THE ORGANIZATION OF THE AUDITORY ORGAN OF THE BLADDER CICADA, *CYSTOSOMA SAUNDERSII*

By JANE M. DOOLAN and D. YOUNG

Department of Zoology, University of Melbourne, Parkville, Victoria, Australia 3052

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[Plates 1–7]

CONTENTS

The auditory organ of *Cystosoma saundersii* consists of 2000–2200 scolopidia arranged in two groups, a dorsal and a ventral group. The dorsal group contains scolopidia orientated along the longitudinal axis of the organ while the ventral group contains scolopidia aligned at right angles to these. On the basis of current theories of sensory transduction, it is possible that these groups may have different intensity characteristics.

The cellular composition of an individual scolopidium was described at the electron microscope level and was found to be similar to that occurring in most other chordotonal organs. Slight differences in fine structure were observed in the structure of the scolopale, the mass and position of the ciliary dilatation and the ciliary root. Differences in these parameters may influence the adequate stimulus needed for a chordotonal organ.

The fine structure of proximal and distal attachments of the scolopidia to the cuticle is similar to that of muscle attachments observed in insects, crustaceans and arachnids.

The central projections of the auditory nerve within the thoracic ganglia are similar to those described for the periodical cicadas.

1. Introduction

Many insects produce sound, especially in the orders Homoptera and Orthoptera. A comparison of the sound-producing mechanisms found throughout these groups shows a remarkable range of structures but the method of sound reception differs little, consisting of a chordotonal organ attached to a tympanum.

Much work has been done recently on the structure of the auditory organs of orthopteran insects (Gray & Pumphrey 1958; Gray 1960; Friedman 1972; Young & Ball 1974; Michel 1974). By comparison, the auditory systems of cicadas (Homoptera) have been little studied. Previous works in this field have been published by Vogel (1923), Myers (1928), Pringle (1954) and Michel (1975), and have shown that the cicada ear is a chordotonal organ composed of more than 1000 scolopidia. Michel (1975) also provided an ultrastructural description of the scolopale region of the component sensillae, and Wohlers *et al.* (1979) gave a description of the auditory projections. In this paper, we describe the auditory organ of the bladder cicada, *Cystosoma saundersii*, paying particular attention to those features that have been neglected in previous studies: the cellular composition and fine structure of the sensory units (scolopidia), the number and orientation of these units and the connections to the cuticle. Young & Hill (1977) have described the exact placement of the auditory organ in relation to the tympanum and tracheal system. Taken together, these papers are intended to provide a reasonably complete anatomy of the auditory system of one species of cicada.

2. Materials and methods

Cicadas were collected by hand at Port Macquarie in New South Wales. For light microscopy, auditory organs and thoracic ganglia were removed and fixed in alcoholic Bouin's: the auditory organs for 2 h and the ganglia for 48 h. They were then dehydrated, cleared and embedded in wax. Sections of 5, 10 and 15 μm thicknesses were cut and stained. Sections of auditory organs were stained with Masson's trichrome stain while those of the thoracic ganglia were silver-stained by the method of Blest (1976).

For electron microscopy, fixative was injected into the auditory capsule, and auditory organs were then dissected out and left in fixative for 1–2 h. The fixative was 2% (by volume) glutaraldehyde and 2% (by volume) formaldehyde in phosphate buffer, pH 7.3. Tissue was post-fixed in osmium tetroxide solution (2 mg/ml) for 2 h, dehydrated and embedded in Araldite. Sections were cut on an LKB Ultramicrotome Mark III with glass and diamond knives, stained with uranyl acetate and lead citrate and viewed on a Jeol JEM 100B electron microscope. Serial sections of 1 μm thickness were cut on the same ultramicrotome, stained with toluidine blue and viewed by light microscopy. These sections and the serial sections through the thoracic ganglia were reconstructed by the method of Pusey (1939).

Scolopale lengths were estimated by two methods: (1) counting the number of transverse sections of 1 μm thickness in which individual scolopales appeared; and (2) direct measurement on longitudinal sections with a Zeiss measuring eyepiece. Since the accuracy of these methods

differ, method 1 yielding quantized lengths, only groups that had been measured by the same method were compared statistically.

Neuron numbers were estimated by three methods: (1) counts of neuronal nuclei from 15 μm serial sections; (2) counts of scolopales from 5 μm and from 1 μm serial sections; and (3) counts of axons from electronmicrograph montages of the auditory nerve.

The projections of the auditory sensory fibres within the thoracic ganglia were investigated by iontophoresis of $CoCl_2$ along the auditory nerve. The thoracic ganglia were dissected out in Eibl's saline (Eibl 1978) and the auditory nerve endings immersed in $CoCl_2$ (25 mg/ml) with bovine serum albumin (1 mg/ml) in distilled water. The $CoCl_2$ was precipitated with ammonium sulphide (20 mg/ml) in Eibl's saline after 16–48 h. Ganglia were then intensified by the method of Pitman (1979), cleared in cedarwood oil and mounted in DPX.

3. Results

(*a*) *Arrangement of scolopidia within the auditory organ*

The auditory organ of *C. saundersii* is contained within an auditory capsule, situated on the second abdominal segment, and is composed of chordotonal scolopidia (Young & Hill 1977).

Proximally, the auditory organ is connected to the tympanum via the tympanal apodeme (figure 1). This is formed by a hollow inpushing of the tympanal ridge, which arises from the

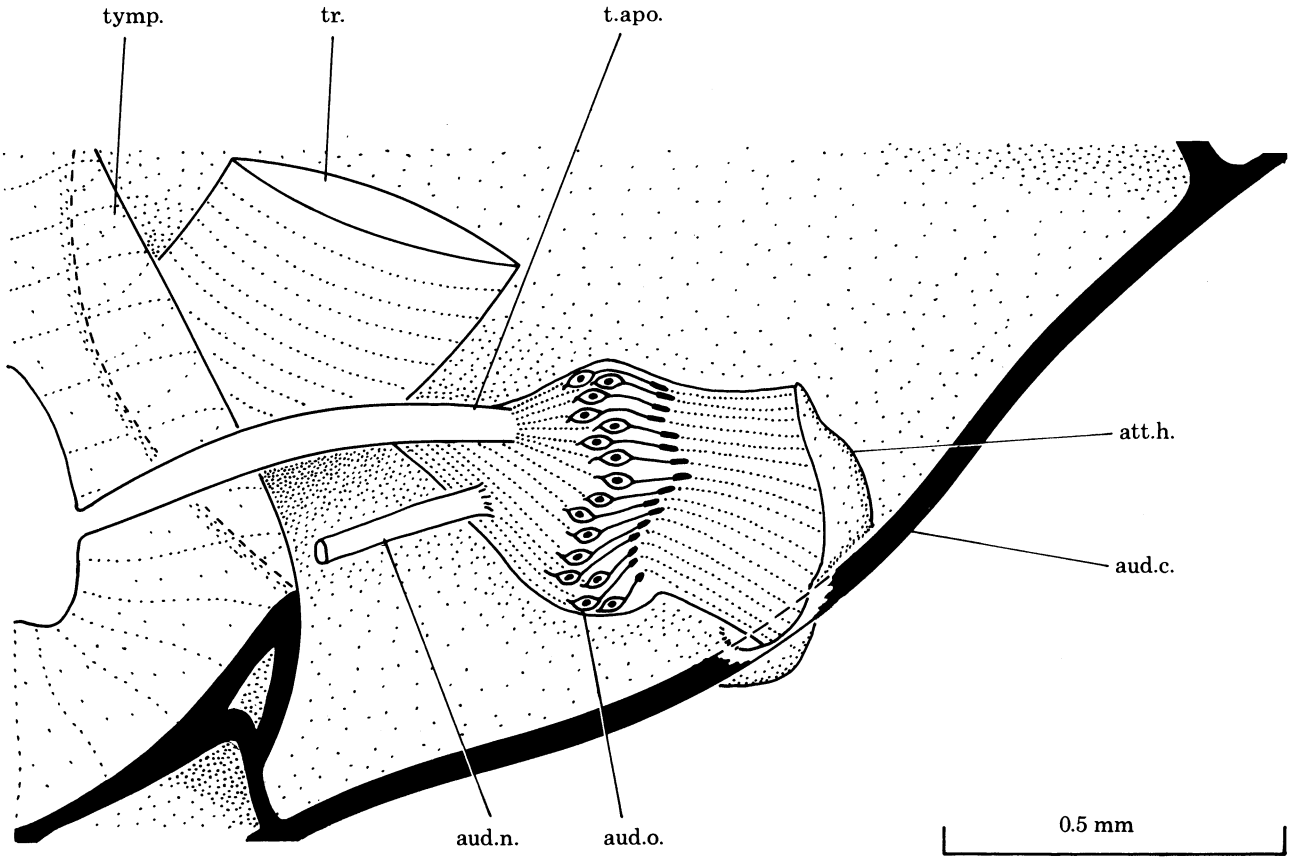


FIGURE 1. Posterior view of the auditory organ (aud.o.) and its cuticular attachments within the auditory capsule (aud.c.); att.h., attachment horn; aud.n., auditory nerve; t.apo., tympanal apodeme; tr., trachea; tymp., tympanum.

tympanal cuticle. In this region, the accessory cells of the scolopidia are packed tightly together and are attached to a small area at the tip of the apodeme (figure 6, plate 1). Distally, the organ is attached to a specialized invagination of the external cuticle (figure 1), the attachment horn (Vogel 1923). Here, the scolopidia spread outward and connect to a cuticular surface larger than that found proximally.

The organ contains 2000–2200 scolopidia, to judge from counts of six organs by the three methods. The results of the three methods are in close agreement (see table 1), and yield a mean of 2135 sensory cells.

TABLE 1. TOTAL NUMBER OF SCOLOPIDIA BASED ON ESTIMATES FROM NEURONAL NUCLEI, SCOLOPALES AND AXONS

preparation	number of neuronal nuclei	number of scolopales	number of axons
CIC 171	2215	2225	—
CIC 172	2116	2026	—
CYS G	—	2091	2125
CYS K	—	·	2171
CYS J	—	—	2100

mean = 2135 standard error = 25.2

Two groups of cells may be distinguished, a dorsal group and a ventral group (figure 2; figure 7, plate 1). The dorsal group contains approximately 1000 scolopidia, all of which are orientated longitudinally, i.e. along the axis of the tympanal apodeme. Distally, the scolopales within this group occur in the centre of the organ and have distal attachment cells approximately 100 μm in length. Proximally, scolopales occur in more dorsal positions and have longer distal attachment cells. Scolopales found on the dorsal surface have distal attachment cells 350 μm in length. The neuron cell bodies of this group are compactly arranged and occur in the same longitudinal orientation as their scolopales (figure 2).

The ventral group constitutes the main body of the organ and consists of approximately 1100 scolopidia. Approximately 900 of these are situated laterally, of which twice as many occur on the posterior side as on the anterior side. The scolopales of these lateral scolopidia are directed inward, at right angles to those of the dorsal group, although some intermediate orientations occur (figures 2, 3, 7). Their cell bodies are found on the periphery of the organ. The remaining 200 are orientated in the same longitudinal direction as the dorsal group and mostly occur in a large clump at the proximal extremity of the organ, although a few may be found distally. The overall effect created by the ventral group when seen in transverse section (figure 7), i.e. at right angles to the tympanal apodeme, is that of a hemisphere of scolopales with the majority directed inwards. Distally, the scolopales of both the dorsal and ventral groups occur in the centre of the organ and consequently the groups cannot be clearly distinguished at that level.

There appears to be no discernible difference in cellular structure between the scolopidia of these two groups. The size of cell bodies varies slightly within each group. However, the within-group variation is the same for both groups, giving an average of 8–10 μm in diameter with a dendrite length of 60 μm. Scolopale lengths show a large degree of variation within each group. Lengths vary between 10 and 25 μm, with a mean of 16.25 μm (s.d. = 3.44 μm), having a normal distribution about the mean (skewness and kurtosis were non-significant). There is

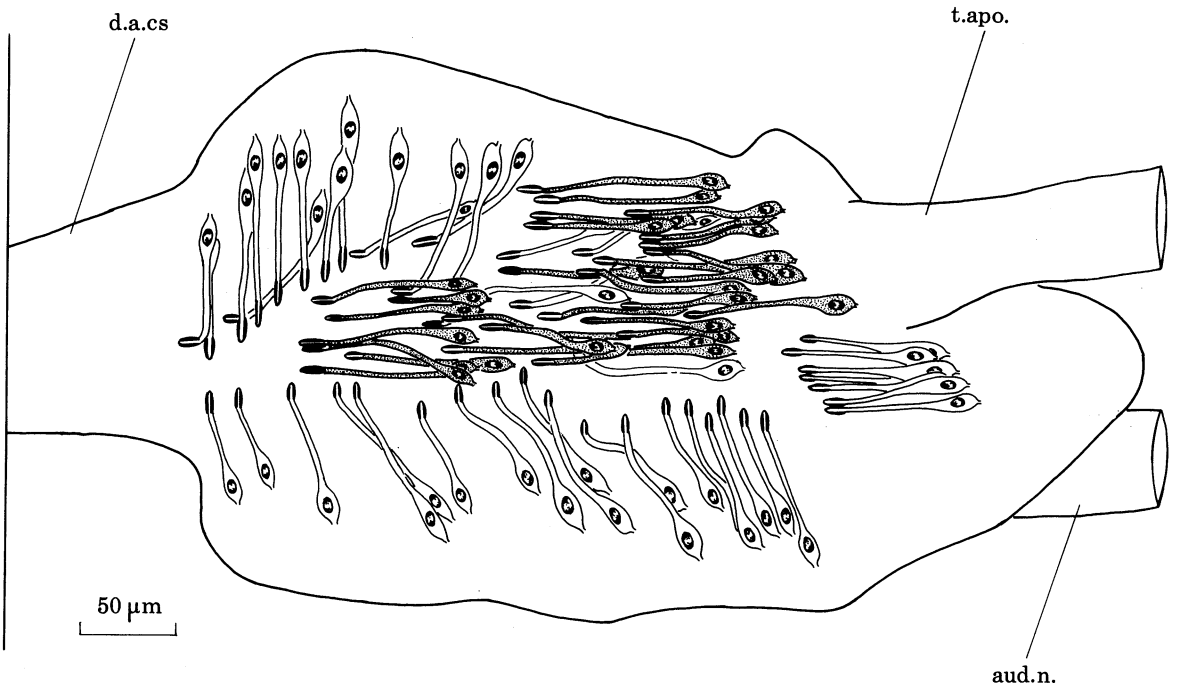


FIGURE 2. Dorsal view of the auditory organ reconstructed from 1 μm serial sections. One scolopidium in 30 has been drawn. The neurons of the dorsal group are stippled. Abbreviations: aud.n., auditory nerve; d.a.cs, distal attachment cells; t.apo., tympanal apodeme.

no significant difference in this respect between the length–frequency distributions of the two groups (the 95 % confidence limits of the means overlap). The structure of the accessory cells and the relationships between these cells show little variation between groups although slight differences in the lengths of the attachment cells occur depending on the position of the scolopidium within the organ. Those in proximal situations have long distal attachment cells and comparatively short proximal attachments (figure 3). Those in the ventral group have a longer distance to traverse to the tympanal apodeme than those of the dorsal group and consequently have longer proximal attachments (figures 2, 3).

The sensory axons collect together in four main aggregations, which fuse to form the auditory nerve. This is situated at the proximal end of the organ, below the tympanal apodeme (figure 1). The axons from the dorsal group clump on the dorsal surface to form one of the aggregations. This gathers axons from centrally positioned neurons as it passes ventrally. It is also joined by two large aggregations that occur laterally, one on each side of the organ, and are composed of the axons from the peripheral cell bodies of the ventral group of sensillae. The last aggregation to pass into the auditory nerve is that of the longitudinally orientated cells of the ventral group. In some cases, this last cluster of axons may occur outside the limits of the membrane encompassing the organ.

(*b*) *Structure of an individual scolopidium*

The scolopidia found in the auditory organ of *C. saundersii* conform to the general design found in most chordotonal organs. Each consists of a bipolar neuron with a dendrite bearing a modified cilium distally (figure 4) and, proximally, an axon that passes out of the auditory

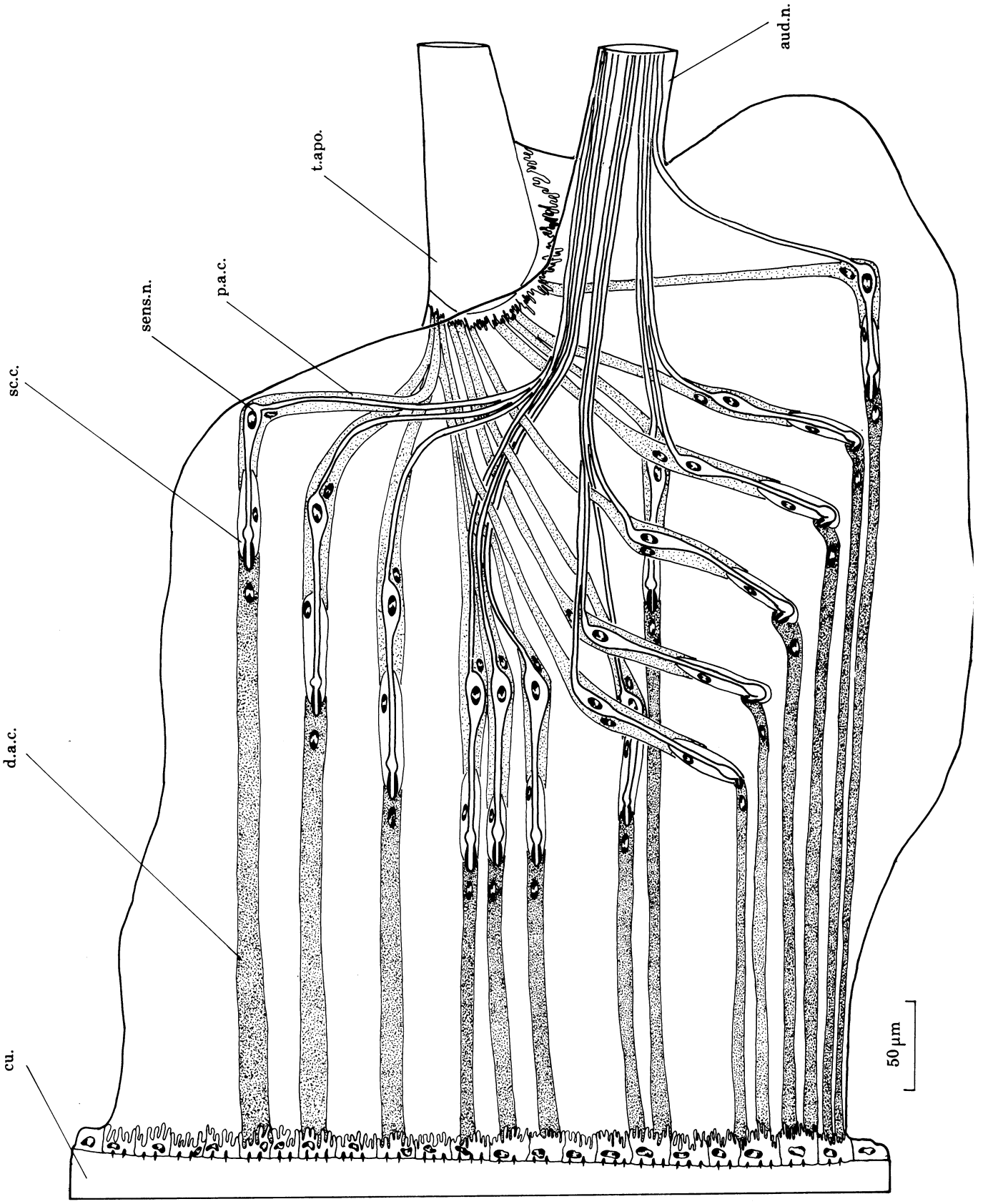


FIGURE 3. For description see opposite.

organ along the auditory nerve. The neuron has four accessory cells: the proximal attachment cell and the distal attachment cell, which attach to the epidermal layers of the cuticle; the scolopale cell, which contains the scolopale; and the Schwann cell, which surrounds the axon. The structure of an individual scolopidium, including accessory cells, is summarized in figure 4.

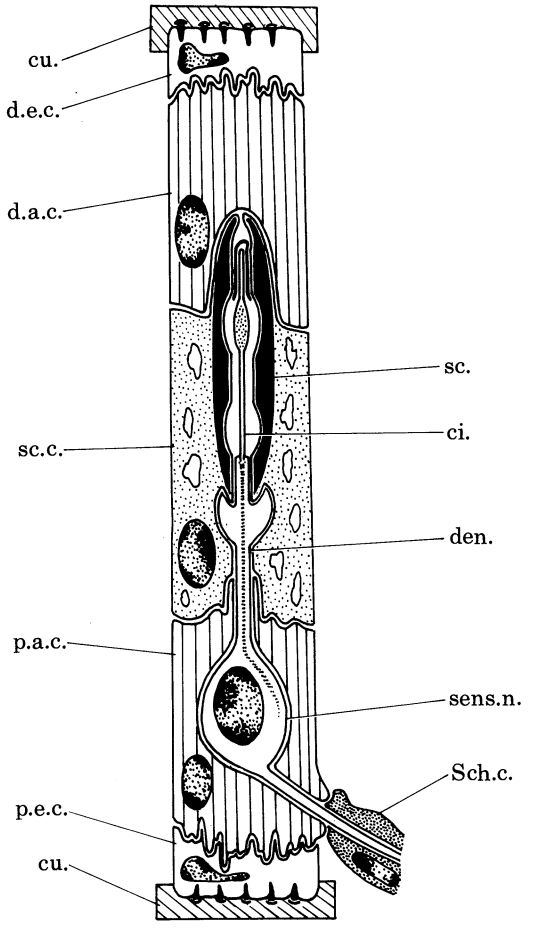


FIGURE 4. Diagram showing the structure of a single scolopidium, including accessory cells and their attachments to the cuticle. Abbreviations: ci., cilium; cu., cuticle; d.a.c., distal attachment cell; d.e.c., distal epithelial cell; den., dendrite; p.a.c., proximal attachment cell; p.e.c., proximal epithelial cell; sc., scolopale; sc.c., scolopale cell; Sch.c., Schwann cell; sens.n., sensory neuron.

(i) *The sensory neuron*

The perikaryon of the sensory cell contains Golgi bodies, endoplasmic reticulum, microtubules and a profusion of mitochondria in random orientations. The cell body and half the length of the dendrite are sheathed by the proximal attachment cell. Distal to this, the dendrite is surrounded by the scolopale cell, and expands into the dendrite dilatation (Gray 1960) immediately proximal to the scolopale (figure 4; figure 12, plate 3).

FIGURE 3. Lateral posterior view of the auditory organ reconstructed from 1 μm serial sections. Selected scolopodia only from both groups have been included. Abbreviations: aud.n., auditory nerve; cu., cuticle; d.a.c., distal attachment cell; p.a.c., proximal attachment cell; sc.c., scolopale cell; sens.n., sensory neuron; t.apo., tympanal apodeme.

The dendrite itself contains longitudinally orientated mitochondria, microtubules, filaments and endoplasmic reticulum. It also contains a large ciliary root, which extends the entire length of the dendrite and down into the cell body. The termination point of the root varies. Many were observed to finish at the axon hillock and some far down into the axon itself (figure 11, plate 3). Michel (1975) states that, in *Cicada orni*, the roots extended only to the level of the cell nucleus. The root is a cross-banded structure with a periodicity of 65–70 nm. It splits along its length in a manner similar to that described by Gray (1960), Slifer & Sekhon (1975), and Michel (1975). In transverse section (figure 9, plate 2), the root consists of densely packed fibrils, some of which project into the surrounding cytoplasm at the periphery of the root. It is surrounded for most of its length by a group of longitudinally aligned microtubules arranged in a concentric ring (figure 9). The density of microtubules varies along the length of the dendrite. Arm-like structures similar to those described by Friedman (1972) link many of the microtubules and occasionally appear between the fibrillar projections of the root and individual microtubules (figure 9, arrowed). Associations between the root and mitochondria occur sometimes, although there appear to be no correlations between the dark bands of the root and the cristae of the mitochondria such as those observed by Olsson (1962). Within the cell body, associations between the root and the nucleus have been observed (figure 11, arrowed), similar to those described by Salisbury & Floyd (1978) for motile cilia.

At the level of the dilatation, the microtubules surrounding the root cease abruptly and a marked increase occurs in the numbers of mitochondria that are randomly orientated (figure 12). Distal to this, the dendrite becomes surrounded by the scolopale. Within this region, the cytoplasmic threads connect the root to the dendritic membrane (figure 8, plate 2), as described by Young (1973). Desmosomes are found between the dendritic membrane and the scolopale cell membrane in the region of the scolopale rods. At the distal end of the dendrite, two basal bodies occur aligned longitudinally. At the level of the proximal basal body, the root breaks into nine rootlets, which pass around this structure and terminate on the distal basal body, from which the cilium originates.

The structure of the modified cilium has been described fully by Young (1973) and only a brief résumé will be given here. The cilium is of the 9×0 configuration. Each doublet consists of a hollow tube and a solid rod with dynein extensions. At its distal end, the cilium dilates for approximately 2 μm. Within this area, the doublets lose the dynein arms. Distal to this, the cilium regains its normal diameter, and becomes surrounded by the cap. At the distal end of the cilium the doublets lose their solidity and progress as hollow tubes (figure 10, plate 2). This arrangement occurs occasionally in the ear of *Cyclochila australasiae* (Young 1973), but is found in large numbers in *C. saundersii*. The cilium is not attached to the cap at its distal end.

(ii) *The accessory cells*

There are four cells that ensheath the neuron at different levels along its length: the distal attachment cell, the proximal attachment cell, the scolopale cell and the Schwann cell. Descriptions of equivalent cells have been published by Gray (1960), Slifer & Sekhon (1975), Michel (1975) and Young (1975), and, for the most part, those occurring in the auditory organ of *C. saundersii* resemble closely those described in the previous studies.

The scolopale occurs within the scolopale cell as several distinct rods, which fuse at the level of the basal bodies to become a continuous tube. This surrounds an extracellular space in the centre of which lies the cilium. The thickness of the scolopale varies along its length, being

thinner in the regions of the ciliary base and dilatation. A range of transverse sections at different levels through the dendrite and cilium are shown in figure 12. At its distal end, the scolopale encompasses the cap, which encloses the cilium. The cap is of the type described by Young (1975) and Michel (1975): a long, thin tube, open at its proximal end and bearing several flanges (figure 10; figure 13, plate 4). Distally it narrows down and converges on the cilium. At its apex, the cap expands outward into a small nodule. At this level, isolated microtubules occur in the scolopale material close to the cap (figure 10).

The scolopale, although totally enclosed by the scolopale cell membrane, is also surrounded by the distal attachment cell for almost half of its length (figure 4). The cytoplasm of the distal attachment cell is clear, with numerous microtubules, few mitochondria and a number of intracellular infoldings of the plasma membrane mentioned by Gray (1960). The nuclei are generally found alongside the cap. The membranes of the distal attachment cell and the scolopale cell are loosely associated with infrequent desmosomes except in the region surrounding the scolopale. In this region, the membranes are in close contact, with cell contact points occurring whenever the distal attachment cell is apposed to a scolopale rod. These contacts have been described by Ashhurst (1970) and Michel (1975) and are composed of small membrane thickenings, with an accumulation and coalescence of microtubules occurring on the side of the distal attachment cell (figure 10, arrowed).

The proximal attachment cell is similar to its distal counterpart. Its cytoplasm is denser and packed with microtubules and granules. There are few mitochondria and none of the membrane thickenings observed in the distal attachment cell. The nuclei generally occur alongside the dendrite dilatation. The proximal attachment cell surrounds the cell body and proximal portion of the dendrite and often part of the axon as well (figure 11; figure 14, plate 4). In the central region of the dendrite, the proximal attachment cell narrows down to 30–40 nm in diameter and becomes surrounded by the scolopale cell. There is no shoulder to the proximal attachment cell but some slight interdigitation between the two cells is evident (figure 4). This is of the order of some three to four fingers of the proximal attachment cell interlocking with extensions of the scolopale cell. Within these regions, septate desmosomes are often seen between the two apposing membranes. They have a periodicity of 22 nm and are of the honeycomb form described by Staehelin (1974). Desmosomes of this type are also found between apposed membranes of the proximal attachment cell, within the mesaxon, between Schwann cell folds and occasionally between the proximal attachment cell and the Schwann cell.

A separate Schwann cell encloses each individual axon (figure 15, plate 4). This occurs for a short length. The axons rapidly clump together and the separate Schwann cells are succeeded by single Schwann cells that sheath individually all the axons in the group. The level at which the Schwann cell enfolds the axon differs with the position of the sensory neuron within the organ and is correlated with differences in the relative distances and courses travelled by the proximal attachment cells and the axons. In general, the axon of a cell will remain surrounded by the proximal attachment cell for the distance over which the courses to the tympanal apodeme and the auditory nerve are similar. When the proximal attachment cell changes course, the axon becomes ensheathed by the Schwann cell. Thus, axons of cells in the dorsal group are surrounded by the proximal attachment cells for a considerable length (figure 3). Within the ventral group, however, the proximal attachment cells bend towards the tympanal apodeme at an earlier stage and, consequently, cell bodies with Schwann cells beginning at the axon hillock and even the nucleus are often observed.

(iii) *The proximal attachments to the cuticle*

Proximally, the organ is attached to the tympanal apodeme. Within a small area at the tip of the apodeme, there is a great concentration of attachment cells (figure 16, plate 5). These connect to the epidermal cells, which form a layer overlying the cuticle (figure 17, plate 5). The attachment cells taper at their proximal ends so that a large number are attached to any one epidermal cell. The method of attachment is one of extensive interdigitation occurring in a manner similar to that seen in the muscle attachments to the epidermis (Lai-Fook 1967; Caveney 1969; Neville 1975) (figure 16). The cells of both types extend in long narrow fingers, that interlock for a length of approximately 2 μm. Using the index of Lai-Fook (1967), we estimate that the interdigitation increases surface area by a factor of 30. Large desmosomes are found on most of the length of the interdigitating finger (figure 16, arrowed). These resemble those found at myo-epidermal junctions and have been identified as fascia adherens or intermediate junctions (Fawcett 1966) (figure 19, plate 6).

The epidermal cell contains a small, granular nucleus and large numbers of microtubules. In the area of attachment to the cuticle, the epidermal cell expands outward in several protuberances, that narrow into the interdigitating fingers. In this area, structures resembling the tonofibrillae of Lai-Fook (1967) are found connecting the epidermal cells to the cuticle (figure 20, plate 6). These structures consist of a cuticular attachment fibre that projects from the cuticle into the epidermal cell (figure 21, plate 6). Hemidesmosomes are found on the epidermal cell membranes when in contact with these cuticular attachment fibres. Within the epidermal cells, microtubules extend from these hemidesmosomes to the fascia adherentes found proximally. The cuticular attachment fibre extends back into the cuticle and becomes associated with a pore canal in a manner similar to that described by Caveney (1969). All the pore canals in the attachment area contain cuticular attachment fibres (figure 22, plate 7). In some cases, two or three have been observed in a single pore canal. The cuticular attachment fibres appear to twist within the twisting pore canals as they penetrate deep into the endocuticle of the tympanal apodeme (figure 22, inset).

(iv) *The distal attachments to the cuticle*

Distally, the auditory organ is attached to a region of cuticle on the external wall of the auditory capsule, the attachment horn. The distal attachment cells do not converge to a small portion of cuticle, as occurs at the tympanal apodeme, but are spread over a region that includes the inner surfaces of the attachment horn, the protuberance and the area between these two (figure 1).

The distal attachment cells connect to the distal epidermal cells, which differ from those found proximally in possessing lysosomes, intracellular vacuoles and fewer microtubules. Outside the attachment zone, the distal epidermal cells are irregularly shaped, terminate in a small number of protrusions and have no specialized connections with the cuticle. In the distal area of attachment, the epidermal cells become enlarged and possess cytoplasmic projections, that extend into adjacent epidermal cells. Cuticular attachment fibres similar to those found proximally are found connecting the epidermis to the cuticle. These also become associated with twisting pore canals and traverse the external cuticle.

The attachment between the distal attachment cells and the epidermal cells is by interdigitation (figure 18, plate 6), which increases the area of cell-to-cell contact by a factor of 30–35, similar to that seen in the proximal attachment. Fascia adherentes are found between

the interdigitating membranes. The microtubules extend from these cell junctions to the hemidesmosomes surrounding the cuticular attachment fibres.

(*c*) *The central projections of the auditory nerve*

The auditory nerve contains fibres from six sources at its point of entry to the metathoracic–abdominal ganglion. These include the 2000 units from the auditory organ, the 500 units from the detensor tympani chordotonal organ (Young 1975) and fibres innervating the detensor tympani muscle, the tymbal muscle (Young 1975) and the muscles of the body wall. The projections of the sensory fibres from the auditory nerve form three distinct, bilaterally symmetrical neuropils, two of which are situated ventrally and the other in an intermediate position (figure 5) within the ganglion.

The majority of fibres contribute to the intermediate neuropil, which extends for most of the length of the metathoracic–abdominal ganglion. This neuropil is metamerically organized with 6–8 finger-like extensions, none of which pass across the midline of the ganglia (figure 23, plate 7). Two of these extensions occur in the metathoracic ganglion and, after Wohlers *et al.* (1979), are termed anterior. The remaining 4–6 extensions occur in the fused abdominal ganglia and are termed posterior. In some cases, the most posterior extension may consist of a single fibre ranging towards the midline.

Several fibres form a second neuropil, which occurs only in the metathoracic ganglion. This overlaps the anterior section of the intermediate neuropil but occurs just below it (figure 5, ventral anterior neuropil). Fibres within this neuropil extend towards the midline and, in some cases, beyond it and do not appear to be metamerically organized.

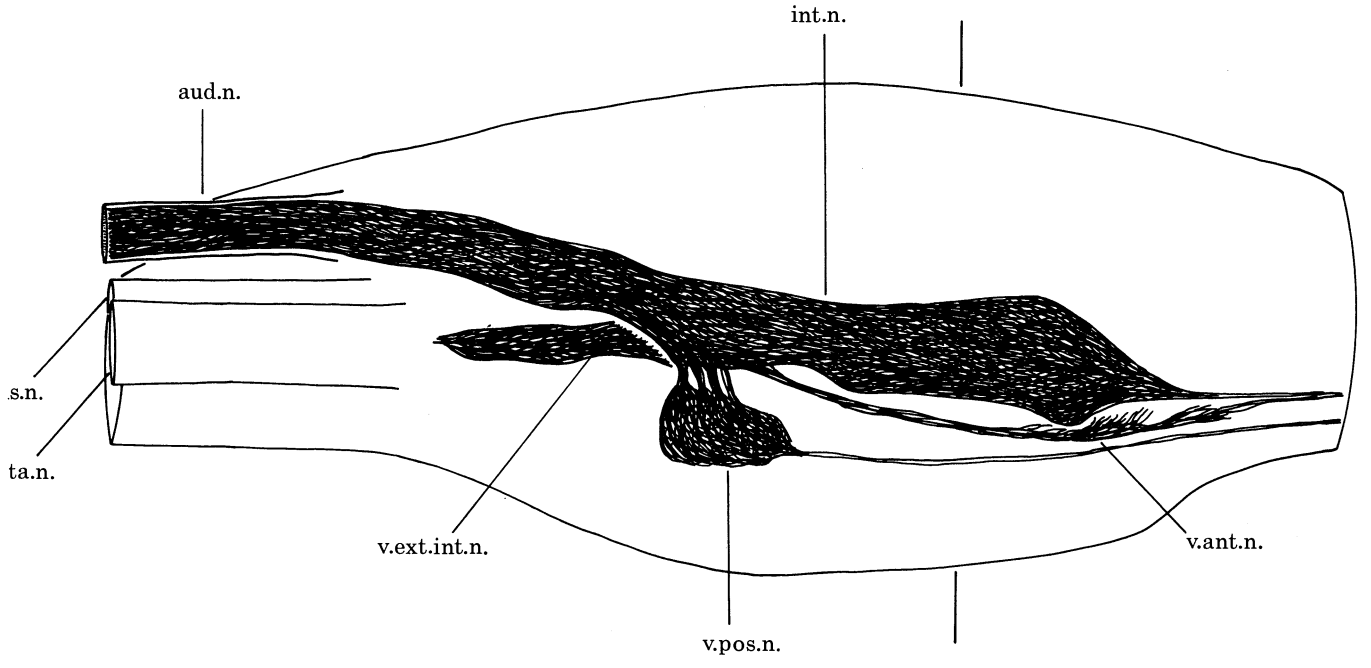


FIGURE 5. Lateral view of the projections of the auditory nerve within the thoracic ganglia reconstructed from 15 μm sections and cobalt fills. Lines indicate the level of the demarcation between the abdominal and metathoracic ganglia corresponding to the small hole in figure 23. Abbreviations: aud.n., auditory nerve; int.n., intermediate neuropil; meta.n., metathoracic nerve; tens.n., tensor nerve; v.ant.n., ventral anterior neuropil; v.ext.int.n., ventral extension of intermediate neuropil; v.pos.n., ventral posterior neuropil.

The third neuropil occurs ventrally to the other two neuropils and is situated in the fused abdominal ganglia (figure 5, ventral posterior neuropil). It does not appear to be metamerically organized, nor are all the fibres ipsilateral. Several may project across the midline.

A bundle of 20–30 fibres collected from the three neuropils projects anteriorly into the mesothoracic ganglion, where approximately half of the fibres terminate on the ipsilateral side. The remainder project forward into the prothoracic ganglion.

Discussion

(*a*) *Comparative anatomy of the auditory organ*

It is apparent that the auditory organ of *C. saundersii* is basically similar in structure and position to the auditory organs of *Cicadetta coriaria* and *Cicada orni* described by Vogel (1923) and Michel (1975).There are, however, some points of difference in the attachments and composition of the organs.

In the cicadas described previously, the region of proximal attachment of the scolopidia covers two-thirds of the tympanal apodeme. In *C. saundersii*, this region is condensed and the apodeme is attached to the sensory units along one-quarter of its length. Michel (1975) mentions that this region of attachment includes three cell types: the attachment cells, the epidermal cells and a layer of small cells underlying the epidermis. The latter was not observed in *C. saundersii*.

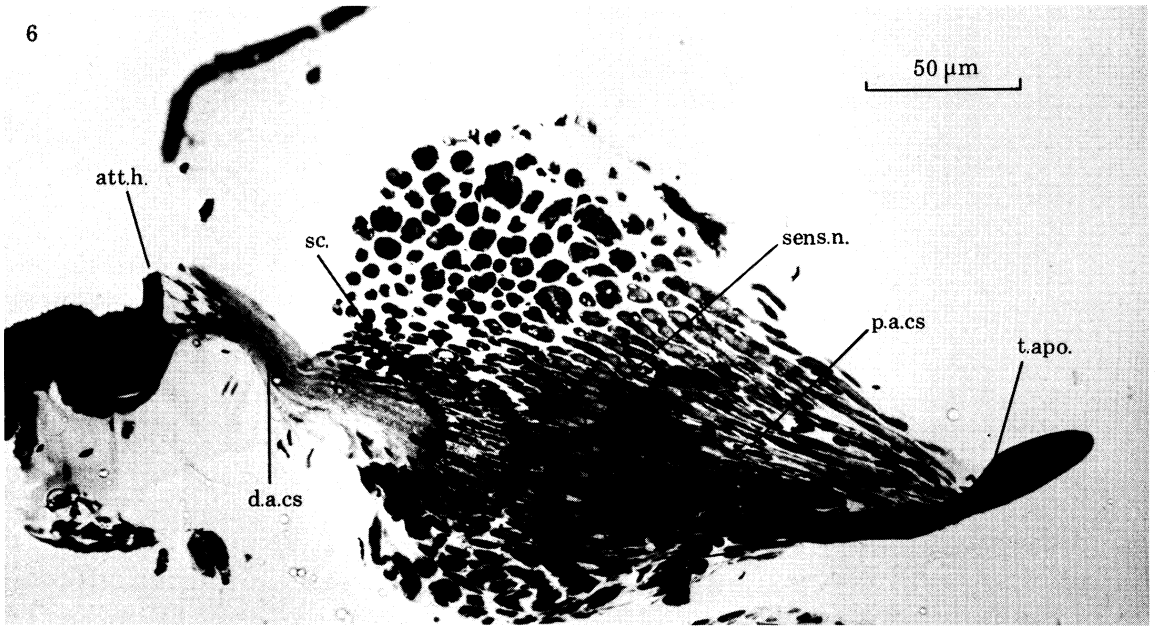
The auditory organ of *C. saundersii* is composed of 2000–2200 scolopidia, a larger estimate than any previously given. In *Cicadetta coriaria*, Vogel (1923) calculated a total of 1500 sensory units, while, in *Cicada orni*, Michel (1975) gives a total of 1300 units, although no indication was given of the methods used to arrive at these estimates. In both these organs, the scolopidia appear to be aligned only along the longitudinal axis of the organ. This is in contrast to *C. saundersii*, where the scolopidia occur in two groups based on their orientations and positions within the organ. We have observed a similar arrangement in two other species of cicada, *Cyclochila australasiae* and *Henicopsaltria eydouxi*, although the demarcation between the two groups is less clearly defined than in *C. saundersii*. It is unknown whether the scolopidia in the auditory organs of *Cicadetta coriaria* and *Cicada orni* occur in different orientations or whether they occur in one group, which may correspond to an enlarged dorsal group of *C. saundersii*. The possible significance of the different orientations of cells will be discussed in a later section.

(*b*) *Comparative fine structure of the scolopidia*

The scolopidia in the auditory organ of *C. saundersii* are similar in structure to those observed in most other chordotonal organs, but are distinctive in the structure of the cap, the mass and position of the ciliary dilatation, the length of the root and the distributions of microtubules and mitochondria.

The cap in *C. saundersii* resembles that described by Young (1973, 1975) in being a long, thin-walled structure that surrounds the cilium and is itself surrounded by the scolopale. This is unlike the scolopidia found in most other chordotonal organs, where the cap is a large, plug-like structure in which the scolopale rods terminate (Gray 1960; Ghiradella 1971; Friedman 1972; Moran *et al.* 1975; Slifer & Sekhon 1975).

The ciliary dilatation in *C. saundersii* possesses a large, amorphous mass in the centre of the doublets. This is situated just below the cap. In other chordotonal organs (Gray 1960; Slifer &



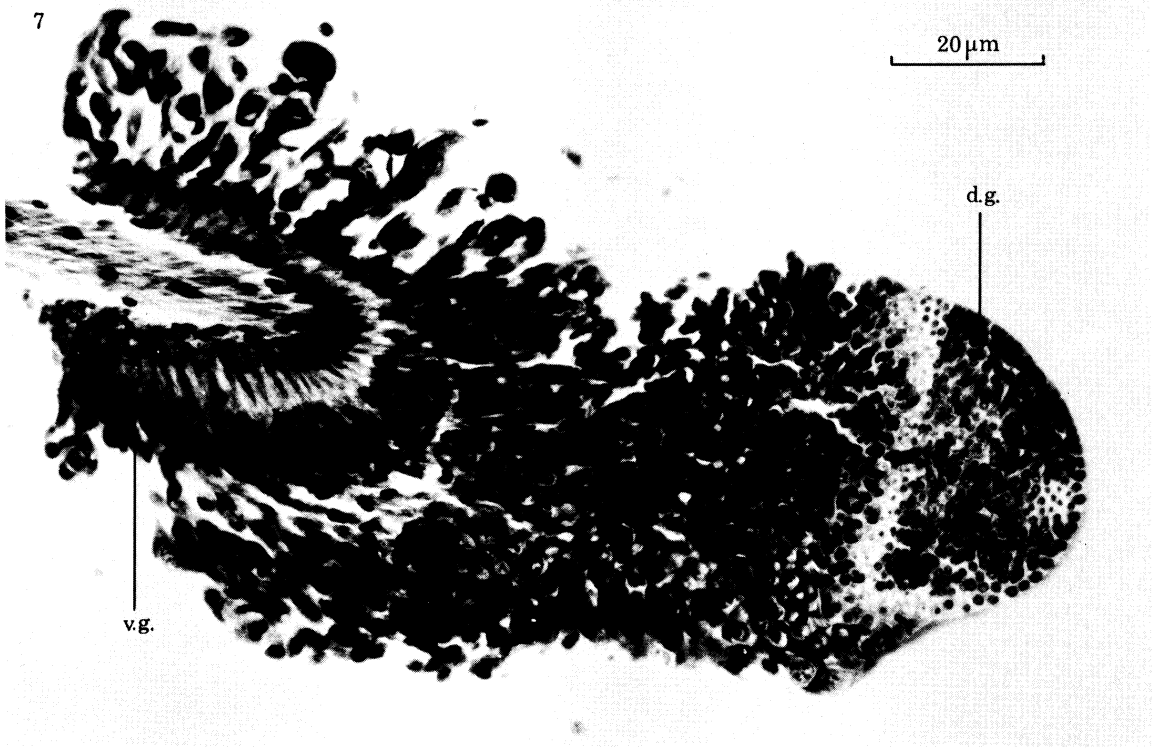


FIGURE 6. Longitudinal, horizontal section (1 μm) through the auditory organ, showing attachments to the cuticle. Abbreviations: att.h., attachment horn; d.a.cs, distal attachment cells; p.a.cs, proximal attachment cells; sc., scolopales; sens.n., sensory neurons; t.apo., tympanal apodeme.

FIGURE 7. Transverse section (15 μm) through the auditory organ showing the two groups of cells: dorsal group (d.g.) with scolopales cut transversely; ventral group (v.g.) with scolopales cut longitudinally and orientated inwards.

(*Facing p.* 536)

Description of plate 2

Figure 8. Longitudinal section through a scolopale. Abbreviations: ci., cilium; d.a.c., distal attachment cell; r., root; sc., scolopale; sc.c., scolopale cell.

Figure 9. Transverse section through the dendrite proximal to the dendrite dilatation. Arrow indicates the link between the root fibrils and microtubules. Abbreviations: r., root; sc.c., scolopale cell.

Figure 10. Transverse section through the cap. Arrow indicates cell contact composed of membrane thickening with an accumulation of microtubules. Note the hollow doublets in the cilium. Abbreviations: ca., cap; d., doublets; sc.r., scolopale rods.

Description of plate 3

Figure 11. Longitudinal section through a neuron cell body showing the root in the axon. Arrow indicates the association between the nucleus and the root. Abbreviations: nuc., nucleus of sensory neuron; p.a.c., proximal attachment cell; r., root.

Figure 12. Transverse section showing different levels through dendrites and scolopale: d.a.c., distal attachment cell; d.d., dendrite dilatation; sc.b., scolopale base with separate scolopale rods; sc.c., scolopale cell; sc.m., scolopale mid-region.

Description of plate 4

Figure 13. Longitudinal section through the distal end of the scolopale showing the cap (ca.); sc.r., scolopale rods.

Figure 14. Longitudinal section through the sensory neuron (sens.n.) showing the axon still surrounded by the proximal attachment cell (p.a.c.).

Figure 15. Transverse section showing four axons sheathed by individual Schwann cells, each sectioned through its nucleus (indicated by arrows).

Description of plate 5

Figure 16. Longitudinal section through the proximal attachment showing the interdigitation between the proximal attachment cells (p.a.c.) and the proximal epithelial cells (p.e.c.). Arrows indicate the fascia adherentes, which occur along most of the interdigitating surfaces.

Figure. 17. Transverse section through the proximal attachment, showing the cuticle (cu.), the proximal epithelial cells (p.e.c.) and proximal attachment cells (p.a.c.). Note the cuticular insertions into the epithelial cells and the fascia adherentes present between the two cellular layers.

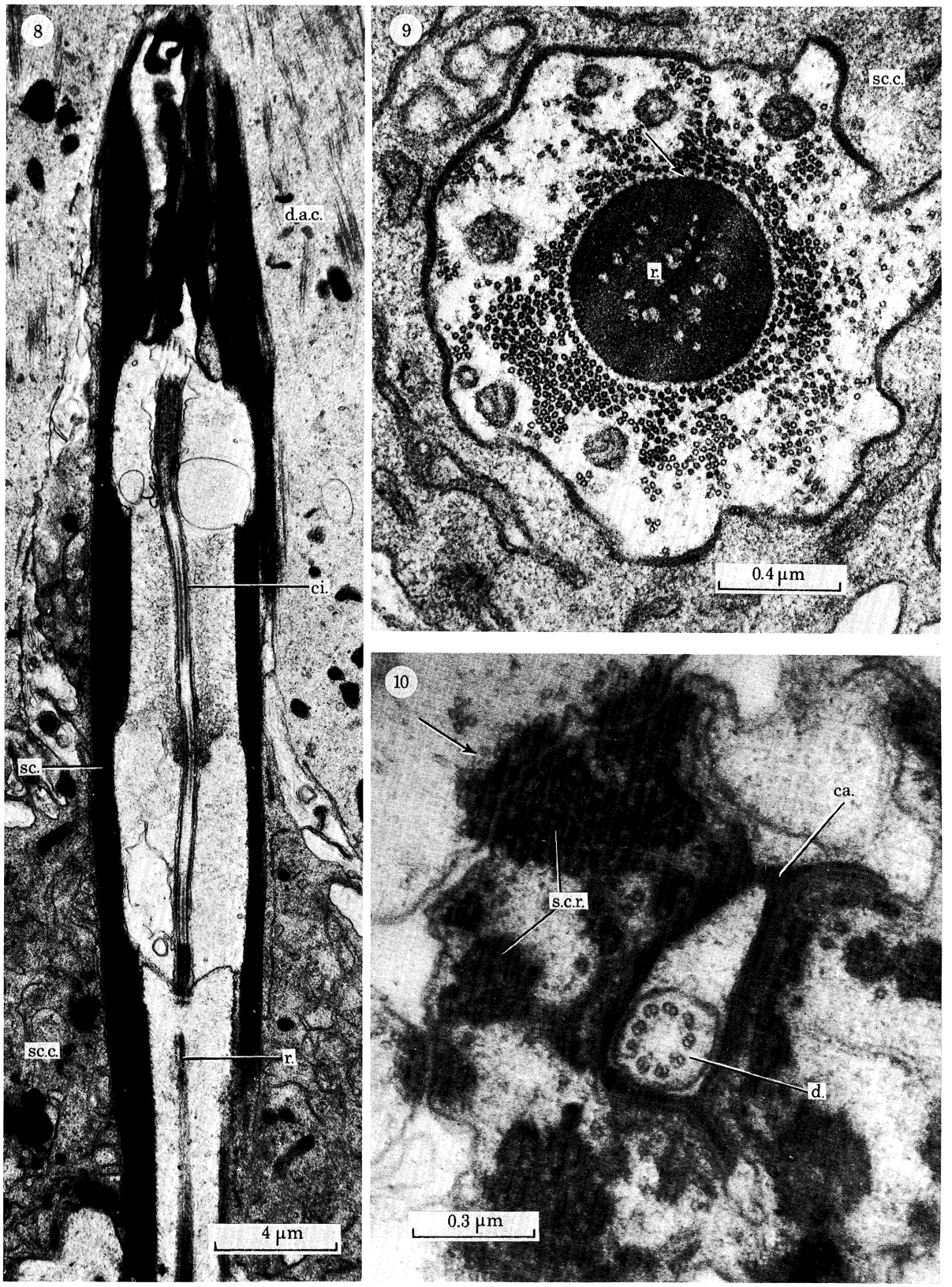
Description of plate 6

Figure 18. Longitudinal section through the distal attachment region, showing the interdigitation between the distal attachment cells (d.a.c.) and the distal epithelial cells (d.e.c.).

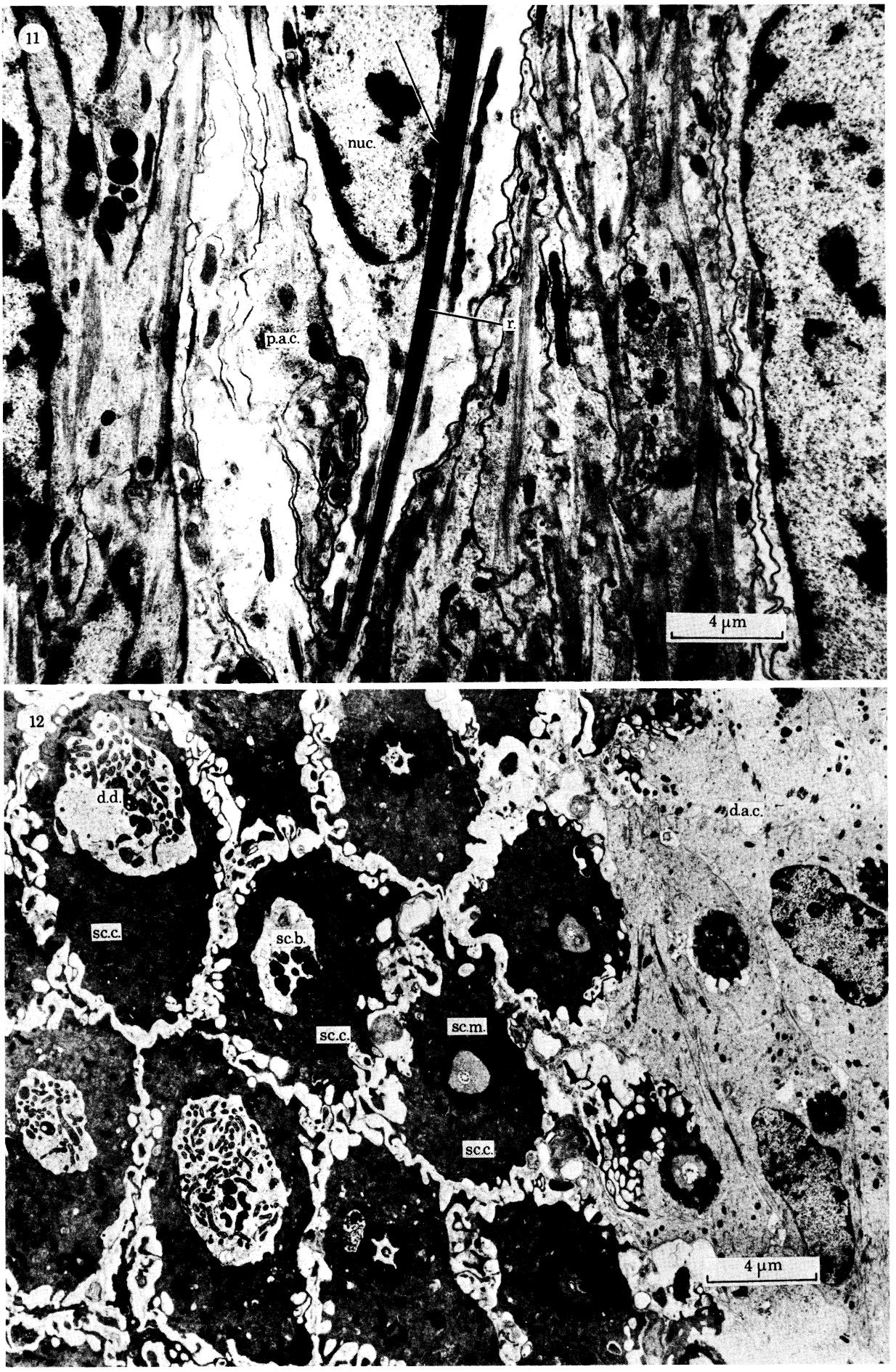
Figure 19. High power electronmicrograph of the fascia adherentes from the interdigitation between proximal attachment cell (p.a.c.) and proximal epidermal cell (p.e.c.). Note the thickened membranes and amorphous material filling the gap between the membranes.

Figure 20. Longitudinal section through the tonofibrillae anchoring the epithelial cells to the cuticle of the tympanal apodeme. Abbreviations: cu., cuticle; p.e.c., proximal epidermal cells.

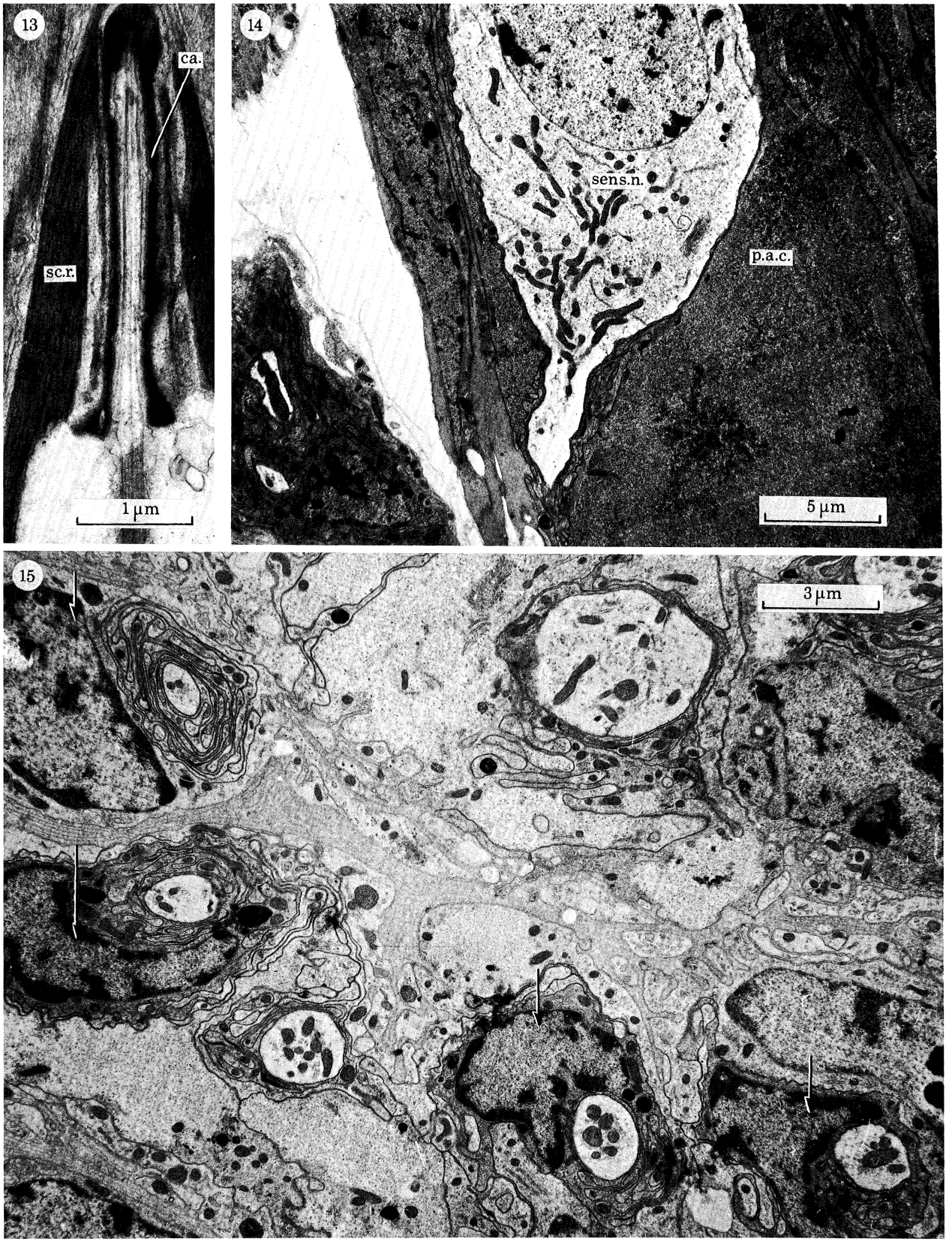
Figure 21. High power electronmicrograph of the tonofibrillae, showing the cuticular attachment fibre (c.u.f.) and the hemidesmosomes (hd.) on the epithelial cell membrane when in contact with the cuticular attachment fibres.



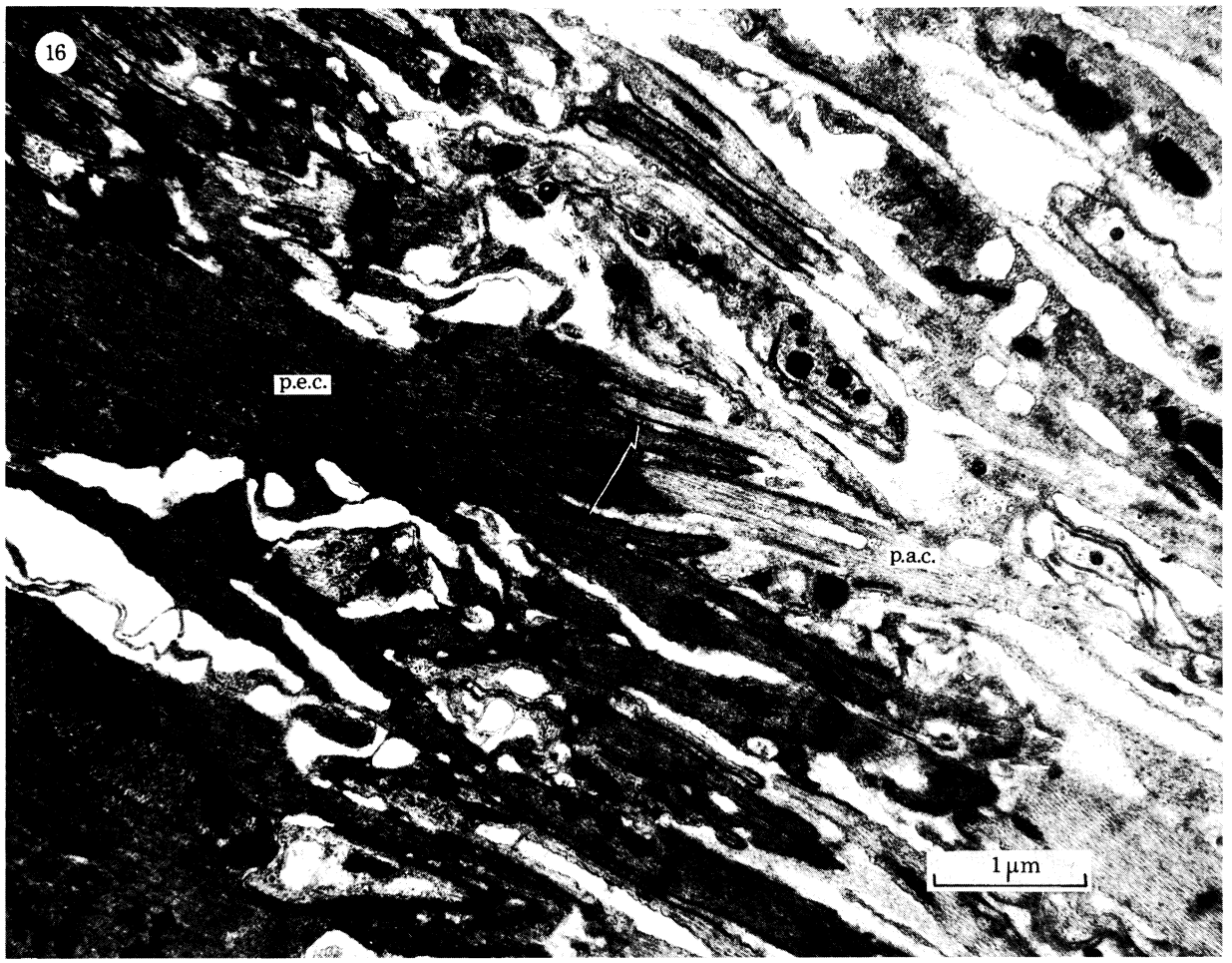
FIGURES 8–10. For description see opposite.



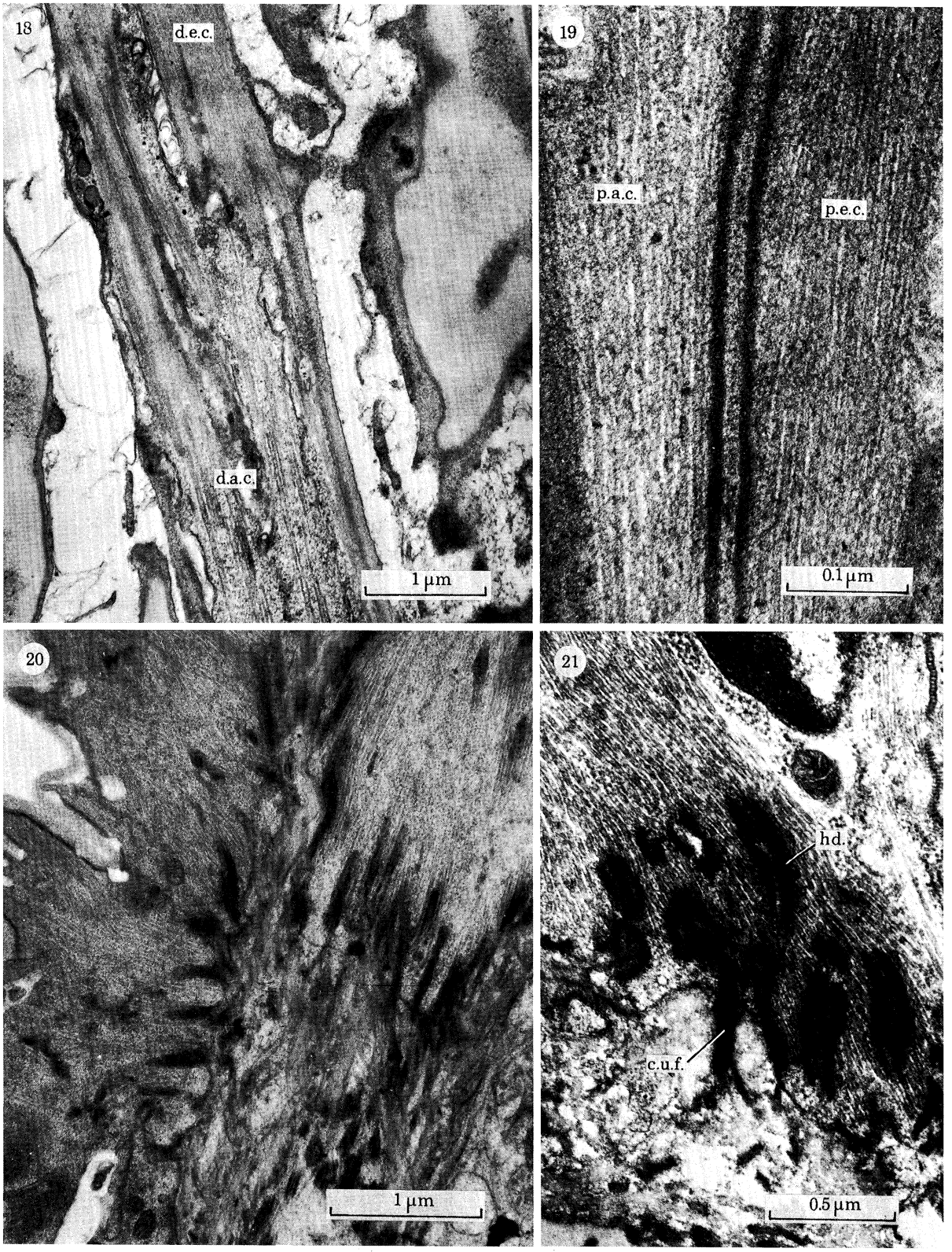
FIGURES 11 AND 12. For description see reverse of plate 1.



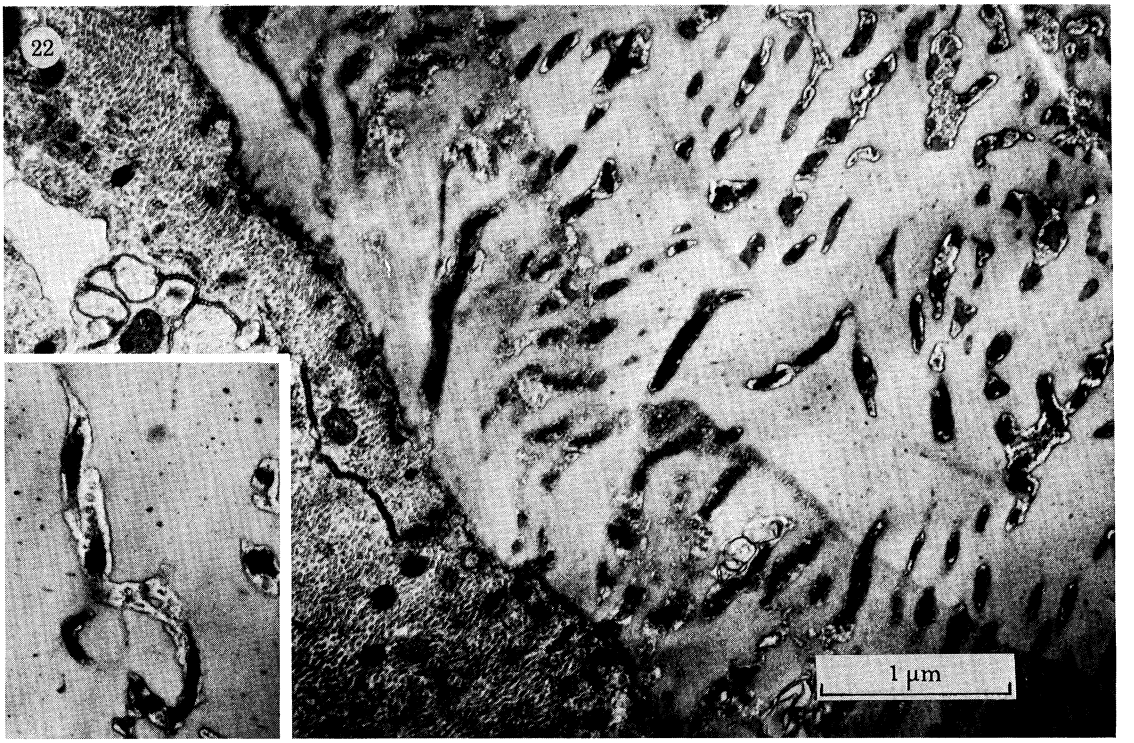
FIGURES 13–15. For description see reverse of plate 1.



FIGURES 16 AND 17. For description see reverse of plate 1.



FIGURES 18–21. For description see reverse of plate 1.



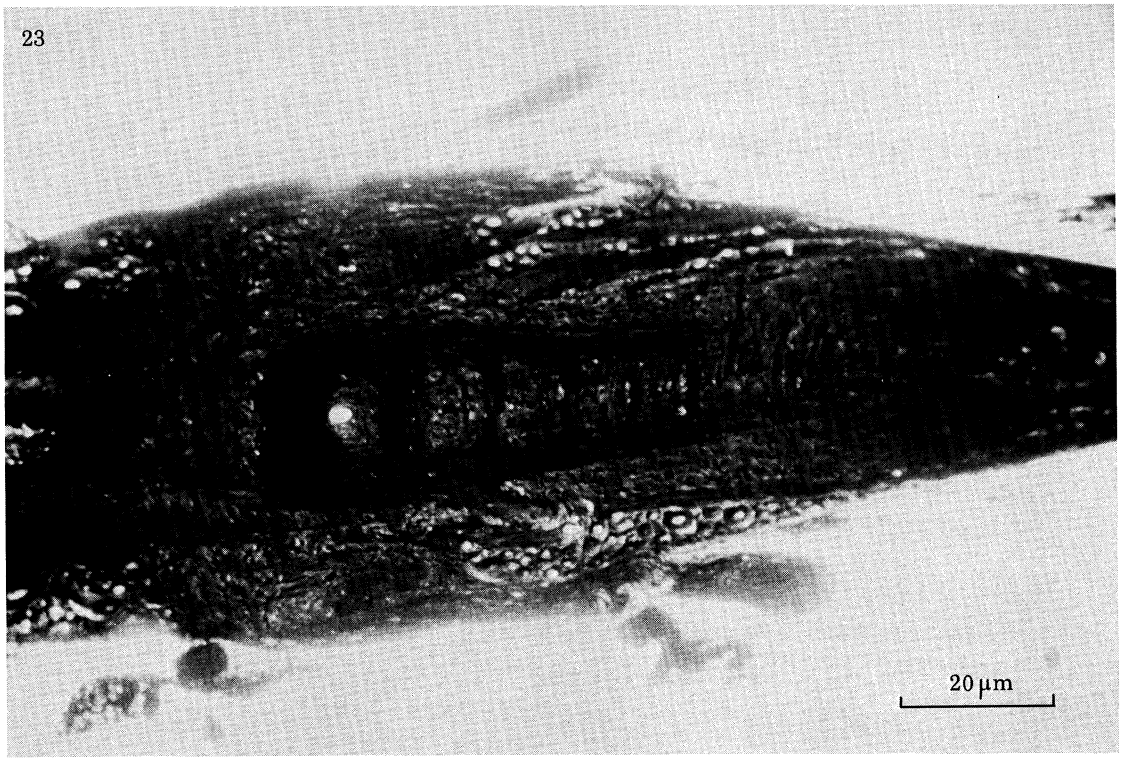


FIGURE 22. Transverse section through the tympanal apodeme, showing the pore canals containing cuticular attachment fibres. Inset shows a longitudinal section through a pore canal containing a twisted cuticular attachment fibre (same magnification).

FIGURE 23. Longitudinal horizontal section (15 μm silver-stained) through the thoracic ganglia, showing the intermediate neuropil with segmentally organized extensions. The small hole represents the demarcation between the metathoracic ganglion and the fused abdominal ganglia.

Sekhon 1975), the mass is reduced and the position of the dilatation varies, in some cases, being found approximately halfway along the cilium.

If current theories of sensory transduction are correct, then it is obvious that differences in the mass of the cap and ciliary dilatation and the position of the ciliary dilatation could become important in defining the necessary stimulus required to activate a chordotonal organ. Howse (1968), Varela *et al.* (1977) and Moran *et al.* (1975, 1977) have suggested that an oblique flexion of the cap is needed to generate an action potential by displacing the tip of the cilium. This is likely to initiate sliding among the doublets of the cilium, which forms a bend in its base and distorts the dendritic membrane. Active sliding in motile cilia requires the movement of the dynein arms of one doublet against the adjacent doublet (Warner & Satir 1974). Therefore, any movement of the cap must also displace the ciliary dilatation to activate sliding since the dynein arms of the doublets are absent above this point.

Within the dendrite, the most obvious feature is the root. In *C. saundersii*, it is of a length comparable to those described by Debaiseaux (1936) and is sometimes associated with other cellular organelles. These associations have been noted in other studies (Olsson 1962; Perry 1968; Friedman 1972; Von Boletsky 1973), although their significance remains obscure. The dendrite itself dilates in an area proximal to the scolopale and contains an increased number of mitochondria. This area may be responsible for the restoration of the resting potential after the distortion of the dendritic membrane, since Atema (1973) suggested that this process would require a large amount of metabolic energy.

The attachments of the scolopidia to the cuticle were found to be similar to the muscle attachments observed in insects (Lai-Fook 1967; Caveney 1969), crustaceans (Bouligand 1962, 1966; Atwood *et al.* 1973; Jahromi & Atwood 1976, 1977*a*, *b*) and arachnids (Smith *et al.* 1969). These similarities are understandable since both muscles and chordotonal organs are under considerable stress and require mechanisms that increase the tensile strength of cells and strengthen cell-to-cell contacts. These mechanisms include the interdigitation of the attachment cells and the epidermal cells and the presence of fascia adherentes between the two, and the tonofibrillae, which anchor the epidermal cells to the cuticle. The tonofibrillae, which consist of the cuticular attachment fibre, the hemidesmosomes and the attached microtubules, appear to have no direct analogue in vertebrates. There is a similarity between vertebrates and invertebrates in the form of the hemidesmosome, which consists of a dense intracellular plaque underlying the membrane. In vertebrates, tonofilaments converge towards the plaque: the tonofilaments are intermediate filaments with diameters of 10 nm, which are thought to have a cytoskeletal function in maintaining cell shape under stress (Lazarides 1980). In the invertebrate situation, these intermediate filaments are absent and are replaced by microtubules with diameters between 21 and 23 nm, which join the hemidesmosomes to the fascia adherentes. These are thought to have the same function as the intermediate filaments in vertebrates in maintaining the mechanical integrity of the cell under stress and tension.

(*c*) *The significance of the orientations of the scolopidia*

Generally, when two or more orientations of scolopidia are observed within an auditory organ, they have been correlated with frequency discrimination after the work of Michelson (1971), who found that the maximum sensitivity of each orientation of cells occurred at the frequencies at which their attachment points to the tympanum vibrated. However, in *C. saundersii*, both orientations of cells attach to the tympanal apodeme and thence to the tympanal

cuticle. Therefore frequency discrimination based on differential vibrations of the tympanum does not seem possible and is not apparent in the tuning curve, which shows a maximum sensitivity at 850 Hz only (Young & Hill 1977). Whether this result is composed of the additive responses of single cells with different scolopale lengths is unknown. However, although frequency discrimination does not appear likely between the two groups, a differential response to intensity could occur with this arrangement. As previously mentioned, the adequate stimulus in chordotonal organs is thought to be the oblique flexion of the cap. This might occur through the flexible joint that is seen between the tympanal ridge and apodeme (Young & Hill 1977). A similar joint is seen in *Cicada orni* (Michel 1975). These structures would transmit a vibration of the tympanum to the auditory organ as an oblique movement, which would tend to displace the caps of the scolopidia orientated longitudinally within the organ. Thus, the scolopidia of the dorsal group would be stimulated readily by a vibration of the tympanum. However, those of the ventral group with their scolopales directed inward, i.e. at right angles to the stimulus, would require a larger vibration of the tympanum to produce the same degree of flexion and would tend to respond only to higher intensities.

(*d*) *The central projections of the auditory nerve*

The central projections of the auditory nerve of *C. saundersii* resemble closely those described by Wohlers *et al.* (1979). There are, however, slight differences. In *Magicicada* spp., only two neuropils appeared to be present, the ventral posterior neuropil, which in this case was metamerically organized, and the intermediate neuropil, which consisted of three anterior extensions and six posterior extensions (Wohlers *et al* 1979). The small, knob-like extension that occurred most anteriorly in the projections described by Wohlers *et al.* (1979) is absent in *C. saundersii.*

The intermediate neuropil is thought to contain the auditory and detensor chordotonal fibres since it is composed of a large number of fibres and shows a high degree of sensory arborization. It occurs in an intermediate situation within the ganglia, in a similar position to the auditory neuropil of orthopterans (Rehbein 1973; Eibl 1976; Eibl & Huber 1979), tettigonids (Kalmring *et al.* 1978) and acridids (Rehbein *et al.* 1974).

Wohlers *et al.* (1979) suggested that their single ventral neuropil was composed of fibres from the body hairs, on the basis of studies by Honegger (1977) and Hustert (1978), which showed that sensory cells from the head and body hairs projected into ventral neuropil. In *C. saundersii* there is an additional anterior ventral neuropil.

It is possible that the two groups of sensory cells in the auditory organ may have different projections within the ganglia. These may account for the differences observed between this study and that of Wohlers *et al.* (1979), although the composition of the ears of the *Magicicada* spp. is unknown.

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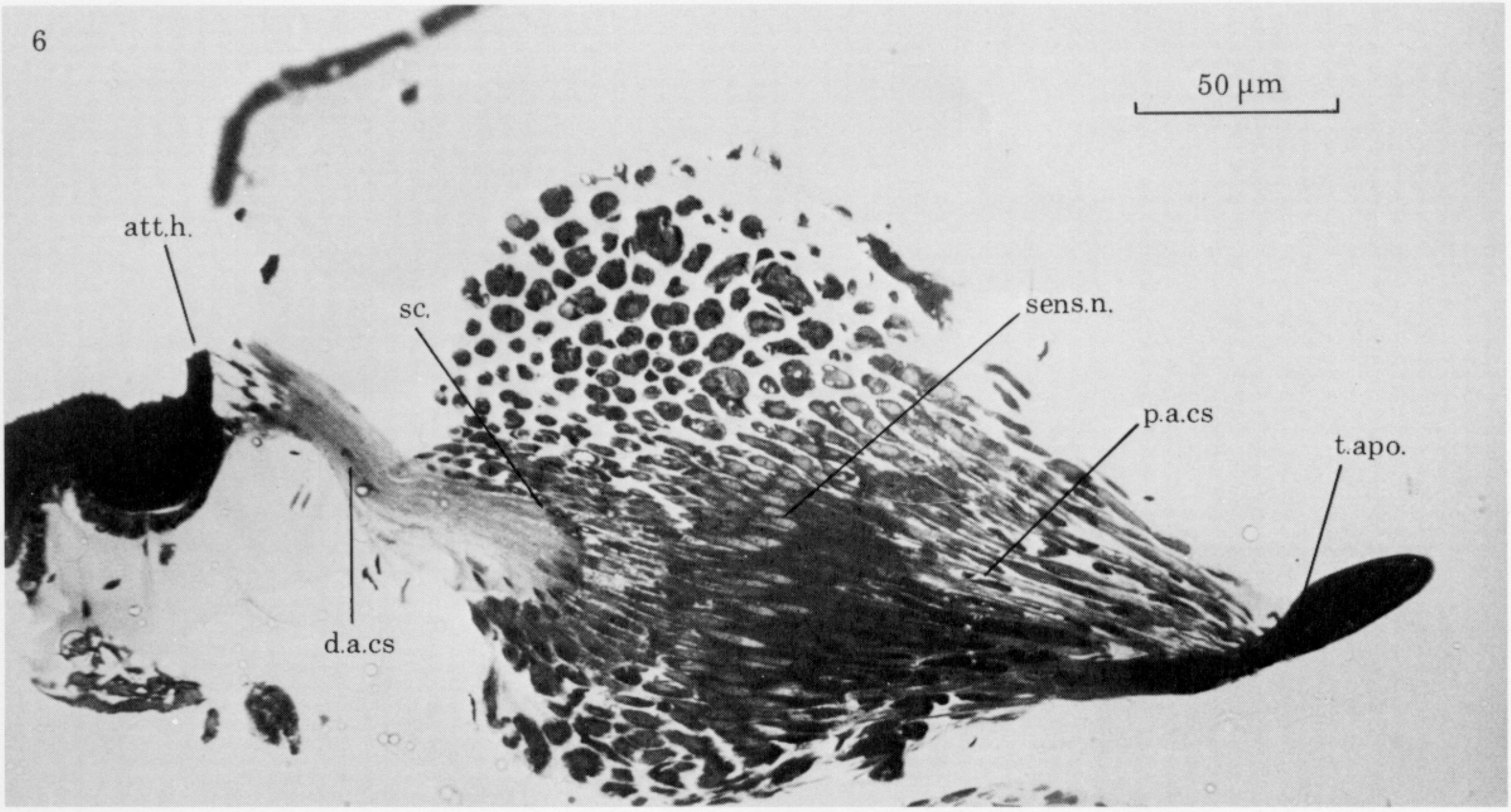


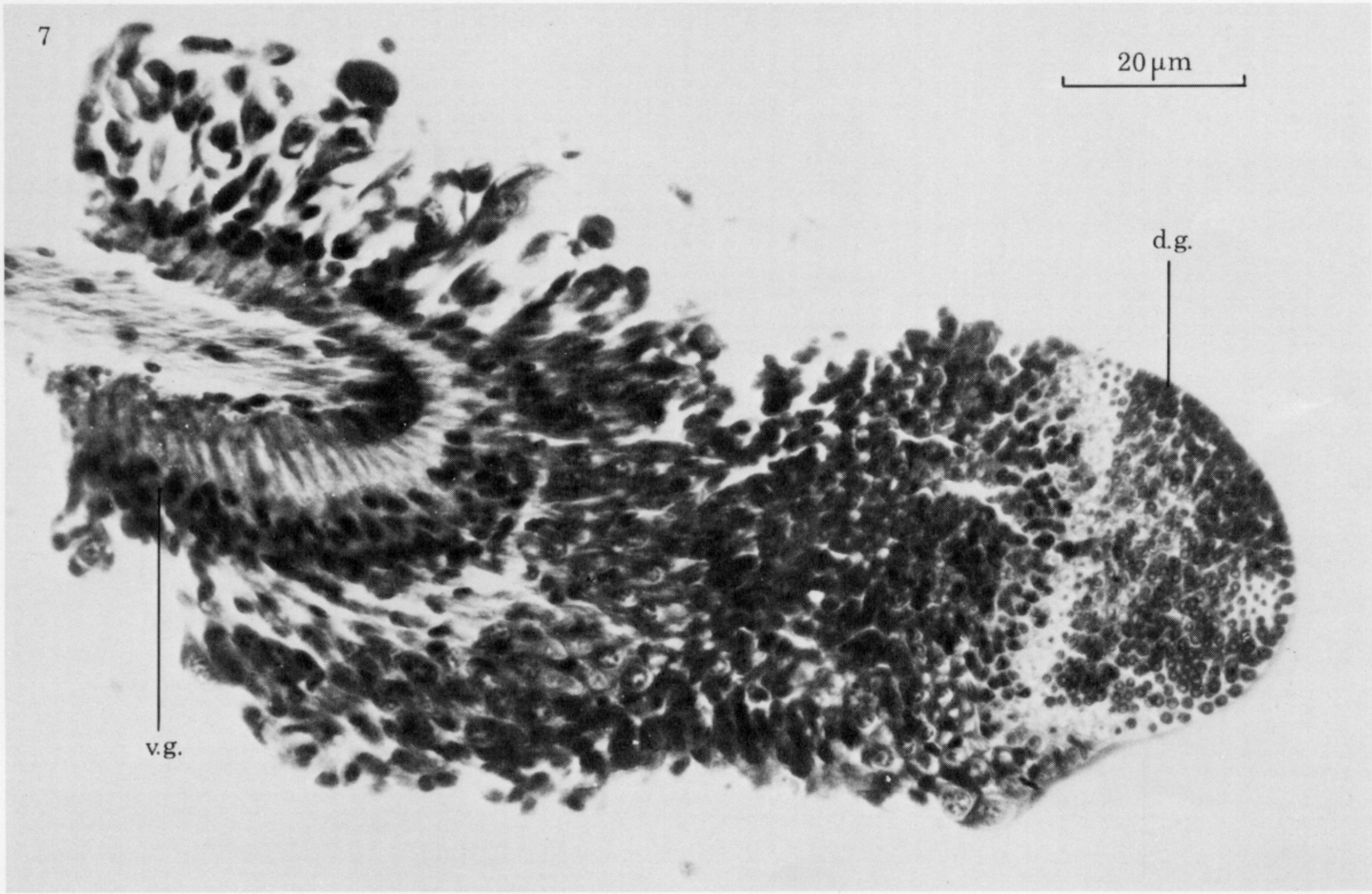
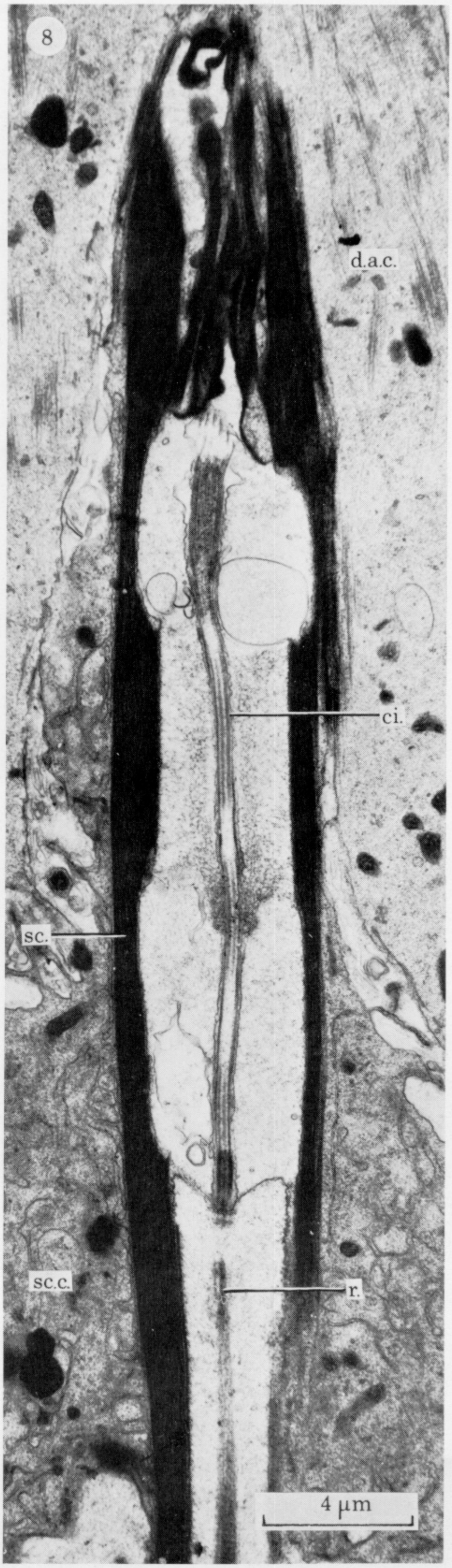
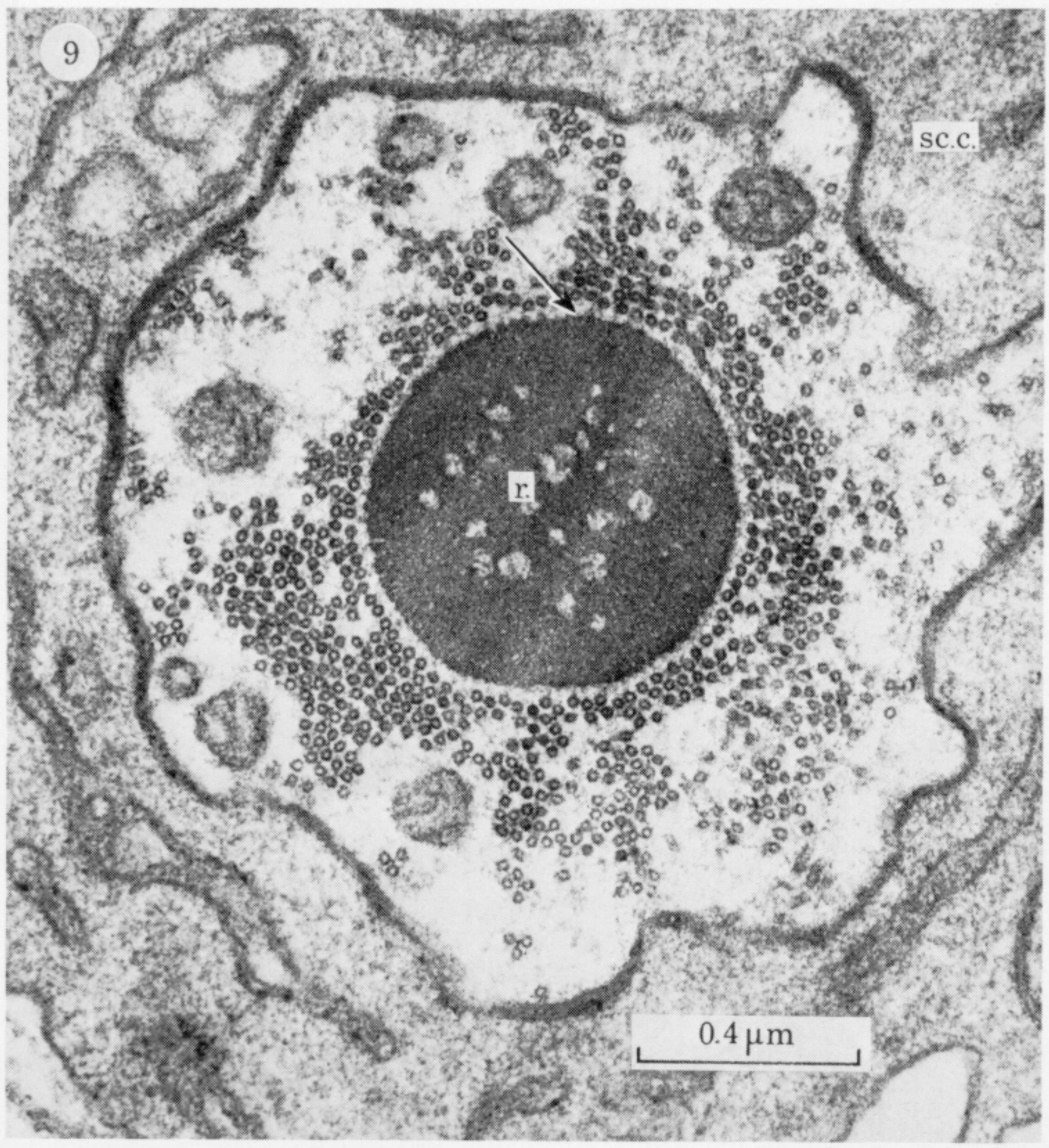
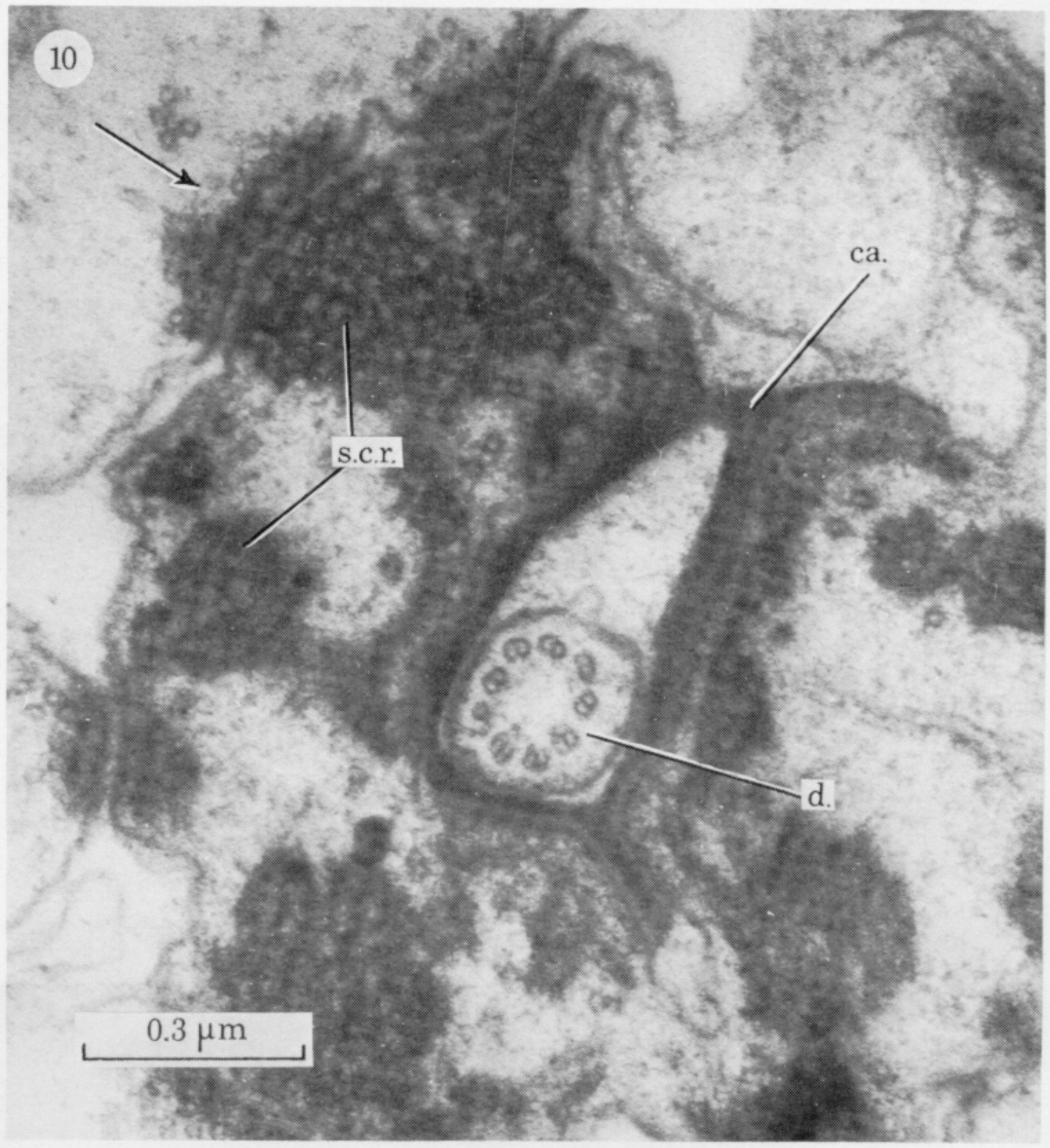


FIGURE 6. Longitudinal, horizontal section (1 µm) through the auditory organ, showing attachments to the cuticle. Abbreviations: att.h., attachment horn; d.a.cs, distal attachment cells; p.a.cs, proximal attachment cells; sc., scolopales; sens.n., sensory neurons; t.apo., tympanal apodeme.

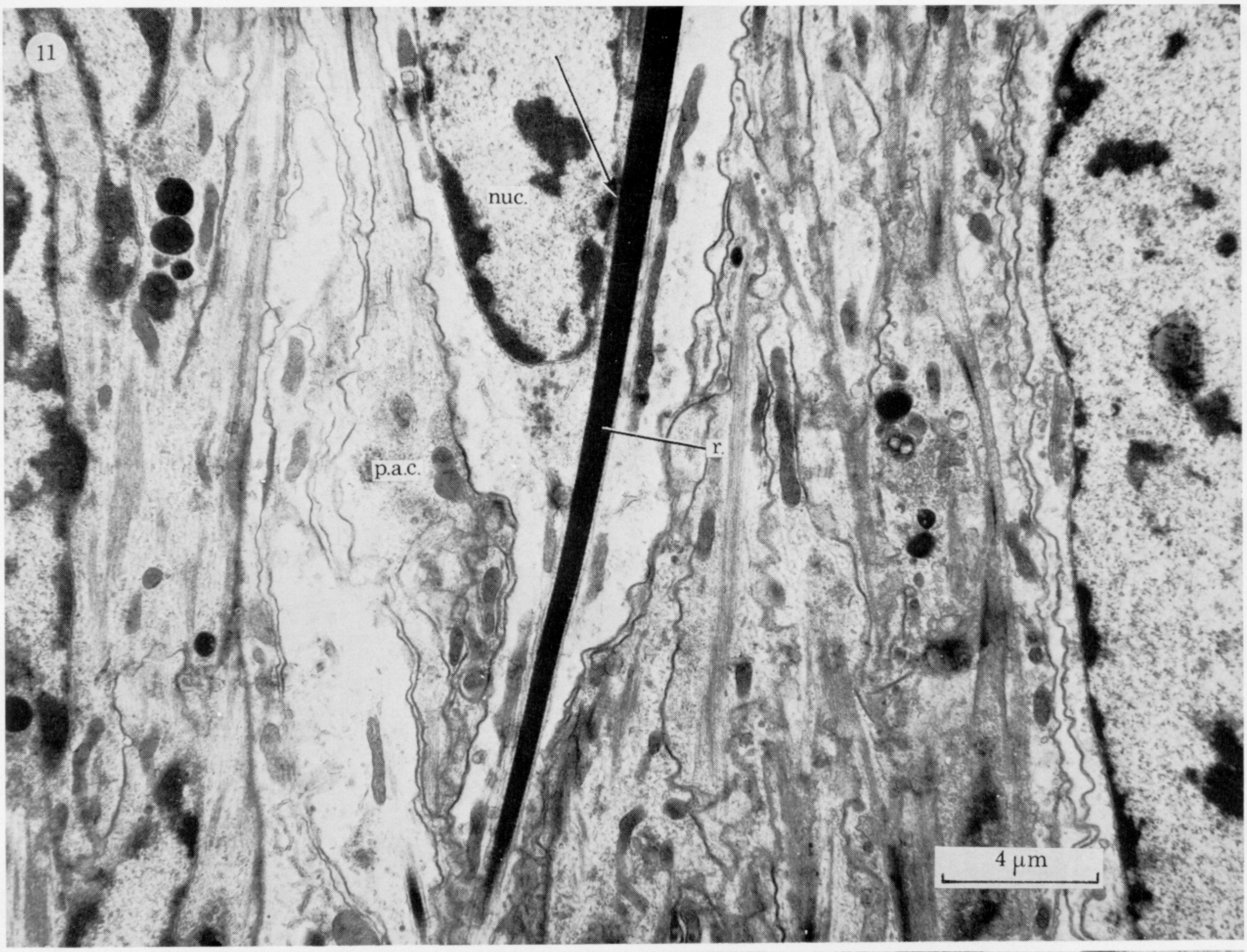
FIGURE 7. Transverse section (15 µm) through the auditory organ showing the two groups of cells: dorsal group (d.g.) with scolopales cut transversely; ventral group (v.g.) with scolopales cut longitudinally and orientated inwards.

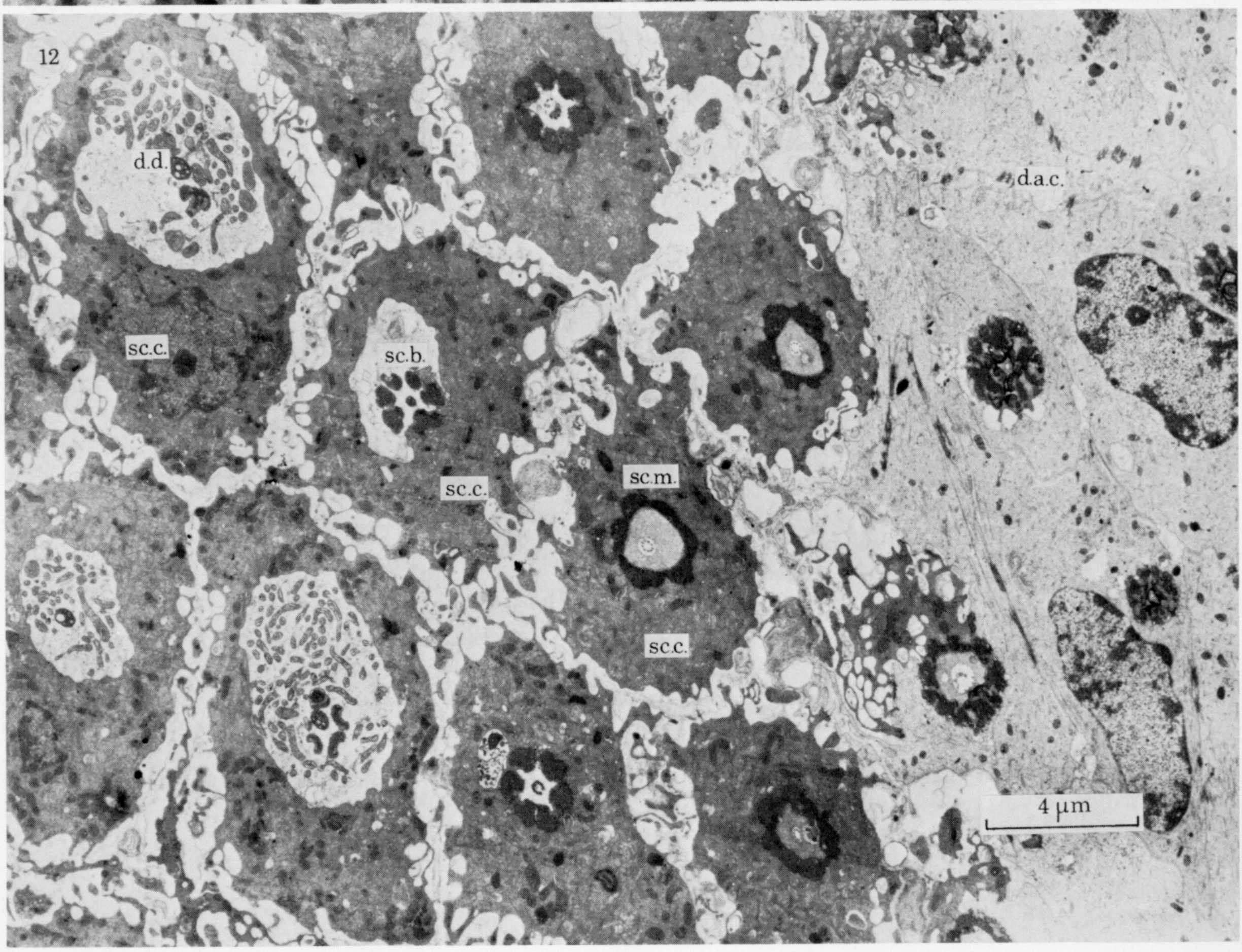




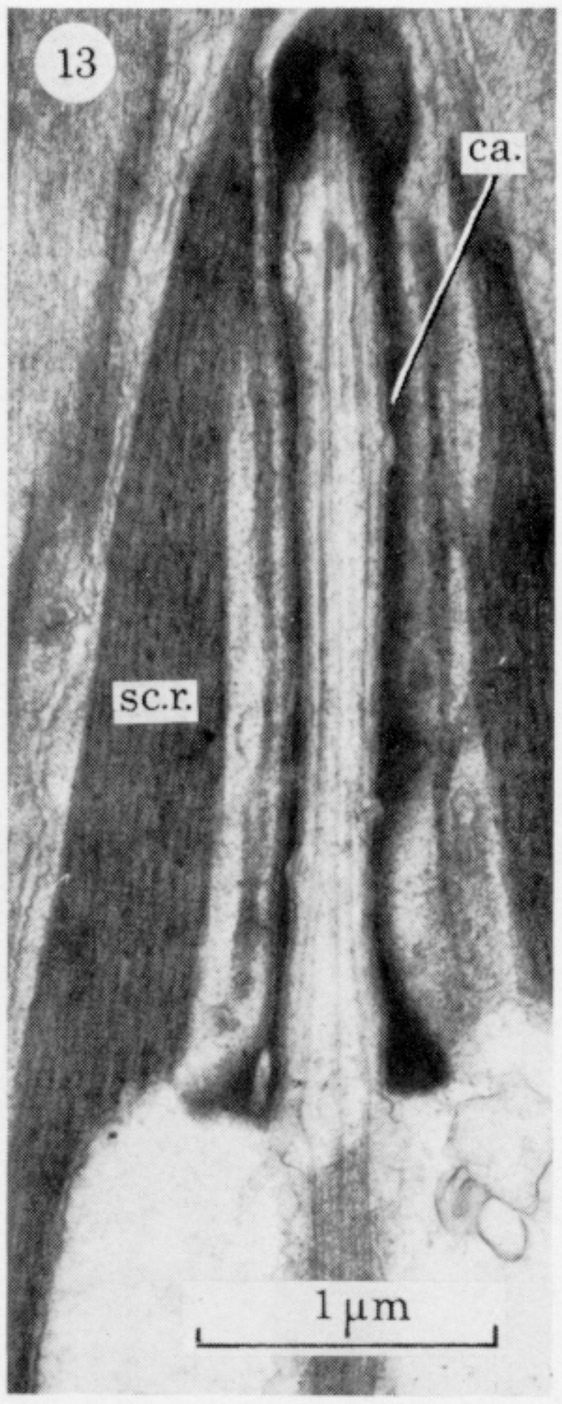


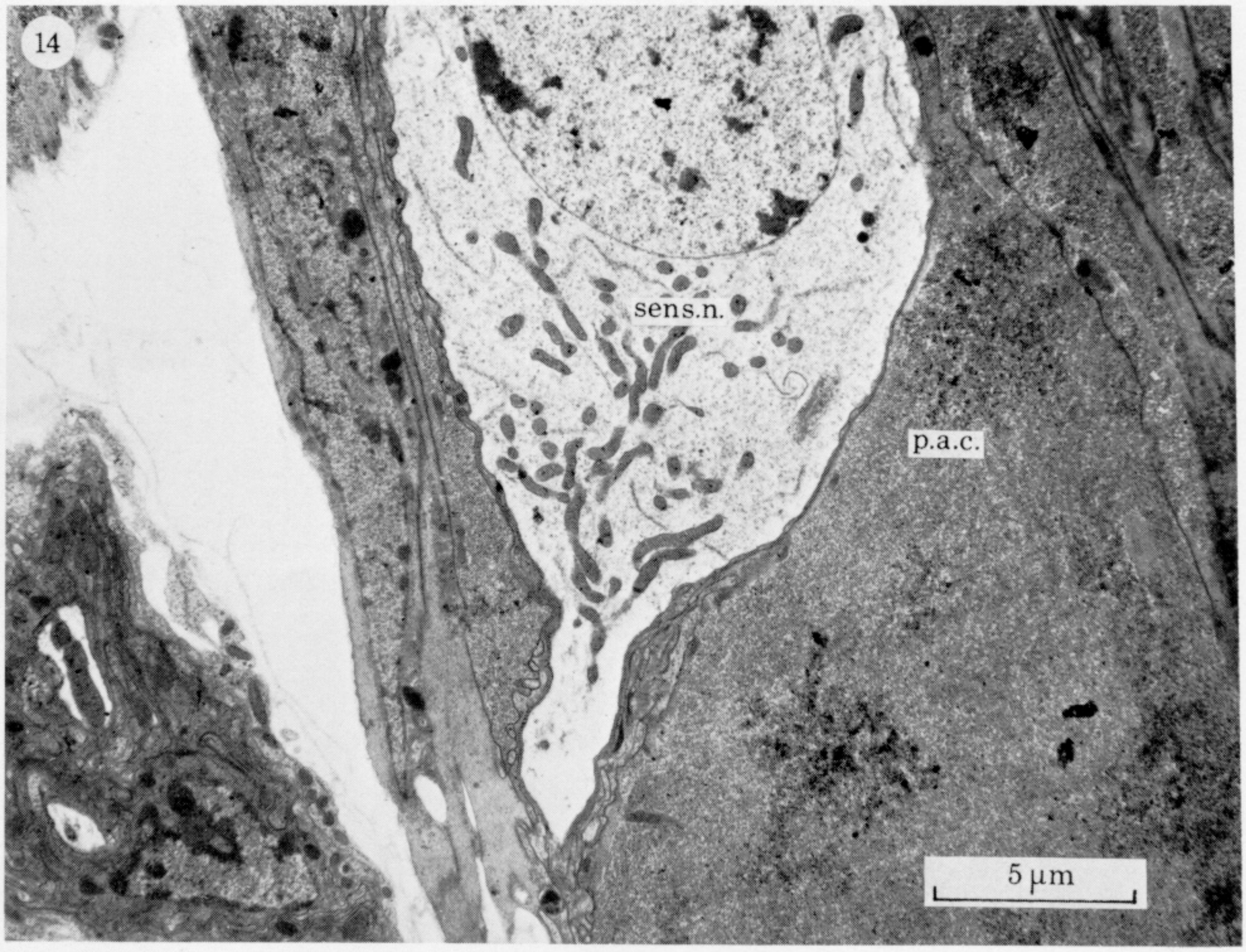
FIGURES 8–10. For description see opposite.

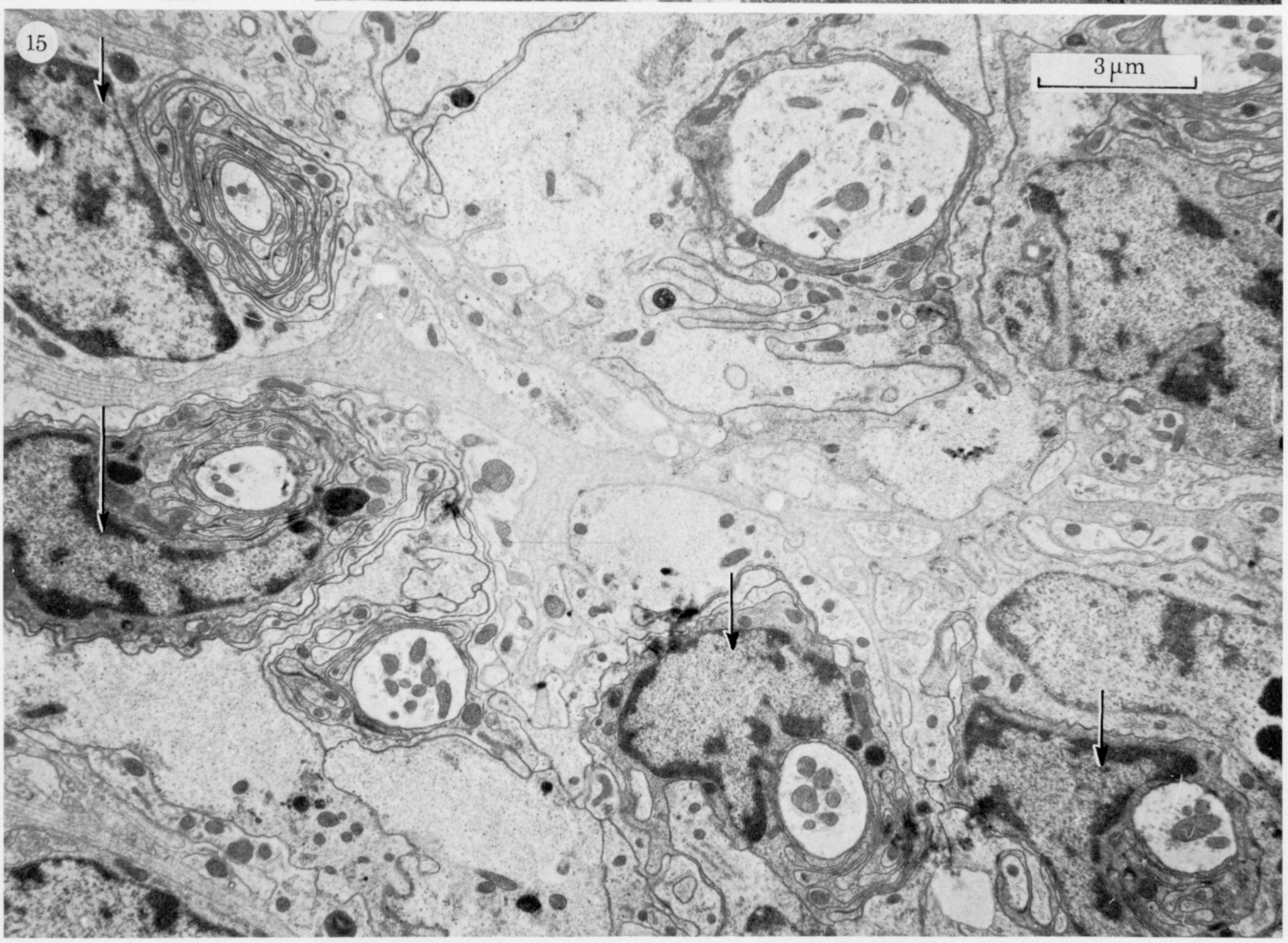




FIGURES 11 AND 12. For description see reverse of plate 1.







FIGURES 13–15. For description see reverse of plate 1.

16

p.e.c.

p.a.c.

1 μm

17

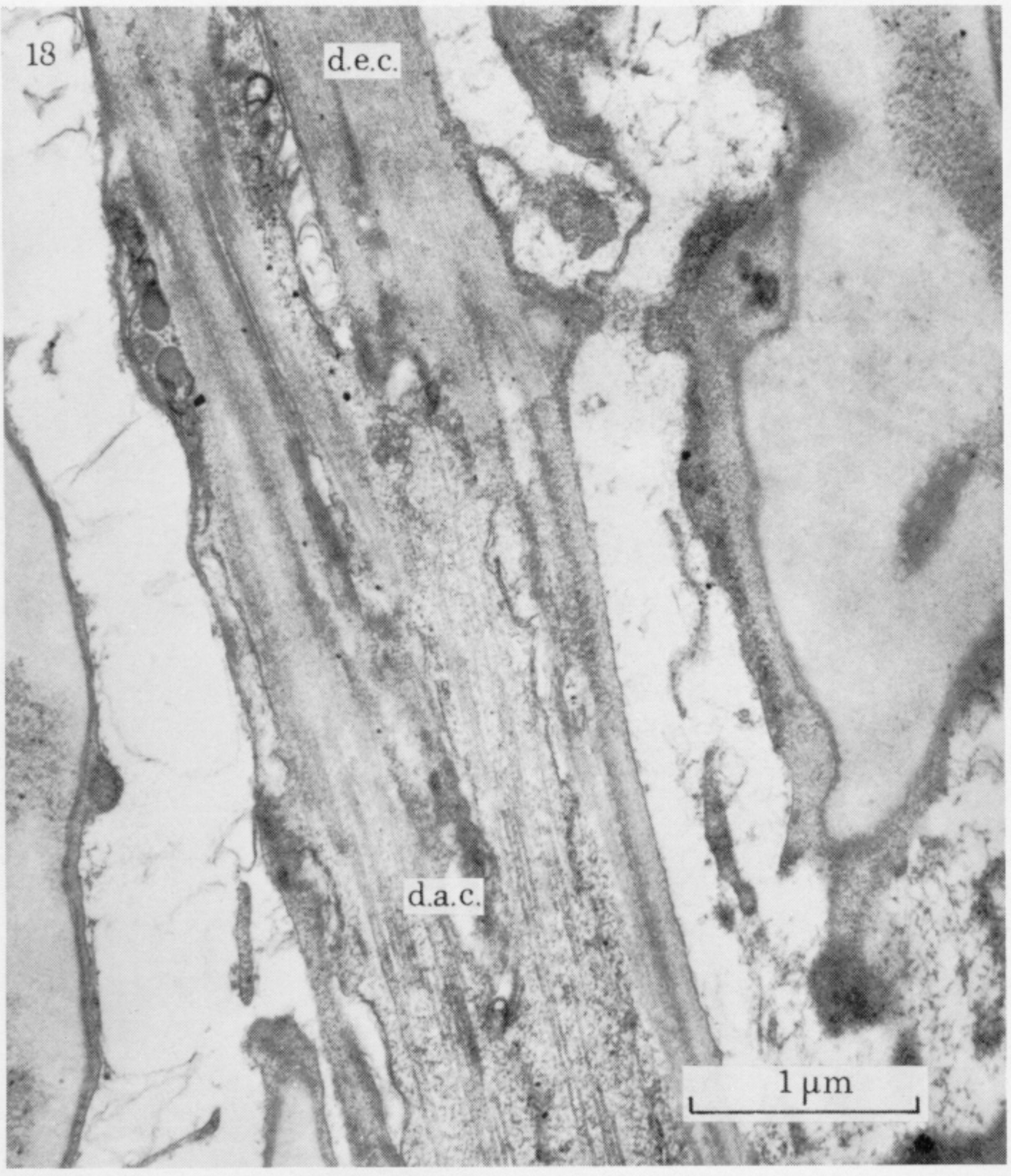
2.5 μm

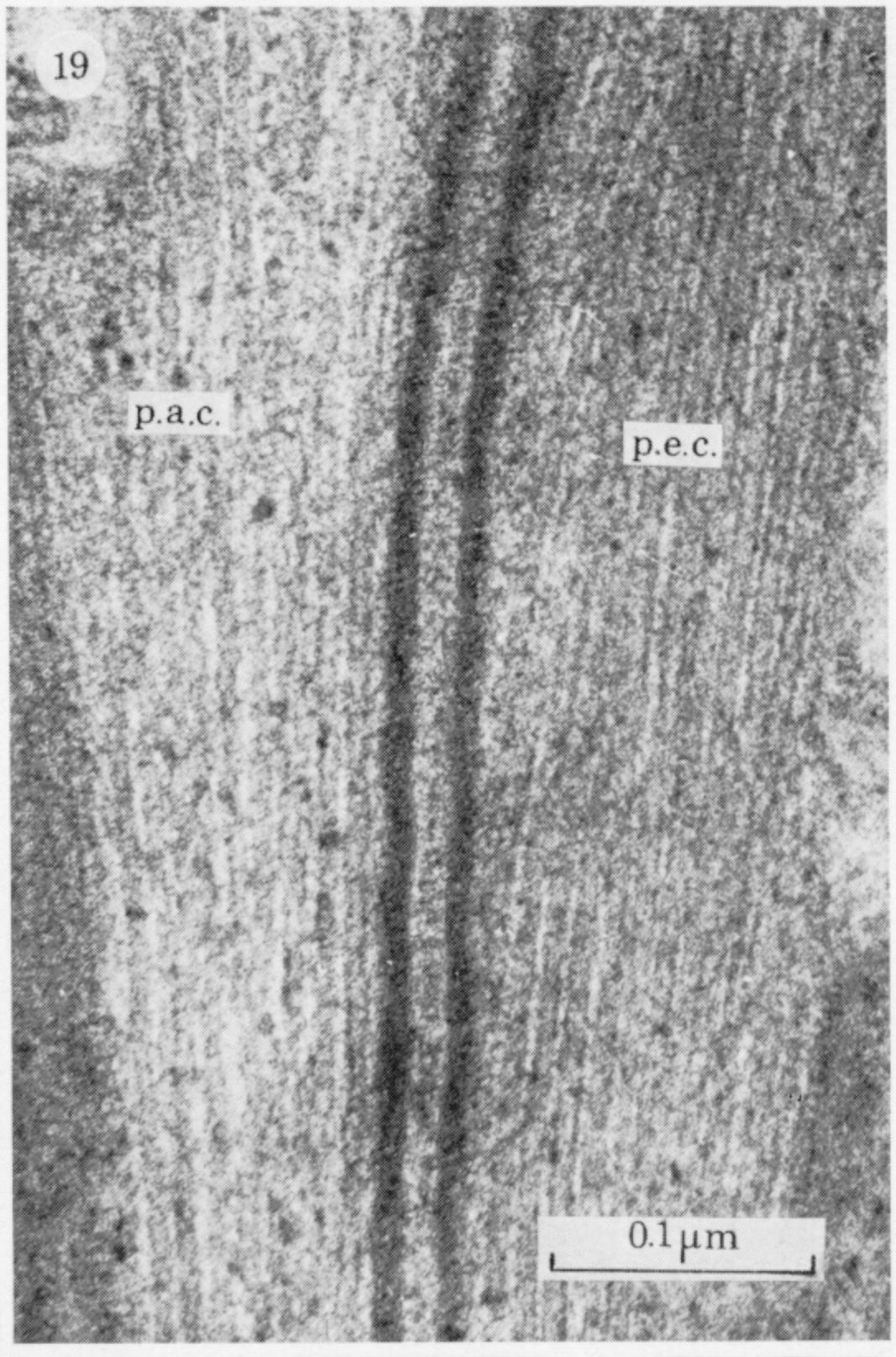
cu.

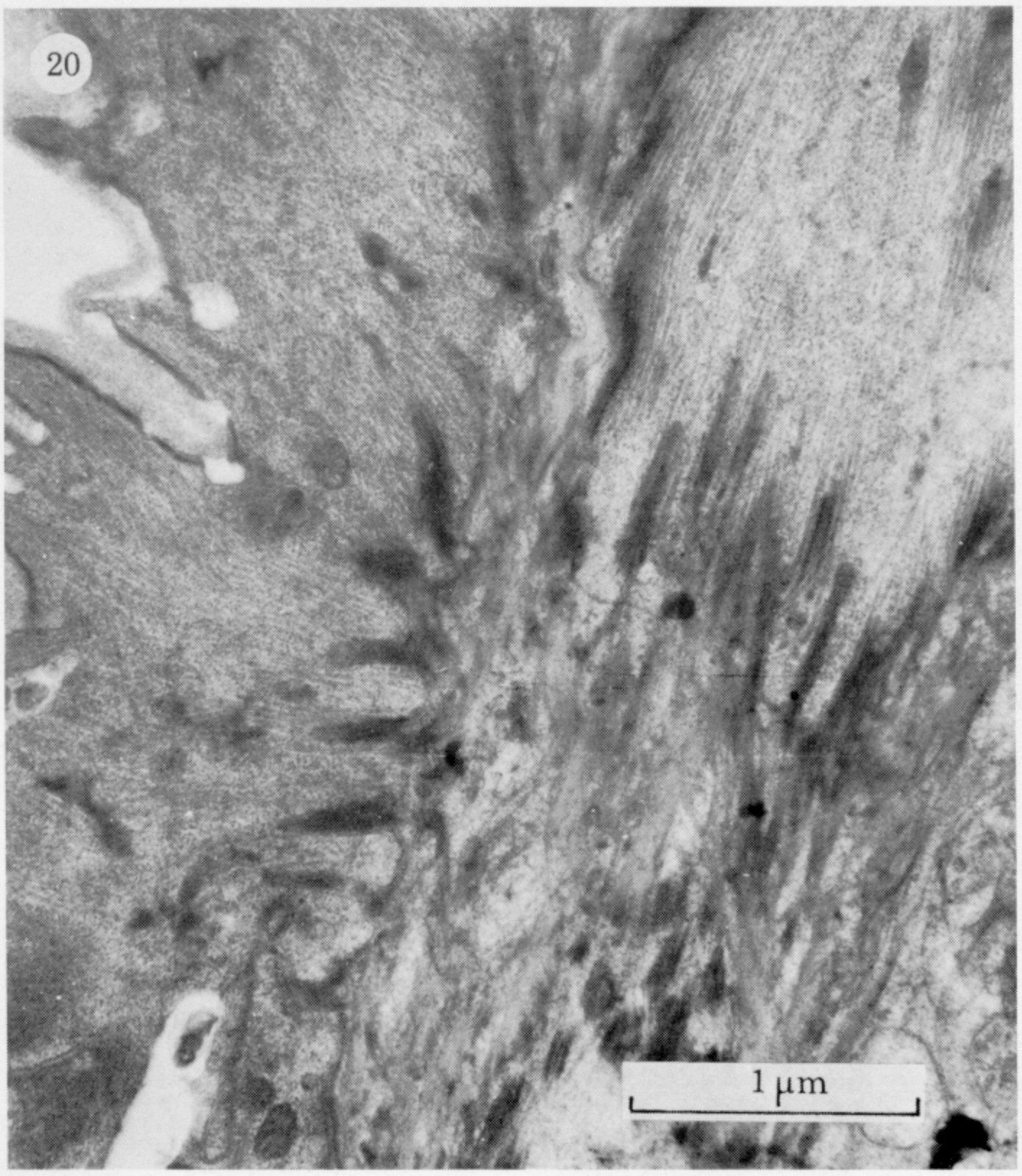
p.e.c.

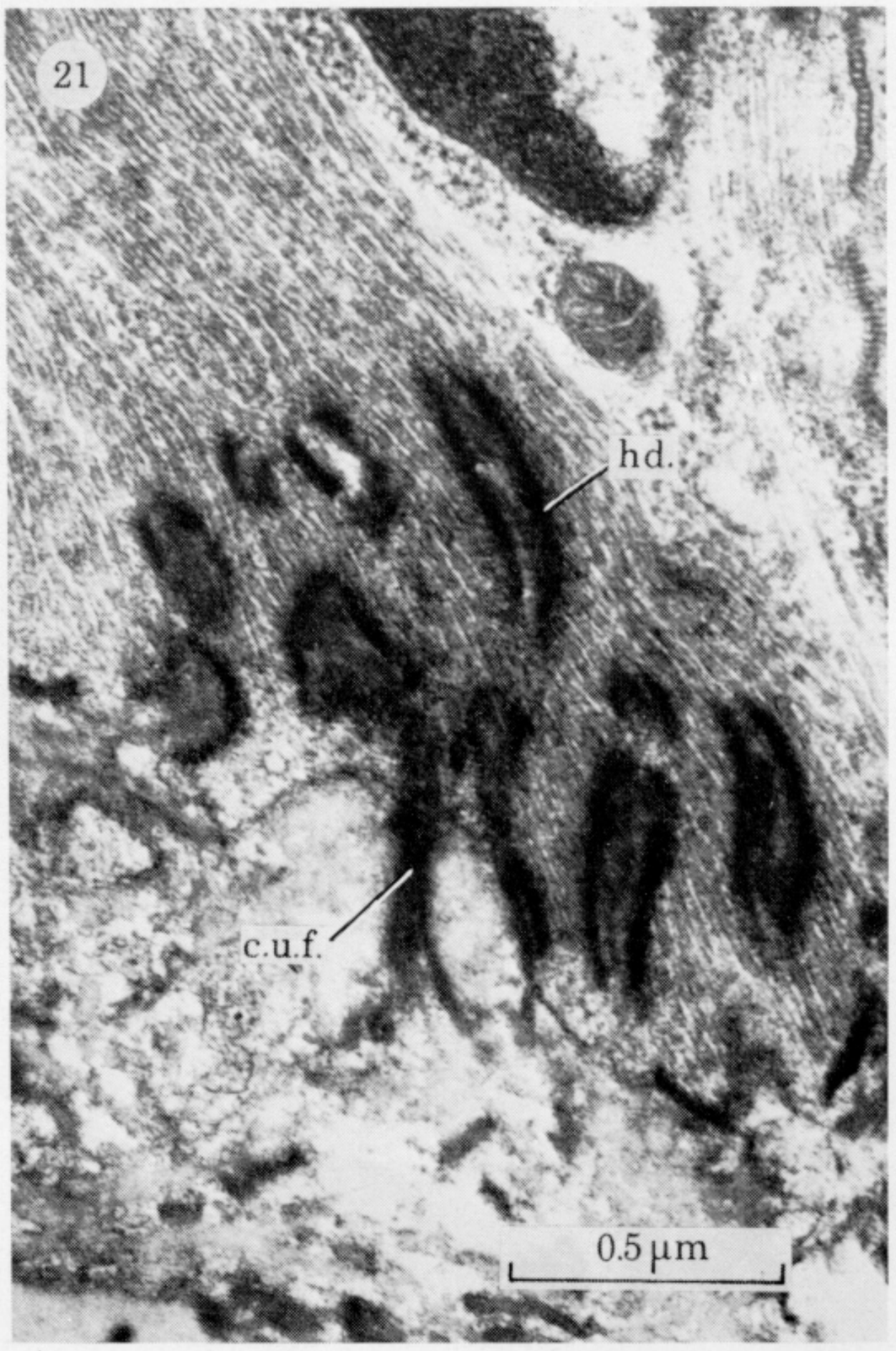
p.a.c.

FIGURES 16 AND 17. For description see reverse of plate 1.



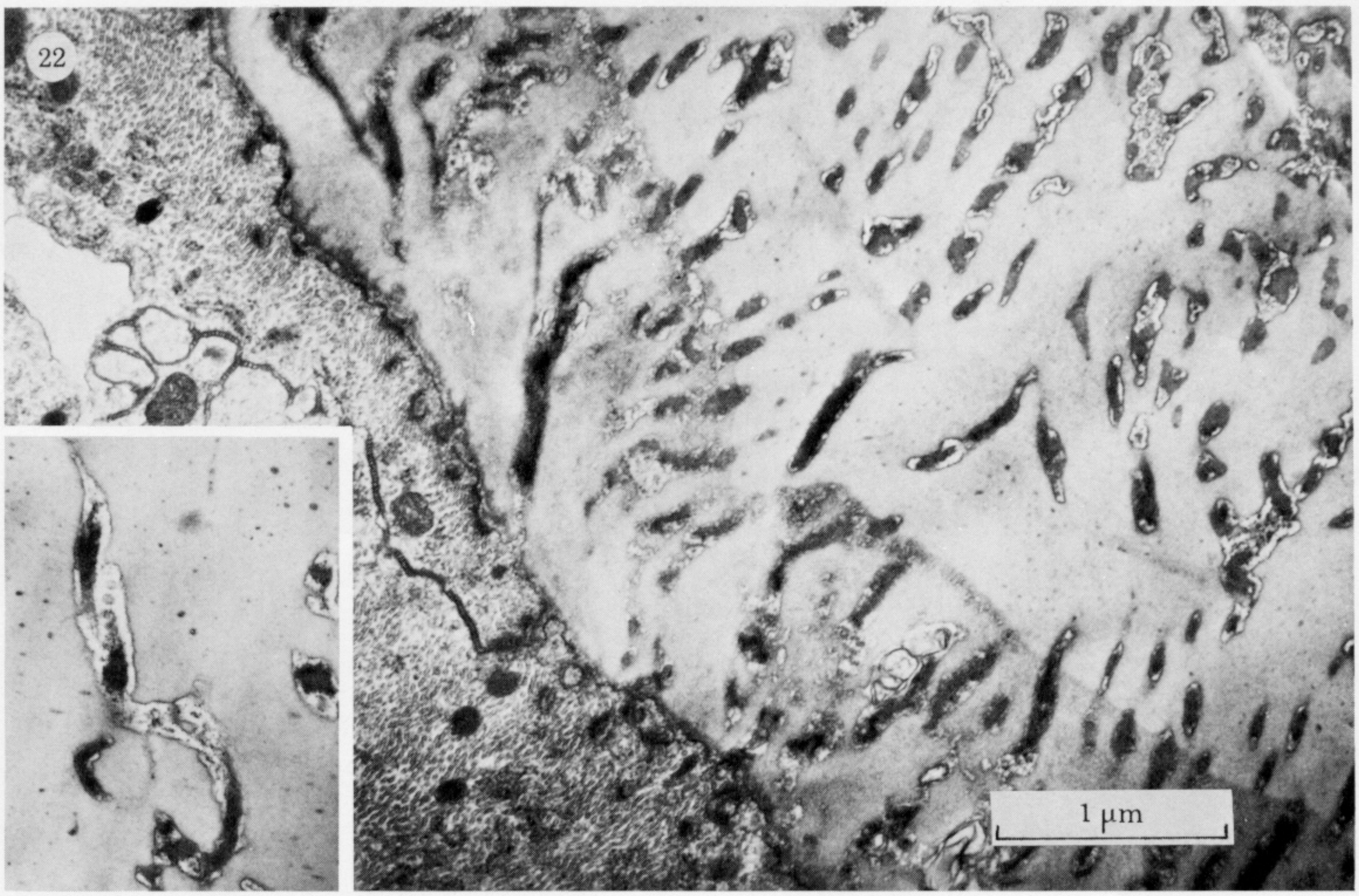






FIGURES 18–21. For description see reverse of plate 1.

22



23

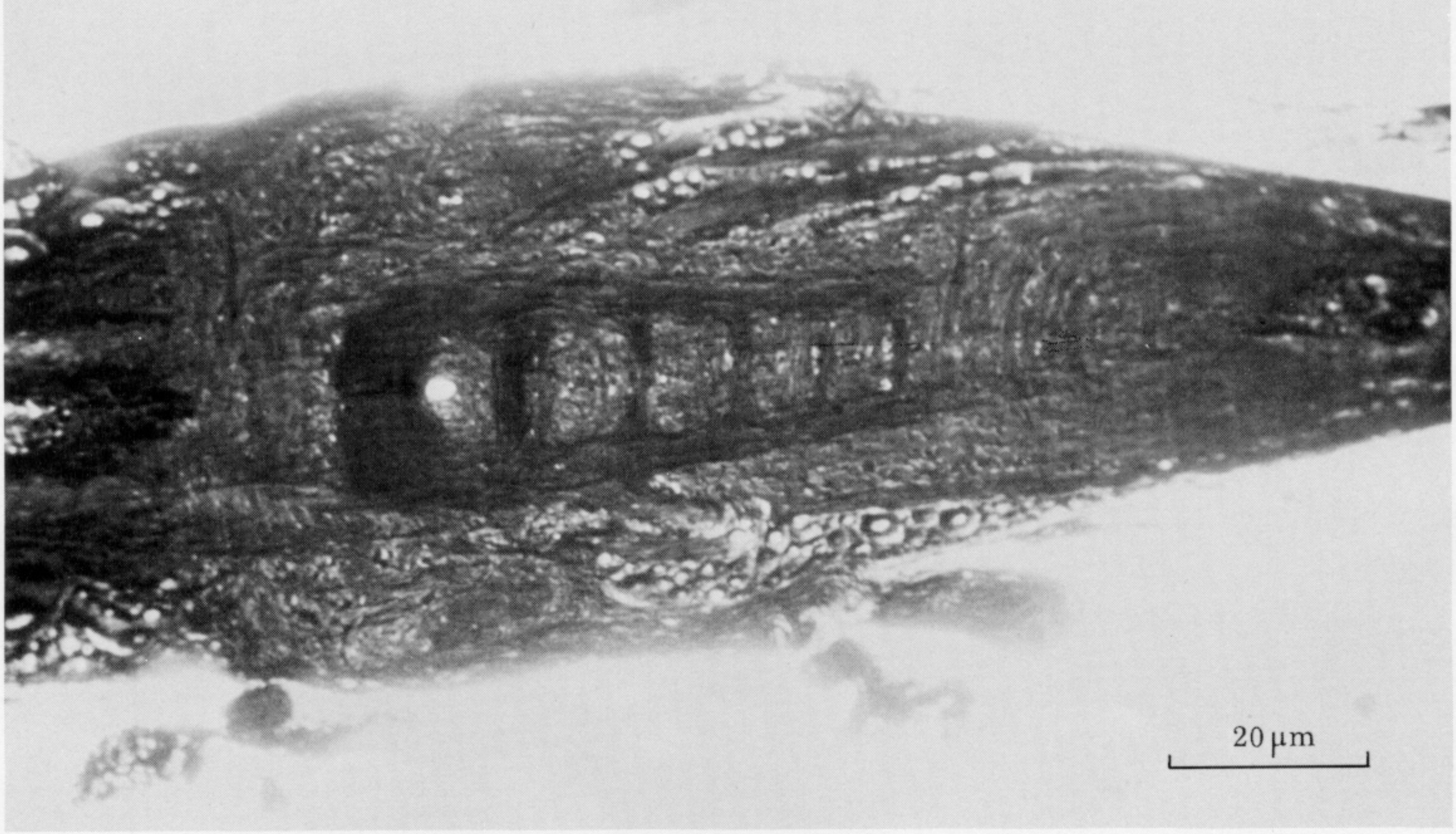


FIGURE 22. Transverse section through the tympanal apodeme, showing the pore canals containing cuticular attachment fibres. Inset shows a longitudinal section through a pore canal containing a twisted cuticular attachment fibre (same magnification).

FIGURE 23. Longitudinal horizontal section (15 µm silver-stained) through the thoracic ganglia, showing the intermediate neuropil with segmentally organized extensions. The small hole represents the demarcation between the metathoracic ganglion and the fused abdominal ganglia.