crustacean chordotonal organs, Taylor (1967) has suggested that unidirectional movement responses, most position responses and responses that are intermediate could arise from a single type of cell and are a function only of the mechanical arrangement between the dendrites and the strand to which they are attached. This suggestion is supported by the work of Hartman & Boettiger (1967), who found that, in the PD organ, extension-sensitive cells and relaxation-sensitive cells insert into different surfaces of the elastic strand, and are operationally independent. They also found that certain movement fibres show position sensitivity at extreme positions of the joint; those at the distal end of the strand are sensitive to the open arc region while those at the proximal end are more sensitive to the closed arc region. The exception to this theory, as Taylor recognized, are the pure position fibres which are associated with smaller cells, situated distally (Wiersma & Boettiger 1959). In the tibio–tarsal

chordotonal organ it has been possible to show that there is a correlation between a difference in cell types and a difference in phasicity. The group 1 scolopidia are identified with large fibres which show a phasic and a tonic response to deflexion of the tarsus and the group 2 scolopidia are identified with the small fibres which show only a tonic response to deflexion.

At least two main causes of receptor adaptation have been distinguished in mechanoreceptors (Mellon 1968). On the one hand, the viscoelastic properties of the tissue linking the receptor neuron to the stimulus source may bring about a decline in the generator potential even though the applied stimulus remains constant. On the other hand, the properties of the spike generating membrane may bring about a decline in spike frequency even though the generator potential remains constant. In this chordotonal organ, the mechanical linkage of the scolopidia, the attachment cell, is the same for both groups at the fine structural level. The attachment cells do differ in length and orientation but this is a graded and not a clear cut difference. On structural grounds, therefore, it is unlikely that the mechanical linkage could account for the differences in phasicity between the two groups. Nakajima & Onodera (1969*a*, *b*) have recently studied this point in the crayfish stretch receptors and have concluded that the marked differences in receptor adaptation between the 'fast' and 'slow' cells are attributable to the differences in the properties of the spike generating membrane rather than to the properties of the generator potentials. Perhaps the same may be true for connective chordotonal organs in respect of the difference between purely tonic (position) fibres and fibres showing phasic responses.

There are some fine structural differences between group 1 and group 2 neurons, of which the most striking lies in the dendrite sheathing. This occurs only around the dendrite and distal part of the cell body and is poorly developed round group 2 neurons but highly developed round group 1 neurons. Thus there would appear to be a correlation between phasicity and the presence of the sheathing.

The Schwann sheathing of group 1 axons is also well developed. This loose wrapping may be of trophic function. In the short chordotonal nerve, only axons of 1 μm or more in diameter have a Schwann cell to themselves. Edwards (1967) has reported that grasshopper peripheral axons do not acquire individual Schwann sheaths during development until they reach 1 μm in diameter. He suggests that their trophic requirements are such that axons need individual support from 1 μm upwards.

(*c*) *The adequate stimulus of the scolopidia*

It is interesting to compare the possible mode of action of the scolopidia in this sense organ with suggestions that have been made for those of other sense organs. Howse (1968) has reviewed earlier suggestions and put forward the general theory that for most scolopidia the initial part of the transduction process is the stretch of the ciliary membrane at the dilatation brought about by flexion between the cap and the scolopale rods.

Now it can be seen that the tibio–tarsal chordotonal organ is so arranged that its strands will be stretched by those movements of the tarsus which pull the intersegmental membrane outwards and that the strands will be relaxed by those movements of the tarsus which fold the intersegmental membrane inwards. Further, the scolopidia all lie parallel to the strands so that they will be subject to the same longitudinal strains as the strands in which they occur. Deflexion of the tarsus within the arc of response has the effect of pulling outwards the intersegmental membrane and so stretching the chordotonal organ, while upward and forward movements have the opposite effect. The recordings show that both classes of fibre respond to deflexion of

the tarsus within the arc of response but are silent when it is returned, that is when the chordotonal organ is being relaxed. From this, it is reasonable to conclude that only extension-sensitive cells are present and that they are stimulated by longitudinal pull.

By comparison, the scolopidia of crustacean connective chordotonal organs are all attached to an elastic receptor strand which consists of a central core of mainly fibrous connective tissue and a peripheral layer of amorphous connective tissue. Taylor (1967) has suggested that scolopidia sensitive to stretch of the strand might lie with their tips across the interface between these two types of tissue and so they would be flexed during stretch of the strand and straightened during relaxation of the strand. However, in the present case there is no separate receptor strand and the organ consists only of scolopidia and surrounding cells, surrounded by the neural lamella. Consequently the longitudinal pull applied to the strands of the organ is necessarily applied directly to the attachment cells of the scolopidia.

Another way in which the scolopidia might be flexed when the strands are stretched has been suggested. Bush (1965*a*) proposed that an elastic fibre with distal and proximal attachments to the scolopidium would tend to flex it one way on relaxation of the receptor strand, while a terminal fibre sharing only the distal attachment would tend to flex the scolopidium the other way during extension of the strand. For this theory to hold it is necessary that some kind of structural component be demonstrated which would fulfil its requirements. But in the tibio–tarsal chordotonal organ there are no suitable components outside of the scolopidia themselves. Within the scolopidia the only potential candidates are the microtubules in the attachment cells but these are distributed evenly around the scolopale and show no preferential point of attachment. Thus it is unlikely that this suggestion can apply to this chordotonal organ.

Thurm (1965) has shown that the adequate stimulus of insect campaniform and hair-plate sensilla is lateral compression of their terminations, produced by caps of elastic material which are situated around the termination. He has equated these caps of elastic material with the cap of the scolopidium and suggested that the latter is stimulated by tension on the cap producing a compression of the ciliary terminal within it. This suggestion also is unlikely to apply to this chordotonal organ because the cap is long and pointed and the scolopale rods penetrate a long way into it. This produces a structure well suited to transmit longitudinal strain and to prevent the flexion suggested by Howse (1968) or the pincer action suggested by Thurm (1965).

Hence the combination of evidence indicates that in the present case the adequate stimulus is an increase in the longitudinal tension of the scolopidia but it is not possible to say, on purely structural evidence, which of the fine structural components of the scolopale unit respond to strain. In this connexion, it is interesting to note two sorts of variation in the scolopale unit. One is that group 3 have only a single dendrite whereas groups 1 and 2 have a pair. The other is that the paired dendrites differ in their ciliary root structures. Both of these variations occur widely in chordotonal organs (Debaiseaux 1936, 1938; Howse 1968). If their functional significance were understood, either or both of these structural facts might provide a useful clue as to the nature of the transducer mechanism.

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References

Ashhurst, D. E. 1959 The connective tissue sheath of the locust nervous system: a histochemical study. *Q. Jl Microsc. Sci.* **100**, 401–412.

Ashhurst, D. E. 1968 The connective tissues of insects. *A. Rev. Ent.* **13**, 45–74.

Baylor, D. A. & Nicholls, J. G. 1968 Receptive fields, synaptic connections and regeneration, patterns of sensory neurons in the CNS of the leech. In *Physiological and biochemical aspects of nervous integration*, ed. F. D. Carlson, pp. 3–16. Englewood Cliffs, N.J.: Prentice-Hall.

Becht, G. 1958 The influence of DDT and lindane on chordotonal organs in the cockroach. *Nature, Lond.* **181**, 777–779.

Burke, W. 1954 An organ for proprioception and vibration sense in *Carcinus maenas*. *J. exp. Biol.* **31**, 127–138.

Bush, B. M. H. 1965*a* Proprioception by the coxo-basal chordotonal organ, CB, in legs of the crab, *Carcinus maenas*. *J. exp. Biol.* **42**, 285–297.

Bush, B. M. H. 1965*b* Proprioception by chordotonal organs in the mero-carpopodite and carpo-propodite joints of *Carcinus maenas* legs. *Comp. Biochem. Physiol.* **14**, 185–199.

Carbonell, C. S. 1949 The thoracic muscles of the cockroach *Periplaneta americana*. *Smithson. misc. Collns* **107**, No. 2.

Chapman, K. M. 1965 Campaniform organs on tactile spines. *J. exp. Biol.* **42**, 191–203.

Debaiseaux, P. 1936 Organes scolopidiaux des pattes d'insectes I. Lepidopteres et Trichopteres. *Cellule* **44**, 271–314.

Debaiseaux, P. 1938 Organes scolopidiaux des pattes d'insectes II. *Cellule* **47**, 77–202.

Debauche, H. 1935 Recherches sur les organes sensoriels antennaires de *Hydropsyche longipennis*. *Cellule* **44**, 43–83.

Dresden, D. & Nijenhuis, E. D. 1953 On the anatomy and mechanism of motion of the mesothoracic leg of *Periplaneta americana*. *Proc. K. ned. Akad. Wet. Ser. C.* **56**, 39–47.

Edwards, J. S. 1967 Some questions for the insect nervous system. In *Insects and physiology*, ed. J. W. L. Beament & J. E. Treherne, pp. 163–173. London: Oliver and Boyd.

Farquhar, M. G. & Palade, G. E. 1963 Junctional complexes in various epithelia. *J. Cell Biol.* **17**, 375–412.

Flock, A. & Duvall, A. J. 1965 The ultrastructure of the kinocilium of the sensory cells in the inner ear and lateral line organs. *J. Cell Biol.* **25**, 1–8.

Glauert, A. M. 1965 The fixation and embedding of biological specimens. In *Techniques for electron microscopy*, ed. D. H. Kay, pp. 166–212. Oxford: Blackwell Scientific Publications.

Glauert, A. M. & Glauert, R. H. 1958 Araldite as an embedding medium for electron microscopy. *J. biophys. biochem. Cytol.* **4**, 191–194.

Gray, E. G. 1960 The fine structure of the insect ear. *Phil. Trans. Roy. Soc. Lond.* B. **243**, 75–94.

Guthrie, D. M. 1967 Multipolar stretch receptors and the insect leg reflex. *J. Insect Physiol.* **13**, 1637–1644.

Guthrie, D. M. and Tindall, A. R. 1968 *The biology of the cockroach*. London: Arnold.

Hartman, H. B. & Boettiger, E. G. 1967 The functional organisation of the propus-dactylus organ in *Cancer irroratus* Say. *Comp. Biochem. Physiol.* **22**, 651–663.

Hess, A. 1958 The fine structure and morphological organisation of the peripheral nerve fibres and trunks of the cockroach (*Periplaneta americana*). *Q. Jl Microsc. Sci.* **99**, 333–340.

Howse, P. E. 1968 The fine structure and functional organisation of chordotonal organs. *Symp. zool. Soc. Lond.* **23**, 167–198.

Lawn, A. M. 1960 The use of potassium permanganate as an electron-dense stain for sections of tissue embedded in epoxy resin. *J. biophys. biochem. Cytol.* **7**, 197–198.

Locke, R. L. 1965 The structure of septate desmosomes. *J. Cell Biol.* **25**, 166–169.

Mellon, de F. 1968 *The physiology of sense organs*. Edinburgh and London: Oliver and Boyd.

Nakajima, S. & Onodera, K. 1969*a* Membrane properties of the stretch receptor neurons of crayfish with particular reference to mechanisms of sensory adaptation. *J. Physiol.* **200**, 161–185.

Nakajima, S. & Onodera, K. 1969*b* Adaptation of the generator potential in the crayfish stretch receptors under constant length and constant tension. *J. Physiol.* **200**, 187–204.

Nijenhuis, E. D. & Dresden, D. 1952 A micromorphological study on the sensory supply of the mesothoracic leg of the american cockroach, *Periplaneta americana*. *Proc. K. ned. Akad. Wet. Ser. C.* **55**, 300–310.

Nijenhuis, E. D. & Dresden, D. 1955 On the topographical anatomy of the nervous system of the mesothoracic leg of the american cockroach, *Periplaneta americana*. *Proc. K. ned. Akad. Wet. Ser. C.* **58**, 121–130.

Pantin, C. F. A. 1946 *Notes on microscopical technique for zoologists*. Cambridge University Press.

Pringle, J. W. S. 1938 Proprioception in insects. II. The action of the campaniform sensilla on the legs. *J. exp. Biol.* **15**, 114–131.

Pringle, J. W. S. 1939 The motor mechanism of the insect leg. *J. exp. Biol.* **16**, 220–231.

Pusey, H. K. 1939 Methods of reconstruction from microscopic sections. *Jl R. microsc. Soc.* **59**, 232–244.

Reynolds, E. S. 1963 The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

Slifer, E. H. 1967 The thin walled olfactory sense organs on insect antennae. In *Insects and physiology*, ed. J. W. L. Beament and J. E. Treherne, pp. 233–245. London: Oliver and Boyd.

Slifer, E. H. & Sekhon, S. S. 1964 The dendrites of the thin walled olfactory pegs of the grasshopper (Orthoptera Acididae). *J. Morph.* **114**, 393–410.

Smith, D. S. & Treherne, J. E. 1963 Functional aspects of the organisation of the insect nervous system. In *Advances in insect physiology*, ed. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth, Vol. 1, pp. 401–484. London and New York: Academic Press.

Snodgrass, R. E. 1926 The morphology of insect sense organs and the sensory nervous system. *Smithson. misc. Collns* **77**, no. 8.

Taylor, R. C. 1967 The anatomy and adequate stimulation of a chordotonal organ in the antennae of a hermit crab. *Comp. Biochem. Physiol.* **20**, 709–717.

Thurm, U. 1964 Mechanoreceptors in the cuticle of the honey bee: fine structure and stimulus mechanism. *Science, N.Y.* **145**, 1063–1065.

Thurm, U. 1965 An insect mechanoreceptor. I. fine structure and adequate stimulus. *Cold Spring Harb. Symp. quant. Biol.* **30**, 75–82.

Usherwood, P. N. R., Runion, H. I. & Campbell, J. I. 1968 Structure and physiology of a chordotonal organ in the locust leg. *J. exp. Biol.* **48**, 305–323.

Wersäll, J., Flock, A. & Lundquist, P. G. 1965 Structural basis for directional sensitivity in cochlear and vestibular sensory receptors. *Cold Spring Harb. Symp. quant. Biol.* **30**, 115–132.

Whitear, M. 1962 The fine structure of crustacean proprioceptors. I. The chordotonal organs in the legs of the shore crab, *Carcinus maenas*. *Phil. Trans. Roy. Soc. Lond.* B **245**, 291–325.

Wiersma, C. A. G. 1959 Movement receptors in decapod Crustaceans. *J. mar. biol. Ass U.K.* **38**, 143–152.

Wiersma, C. A. G. & Boettiger, E. G. 1959 Unidirectional movement fibres from a proprioceptive organ of the crab, *Carcinus maenas*. *J. exp. Biol.* **36**, 102–112.

Wigglesworth, V. B. 1959*a* The histology of the nervous system of an insect, *Rhodnius prolixus* (Hemiptera). I. The peripheral nervous system. *Q. Jl microsc. Sci.* **100**, 285–298.

Wigglesworth, V. B. 1959*b* The histology of the nervous system of an insect, *Rhodnius prolixus* (Hemiptera). II. The central ganglia. *Q. Jl microsc. Sci.* **100**, 299–313.

Wood, R. L. 1959 Intercellular attachments in the epithelium of *Hydra* as revealed by electron microscopy. *J. biophys. biochem. Cytol.* **6**, 343–351.

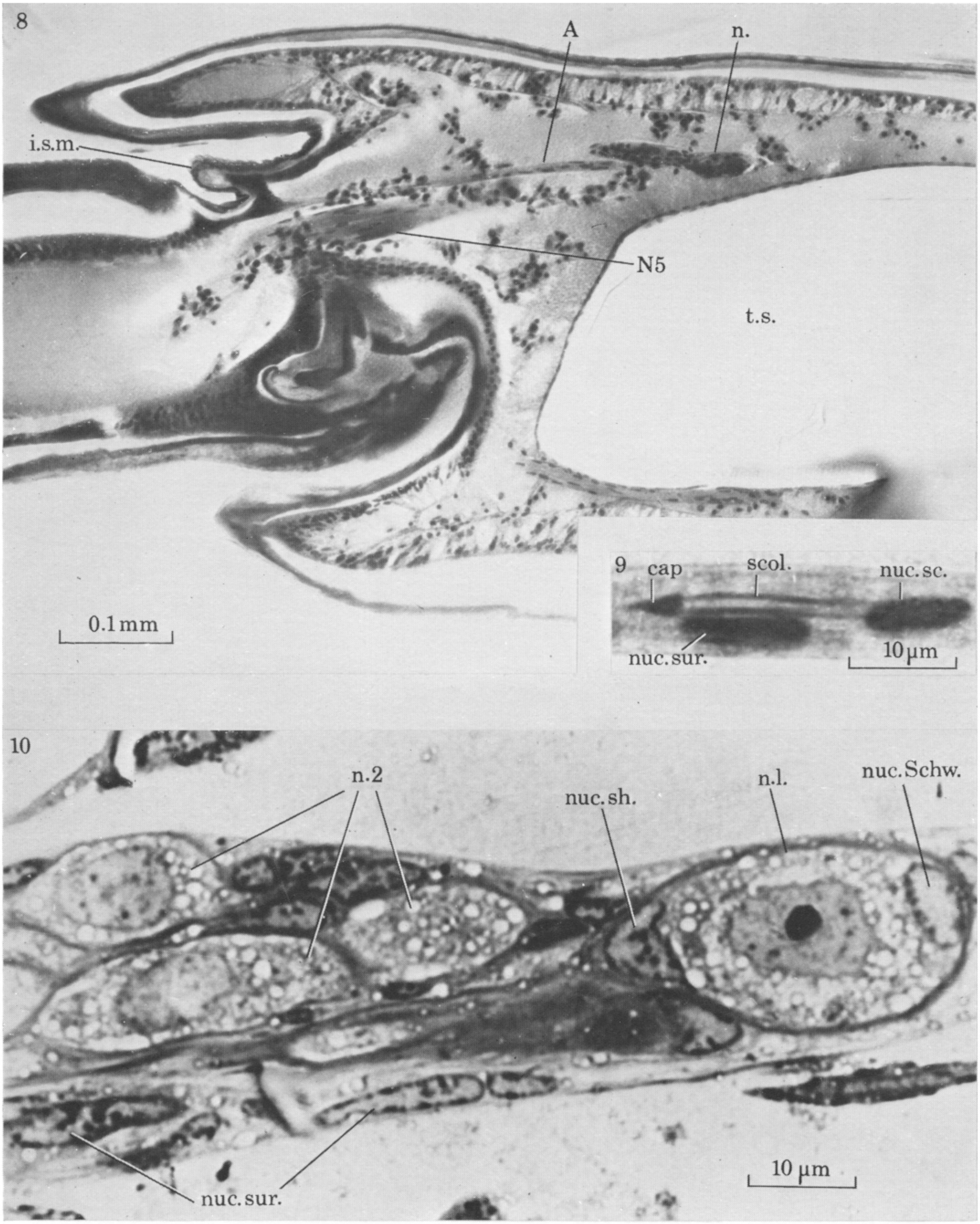
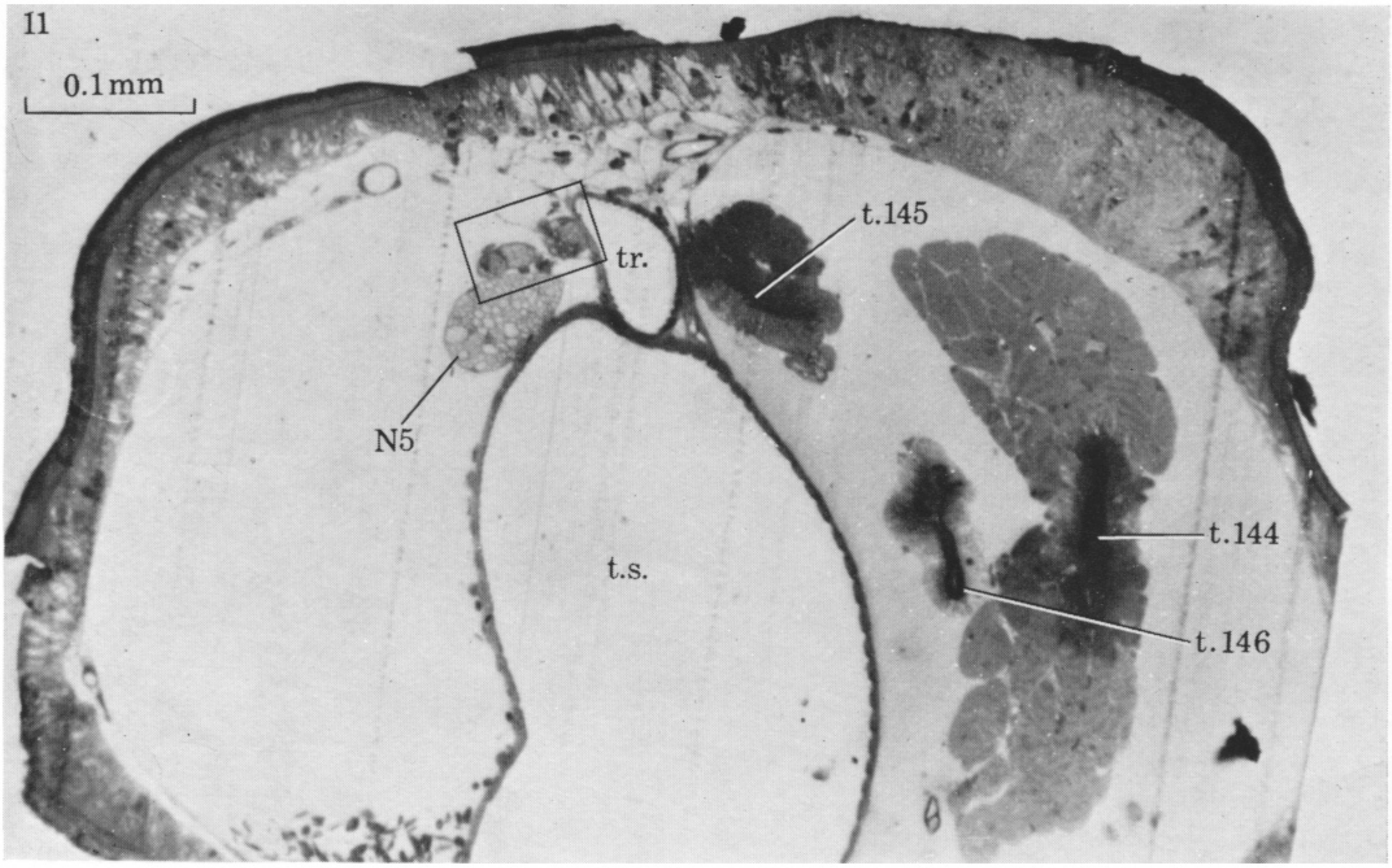


FIGURE 8. One of the longitudinal wax sections, 15 μm thick, on which the reconstruction in figure 4 is based. Some of the bipolar neurons (n.) are seen near the tracheal sac (t.s.). Side branch A can be seen along its whole length from the neurons to its insertion on the intersegmental membrane (i.s.m.). N5, the fifth nerve trunk.

FIGURE 9. Scolopale of side branch A enlarged from figure 8, showing the scolopale rods (scol.) and the pointed cap. nuc. sur., surrounding cell nucleus; nuc. sc., scolopale cell nucleus.

FIGURE 10. A longitudinal Araldite section, 1 μm thick, through the sensory neurons. This section passes through the nucleus of a group 1 neuron (n.1.) and through the nuclei of its Schwann cell (nuc. Schw.) and its sheath cell (nuc. sh.). On the left are three group 2 neurons (n.2.). nuc. sur., surrounding cell nucleus.



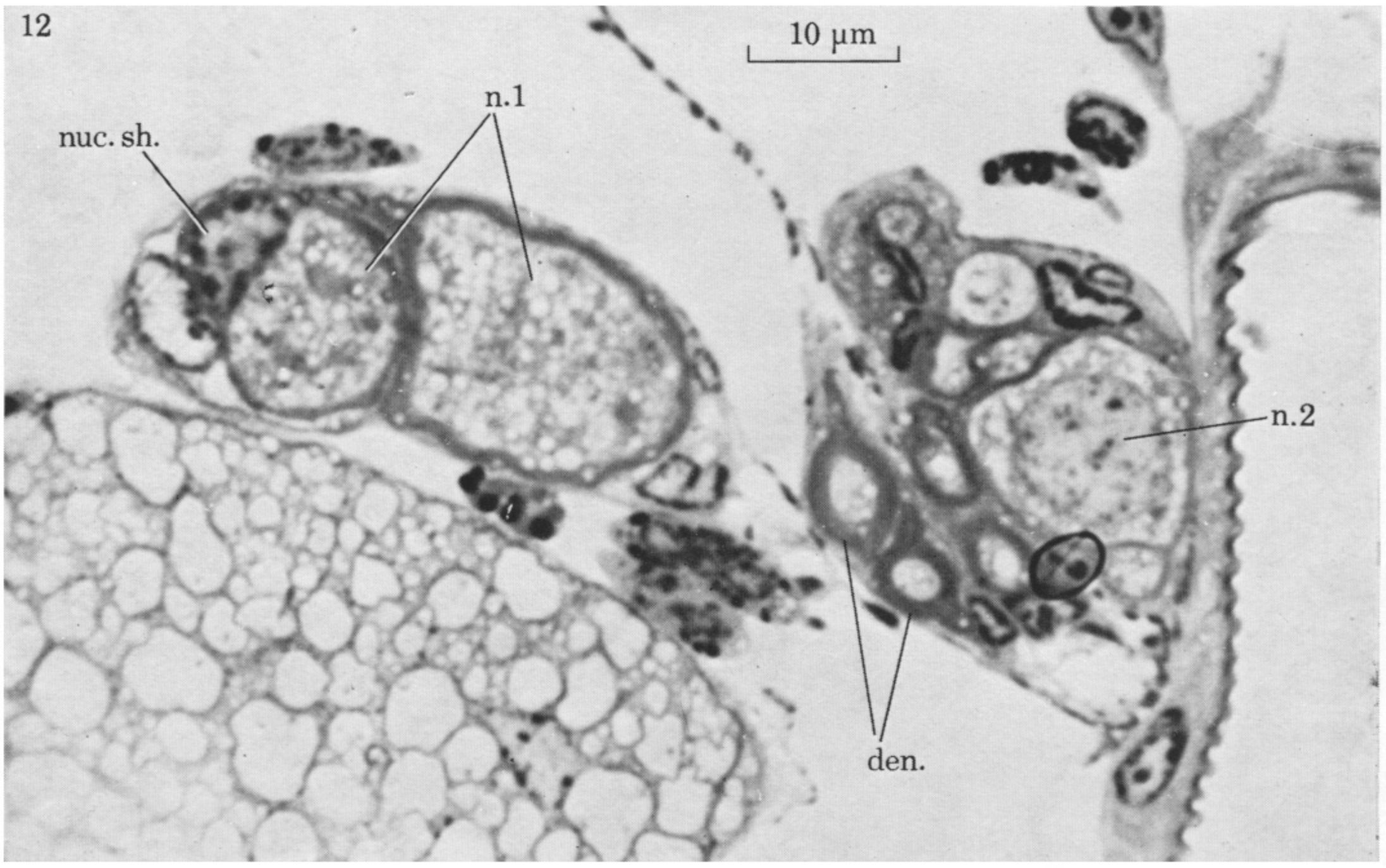


FIGURE 11. Transverse Araldite section through the tibia of the right mesothoracic leg of *Periplaneta*. The chordotonal organ (within the rectangle) is shown at higher magnification in figure 12. Note that the chordotonal organ is closely accompanied by a tracheole (tr.) and the tendon of muscle 145 (t.145). N5, the fifth nerve trunk; t.s., tracheal sac; t.144, tendon of muscle 144; t.146, tendon of muscle 146.

FIGURE 12. The tibio-tarsal chordotonal organ enlarged from figure 11. The level at which this section is cut is shown in figure 5 by a pair of numbered arrows. On the left are shown the two group 1 neurons (n.1.) which supply the single scolopale of side branch B. On the right is the main branch showing a group 2 neuron (n.2.) cut through its nucleus and pairs of group 1 dendrites (den.), the labelled pair cut through the region of heaviest sheathing. nuc.sh., nucleus of sheath cell.

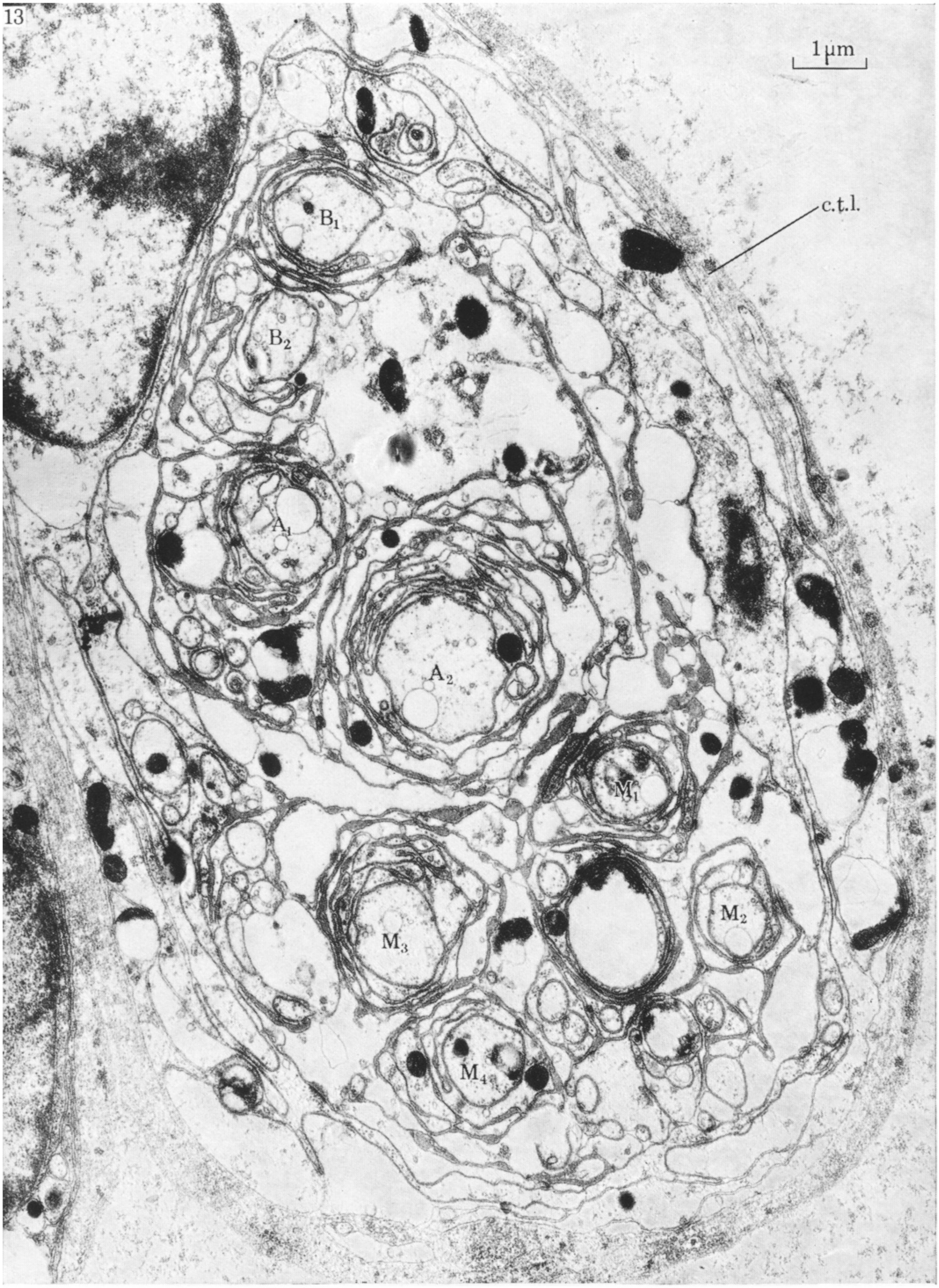


FIGURE 13. An electronmicrograph of a transverse section of the chordotonal nerve at the point where it leaves the main nerve trunk, as shown by the numbered arrow in figure 4. There are about two dozen axons, varying from 0.2 to 1.5 μm in diameter, intermingled with Schwann cell cytoplasm. The nerve is surrounded on the outside by a connective tissue layer (c.t.l.). The axons of group 1 (A_1A_2,B_1B_2,M_1–M_4) can be individually identified and are labelled here according to which neurons they derive from. This labelling is the same as that in figure 5.

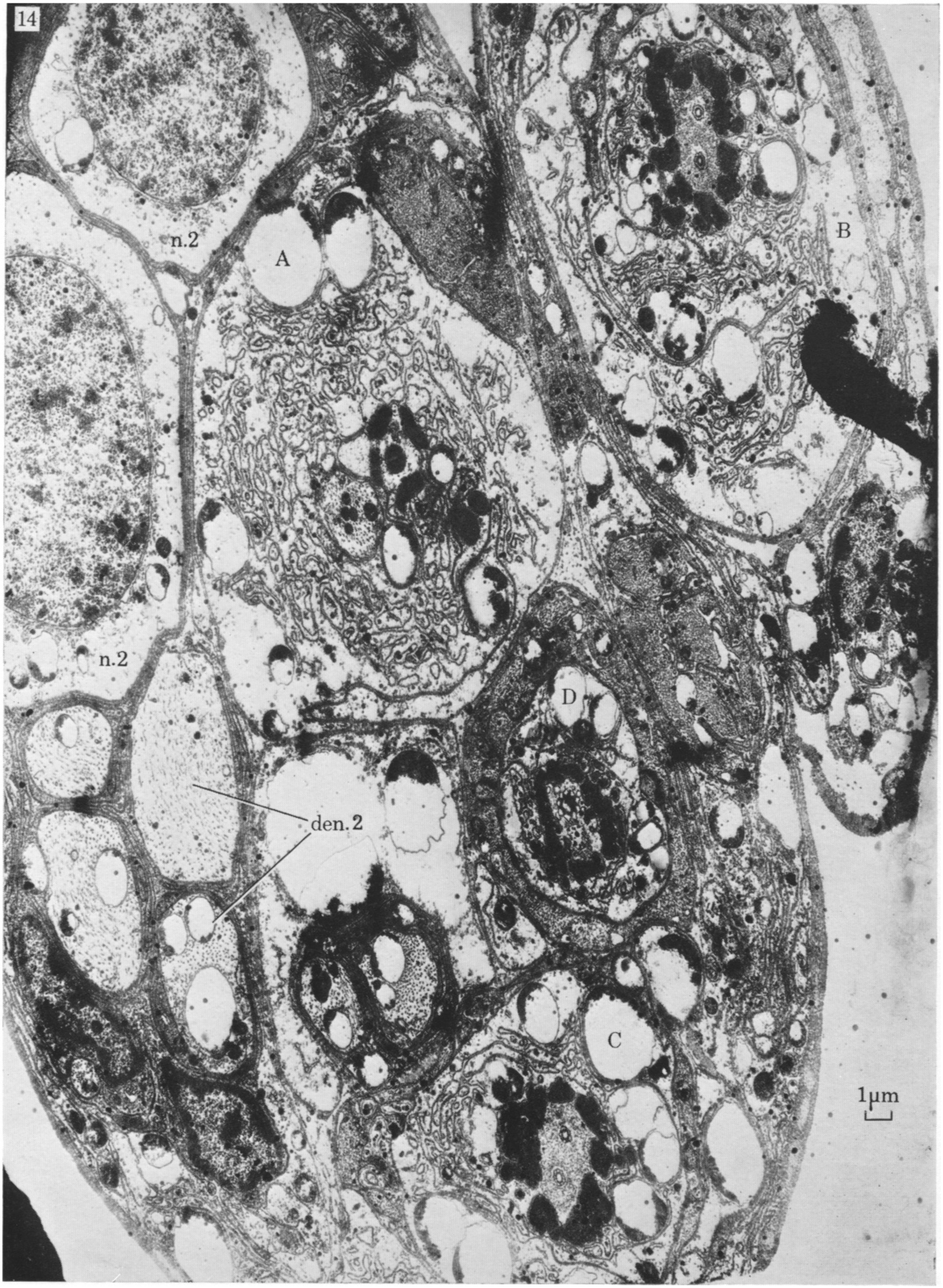


FIGURE 14. An electronmicrograph surveying a transverse section of the chordotonal organ at the point where side branch B leaves the main branch. The approximate level of section is shown by a pair of numbered arrows in figure 5 but this specimen shows a greater degree of overlap between the groups of scolopidia in the proximo–distal axis than does that in figure 5. The section shows four scolopale cells (A, B, C, D) cut at different levels: A, a group 2 scolopale cut through the base of the scolopale; B, the group 1 scolopale of side branch B cut at the level of the ciliary base: C, a group 2 scolopale cut through the mid region; D, a group 1 scolopale cut at the level of the ciliary dilatation. den. 2, group 2 dendrites; n.2., group 2 neurons.

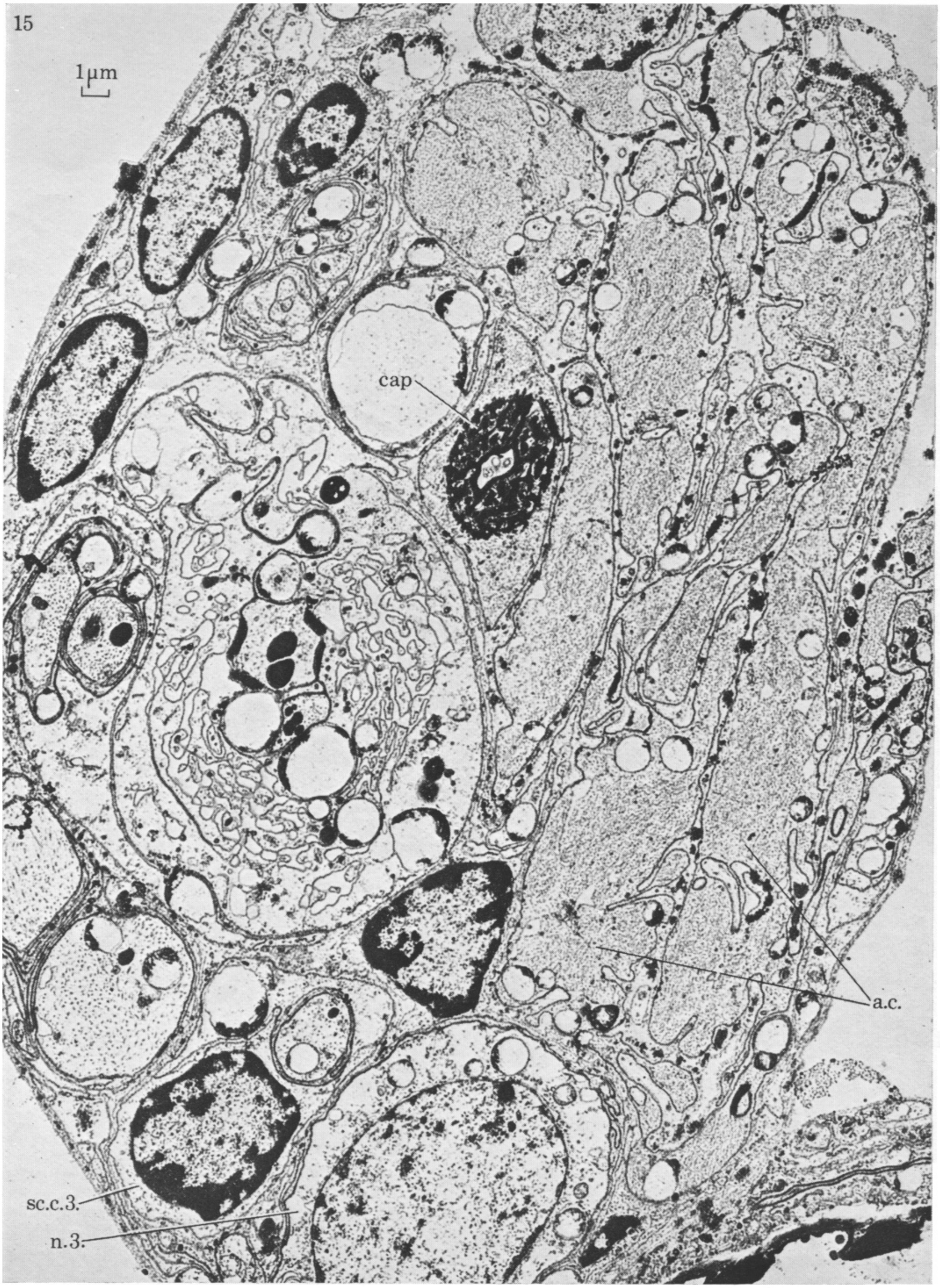
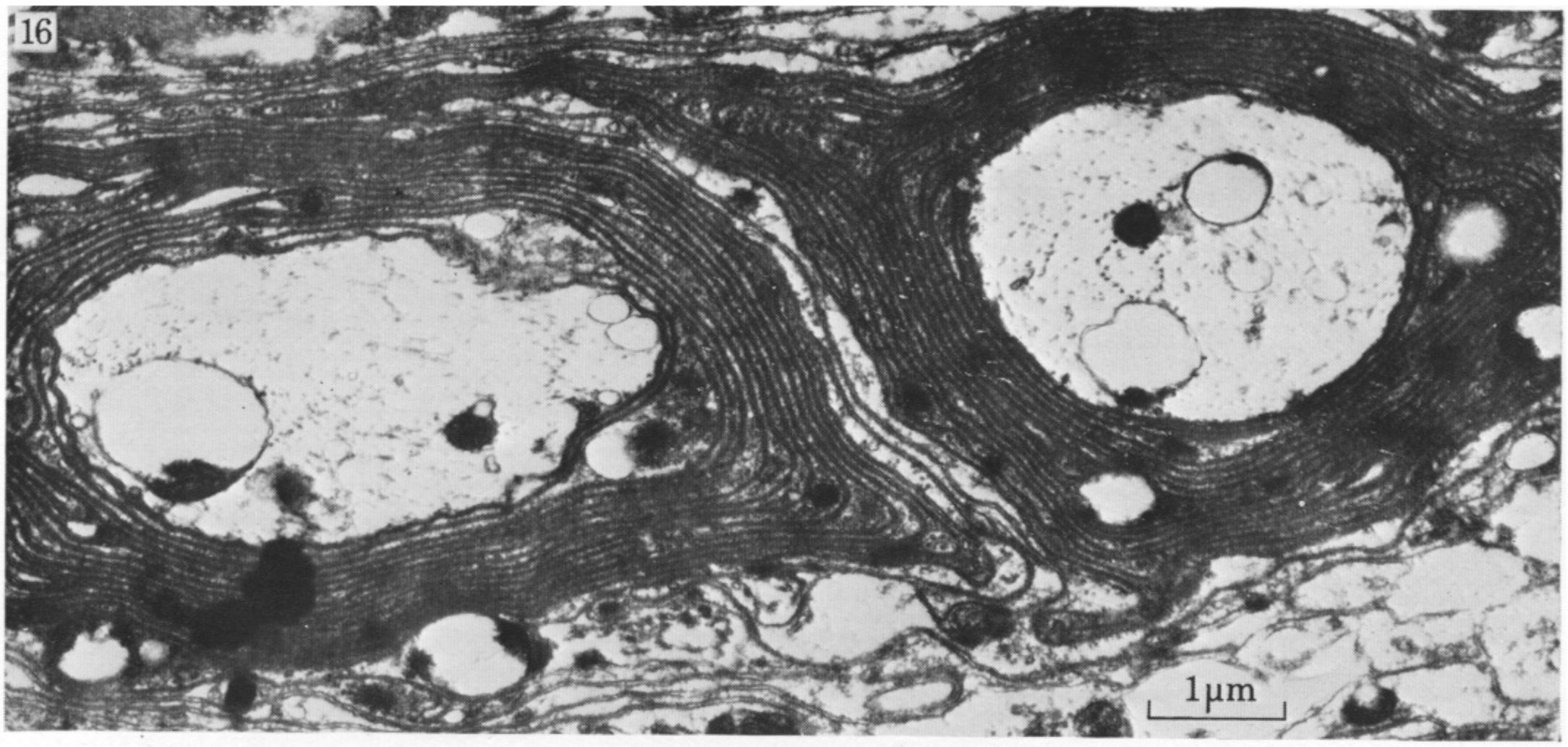


FIGURE 15. An electronmicrograph surveying the chordotonal organ at a level indicated in figure 5 by a pair of numbered arrows. This spciemen shown a greater degree of overlap between the groups of scolopidia in the proximo–distal axis than does that in figure 5. At this level the chordotonal organ main branch consists mostly of attachment cells (a.c.). There are a few group 2 dendrites and scolopales, especially one through the cap. Also at this level can be seen a group 3 neuron (n.3.) and a group 3 scolopale cell, (sc.c.3), cut through its nucleus and enclosing a single dendrite.



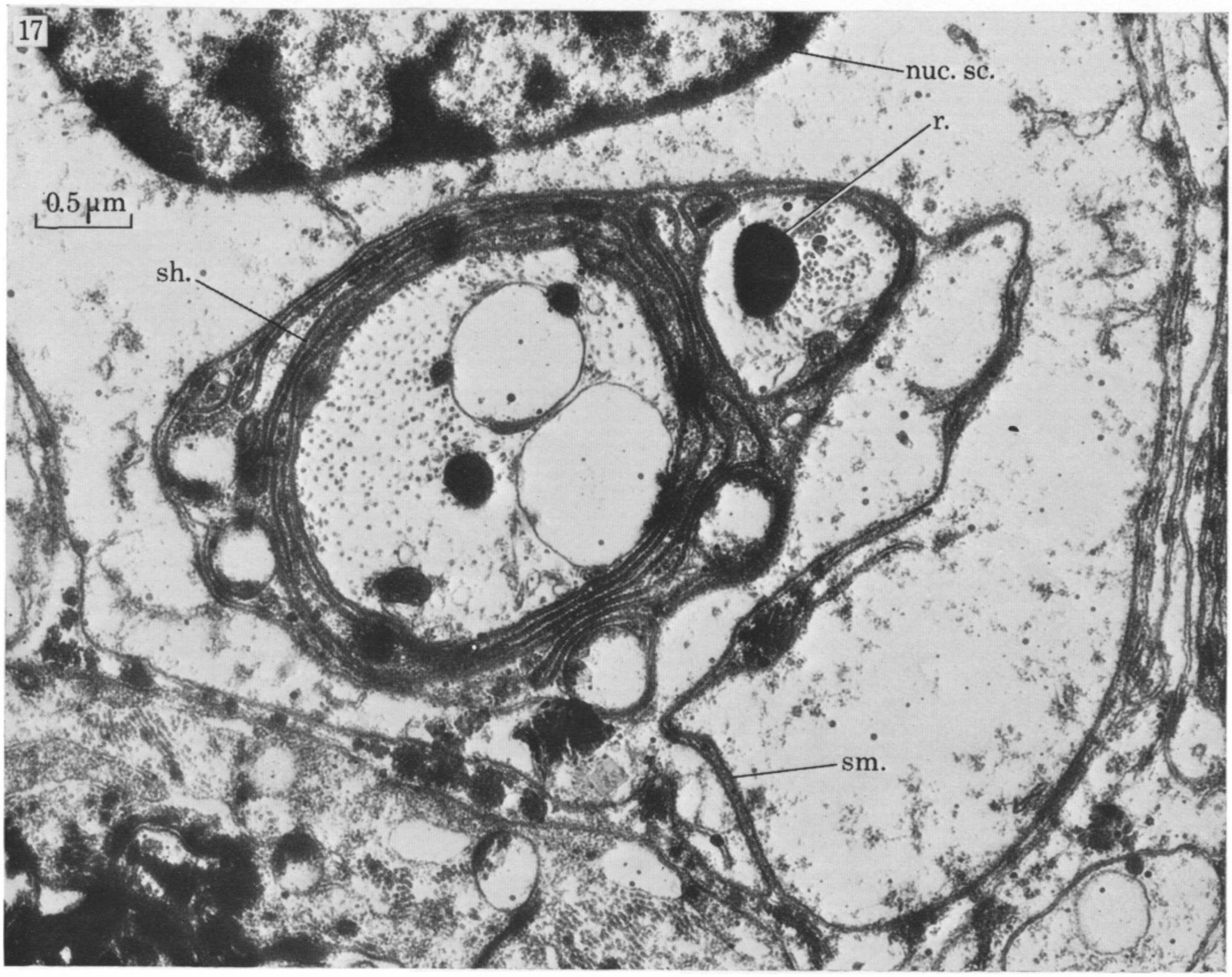


FIGURE 16. Electronmicrograph of a thin section from the same specimen as the thick section shown in figure 12. This section, cut at a short distance distally to that in figure 12, shows the same pair of dendrites which are labelled (den.) in figure 12. The thick sheath can be seen to consist of ten to twelve densely wound folds of the double surface membrane of the sheath cell.

FIGURE 17. Transverse section through a group 1 scolopidium at the level of the scolopale cell nucleus (nuc.sc.) as shown by the numbered arrow in figure 6. An infolding of the surface membrane (s.m.) of the scolopale cell encloses the two dendrites. At this level the sheathing (sh.) of the dendrites has dwindled, more so on the right hand dendrite than on the left hand one. The right hand dendrite contains the large root (r.) of its cilium. Both dendrites contain neurotubules.

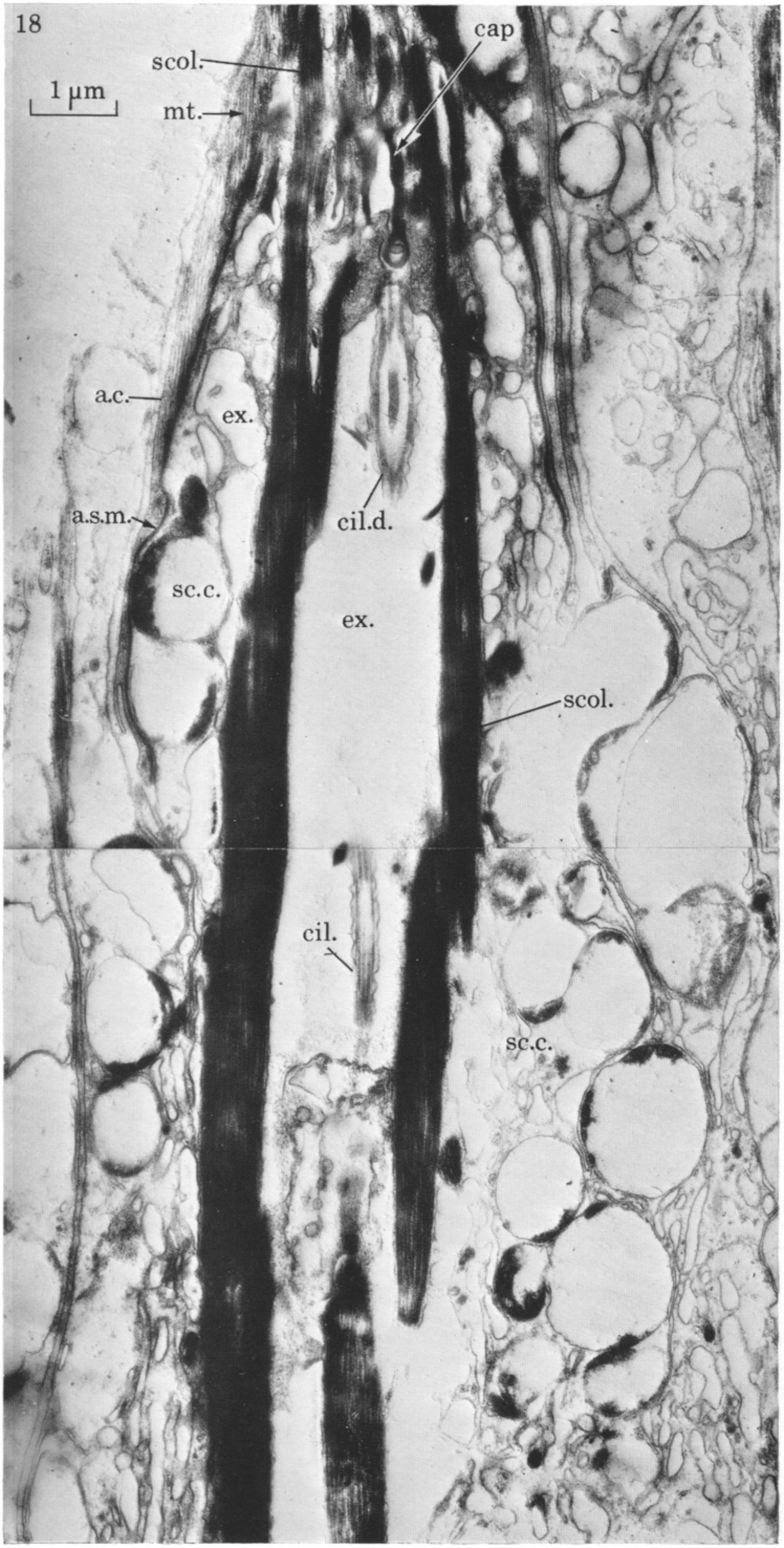
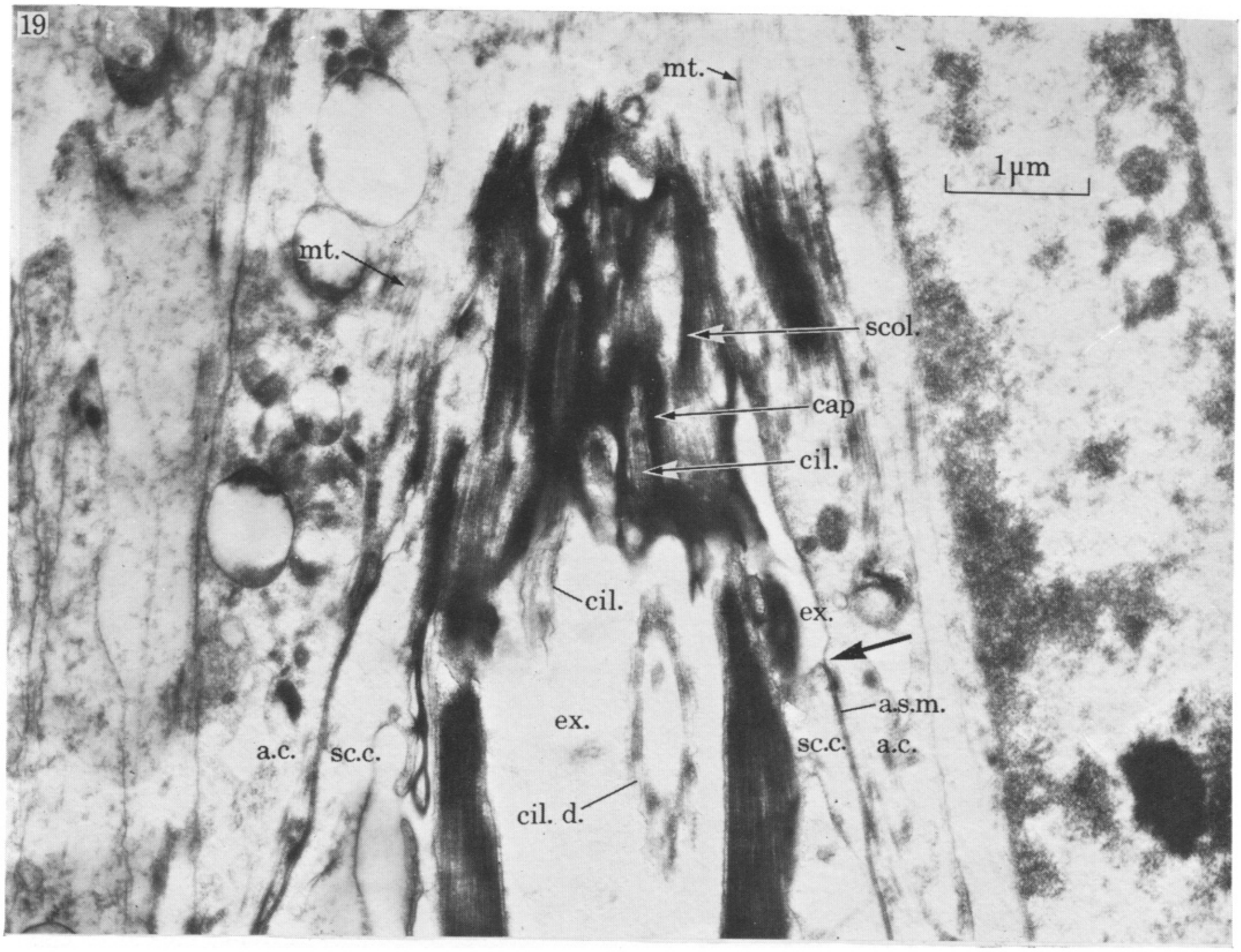
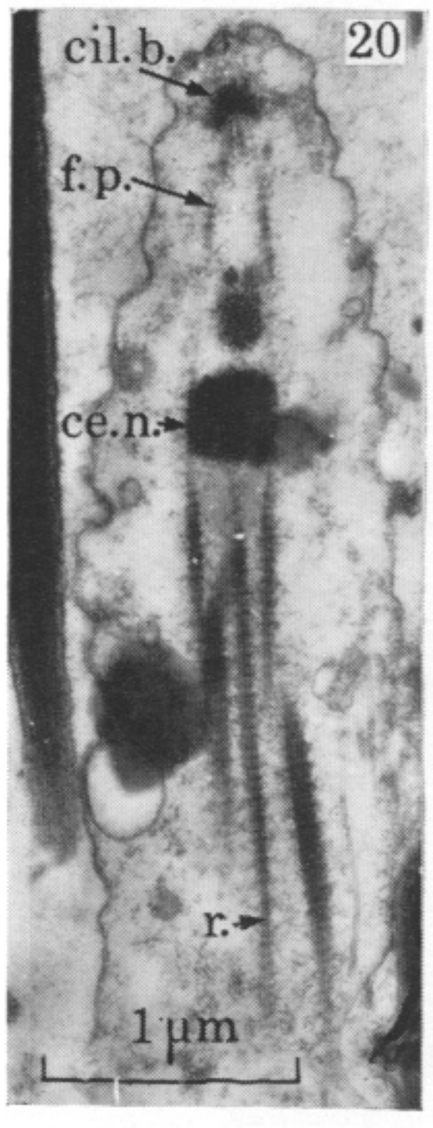
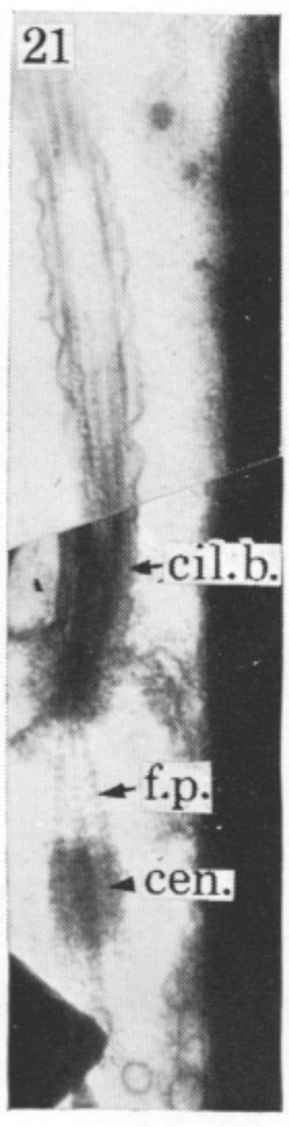
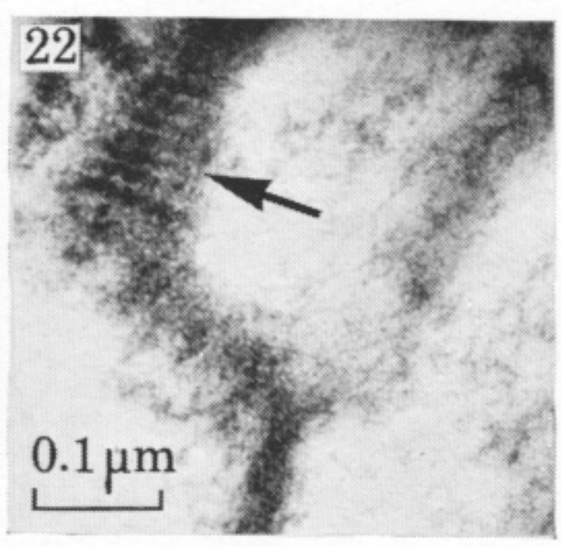


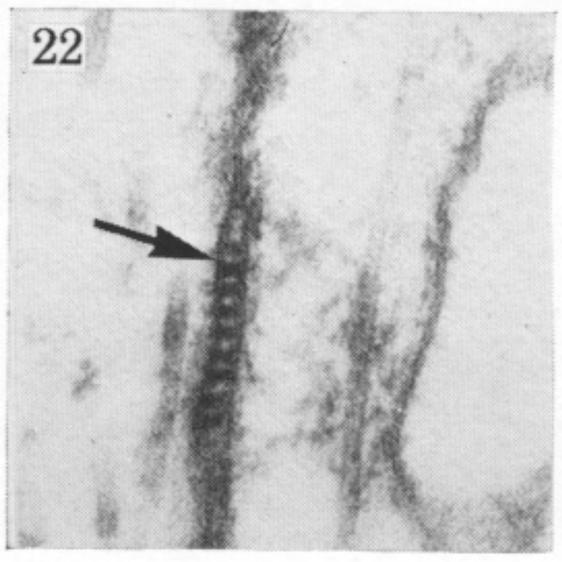
FIGURE 18. Longitudinal section of the scolopale unit, passing through the base of one cilium (cil.) and through the dilatation (cil. d.) of the other cilium. The scolopale rods (scol.) are surrounded by the scolopale cell (sc.c.), whose surface membrane is closely apposed (a.s.m.) to that of the attachment cell (a.c.), which contains many microtubules (mt.). den., dendrite; ex., extracellular space.











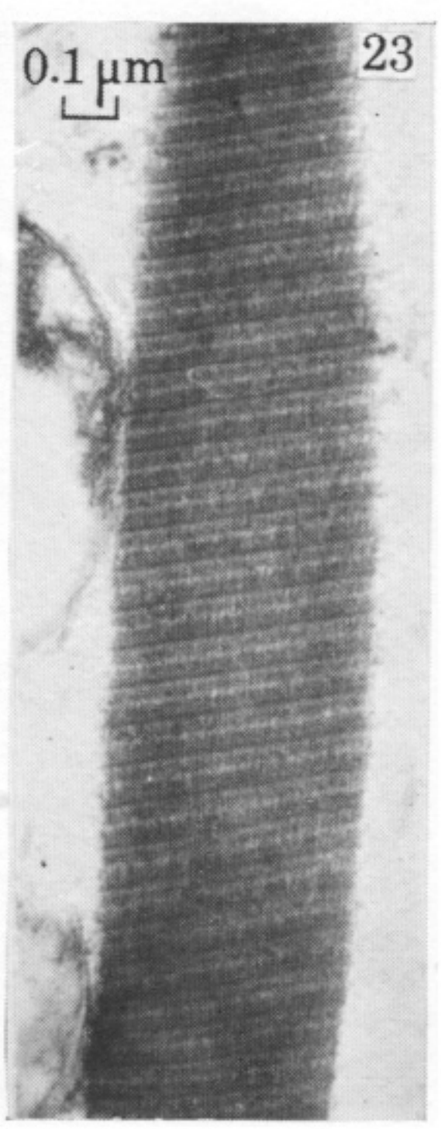


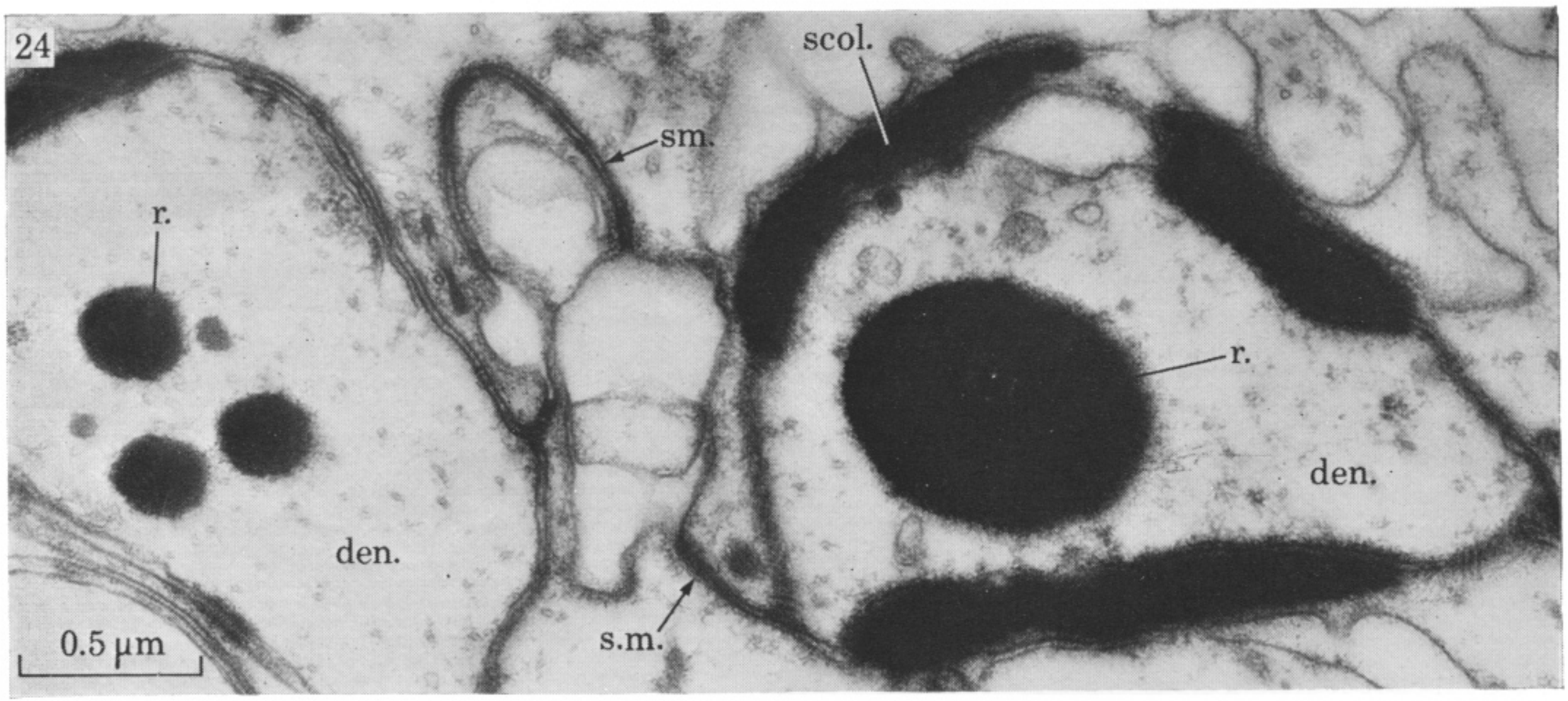
FIGURE 19. Longitudinal section of the distal part of the scolopale unit. The arrow on the right indicates the point where the apposed surface membranes (a.s.m.) of the scolopale cell (sc. c.) and attachment cell (a.c.) separate to pass around the cap material, which is situated in the extracellular space (ex.). The scolopale rods (scol.) penetrate a long way into the cap. The two cilia (cil.) also penetrate well into the cap, fitting tightly into clefts in the cap material. Compare with figures 30 and 31. cil. d., ciliary dilatation; mt., microtubules.

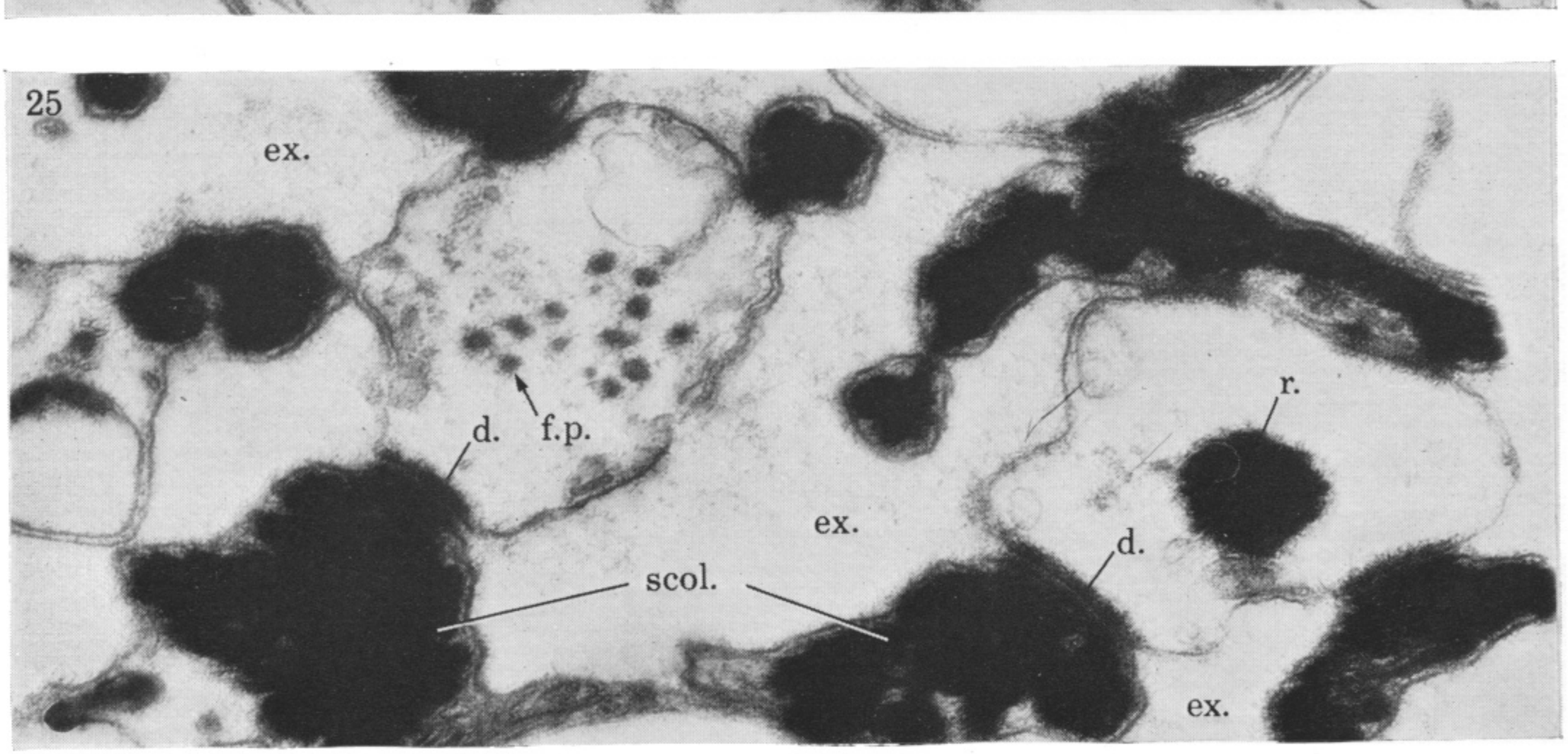
FIGURE 20. Longitudinal section of the centriolar region of the dendrite with rootlets. ce.n., centriole; cil. b. ciliary base; den., dendrite; f.p., finger processes; r., rootlet.

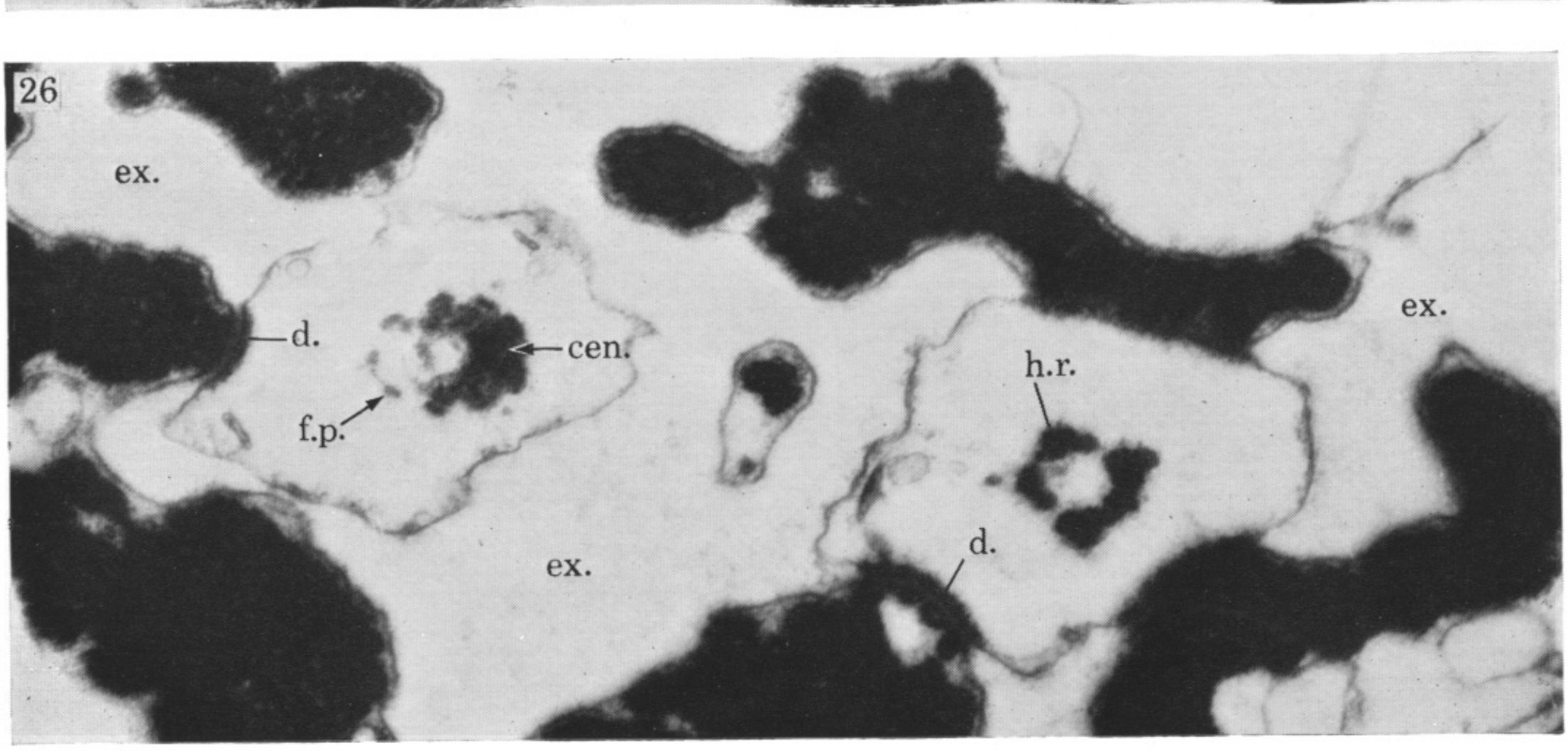
FIGURE 21. Montage of two consecutive longitudinal sections of the centriolar region of the dendrite with the single, large root. Magnification and labelling as in figure 20.

FIGURE 22. Section showing septate desmosomes between the apposed membranes of the dendrite and scolopale cells. The lower part of the picture shows the typical appearance of cross-bridges (arrow) between the apposed membranes. In the upper part of the picture this same pair of membranes have been cut tangentially, showing the cross-bridges to be composed of an hexagonal array (arrow).

FIGURE 23. Longitudinal section through the single, large root, showing the pattern of cross striation.





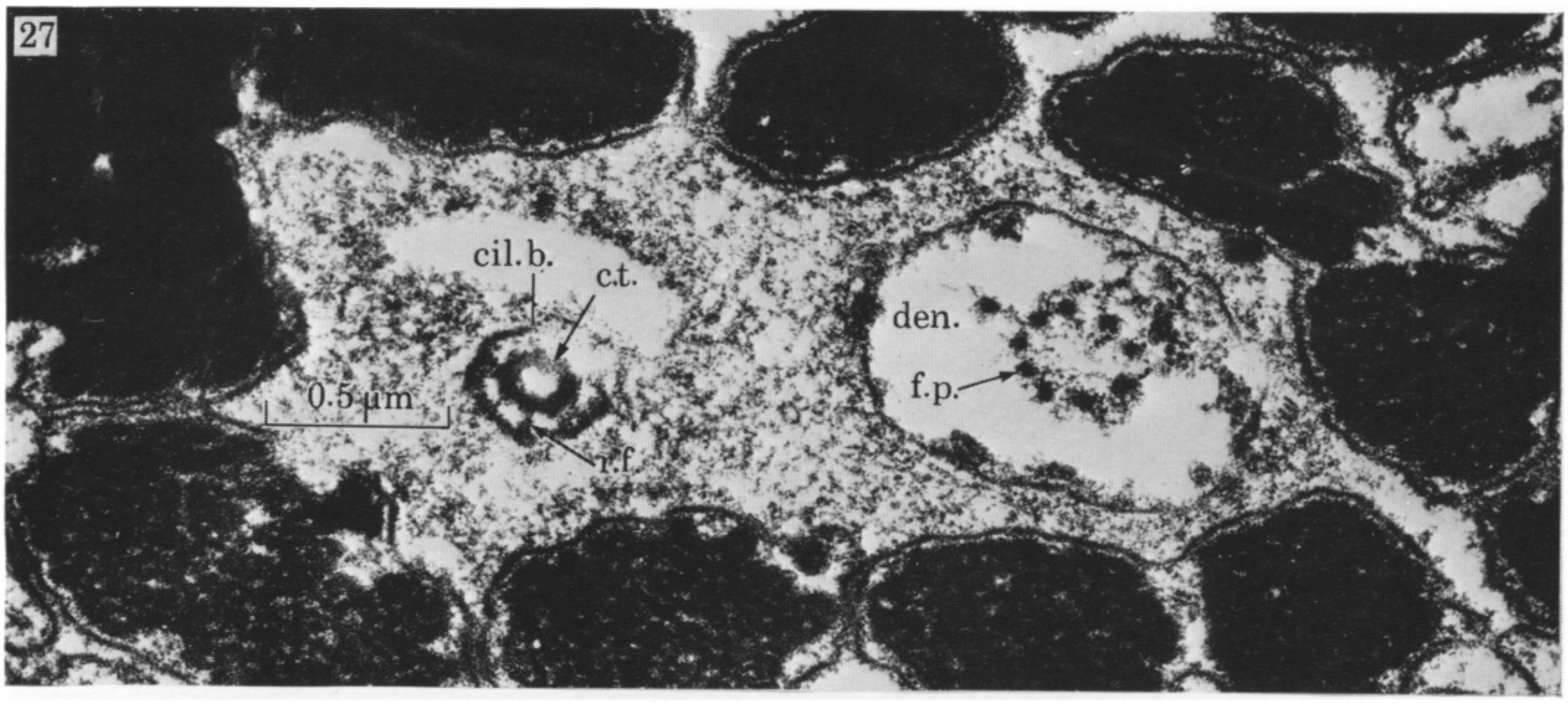


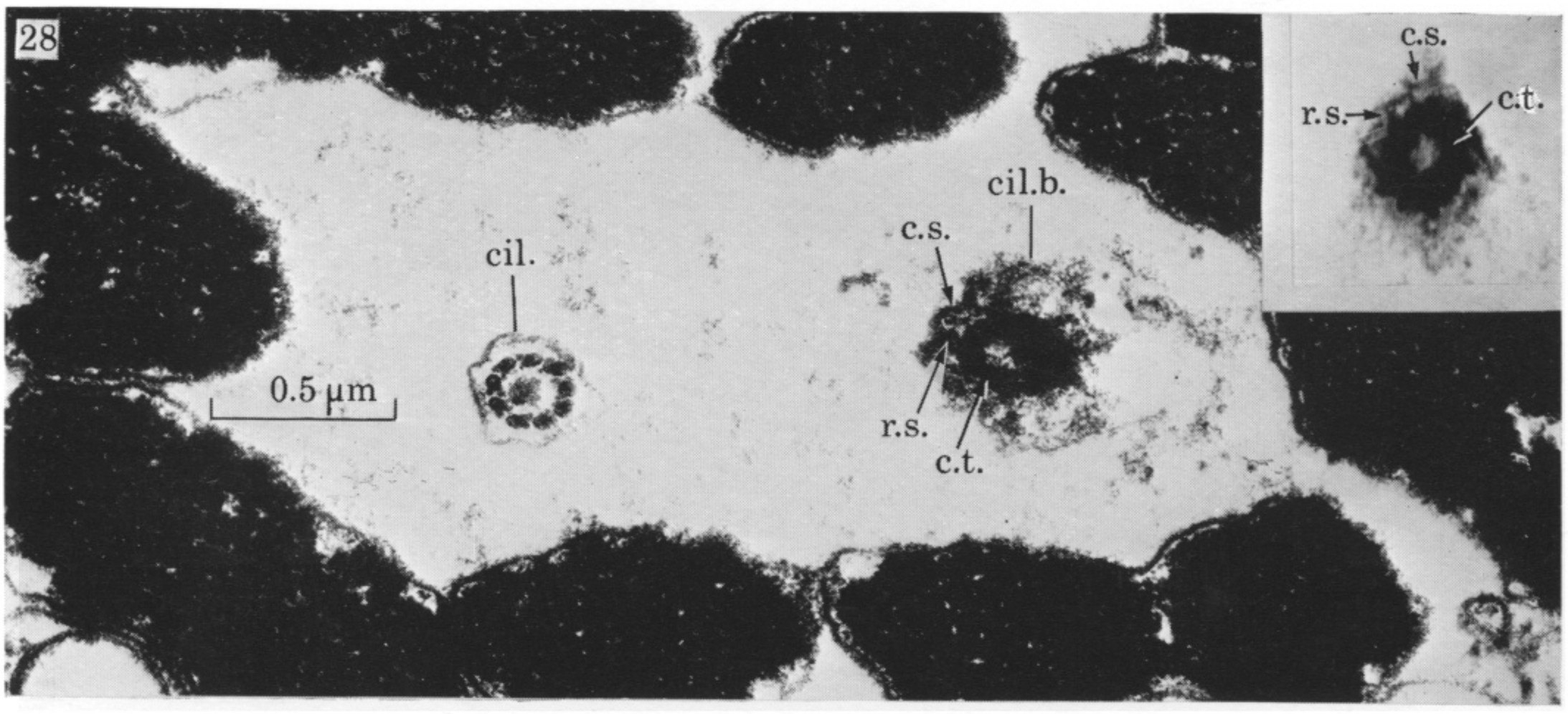
FIGURES 24, 25 and 26 are electronmicrographs of transverse sections taken at short intervals along the same scolopidium, at the base of the scolopale unit. The level at which each is cut is shown by a corresponding numbered arrow in figure 7.

FIGURE 24. The two dendrites (den.) are surrounded only by the scolopale cell, whose surface membrane fits closely round them and meets itself between them (arrow, s.m.). The dendrite on the left contains three rootlets (r.) and that on the right a single large root (r.). A few scolopale rods (scol.) make their appearance in the scolopale cell.

FIGURE 25. The two dendrites have shrunk away from the scolopale cell leaving an extra-cellular space (ex.) between them. Contact is maintained where scolopale rods (scol.) occur in the scolopale cell and at these points desmosomes (d.) can be clearly seen in the dendrites. On the left the three rootlets have split into bundles of finger processes (f.p.) and on the right the single root (r.) has narrowed considerably. More scolopale rods are now present.

FIGURE 26. The finger processes (f.p.) of the left hand dendrite have become arranged as a ring of nine around the centriole (cen.). This section glances across the top of the centriole so that only some of the finger processes are seen connected to the central tube of electron-dense material. On the right, the single root has become hollow (h.r.) and is just dividing into finger processes.





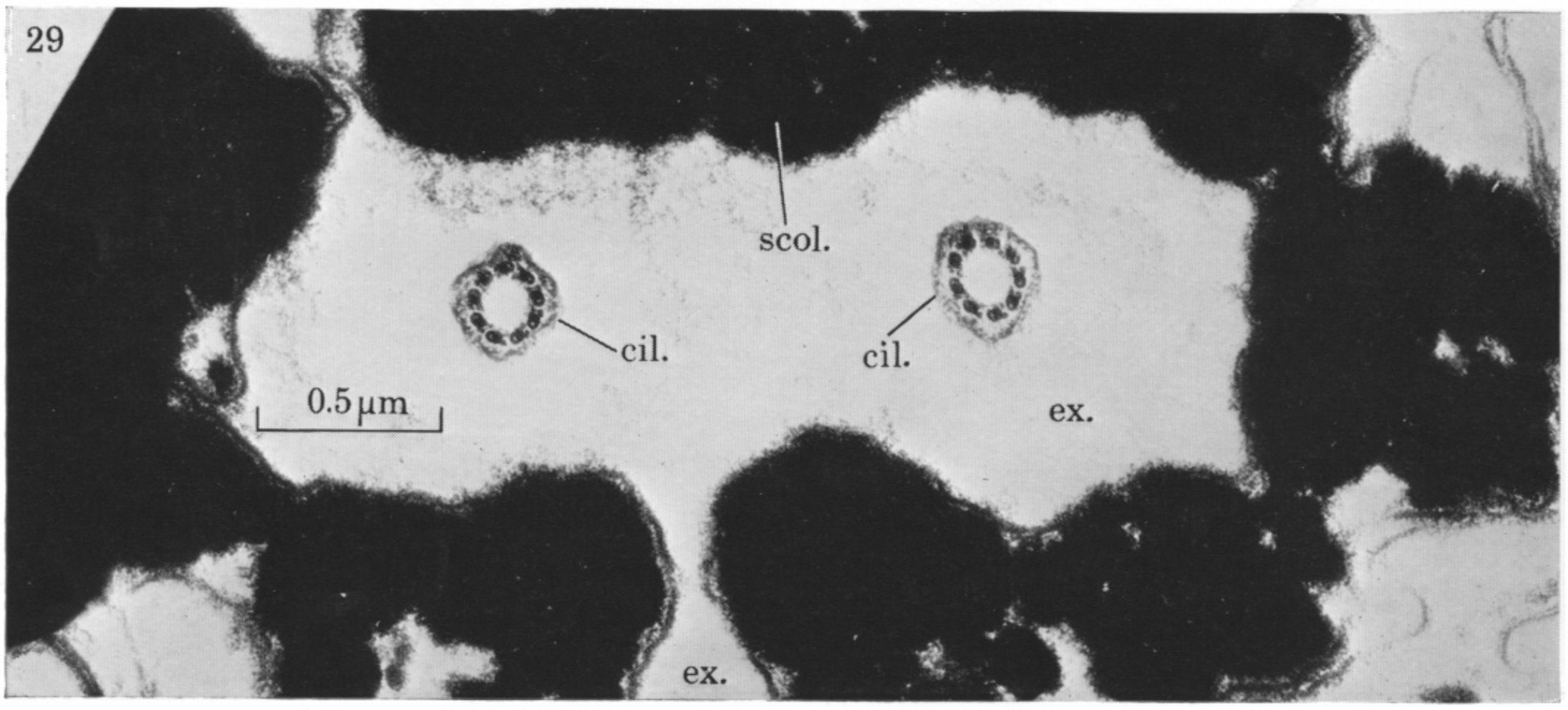
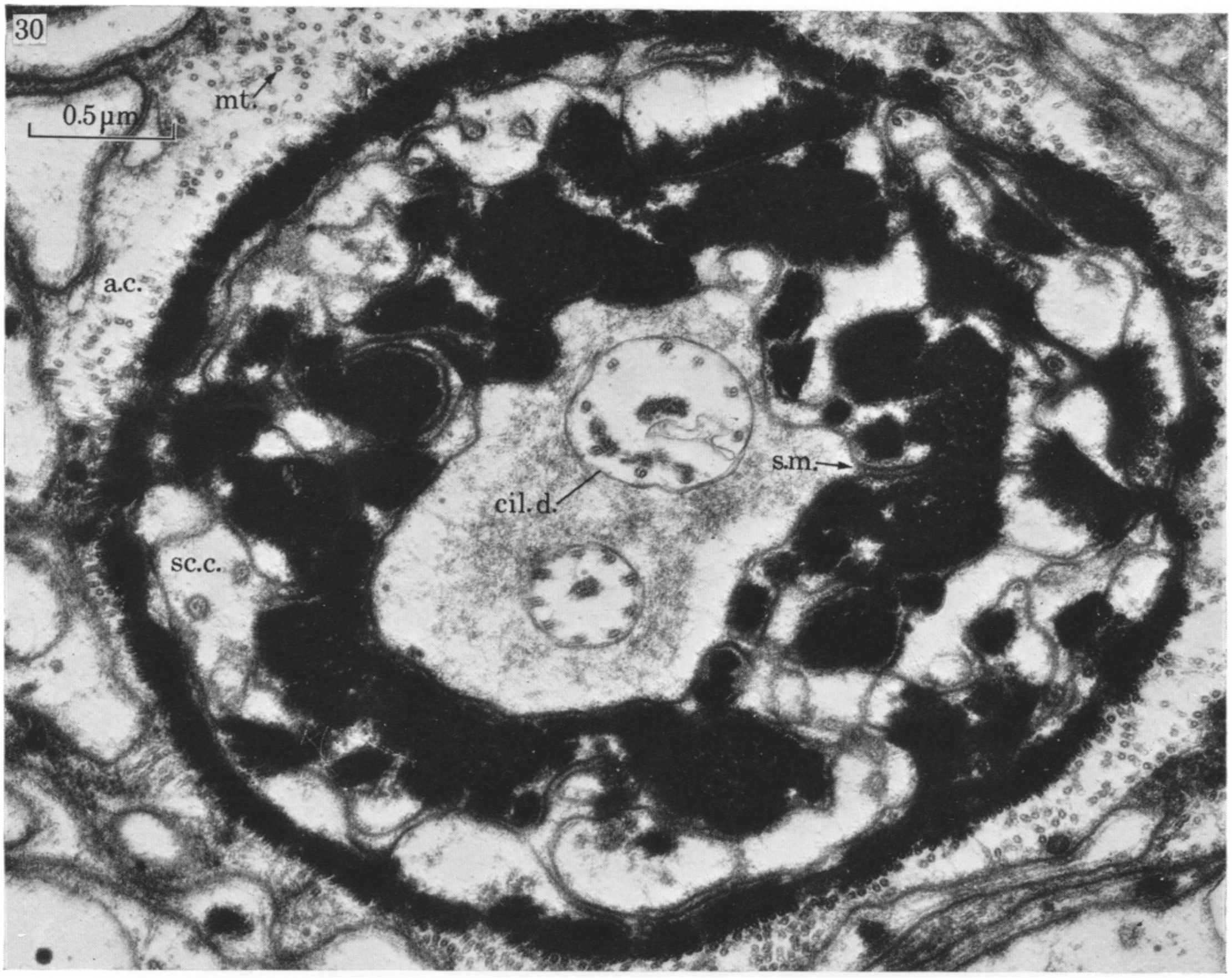


FIGURE 27. Transverse section of the scolopale unit at the level indicated by the numbered arrow in figure 7. On the left, the section passes through the ciliary base (cil. b.) just distal to the point where the ciliary shaft emerges from the surface of the dendrite. At this level, the central tube (c.t.) of the ciliary base is joined to the outer membrane by radial filaments (r.f.). On the right, the dendrite (den.) is cut through the ring of finger processes (f.p.) just below the centriole.

FIGURE 28. The same specimen as shown in figure 27 cut at the level indicated by the numbered arrow in figure 7. On the left, the section passes through the ciliary shaft (cil.). On the right, the section passes through the ciliary base (cil. b.) at the point where the ciliary shaft emerges from the surface of the dendrite. At this point, the central tube (c.t.) bears nine radiating spokes (r.s.), which are cross-connected by oblique strands (c.s.). *Inset.* Another section through the ciliary base at the point where the ciliary shaft emerges from the surface of the dendrite, showing the radiating spokes and their cross-connecting strands. Magnification and labelling as in the main figure.

FIGURE 29. Transverse section through the mid region of the scolopale unit, as indicated by the numbered arrow in figure 7. The two cilia (cil.) are alike, each containing a ring of nine ciliary fibrils. The cilia are surrounded by the extracellular space (ex.) which extends out between the scolopale rods (scol.) in a few places.



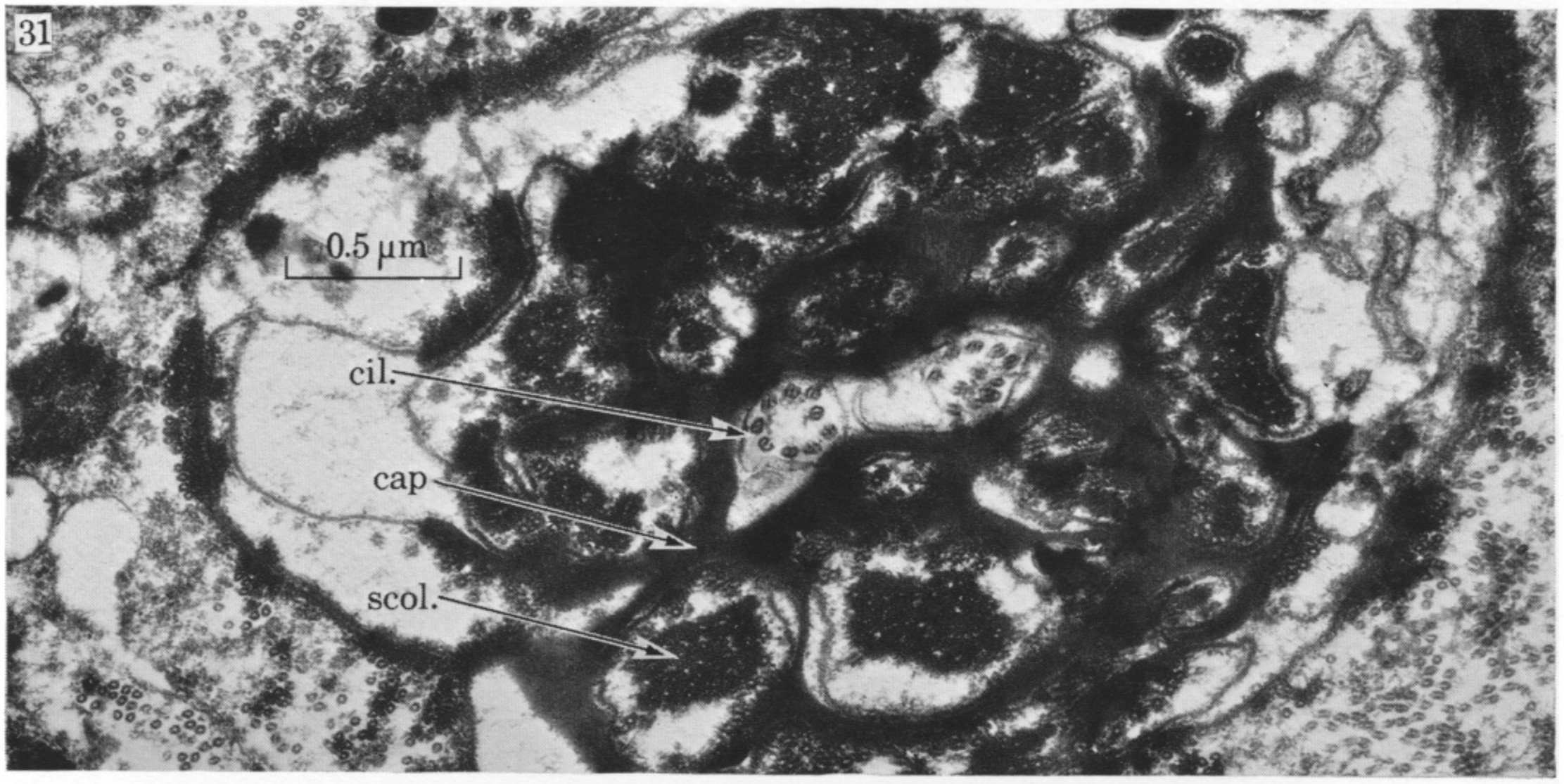
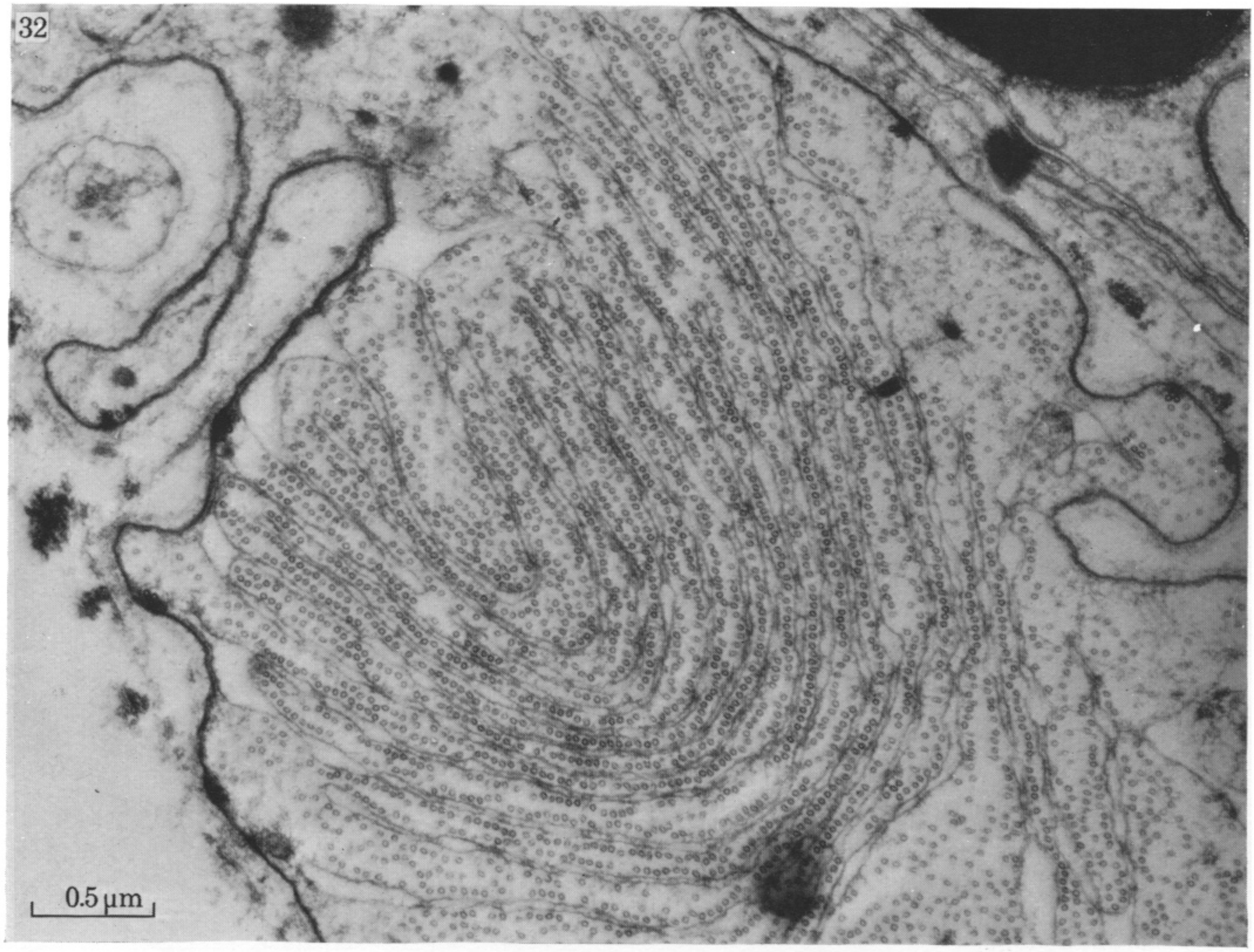
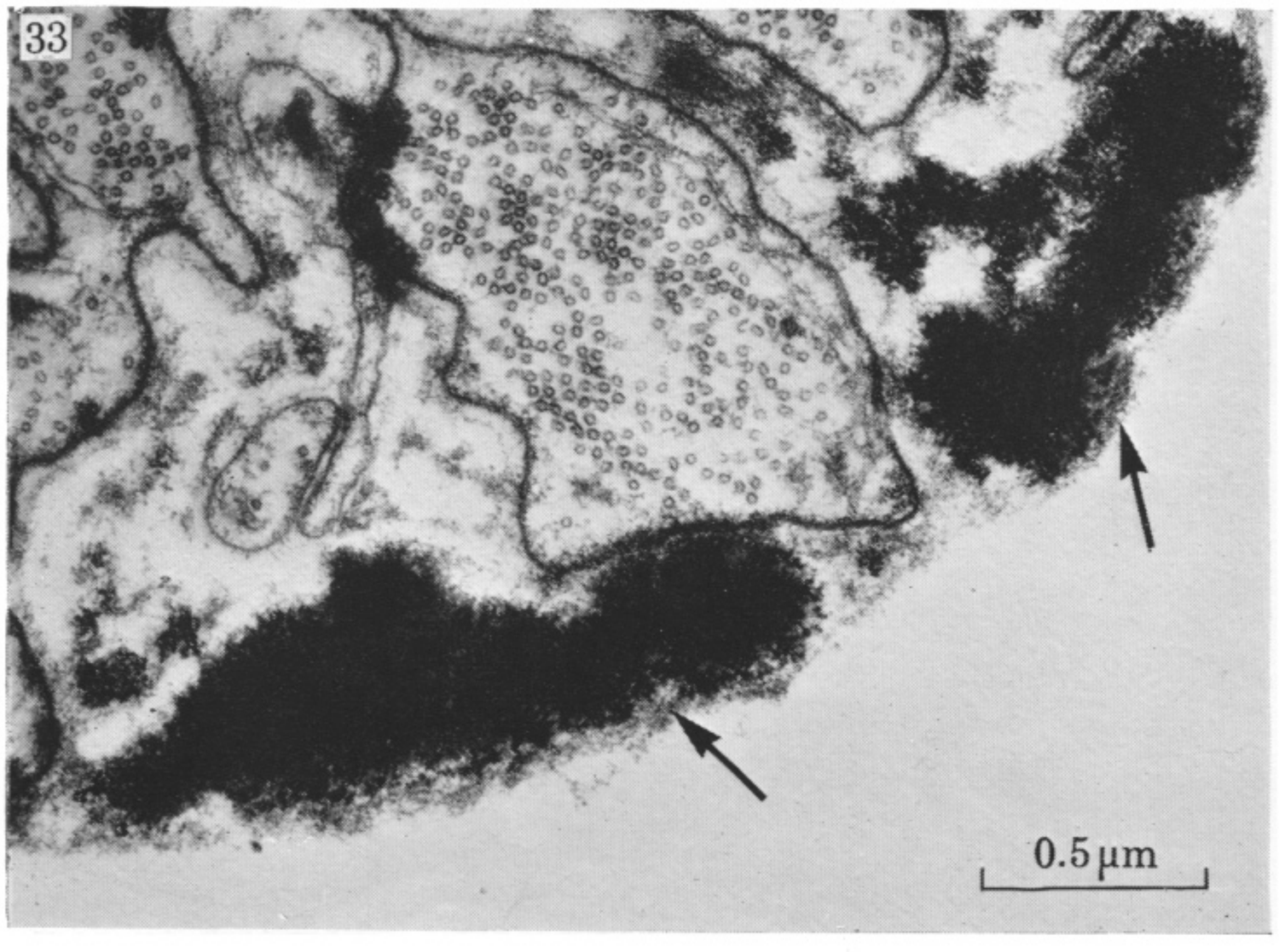


FIGURE 30. Transverse section through the ciliary dilatation (cil. d.) at a level indicated by the numbered arrow in figure 7. At this level the ring of scolopale rods is largely fused except where the surface membrane (s.m.) of the scolopale cell passes through. The scolopale cell (sc. c.) is greatly narrowed and is surrounded by the attachment cell (a.c.) which contains numerous microtubules (mt.).

FIGURE 31. Transverse section through the scolopale cap at a level indicated by the numbered arrow in figure 7. The tips of the two cilia (cil.), each bounded by the nerve cell membrane, are wedged in a cleft in the cap. The tips of the scolopale rods (scol.), each surrounded by the scolopale cell membrane, are inserted into the cap. The cap material itself is extra-cellular. A similar section through a cap can be seen at lower magnification in figure 15.





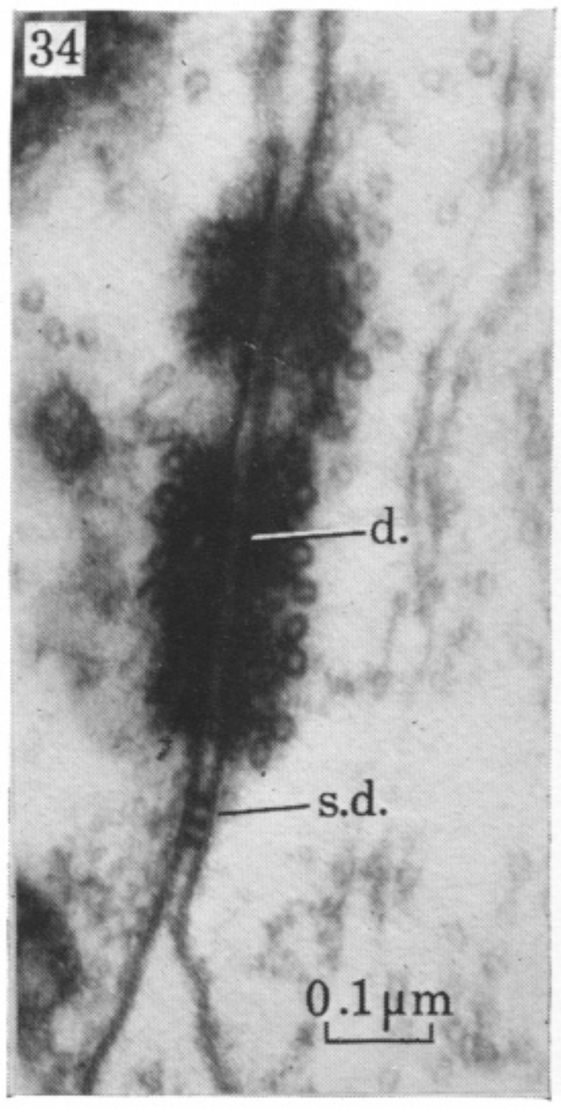


FIGURE 32. Transverse section through the attachment cell some distance beyond the cap. Many of the microtubules are arranged rather regularly in rows along infoldings of the surface membrane.

FIGURE 33. Transverse section through the neural lamella in the distal region of the chordotonal organ, showing the electron dense material (arrows) embedded in the neural lamella.

FIGURE 34. Transverse section through a region where infoldings of the attachment cell surface membrane are closely apposed to each other. Desmosomes (d.) and a small septate desmosome (s.d.) can be seen.