

THE FINE STRUCTURE OF THE CENTRIOLAR APPARATUS AND
ASSOCIATED STRUCTURES IN THE COMPLEX FLAGELLATES
TRICHONYMPHA AND *PSEUDOTRICHONYMPHA*

By A. V. GRIMSTONE

Department of Zoology, University of Cambridge

AND I. R. GIBBONS

Biological Laboratories, Harvard University, Cambridge, Massachusetts, U.S.A.

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The centriolar apparatus in the flagellate genera *Trichonympha* and *Pseudotriconympha* is located at the anterior end of the cell, in the rostrum. It forms part of a complex system of structures which includes the rostral tube, inner and outer caps, and the rostral flagella. The fine structure of these organelles is described in detail on the basis of an electron-microscope study of sectioned and negatively stained material.

In *Trichonympha* the rostral tube is a hollow cylinder, made of a cross-striated protein with a periodicity of about 450 Å. This is organized into radially arranged lamellae, which continue posteriorly as the parabasal filaments. The tube is continuous anteriorly with two finely striated crescentic bodies, which correspond to the so-called short centrioles of some previous workers. There is no evidence that they are centriolar in function. In the interphase cell the centriolar apparatus consists principally of a long centriolar rod, of complex fine structure, lying in the anterior end of the rostral tube. There is no evidence of typical centriolar structure in this, but at division an aster forms around one end of it. Surmounting the apex of the rostral tube is a dense, finely fibrous body, the inner cap. Lying within this there is a typical centriole (similar in structure to a basal body), and also the basal body of one flagellum, which appears to be distinct from all the rest. The functions of these two structures are not known. The margin of the inner cap connects with the complex system of delicate fibres which links the basal bodies of the rostral flagella. The function

of the fibres, and possibly also of the inner cap, may be to coordinate the activities of the rostral flagella. The outer cap is composed mainly of tubules, 250 Å in diameter, but shows variations in structure in different species.

The structures in *Pseudotriconympha* which presumably serve similar functions are in many respects very differently organized. The rostral tube is more complex, with distinct inner and outer walls of different fine structure. There are also complex inner and outer surface layers. A striking feature is that although the various components of the tube are quite different in structure, they display a common periodicity in their organization. The centriolar apparatus appears to consist principally of two dense bands running along the inner wall of the tube, connecting anteriorly to an extended layer of centriolar material to which spindle fibres are attached in radially polarized fashion throughout interphase. There is no centriolar rod or typical centriole, such as is found in *Triconympha*. Very elaborate systems of fibres are associated with the inner cap and the anterior end of the rostral tube.

The two genera are compared, and the findings related to knowledge of centriolar structures in other types of cell. Possible evolutionary explanations for the complexity and variation in fine structure in these flagellates are considered.

INTRODUCTION

The organisms with which this paper is concerned are the flagellates *Triconympha* and *Pseudotriconympha*. The fine structure of the flagella of these organisms has been described previously (Gibbons & Grimstone 1960); in this paper we present an account of their centriolar apparatus and certain associated organelles. A detailed account of these complex structures can be justified on several grounds: firstly, present knowledge of the structure of centrioles, and particularly those of protozoa, is extremely limited; secondly, the degree of elaboration and the precision of organization exhibited by the structures to be described is possibly greater than has previously been found at the subcellular level of organization, and is intrinsically interesting as a demonstration of the structure-building capacities of cells; and thirdly, the two genera considered, although superficially similar in their general organization, show distinct differences at the fine-structural level in the morphology of organelles apparently serving similar functions.

MATERIALS AND METHODS

Triconympha occurs as a symbiont in various termites and in the wood-feeding cockroach, *Cryptocercus punctulatus*. The species studied in detail were *Triconympha campanula*, *T. collaris* and *T. sphaerica*. These all occur in the termite *Zootermopsis angusticollis*, colonies of which were obtained from various localities in California and British Columbia. The members of the genus which occur in *Cryptocercus*, with the exception of *T. grandis*, were also studied, though in less detail and for the most part without attempting to distinguish between the numerous species which have been described from this host (Cleveland, Hall, Sanders & Collier 1934). *Cryptocercus* was collected at Mountain Lake Biological Station, Virginia, U.S.A.

The species of *Pseudotriconympha* examined was an undescribed one used in previous studies of flagella (Gibbons & Grimstone 1960). A description of this organism will be given elsewhere. For the time being it is unambiguously identified as being the only species of the genus occurring in *Proterhinotermes simplex*. Colonies of this termite were collected in Florida.

Termites and *Cryptocercus* were fed in the laboratory on wood or paper towelling. Flagellates were obtained by gently squeezing the abdomen of the host or by removing and opening the hind gut. The methods of examination used were for the most part conventional ones and demand no detailed description. Material was prepared for electron microscopy by techniques which have been fully described elsewhere (Gibbons & Grimstone 1960). Organisms were fixed in a buffered solution of osmium tetroxide, embedded in Araldite epoxy-resin, sectioned, and stained with uranyl acetate. Accurately oriented serial sections in both transverse and longitudinal planes were obtained by cutting single organisms, selected under phase contrast after flat embedding. This was done for a number of individuals of both genera. The information so obtained was supplemented by examination of a large number of randomly oriented sections. The digitonin technique (Child & Mazia 1956) was used to obtain isolated parts of cells for examination by phase contrast and electron microscopy. For the latter the fragmented material was negatively stained by mixing with 2% sodium phosphotungstate at pH 7.0 after washing with distilled water. The electron microscopes used were a Philips EM 200 and an RCA EMU-3D.

TRICHONYMPHA

General morphology and division

Detailed accounts of the structure of *Trichonympha* from termites have been given by Kirby (1932, 1944), and of the species from *Cryptocercus* by Cleveland *et al.* (1934). The main features of *T. campanula* are shown in figure 1. It is a large cell, on average about 220 μm long. The anterior region of the body bears numerous longitudinal rows of flagella, the bases of which lie in longitudinal grooves in the cell surface. The anterior end of the flagellated region is termed the *rostrum*, and it is with this that the present paper is chiefly concerned. The rostrum has only about half as many rows of flagella as the rest of the body. It contains a central *rostral tube*, surrounded by the tightly packed basal bodies of the rostral flagella, and is surmounted by a dome-shaped *outer cap* (figure 5, plate 22). Lying over the anterior end of the rostral tube there is a hemispherical structure called the *inner cap*, within and below which lies the *centriolar apparatus*. On the basis of light-microscope studies Cleveland (1949*a*, 1960*a*, *b*) describes the latter as consisting of two *centrioles*, one long, one short. The former is a rod extending down into the rostral tube (figure 6, plate 22) and, according to Cleveland, it has at its anterior end a granule exactly similar to the so-called short centriole (figure 2). A somewhat different interpretation of these structures will be presented later, on the basis of our electron-microscope observations, and in this paper we shall refer to the long centriole as the *centriolar rod*, and to the short ones as the *crescentic bodies*. The only other structures which need be mentioned here are the *nucleus*, and the *parabasal bodies* which lie around it (figure 1). The latter may number sixty or more and are connected to the base of the rostrum by the *parabasal filaments*.

The species of *Trichonympha* differ from one another chiefly in size and proportions and in characters such as the number and arrangement of the parabasal bodies. Few significant differences have been found in the fine structure of the rostrum and centrioles in the various species studied and, unless otherwise indicated, the description given in this paper may be taken as applying to all of them.

The intricate structure of the rostrum and centrioles of *Trichonympha* cannot be fully appreciated without some knowledge of the method of division. The following brief outline is based on the descriptions of Cleveland (1949*a*, 1960*a*).

As will be described more fully later, the rostral tube is a bilaterally symmetrical structure, made up of two half tubes. Early in division these separate, together with their associated flagella. A second centriolar rod develops from the region of the inner cap (according to Cleveland it arises from what he terms the short centriole), and elongates until it is as long as the first. Asters develop around the posterior ends of the centriolar rods, and the mitotic spindle is formed by overlapping and fusion of astral fibres (figure 3). The spindle increases in length and thickness and eventually makes contact with the nucleus, following the rounding up of the cell and further separation of the two halves of the rostrum (figure 4). The details of mitosis have been fully described by Cleveland but are irrelevant

EXPLANATION OF PLATES 22 TO 33

TRICHONYMPHA

Unless otherwise stated, all figures show *Trichonympha* from *Zootermopsis*. Figures 5 and 6, plate 22, are phase-contrast photomicrographs; all other figures are electron micrographs. With the exception of figure 14, plate 25, which shows negatively stained material, all electron micrographs are of sections, stained with uranyl acetate.

Abbreviations

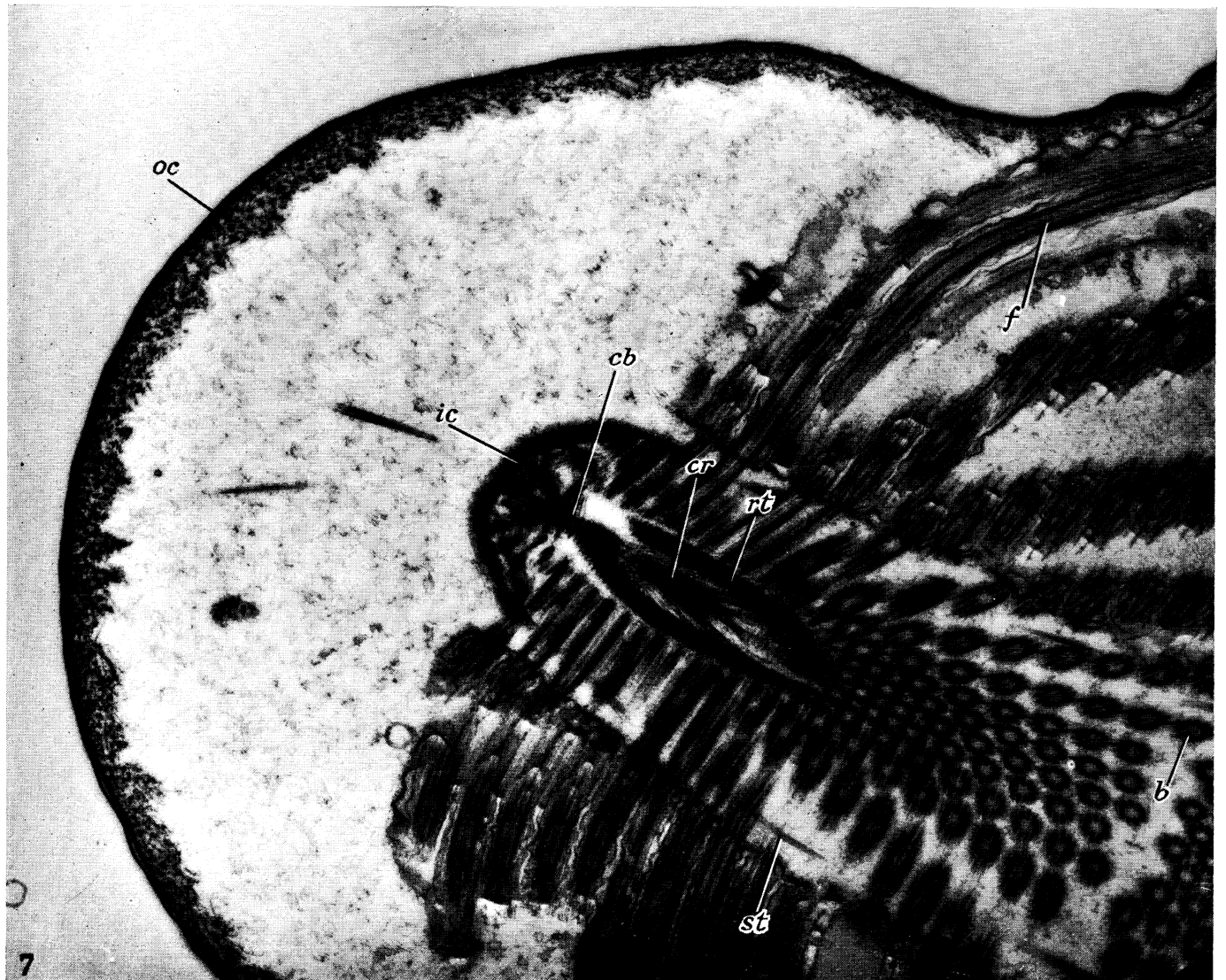
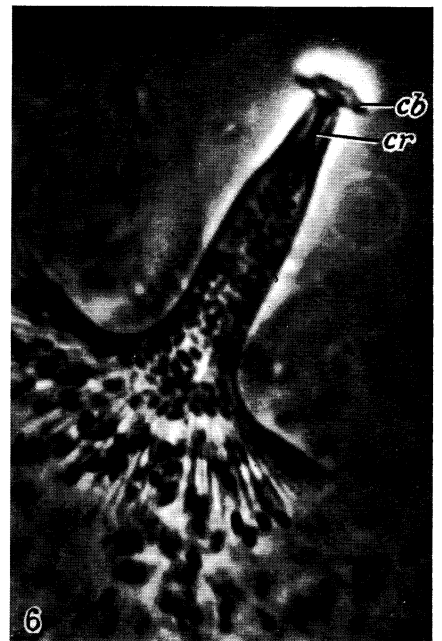
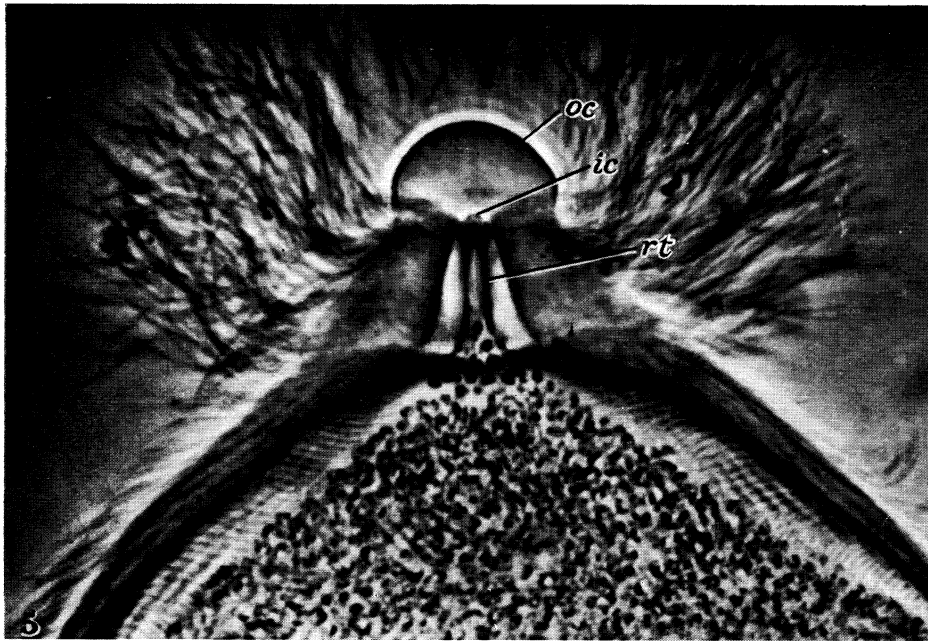
<i>b</i>	basal body	<i>is</i>	inner surface layer of rostral tube
<i>ba</i>	basal body of anterior flagellum	<i>oc</i>	outer cap
<i>bm</i>	bacterium	<i>os</i>	outer surface layer of rostral tube
<i>brt</i>	basal body within rostral tube	<i>pf</i>	parabasal filament
<i>c</i>	typical centriole	<i>rn</i>	radial net
<i>cb</i>	crescentic body	<i>rt</i>	rostral tube
<i>cr</i>	centriolar rod	<i>sr</i>	striated ribbon
<i>f</i>	flagellum	<i>st</i>	strand arising from inner cap
<i>g</i>	granule	<i>v</i>	vesicle
<i>ic</i>	inner cap		

DESCRIPTION OF PLATE 22

FIGURE 5. Phase-contrast photomicrograph of the anterior end of *T. campanula* after osmium fixation. The light area on either side of the rostral tube is made up of closely packed basal bodies ($\times 1000$).

FIGURE 6. Photomicrograph of the rostral tube of *T. collaris*, isolated by the digitonin technique, with the crescentic bodies remaining attached to its anterior end. Note that the tube frays out posteriorly into separate fibres, which are the parabasal filaments. The centriolar rod with its funnel-shaped posterior end is clearly visible ($\times 2500$).

FIGURE 7. Low-magnification electron micrograph of the anterior end of the rostrum of a species of *Trichonympha* from *Cryptocercus*. Note particularly the outer cap, the strands passing back from the margin of the inner cap, and the fibres running from the centriolar rod to the rostral tube ($\times 18000$).



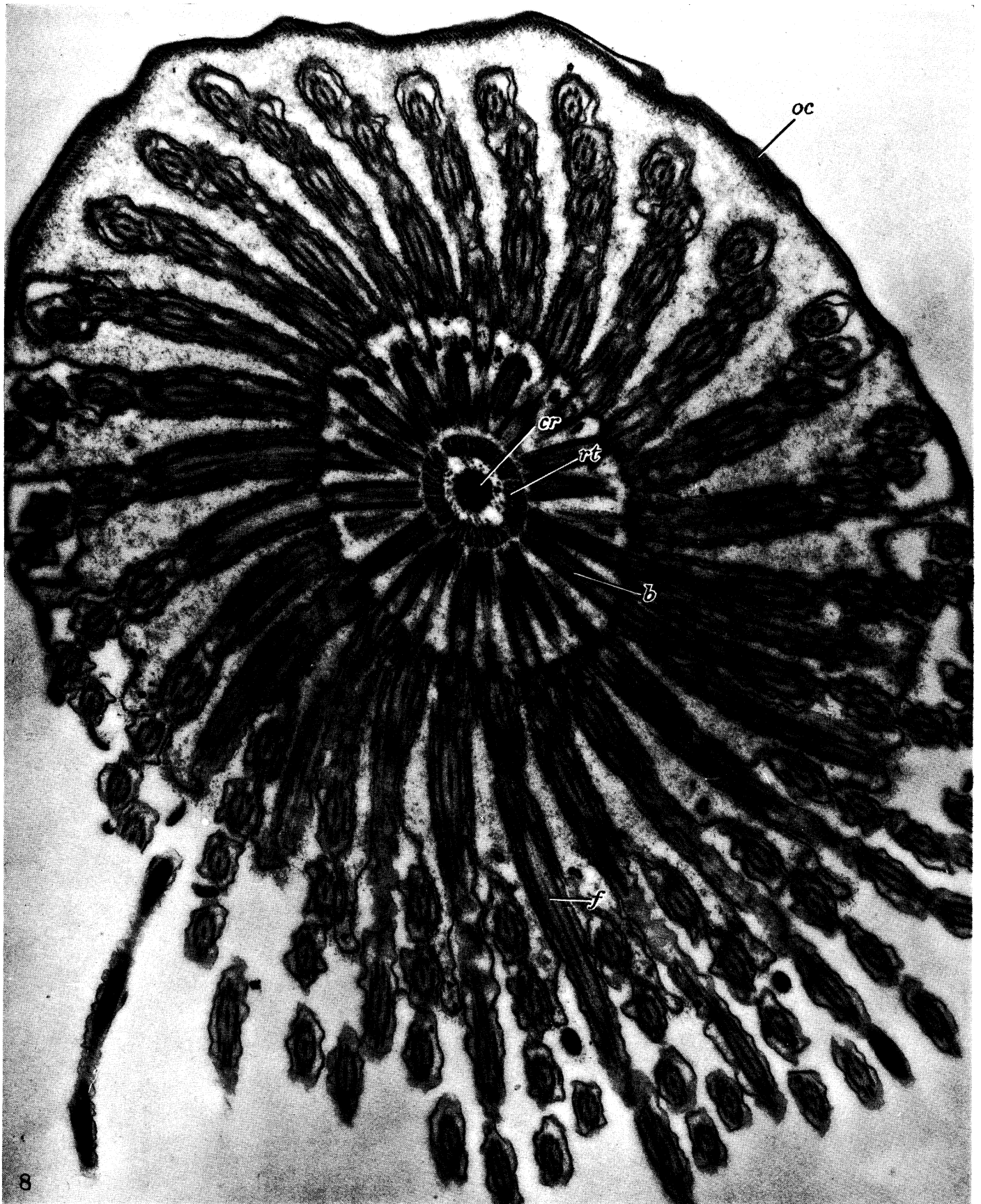


FIGURE 8. Transverse section of the rostrum of a species from *Cryptocercus*. The section is slightly oblique: at the top of the picture it passes through the outer cap, and at the bottom through the flagellar grooves. Note that with the exception of the rostral tube, most of the cytoplasm is occupied by flagella and their basal bodies. A whorled arrangement of dense lamellae is visible in the central region of the centriolar rod ($\times 25000$).

FIGURE 11. Serial section to that shown in figure 10. This shows the basal body of the anterior flagellum, lying between the two crescentic bodies, and also one of the striated ribbons ($\times 30000$).

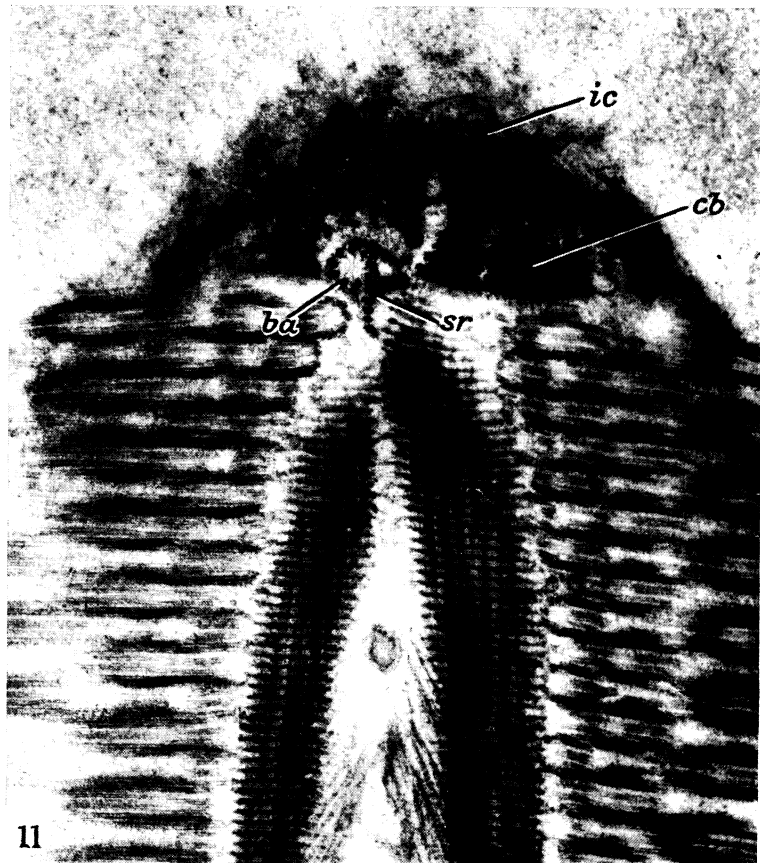
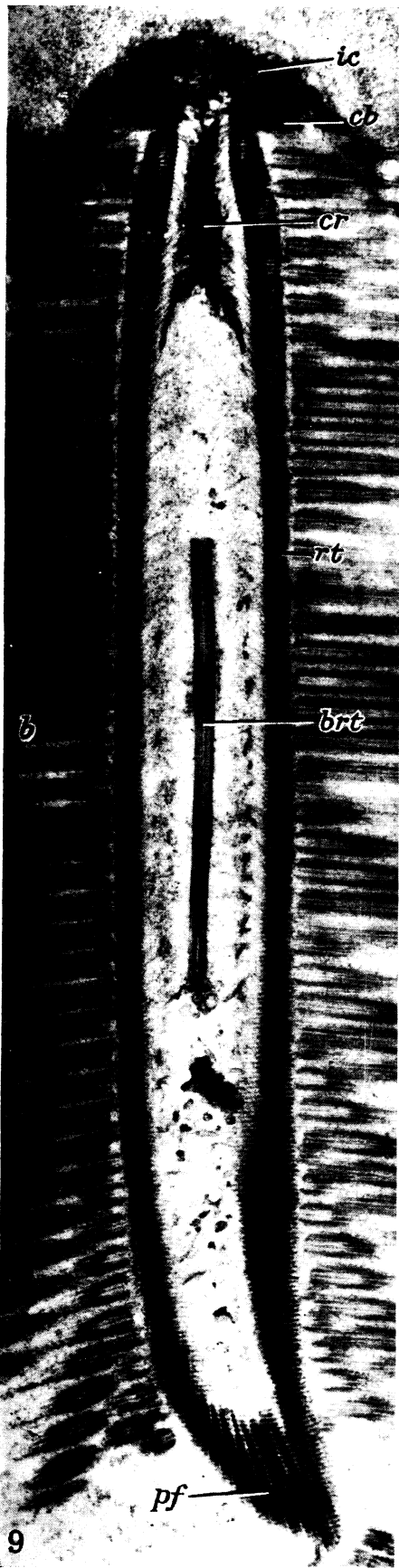
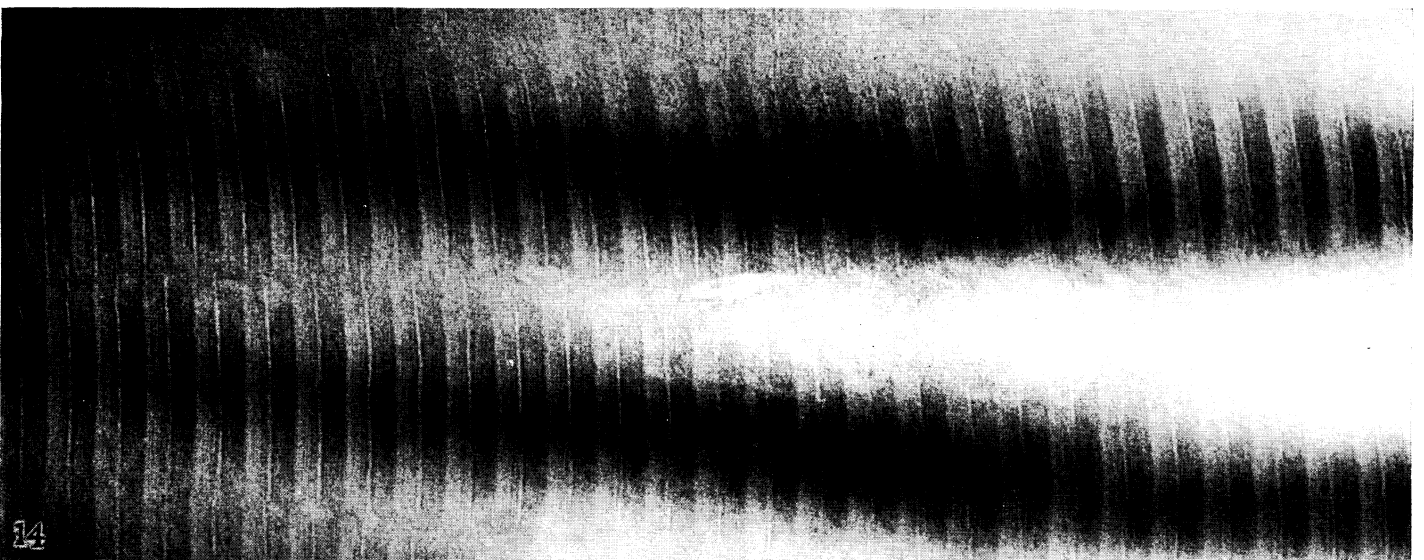
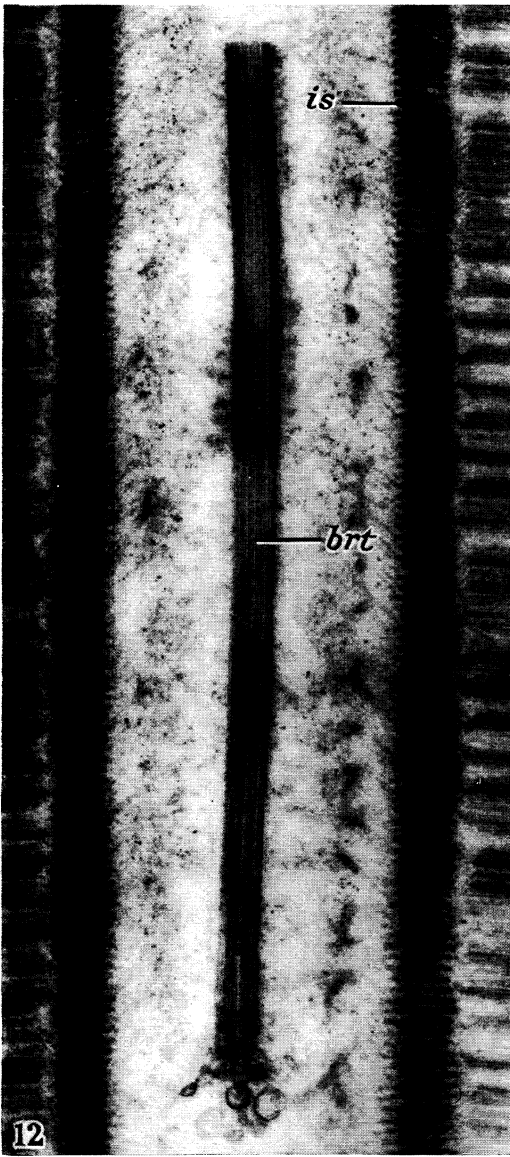


FIGURE 9. Longitudinal section showing the full length of the rostral tube. At the bottom of the picture the separate parabasal filaments can be seen. Note the rows of close-packed basal bodies, the inner cap, crescentic bodies, centriolar rod and the long basal-body-like structure in the rostral tube. This figure may be compared with figure 41, plate 34 ($\times 11\,000$).

FIGURE 10. Enlargement from figure 9. The regularly arranged delicate fibres connecting the centriolar rod to the inner surface layer of the rostral tube are clear. Note the striated crescentic bodies, and the fine fibres running radially through the inner cap ($\times 30\,000$).

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here. Each daughter cell eventually receives half of the organelles of the parent cell and forms a new half set. The new half rostra (flagella, half rostral tubes, etc.) begin to develop in connexion with the anterior ends of the old half rostra, growing out from the region which contains the old crescentic body, the half inner cap and the anterior end of the centriolar rod (figure 4).

Rostral tube and parabasal filaments

Preliminary descriptions of the fine structure of the rostrum of *Trichonympha* have been given by Pitelka & Schooley (1958) and by Grimstone (1961). These accounts are largely correct as far as they go, but they are incomplete and based on relatively low-resolution electron micrographs. It is proposed to re-describe the structures completely, beginning with the rostral tube.

Figure 6, plate 22, shows a rostral tube of *T. collaris* isolated by the digitonin technique. It is a short, hollow cylinder, about 20 μm long and 2 μm in diameter in this species, tapered anteriorly and widening posteriorly before fraying out into a number of separate fibres. It is refractile and always clearly visible in living organisms, and it stains heavily with iron haematoxylin and with stains for protein such as brom-phenol blue. The tube is shown at low magnification in transverse section in figure 8, plate 23, and in longitudinal section in figure 9, plate 24; its formation from two similar half tubes is apparent in the former.

The shape and texture of the walls of the tube change markedly along its length. These changes, which are seen most clearly in transverse sections, are illustrated in figures 19 to 26, plates 27 and 28.

At the apex of the tube the walls are thick (about 0.4 μm at maximum) and the two halves are crescent-shaped in cross section (figure 19). They are made up of a number of delicate, radiating lamellae, set in a less dense matrix. Some of the lamellae are split peripherally, giving them a Y-shaped appearance in cross section, and between them there are rather irregular rows of small dense dots, possibly representing fine fibres. Slightly further back, about half-way along the length of the centriolar rod, the walls are thinner and the tube is more nearly circular in cross section (figure 20). Figure 21, close to the posterior end of the centriolar rod, presents a similar appearance. The number of lamellae increases in this region, the additional ones probably being formed by bifurcation of some

DESCRIPTION OF PLATE 25

FIGURE 12. Enlargement of part of figure 9 to show the long basal-body-like structure in the rostral tube. Regular rows of fine fibres can be seen connecting the inner surface layer of the rostral tube to small dense patches of material. The latter are sections through sinuous strands of peripheral material running back from the centriolar rod (compare figure 9) ($\times 23\,000$).

FIGURE 13. Longitudinal section through the base of the rostral tube showing the origin of the parabasal filaments ($\times 10\,000$).

FIGURE 14. Part of a negatively stained rostral tube isolated by the digitonin method. The micrograph shows the base of the tube at the level where it is fraying out into separate parabasal filaments. Note the complex banding. The staining in this preparation is probably partly positive, partly negative ($\times 123\,000$).

of the more anterior ones. Posterior to the centriolar rod the tube is quite circular in section and its walls are still thinner (figures 22 and 23). In this region the lamellae gradually become more clearly differentiated from the matrix and are transformed from delicate, sheet-like structures into substantial fibres, approximately rectangular in section,

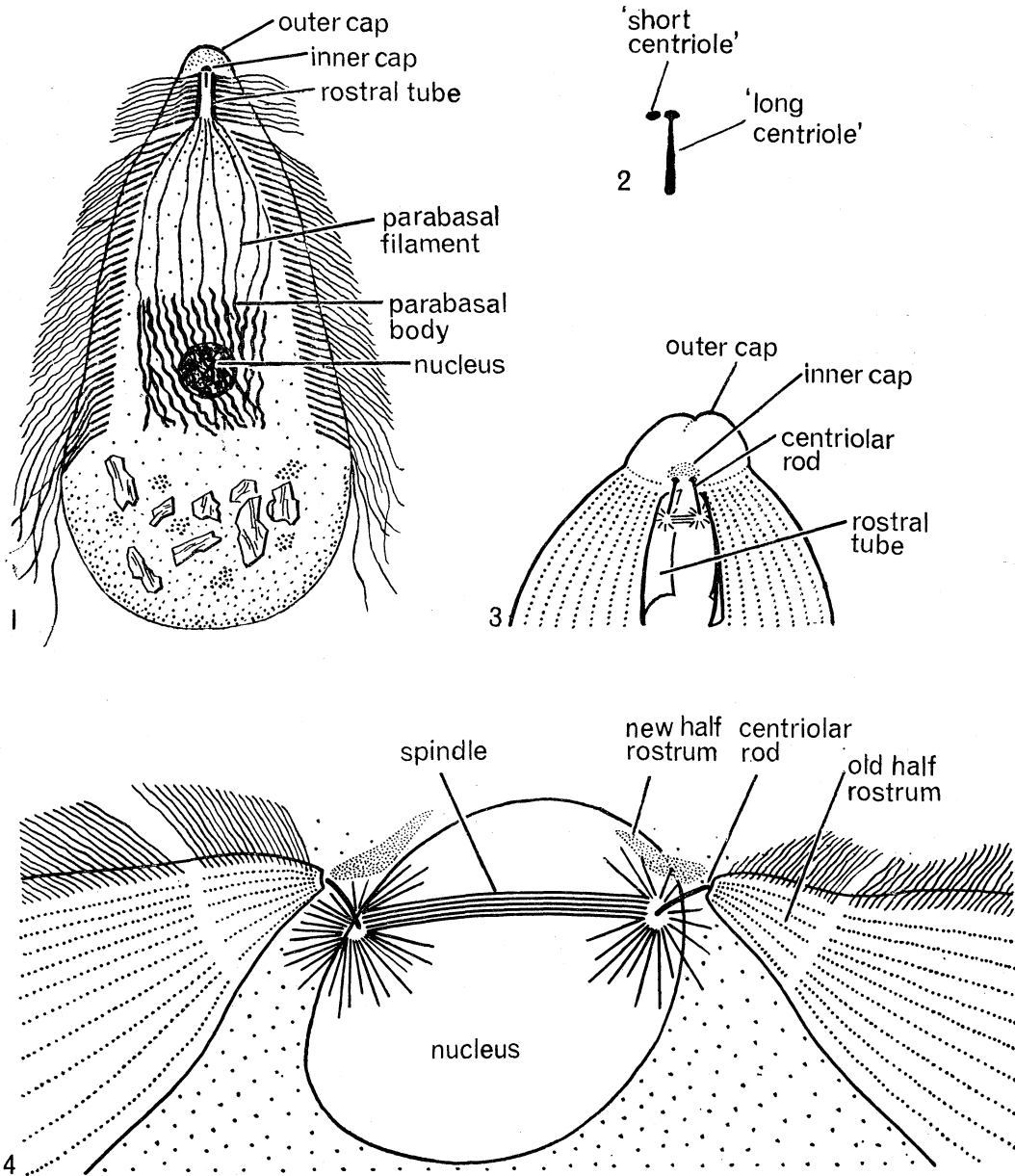


FIGURE 1. Diagram of *Trichonympha campanula*.

FIGURE 2. Diagram showing the structure of the centrioles according to the interpretation of Cleveland (1960a).

FIGURE 3. An early stage in mitotic division. The two halves of the rostrum have separated and the second centriolar rod has developed. Asters have formed around the distal ends of the rods and a small mitotic spindle has formed between them (modified after Cleveland 1960a).

FIGURE 4. A later stage in division, showing the fully formed spindle applied to the nucleus. The two halves of the rostrum are fully separated and two new halves are developing. Chromosomes are omitted (modified after Cleveland 1960a).

which become increasingly distinct in passing backwards (figures 24 and 25). Towards the base of the rostrum the two halves of the tube usually begin to separate slightly and may become somewhat out of alinement.

At the posterior end of the rostrum the rostral tube loses its coherence and the fibres, previously united in a matrix, become free (figure 26). The fibres are thickened in this region by the addition to the inner margin of each of them of a solid, wedge-shaped element, so that the fibres are duplex structures at this level. The wedge-shaped thickenings are present only at the junction of the rostrum with the rest of the body. Posterior to this the thickenings stop and the diameter of the fibres diminishes rather abruptly. The fibres run backwards, at first close below the basal bodies of the post-rostral flagella and then passing deeper into the cytoplasm where they constitute the *parabasal filaments* (figure 13, plate 25). These connect with and extend along the whole length of the parabasal (or Golgi) bodies, as previously described (Grimstone 1959*a*). The continuity of the parabasal filaments with the fibres of the rostral tube was first reported by Pitelka & Schooley (1958) and has been confirmed in the present study.

Longitudinal sections show that the walls of the rostral tube are cross-striated along their whole length (figures 9, 10 and 11, plate 24; figure 27, plate 29), as also are the parabasal filaments (figure 13, plate 25). The periodicity of the striation apparently varies in different preparations, a feature noted by Pitelka & Schooley (1958). In most micrographs the periodicity in the rostral tube itself is about 450 Å, but values as low as 350 Å have been found. The periodicity of the striation of the parabasal filaments also apparently varies between 250 and 450 Å. The striation is complex and there are several sub-bands in each major period, a feature which is shown particularly clearly in negatively stained preparations of isolated rostral tubes (figure 14, plate 25). The striation of the rostral tube is not restricted to the lamellae and fibres: in the anterior part of the tube at least, it is clearly continuous across the gaps between adjacent lamellae, and the striations of all the lamellae are in register (figure 11, plate 24; figure 33, plate 31). This suggests that the walls of the tube at this level should be thought of as solid structures, in which the lamellae form denser regions. The rows of dots between the lamellae, noted earlier in transverse sections, may represent an aspect of the inter-lamellar striation.

Transverse sections of the rostral tube show a distinct, dense *inner surface layer* on its inner face (figures 21 and 22, plate 27). This extends the whole length of the tube, to the base of the rostrum, though it is more prominent anteriorly. In longitudinal sections it appears as a series of dense dots, about 250 Å in diameter and probably representing transversely oriented fibres. These are spaced with precisely the same periodicity as that of the striations of the tube and are alined with the major banding (figure 10, plate 24). There is a similar *outer surface layer* on the exterior of the tube, but this is less prominent and does not extend much beyond the posterior end of the centriolar rod (figure 10).

Apart from the centriolar rod, described in the next section, the contents of the rostral tube vary somewhat from one species to another. In some cases the micrographs show nothing except an amorphous ground substance, sometimes containing a few small vesicles. In others, and particularly in the species from *Zootermopsis*, a variety of granules may be present (figure 6, plate 22). In *T. collaris*, but probably not in other species, some of the granules are bacteria, distinguished by typical bacterial features such as a cell wall and

chromatin bodies (figure 25, plate 28). Another type of granule, found in this and other species, lacks these characteristics. This type is irregularly shaped and has moderately dense, uniform contents, bounded by a membrane (figure 26, plate 28). Similar granules occur elsewhere in *Trichonympha* and are often particularly abundant in the cytoplasm immediately below the basal bodies. Their functions are not known, but their location close to the flagella suggests that they might be concerned with energy release. *Trichonympha*, an obligate anaerobe, contains no typical mitochondria.

The only other noteworthy structure in the rostral tube has so far been found in only three organisms, all from *Zootermopsis*, and is certainly not always present. It is shown in longitudinal section in figure 9, plate 24 and figure 12, plate 25, and in transverse section in figure 26, plate 28, and takes the form of a single, isolated basal body about 5 μm long, running longitudinally through the lower half of the rostral tube. No flagellum is attached to it. This organelle has not previously been described and its significance is not known.

Centriolar apparatus

The centriolar apparatus is embedded in or attached to the so-called *inner cap*, which is the dense, hemispherical mass of material surmounting the rostral tube (figures 6 and 7, plate 22; figure 9, plate 24; figure 32, plate 31). The inner cap should perhaps itself be regarded as part of the centriolar apparatus, though there is as yet no indication about its possible role in division and morphogenesis. It is a solid structure and not delimited from the overlying cytoplasm by a membrane, so that the term 'cap' is not entirely appropriate. It is well established, however, and will be retained here.

The diameter of the inner cap, about 4 μm , is greater than that of the anterior end of the rostral tube, and its margins overlie the proximal ends of the most anterior basal bodies (figure 7, plate 22; figure 9, plate 24). Its appearance varies somewhat in different individuals and it seems likely that it rather readily undergoes alterations in the course of fixation and embedding. The central region is usually coarsely fibrous in texture, often with a spongy appearance, and large cavities are often present in the species from *Cryptocercus* (figure 7; figure 27, plate 29). A less dense, more homogeneous layer, sometimes with a finely fibrous texture, is often present on the outer surface (figure 10, plate 24; figure 15,

DESCRIPTION OF PLATE 26

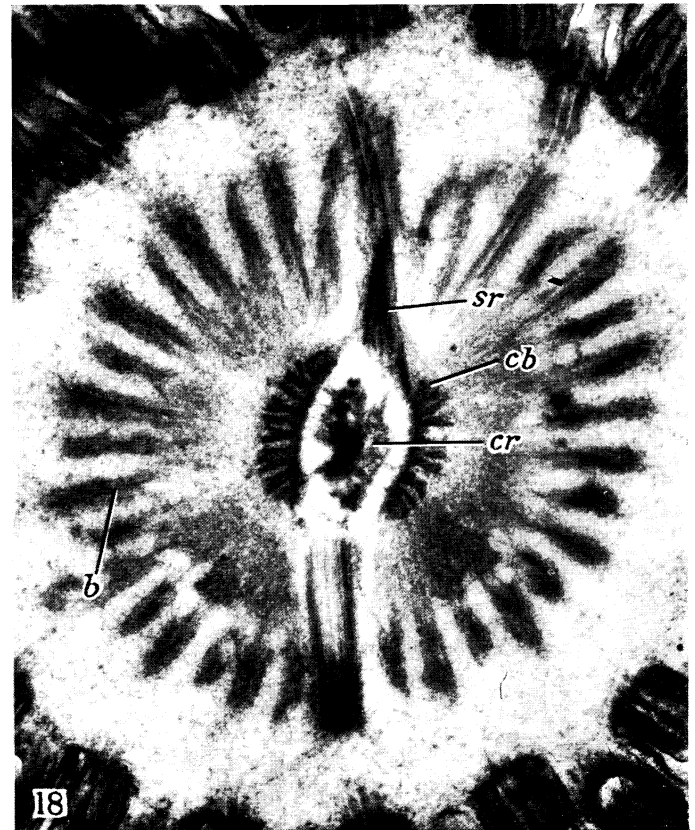
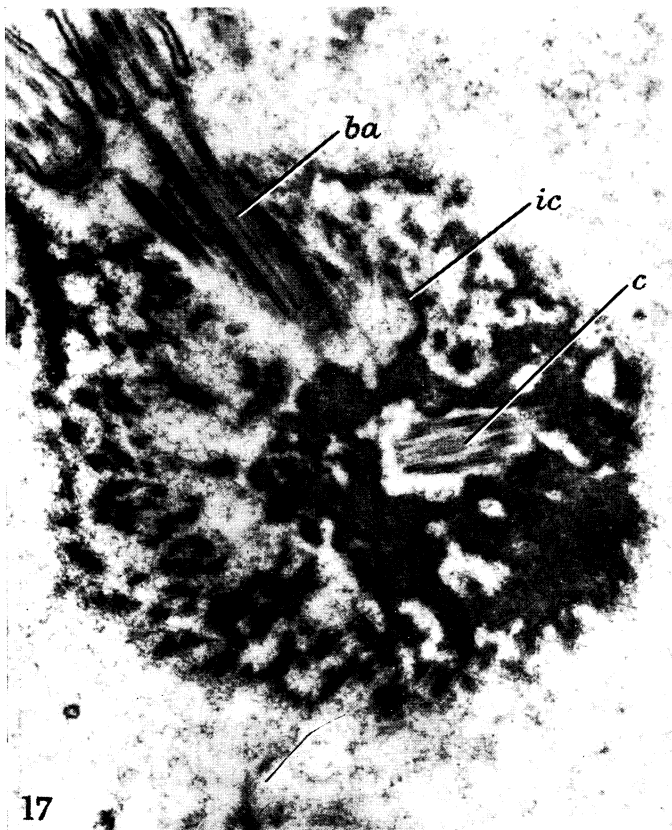
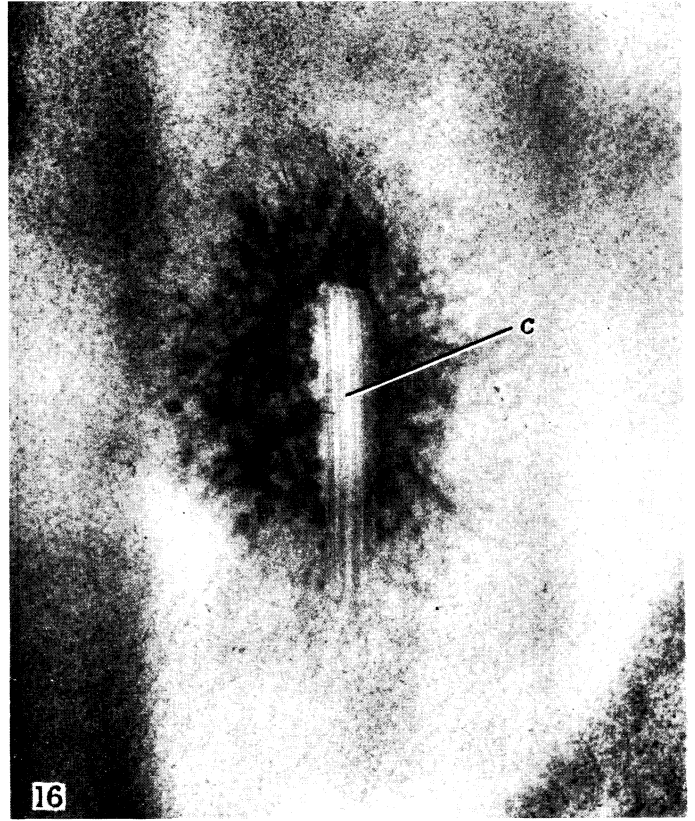
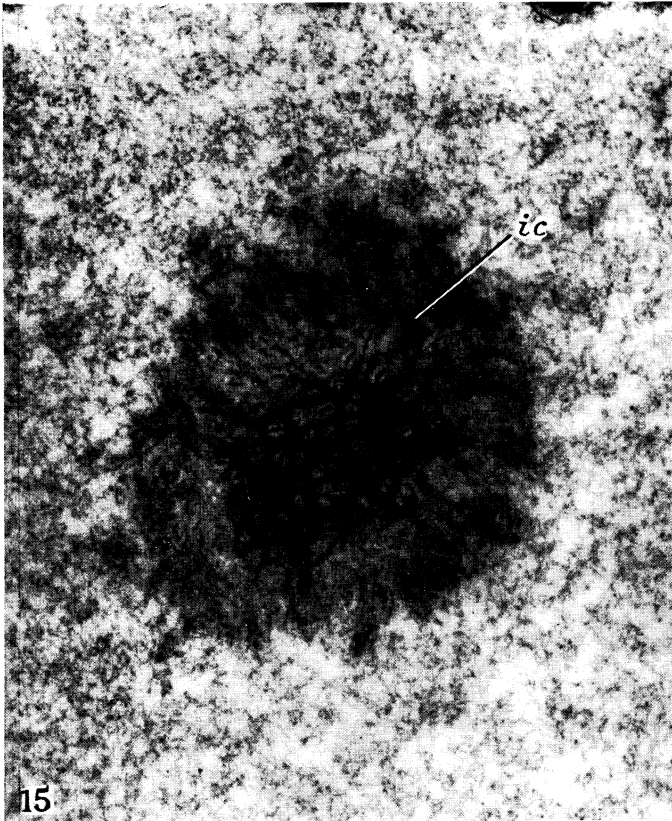
FIGURES 15 TO 26, PLATES 26 TO 28, show a series of transverse sections through the inner cap and rostral tube of different organisms, arranged in sequence and starting anteriorly.

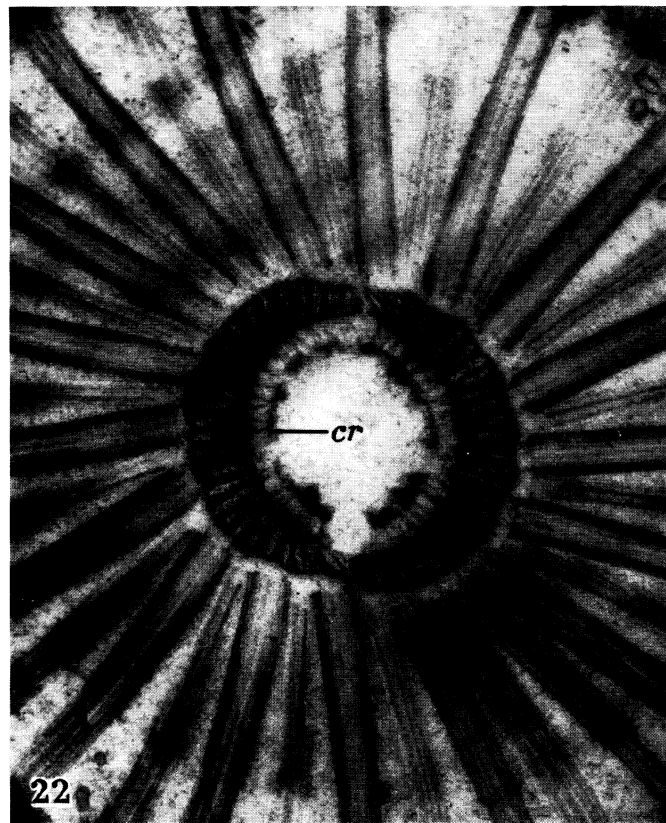
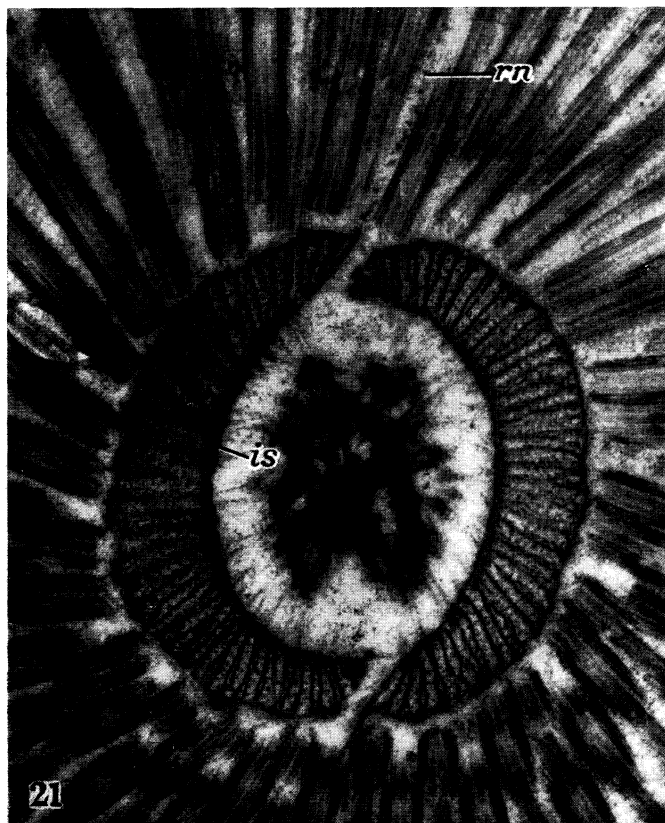
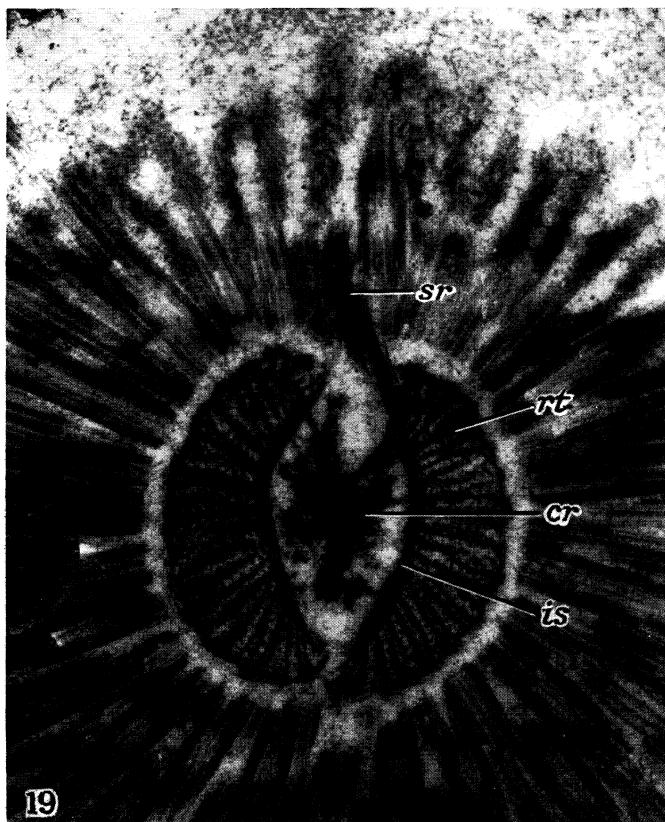
FIGURE 15. Inner cap, showing central coarse reticulum and less dense periphery. The dark lines in the interstices of the reticulum are interpreted as sections through ribbon-shaped fibres passing radially through the inner cap ($\times 29\,000$).

FIGURE 16. Inner cap, showing the typical centriole ($\times 29\,000$).

FIGURE 17. Section through the inner cap of an organism from *Cryptocercus*. It is slightly oblique and includes both the typical centriole and the anterior flagellum with its basal body ($\times 38\,000$).

FIGURE 18. Section through the base of the inner cap, including the two crescentic bodies, the tip of the centriolar rod, the basal body of the anterior flagellum, and one of the striated ribbons. The radiating structures (*b*) are the anterior edges of the basal bodies of the rostral flagella, together with marginal inner cap material (compare figure 29) ($\times 29\,000$).



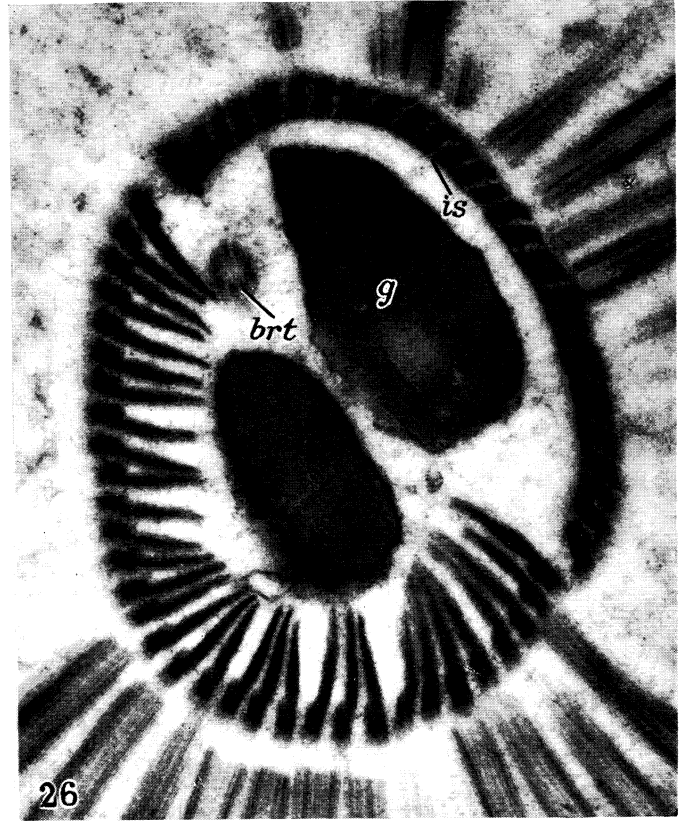
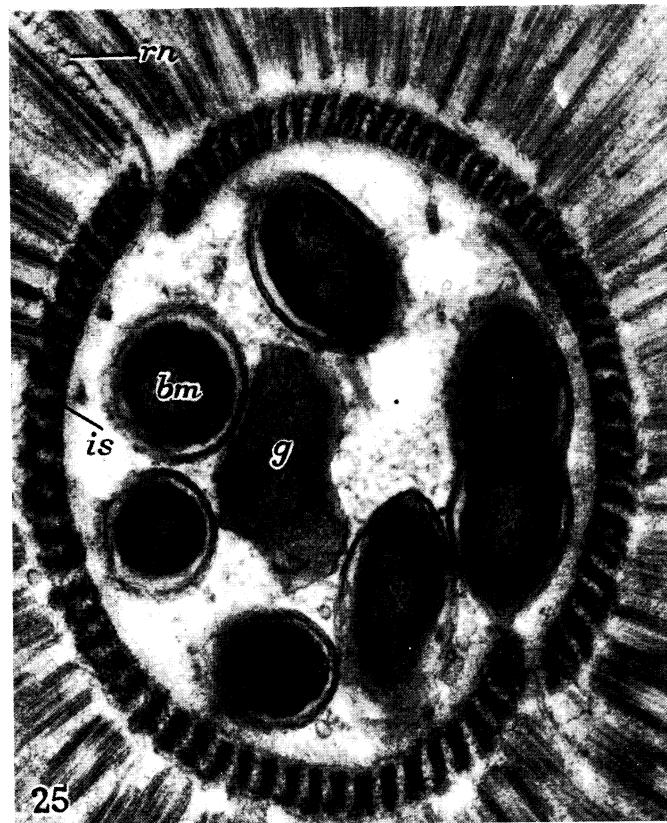
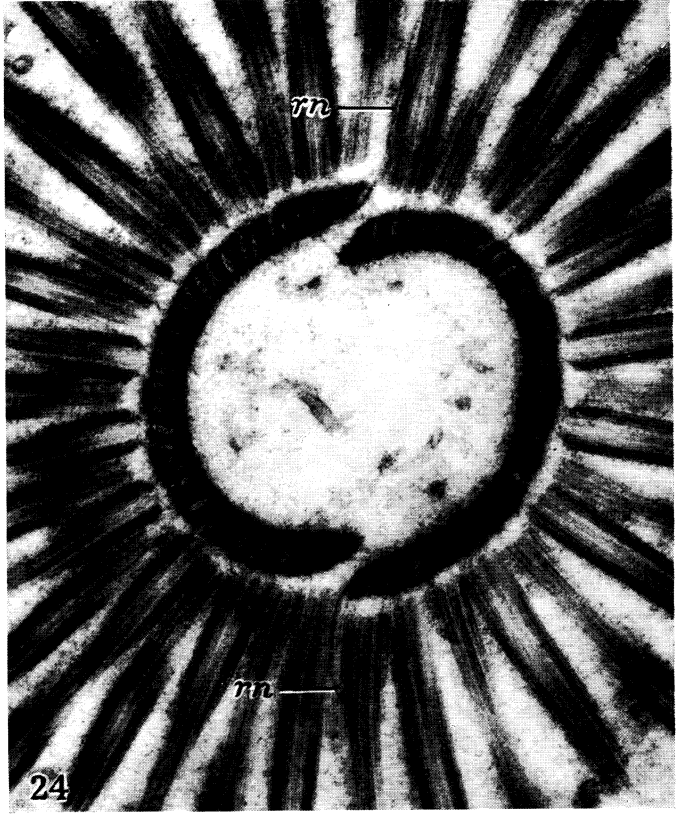
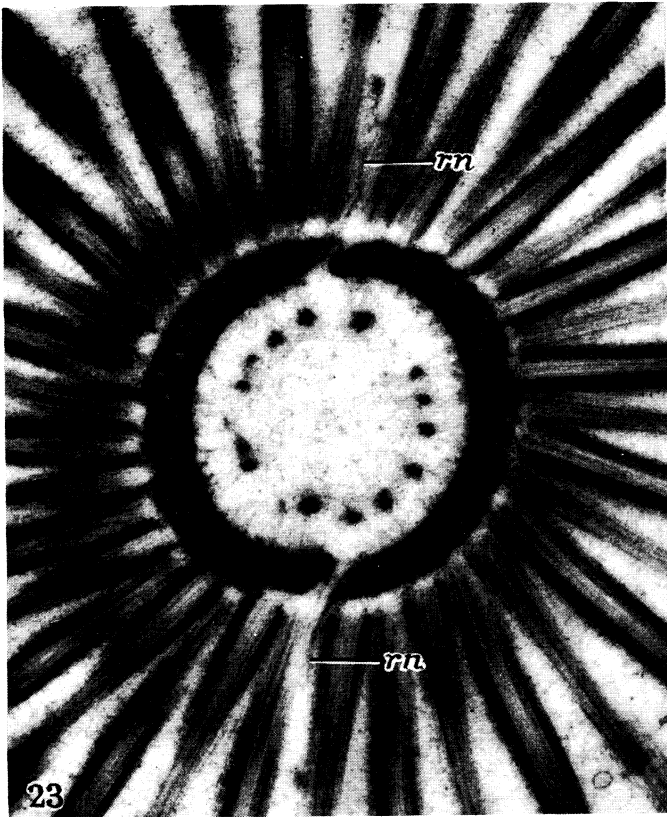


DESCRIPTION OF PLATE 27

- FIGURE 19. This shows the typical structure of the centriolar rod and the origin of the one of the striated ribbons from the inner surface layer of one of the half rostral tubes ($\times 29\,000$).
- FIGURE 20. Section similar to that shown in figure 19, but with the half rostral tubes more nearly semi-circular in section. Part of one of the striated ribbons is visible ($\times 29\,000$).
- FIGURE 21. Section showing the typical appearance of the rostral tube towards the posterior end of the centriolar rod. The lamellae and the rows of dots between them are clear, as is the inner surface layer of the tube. One of the radial nets is visible ($\times 29\,000$).
- FIGURE 22. Section posterior to the main body of the centriolar rod, including its funnel-shaped end, which appears here as an irregular circle. Fibres connect this to the inner surface layer of the rostral tube. Note that the lamellae of the latter appear more substantial than in the more anterior sections ($\times 29\,000$).

DESCRIPTION OF PLATE 28

- FIGURE 23. Section slightly posterior to that in figure 22, in which the peripheral material of the centriolar rod is represented by separate strands. The rostral tube has well defined lamellae. Both radial nets can be seen, each connected to one of the half rostral tubes ($\times 29\,000$).
- FIGURE 24. Similar to figure 23, but posterior to the centriolar rod. The two halves of the rostral tube are not perfectly aligned. Note the radial nets ($\times 29\,000$).
- FIGURE 25. Section near the base of the rostral tube, in which the lamellae have become distinct fibres, rectangular in section, but still united in each half tube by the inner surface layer. One of the radial nets clearly shows fibres in longitudinal and transverse section. The bodies in the rostral tube are bacteria, except for one of the dense granules characteristic of the cytoplasm of *Trichonympha* ($\times 29\,000$).
- FIGURE 26. Oblique section at the base of the rostral tube. In the half tube in the lower part of the picture the fibres have separated and are thickened by the addition of wedge-shaped elements on their inner faces. The other half is cut at a more anterior level and still has a structure similar to that in figure 25. A basal-body-like structure is visible in the lumen of the tube, cut in transverse section ($\times 29\,000$).



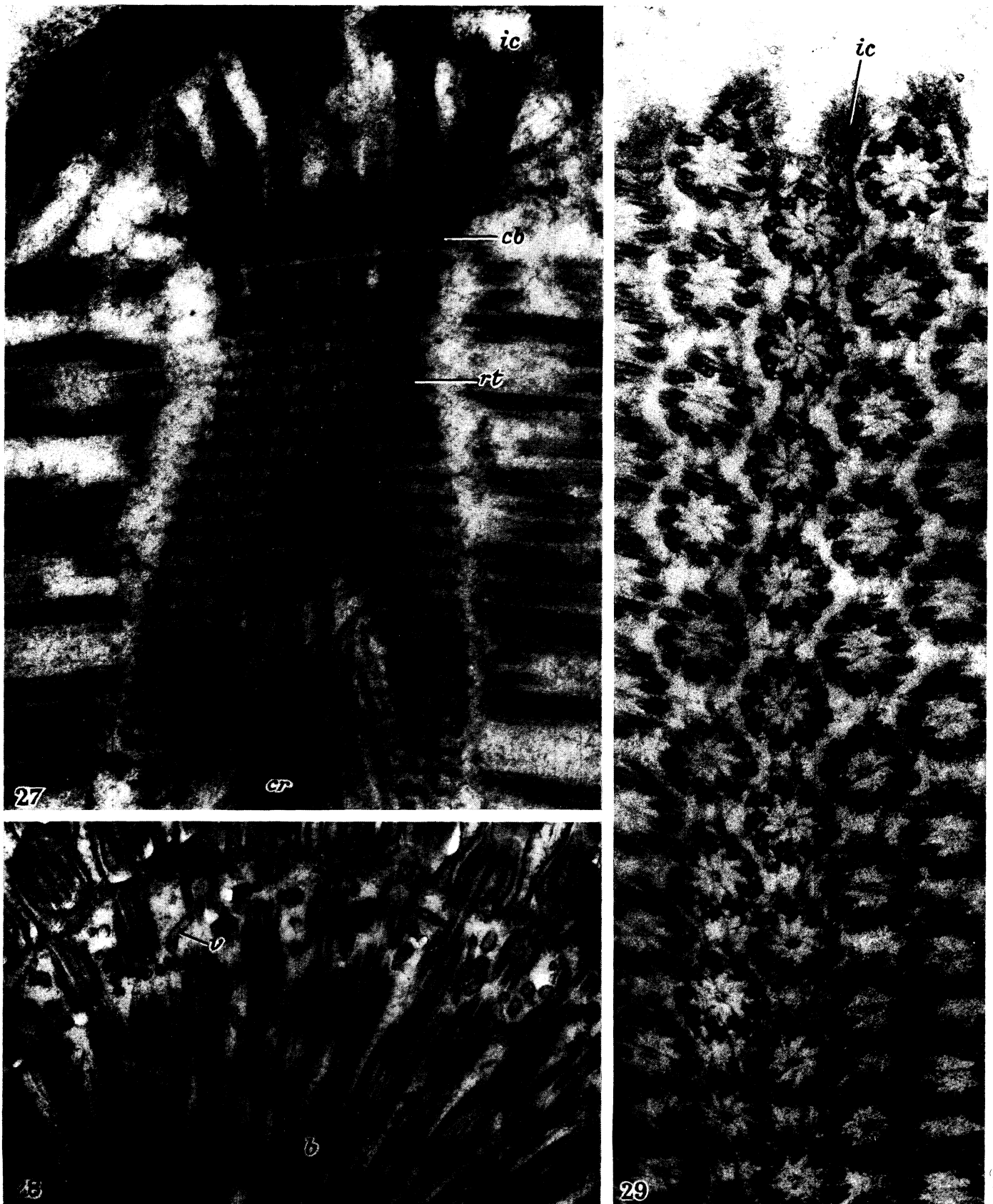


FIGURE 27. Longitudinal section of an organism from *Cryptocercus*, showing the anterior end of the rostral tube, which is continuous with one of the crescentic bodies. Note the complex striation of the latter, which is distinct from that of the rostral tube and extends faintly into the inner cap. At the bottom of the picture the fibres connecting the centriolar rod to the rostral tube can be seen ($\times 94\,000$).

FIGURE 28. Section showing the tubules and vesicles in the cytoplasm between the distal ends of the rostral basal bodies ($\times 36\,000$).

FIGURE 29. Section through five rows of basal bodies at the anterior end of the rostrum. Only in the central one are the basal bodies cut exactly transversely. The fine fibres between them are clear and the connexion of the fibres to the inner cap material at the top of the picture is evident ($\times 80\,000$).

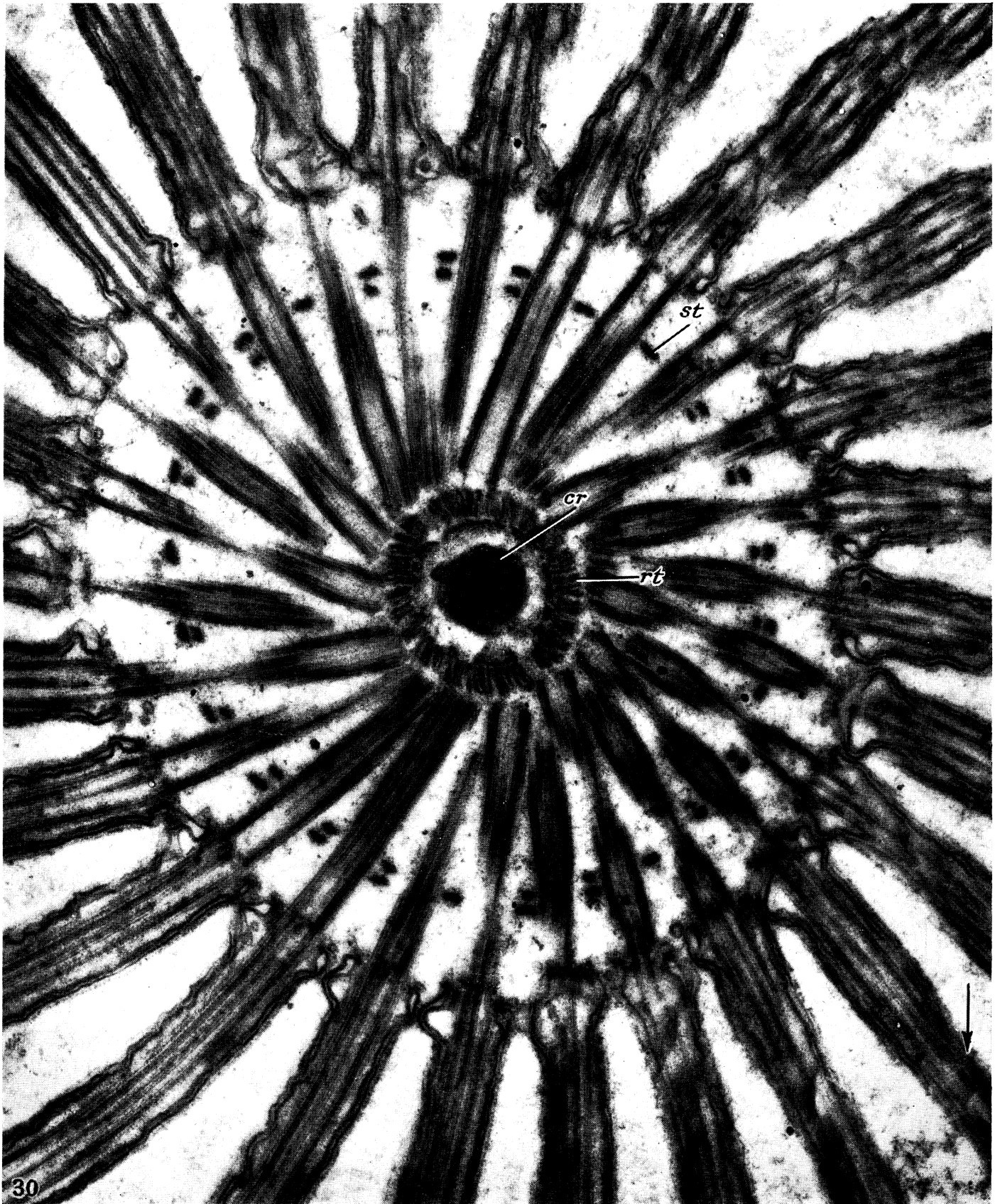
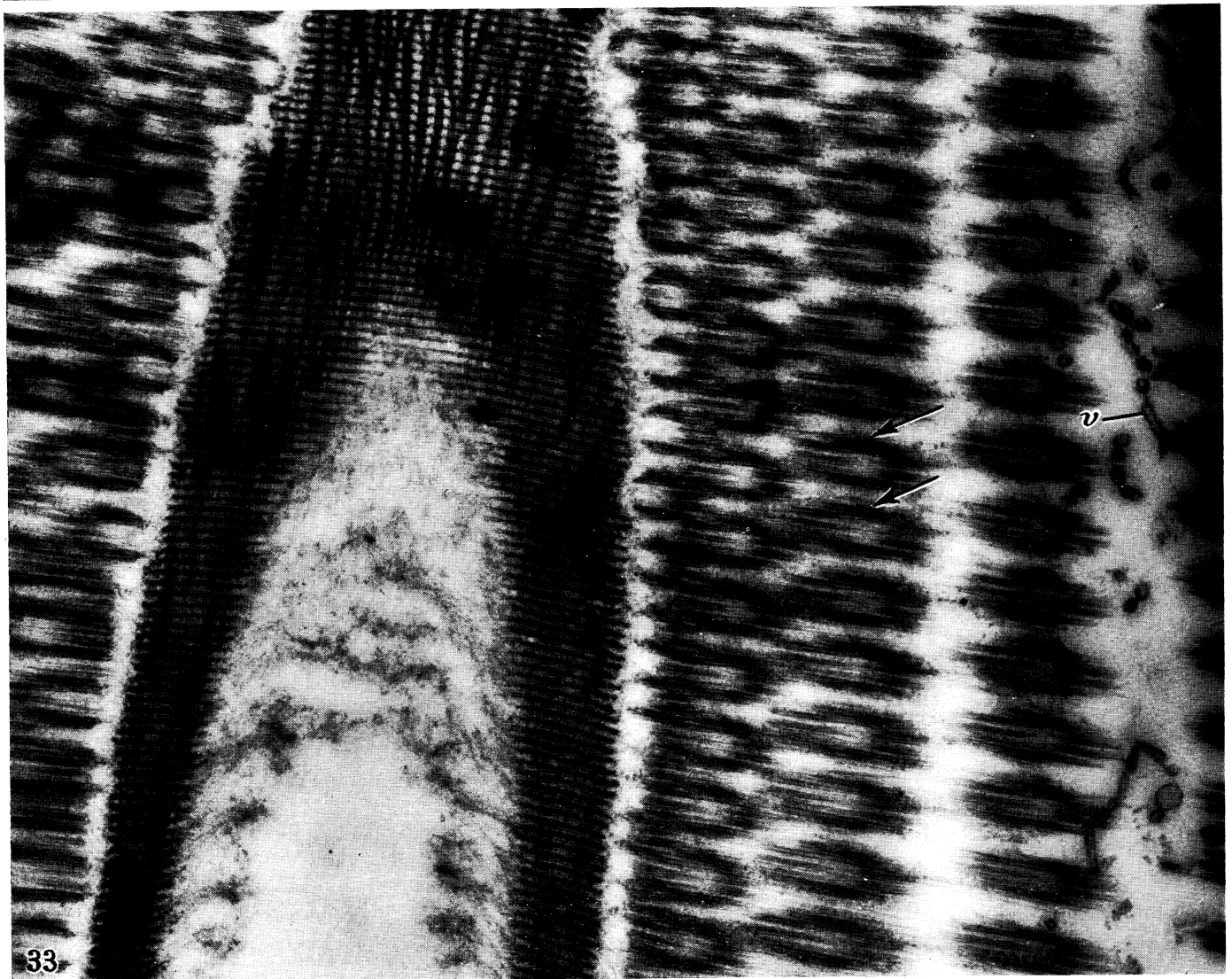
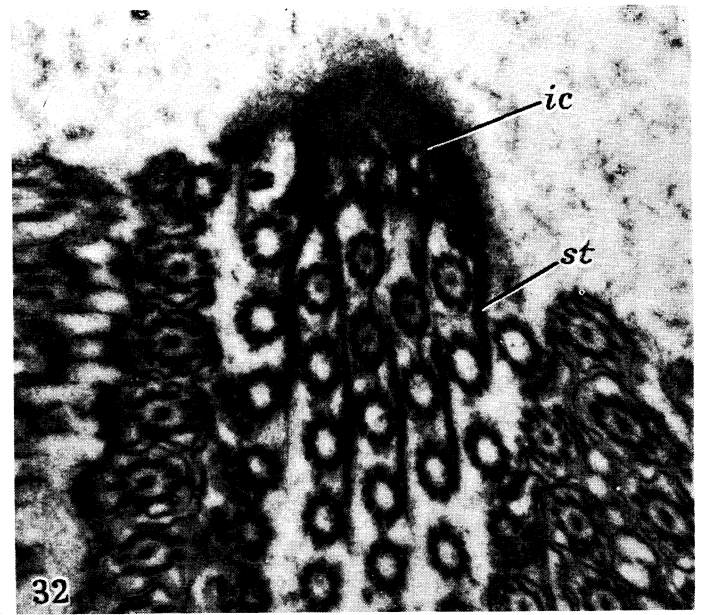
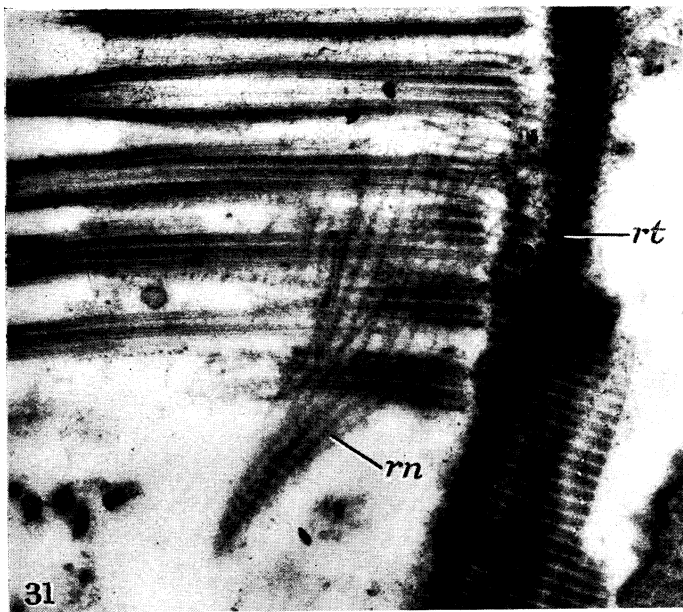


FIGURE 30. Transverse section of the rostrum of an organism from *Cryptocercus*, to show particularly the circle of dense strands running between the basal bodies of the flagella. These are the strands which originate from the margin of the inner cap (compare figure 32). One of the flagella (arrow) shows a prominent striation along one edge. The lumen of the rostral tube is largely occupied by the centriolar rod ($\times 44\,000$).



DESCRIPTION OF PLATE 31

FIGURE 31. Longitudinal section showing the wall of the rostral tube near its base, and one of the radial nets in surface view. The transverse and longitudinal fibres of which the net is composed can be seen ($\times 36\,000$).

FIGURE 32. Longitudinal section of an organism from *Cryptocercus*, showing the inner cap and the strands which arise from its margin ($\times 29\,000$).

FIGURE 33. Longitudinal section showing the striated, bifurcating lamellae making up the wall of the rostral tube. The proximal ends of the basal bodies appear to be connected to the outer surface of the tube by fine fibres. Note the striations along the anterior margins of some of the basal bodies (arrows). Peripherally some of the vesicles and tubules running between the rostral basal bodies can be seen ($\times 36\,000$).

DESCRIPTION OF PLATE 32

FIGURE 34. Low-magnification micrograph of a section of an organism from *Cryptocercus*, passing longitudinally through the rostrum and its junction with the post-rostral region. Note the very regular rows of basal bodies and flagella, the latter running in grooves in the cell surface. Part of the outer cap is visible at the top, right. The strands of material which originate at the margin of the inner cap can be seen passing between the rows of basal bodies ($\times 20\ 000$).



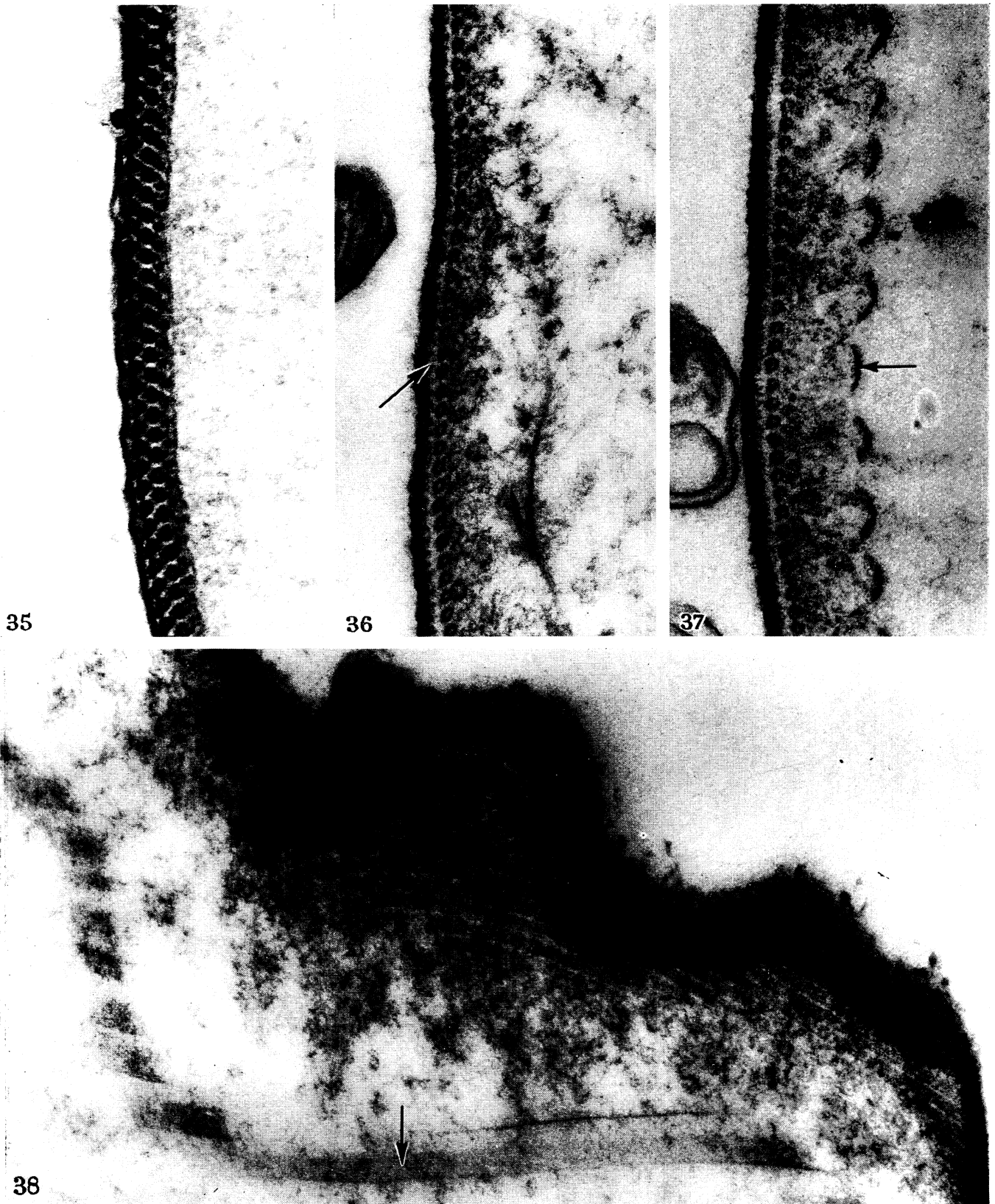


plate 26). A constant feature of the inner cap is the presence of radiating fibres passing through its substance and out into the cytoplasm above (figure 10). These are visible in transverse sections through the upper parts of the inner cap, where they appear as thin bands running through lighter spaces (figure 15).

The account of the *centrioles* to be given here is in some respects at variance with that of Cleveland (1949*a*, 1960*a*), based on light-microscope studies. According to Cleveland, as already noted, in the non-dividing cell there are two granules at the anterior end of the rostral tube, to one of which a rod is attached. These constitute the short and long centrioles (see figure 2). The appearance of the centrioles as it can be seen in living organisms is shown in the photomicrographs published by Cleveland (1960*b*); figure 6, plate 22, in this paper is comparable.

The only structures which can be identified in the electron micrographs as Cleveland's short centriole and the expanded end of the long one are shown in figures 9, 10 and 11, plate 24, and figure 18, plate 26. They are situated immediately above the anterior end of the rostral tube, embedded in the lower surface of the inner cap, and are crescent-shaped in transverse section (figure 18). Kirby (1944), who first described these structures, called them *crescentic bodies* and this term will be used here. There are two crescentic bodies, each of which lies immediately over one of the half rostral tubes. The substance of the crescentic bodies is continuous with that of the rostral tube (figure 10), to which they remain attached in fragmented preparations (figure 6). They extend laterally almost to the margin of the inner cap, so that they are wider than the anterior end of the rostral tube. In cross section they are approximately rectangular and appear dense and prominently cross-striated (figures 10 and 11). The striation is highly complex and different from that of the rostral tube, being made up of many alternating light and dark bands, of varying thickness and spacing, in which no clear repeating pattern can be made out (figure 27, plate 29). The striation extends above the crescentic bodies into the lower part of the inner cap, in the form of faint bands. Posteriorly the striation merges with that of the rostral tube. The detailed form of the banding apparently varies from one species to another. Longitudinal sections show occasional discontinuities in the substances of the crescentic bodies (figure 27). There is no evidence that the two bodies are connected to each other, though their lateral margins may be in fairly close contact.

The *centriolar rod* (that is, Cleveland's long centriole) is shown in phase contrast in figure 6, plate 22. It lies in the anterior end of the rostral tube and is about 2 μm long,

DESCRIPTION OF PLATE 33

FIGURE 35. Typical appearance of the outer cap in species of *Trichonympha* from *Zootermopsis*, consisting chiefly of two rows of tubules, here cut transversely. Note that the tubules have small lateral projections ($\times 100\,000$).

FIGURE 36. Outer cap of an organism from *Cryptocercus* with a distinct annular layer (arrow), a single layer of tubules, and an irregular fibrous layer ($\times 100\,000$).

FIGURE 37. Similar to figure 36, but showing more clearly the row of curved ribbons (arrow) ($\times 100\,000$).

FIGURE 38. Tangential section of outer cap of an organism from *Cryptocercus*, showing the honeycomb appearance of the annular layer in surface view, and also the ribbons (arrow) ($\times 76\,000$).

slightly tapered anteriorly and with a wide posterior end. Electron micrographs show that its anterior tip lies immediately below the inner cap, to which it appears to be connected by irregular fibrous strands (figures 9 and 10, plate 24). There is no evidence of its attachment to either of the crescentic bodies. The cross-sectional shape of the rod conforms approximately to that of the lumen of the rostral tube: that is, it is somewhat flattened anteriorly and more or less cylindrical further back (see figures 19, 20 and 21, plate 27). It consists of a compact central part surrounded by less dense fibrous or spongy peripheral material. The central region always contains a number of extremely dense, longitudinally oriented lamellae, about 200 Å thick, set in a less dense matrix. In the species of *Trichonympha* from *Zootermopsis* there may be about twelve of these lamellae, arranged parallel to each other in a single row (figure 19, plate 27). In species from *Cryptocercus* the lamellae usually present an irregularly whorled arrangement (figure 8, plate 23). The peripheral material of the rod somewhat resembles that of the inner cap in texture. It flares out posteriorly to form the wide, funnel-shaped end of the rod (figure 10, plate 24) and strands of it extend for a considerable distance down the rostral tube (figures 19 and 22, plate 27; figure 23, plate 28). As with the inner cap, fine fibres extend from it, to connect, in this case, with the inner surface layer of the rostral tube (figure 10; figures 21 and 22, plate 27; figure 33, plate 31). These fibres are about 150 Å in diameter and cross-banded, with a periodicity of about 300 Å. Longitudinal sections show that they are regularly arranged, sloping forwards around the anterior half of the centriolar rod, and backwards posterior to this. They connect precisely with the fibres forming the inner surface layer of the rostral tube (figure 10, plate 24). The fibres occur not only at the level of the main body of the rod but also posterior to it, arising from the backwardly extending strands of peripheral material (figure 12, plate 25).

None of the structures so far described is in any way similar in fine structure to the characteristic type of centriole described in other cells, which resembles a basal body. It was reported previously, however, that such a typical centriole does occur in *Trichonympha*, embedded in the inner cap (Grimstone 1961). The original identification of this structure was based on a small number of micrographs, which permitted only a tentative description. Examination of large numbers of serial sections in the present study has confirmed the original observation but has shown that the situation is more complex than originally supposed. Figure 16, plate 26, shows a transverse section through the inner cap. The latter is surrounded by homogeneous cytoplasm and the section is therefore cut above the level of the basal bodies of the rostral flagella (compare with figure 9, plate 24). In the inner cap in this section there is a structure resembling a basal body. Examination of similar sections confirms the constant presence of this organelle but provides no evidence that it bears a flagellum, and it is therefore thought to be a typical *centriole*. In addition to this, however, there is a second basal body in the inner cap, located nearer the lower surface and lying between the two crescentic bodies (figure 11, plate 24). This has all the typical fine-structural features of a basal body, including triplet outer fibres and a 'cartwheel' (see Gibbons & Grimstone 1960), and serial sections show that it bears a typical flagellum. This basal body differs from the others of the rostrum, therefore, only in its unusual location. No other flagellum is found so far forward as this, and no other has its basal body extending into the inner cap. It will therefore be called the *anterior flagellum*. Its significance is not

known (but see Discussion). Figure 17, plate 26 shows an oblique section through the inner cap which includes both the anterior flagellum and the centriole.

The centriole and anterior flagellum are, of course, extremely small in comparison with the volume of the whole cell and it is difficult to be certain that the description of their fine structure given here is correct in all respects. The account is consistent, however, with all observations made so far.

The remaining elements to be described in connexion with the centriolar apparatus are two, dense, cross-striated ribbon-shaped structures, curved in section, which extend radially from between the crescentic bodies to the distal ends of the neighbouring rostral basal bodies (figure 11, plate 24; figure 18, plate 26). These are distinct from the radial nets, to be described below, but run in the same plane and, like them, appear to arise from the inner surface layer of the rostral tube. Figure 11 shows that one of the striated ribbons runs close to the basal body of the anterior flagellum, to which it is apparently connected by a dense fibre.

Radial nets

The formation of the rostral tube from two half tubes gives the rostrum a plane of bilateral symmetry. The anterior flagellum and the striated ribbons, just described, both lie along this plane, and so do the two structures which Pitelka & Schooley (1958) termed radial membranes. These arise from the junctions between the halves of the rostral tube and extend outwards between the basal bodies of the rostral flagella (figure 21, plate 27; figures 24 and 25, plate 28). They mark the plane along which the two halves of the rostrum separate at division. Anteriorly they extend out as far as the distal ends of the basal bodies, but towards the base of the rostrum they do not reach as far. They do not appear to extend posterior to the rostrum. The term 'radial membrane' applied to these structures by Pitelka & Schooley is inappropriate, since they are not continuous membranes but consist of a lattice made up of two sheets of fine fibres, in one of which the fibres run longitudinally and in the other transversely (figure 31, plate 31; figure 25, plate 28). For this reason the term *radial net* is preferred here. The nets arise from the inner surface layers of the rostral tubes, one being associated with each half tube (figures 24 and 25, plate 28), and the transverse fibres of the nets are probably continuous with those making up the surface layers.

Rostral flagella

The arrangement and fine structure of the rostral flagella have been described previously in detail (Gibbons & Grimstone 1960) and only certain new information will be included here.

As previously described, the proximal regions of the basal bodies in the rostrum are connected to each other by elaborate, delicate systems of fine fibres, of asymmetrical but highly constant form (figure 29, plate 29). Each basal body is connected by these to its closest neighbours in the same and in the two adjacent rows. There are many such sets of fibres associated with each basal body, evenly spaced along its proximal region and all apparently identical and with the same orientation. They account for the denser appearance, in low-power micrographs, of the ground substance between the proximal ends of the basal bodies. The present study has shown that the fibres are present in all species

of *Trichonympha* examined and that they are always similarly arranged. It has also revealed that the fibres associated with the most anterior basal bodies are intimately connected with the lower surface of the inner cap: figure 29 shows that fibres and inner cap merge almost imperceptibly into each other. It appears, therefore, that all the rostral basal bodies are connected to each other, and ultimately to the inner cap. The functional significance of this arrangement is considered in the Discussion.

In species of *Trichonympha* from *Cryptocercus* there also arise from the margin of the inner cap a number of long, rather thick strands of finely fibrous material which run backwards in the gaps between the rows of basal bodies (figure 32, plate 31; figure 7, plate 22). These strands are about 500 Å in diameter and there may be either one or two in each gap. All of them are situated at about the same distance from the rostral tube, so that they form a circle in transverse sections (figure 30, plate 30). They extend into the post-rostral region (figure 34, plate 32) but their ultimate fate has not been traced. No such strands are present in the species from *Zootermopsis*. Since the species from *Cryptocercus* possess a nuclear sleeve (see Cleveland, 1949*a*), while those from *Zootermopsis* do not, it is possible that the fibrous strands represent the anterior part of the nuclear sleeve.

The proximal ends of the basal bodies of the rostral flagella in all species appear to be connected to the outer surface of the rostral tube by delicate fibres (figure 33, plate 31).

A feature of the flagellar apparatus which has been newly observed is the frequent presence of small tubules lying in the cytoplasm around the distal ends of the basal bodies (figure 28, plate 29; figure 33, plate 31). The tubules are bounded by unit membranes and are about 500 Å in diameter, sometimes scattered through the cytoplasm near the ends of the basal bodies but more often localized around the junctions of the basal bodies and the flagella, close to the point of origin of the flagellar membranes. In their size and general appearance these tubules are reminiscent of the sarcoplasmic reticulum of striated muscle fibres.

No new structures have been detected in the flagella themselves, with the exception of a distinct striation sometimes seen in longitudinal sections, apparently running along one side of the cylinder of outer fibres in both flagella and basal bodies (figure 30, plate 30; figure 33, plate 31). The precise location of this striation has not been determined, but it is clearly distinct from and more prominent than the faint cross-banding, previously described in the outer fibres themselves (Gibbons & Grimstone 1960).

It may be noted at this point that the so-called 'circular fissure', which Kirby (1944) and subsequent workers believed to be a deep cleft at the junction of the rostrum with the rest of the body, extending inwards from the cell surface to the rostral tube, is in fact merely a gap, of varying extent and filled with optically empty cytoplasm, between the rostral and post-rostral flagella and basal bodies. This may become prominent when the rostral flagella are directed anteriorly, or when the rostrum is bent to one side, but the electron micrographs show quite clearly that there is no invagination of the cell membrane at this point (figure 13, plate 25; figure 34, plate 32).

Outer cap

The outer cap is a substantial, refractile, dome-shaped structure, very readily visible in living organisms (figure 5, plate 22). The flagella at the anterior end of the rostrum emerge from under its rim, which is drawn out into small projections extending over the

ridges separating the grooves in which the bases of the flagella lie. The appearance of the cap in a low-magnification electron micrograph is shown in figure 7, plate 22.

In the species of *Trichonympha* from *Zootermopsis* the structure of the cap is relatively simple. It consists of an outer membrane, continuous with the plasma membrane of the rest of the cell, beneath which are two layers of tubules (figure 35, plate 33). The latter are circular in cross-section, about 250 Å in diameter, and sometimes have small lateral projections. The tubules in each layer are evenly spaced and run parallel to each other. Contrary to the findings of Pitelka & Schooley (1958), the tubules in the two layers appear to run in the same direction. Their orientation with respect to the cap as a whole has not been determined. Below the tubules, separating them from the contents of the cap, there is a tenuous membrane.

The caps of the species from *Cryptocercus* are more complex. There is an outer plasma membrane, as in the species just described, and below this there is a layer which appears in tangential sections as an array of hexagonally packed annuli (figure 38, plate 33). The annuli are about 250 Å in diameter and in sections normal to the cap their walls appear as a single row of short dark lines running perpendicular to the surface (figure 36, plate 33). Below this there is a single row of tubules, similar to those in the species from *Zootermopsis* (figures 36 and 37, plate 33). The tubules run parallel to each other over considerable areas of the cap, but there are indications that tubules running in one direction may abut onto others running in a different one. Below them there is a thick layer with a rather irregularly fibrous texture (figure 7, plate 22), and below this again there is usually a series of long ribbons, curved in section, which run parallel to each other and to the surface of the cap (figures 37 and 38). (These are apparently not present in all individuals (figure 7).) There may, therefore, be as many as five distinct layers in the cap of the species from *Cryptocercus*, of which only the tubules and the plasma membrane are also present in the species from *Zootermopsis*.

The tubules of the outer cap are similar in dimensions and appearance to the supposedly contractile tubules found in a variety of organelles in protozoa (see Grimstone 1961; Grimstone & Cleveland 1965). There is, however, no evidence that the cap of *Trichonympha* can change shape. Its function is usually thought to be protective and at present no explanation can be given of its elaborate and variable ultrastructure.

The characteristic texture of the material below the cap is shown in figure 7, plate 22. Apart from the fibres radiating from the inner cap it is usually homogeneous and amorphous.

PSEUDOTRICHONYMPHA

General morphology and division

The most recent accounts of the structure of *Pseudotriconympha* as it can be seen in the light microscope are those of Grassé & Hollande (1951) and Grassé (1952). Compared with *Trichonympha*, this organism is rather imperfectly known. In shape and superficial appearance most members of the genus are somewhat similar to the large species of *Trichonympha* and, like them, are covered with longitudinal rows of flagella (figure 39). These extend over almost the whole cell surface, however, leaving only a very small non-flagellated area at the posterior end. The species studied in the present work is about

350 μm long. The rostrum bears the same number of rows of flagella as the rest of the body and there is no sharp discontinuity between the two; it is distinguished morphologically only by the presence of a rostral tube. An outer cap is present, as in *Trichonympha*, and

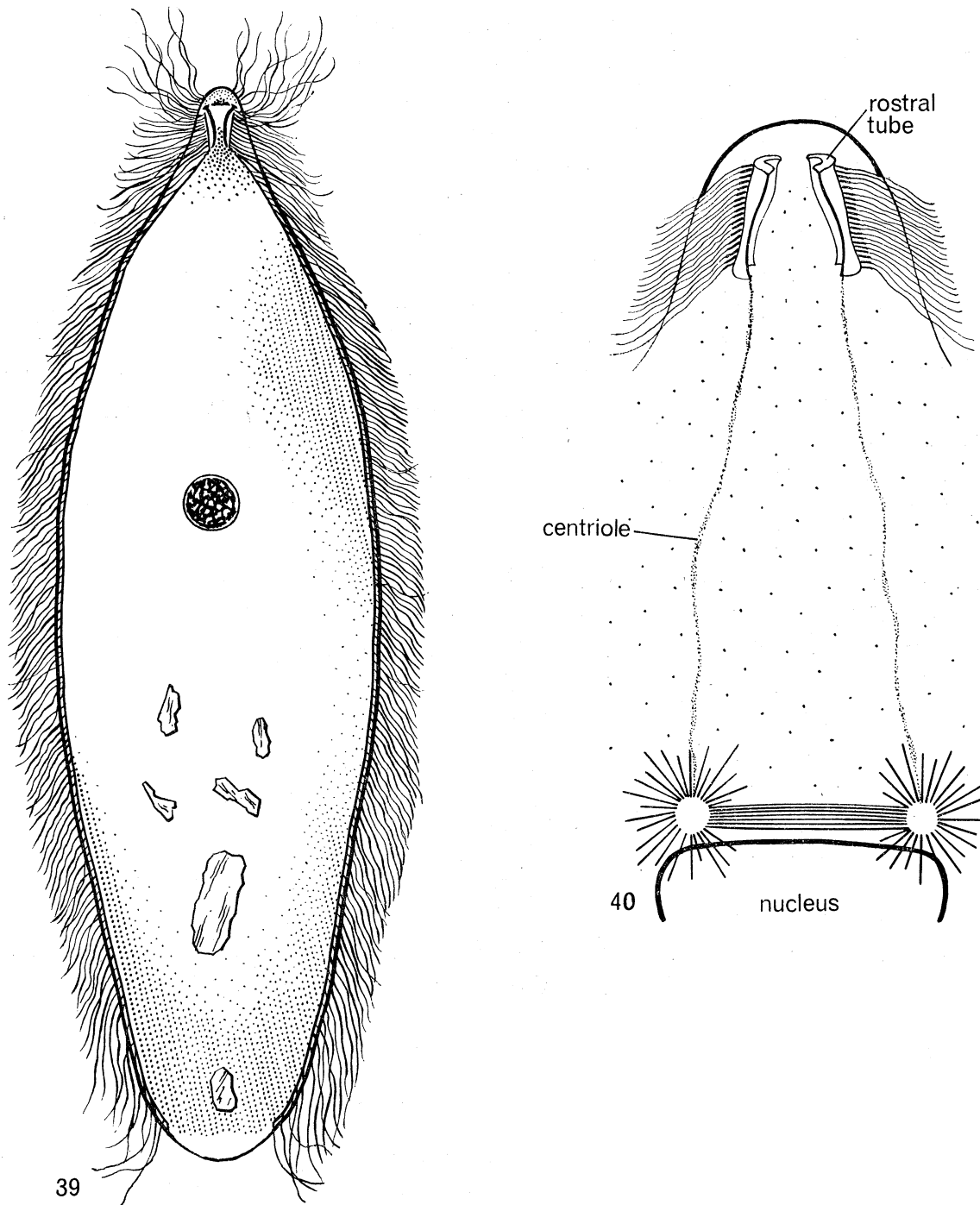


FIGURE 39. Diagram of *Pseudotriconympha*.

FIGURE 40. Diagram showing the formation of the mitotic apparatus in *Pseudotriconympha*. Spindle fibres develop around the posterior ends of the centrioles, which grow out at division from the base of the rostral tube. The two halves of the latter have separated. (Modified after Cleveland (1935).)

there is also an inner cap. There is no centriolar rod in the non-dividing cell but two small supposed 'centrioles' have been described by some authors at the anterior end of the rostral tube. Parabasal bodies are absent and are replaced by numerous dictyosomes scattered throughout the cell.

Division in *Pseudotriconympha* has been described by Cleveland (1935, 1957) and by Grassé & Hollande (1951). According to their accounts two long centrioles grow out posteriorly from the base of the rostral tube. An aster develops around the distal end of each, as in *Triconympha*, and a small spindle is formed by the overlapping and fusion of astral rays. The spindle increases progressively in length and thickness as the asters develop. The long centrioles continue to elongate until the spindle reaches the nucleus (figure 40); they may ultimately attain a length of about 50 μm . The details of mitosis are not relevant here, but as in *Triconympha* the rostrum eventually splits longitudinally and is shared, together with the other cytoplasmic organelles, between the two daughter cells. The spindle and the long centrioles break down following division.

Rostral tube and contents

The rostral tube differs markedly from that of *Triconympha*. Its fine structure, in particular, is extremely elaborate and a greater number of distinct morphological components is present than in *Triconympha*. Comparison of figure 41, plate 34, with figure 9, plate 24, which is at approximately the same magnification, gives a good impression of the size and shape of the tube in the two genera.

The rostral tube in *Pseudotriconympha* is about 10 μm long and circular in cross-section, with thick walls. It is made up of two similar half tubes. Externally the diameter increases uniformly from about 3 μm at the base to 6 μm anteriorly. Internally, however, the posterior two-thirds are more or less uniform in diameter, while the anterior end is expanded into a broad funnel, with a prominent thickened rim (figure 41, plate 34). The difference between the internal and external shape is accounted for by variation in the thickness of the walls. The two halves of the tube do not usually quite meet but are separated by small gaps (figure 49, plate 42). They are, however, precisely aligned with respect to each other, and throughout its length the tube as a whole is more nearly circular in cross-section than that of *Triconympha*.

The wall of the tube is made up of two distinct layers, differing markedly in fine structure, which will be termed the *inner* and *outer walls* (figure 41).

The *inner wall* has a fairly uniform thickness of about 0.2 μm , except at the extreme anterior end where it widens to form the rim of the tube. The rim has a rounded margin (figure 42, plate 35). In longitudinal sections, the inner wall has a very striking periodic structure, consisting of alternating light and dark layers arranged with great regularity along the whole length of the tube, and with a constant periodicity of about 220 Å (figure 43, plate 36). The light and dark layers apparently extend uninterruptedly around each half tube, and for the most part they appear to be quite homogeneous, in both longitudinal and transverse sections (figure 43; figure 44, plate 37; figure 50, plate 43). In the rim, however, a well-defined radial structure is visible in both transverse (figure 47, plate 40) and tangential longitudinal sections (figure 55, plate 48).

The *outer wall* of the tube is for the most part thicker than the inner and in transverse

sections appears to be made up of a large number of longitudinal, radially arranged lamellae (figure 50, plate 43; figure 51, plate 44). In this respect it resembles the whole rostral tube of *Trichonympha*. The lamellae are relatively thin and appear striated in transverse sections, with a radial periodicity of about 350 Å. There are faint indications of material with a similar periodicity in the gaps between the lamellae. Towards the base of the tube the lamellae may be curved about a longitudinal axis (figure 51), but elsewhere they are usually more or less straight. The lamellae are most numerous in the anterior part of the tube, where there may be up to 100 in each half tube. Lamellae may bifurcate (figure 53, plate 46). In longitudinal sections cut perpendicular to the plane of the lamellae the latter appear as continuous, cross-striated structures (figure 53). The striation is complex, with a number of bands in each segment, and has a major periodicity of 440 Å. A quite different appearance is seen in longitudinal sections cut parallel to the face of the lamellae (figure 43, plate 36). Here there is also a well-defined periodic structure, but made up of a repeating pattern of short lines and dots arranged in double rectangular array. The lines and dots appear to be joined to their nearest neighbours by fine fibres. The pattern is extremely regular and is shown diagrammatically in figure 57. The clarity with which either of the repeating patterns can be seen depends on the orientation of the section. Partial or complete transitions from one to the other can be found (figure 44, plate 37; figure 53, plate 46). It is notable that the repetitive structures of the outer and inner walls occur in register, the line elements of the former lying exactly opposite alternate dense layers of the latter (figure 43, plate 36). The inner and outer walls, therefore, although markedly different in ultrastructure, display a common fundamental periodicity in their organization. This periodicity is also manifest in the structures which occur on the outer and inner surfaces of the tube, now to be described.

EXPLANATION OF PLATES 34 TO 48

PSEUDOTRICHONYMPHA

All figures are electron micrographs of sectioned material, stained with uranyl acetate.

Abbreviations

<i>b</i>	basal body	<i>os</i>	outer surface layer
<i>cbd</i>	centriolar band	<i>ow</i>	outer wall of rostral tube
<i>fr</i>	fibrous ribbon	<i>r</i>	rostral rod
<i>ic</i>	inner cap	<i>m</i>	radial net
<i>isI</i>	inner surface layer I	<i>s</i>	seam of rostral tube
<i>isII</i>	inner surface layer II	<i>t</i>	tubule (presumed spindle fibre)
<i>iw</i>	inner wall of rostral tube	<i>tl</i>	tubule-bearing layer
<i>oc</i>	outer cap	<i>v</i>	vesicle

DESCRIPTION OF PLATE 34

FIGURE 41. Low-magnification electron micrograph showing the rostral tube in longitudinal section.

Details of fine structure are shown more clearly in subsequent figures. Note the overall shapes of the rostral tube and the inner and outer caps. The rostral tube is closely surrounded by the basal bodies of the rostral flagella (the flagella themselves are damaged in this section). The posterior part of the tube and the post-rostral cytoplasm contain many dense granules. This figure may be compared with figure 9, plate 24 ($\times 10000$).



(Facing p. 230)

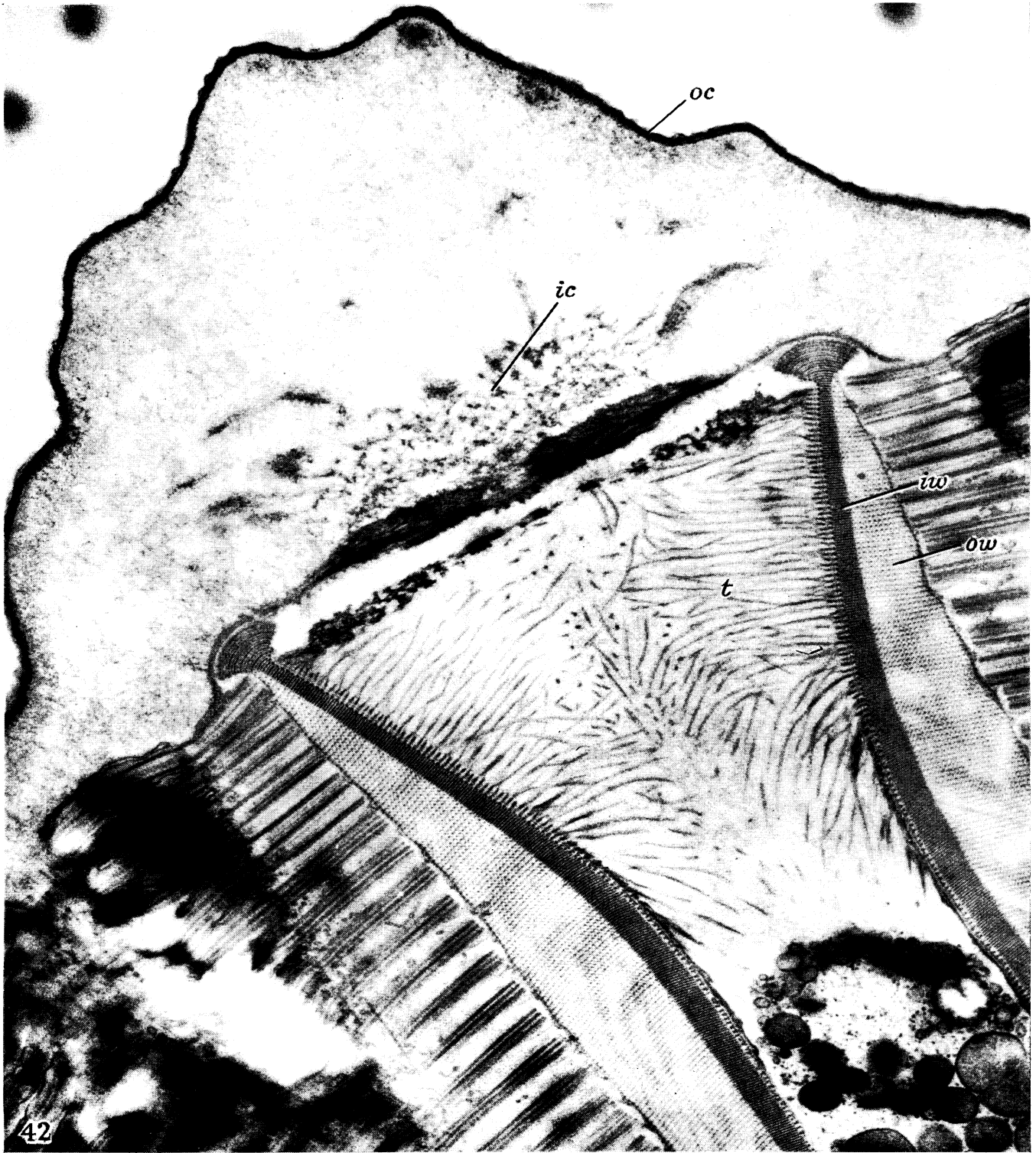


FIGURE 42. Enlargement from figure 41, showing the anterior end of the rostral tube. Note the inner and outer walls and the thickened rim. The general disposition of the inner cap and associated structures can be seen but details of fine structure are not clearly visible (see figure 43). In the lumen of the rostral tube note the restriction of the tubules to the wide anterior part ($\times 21\,000$).

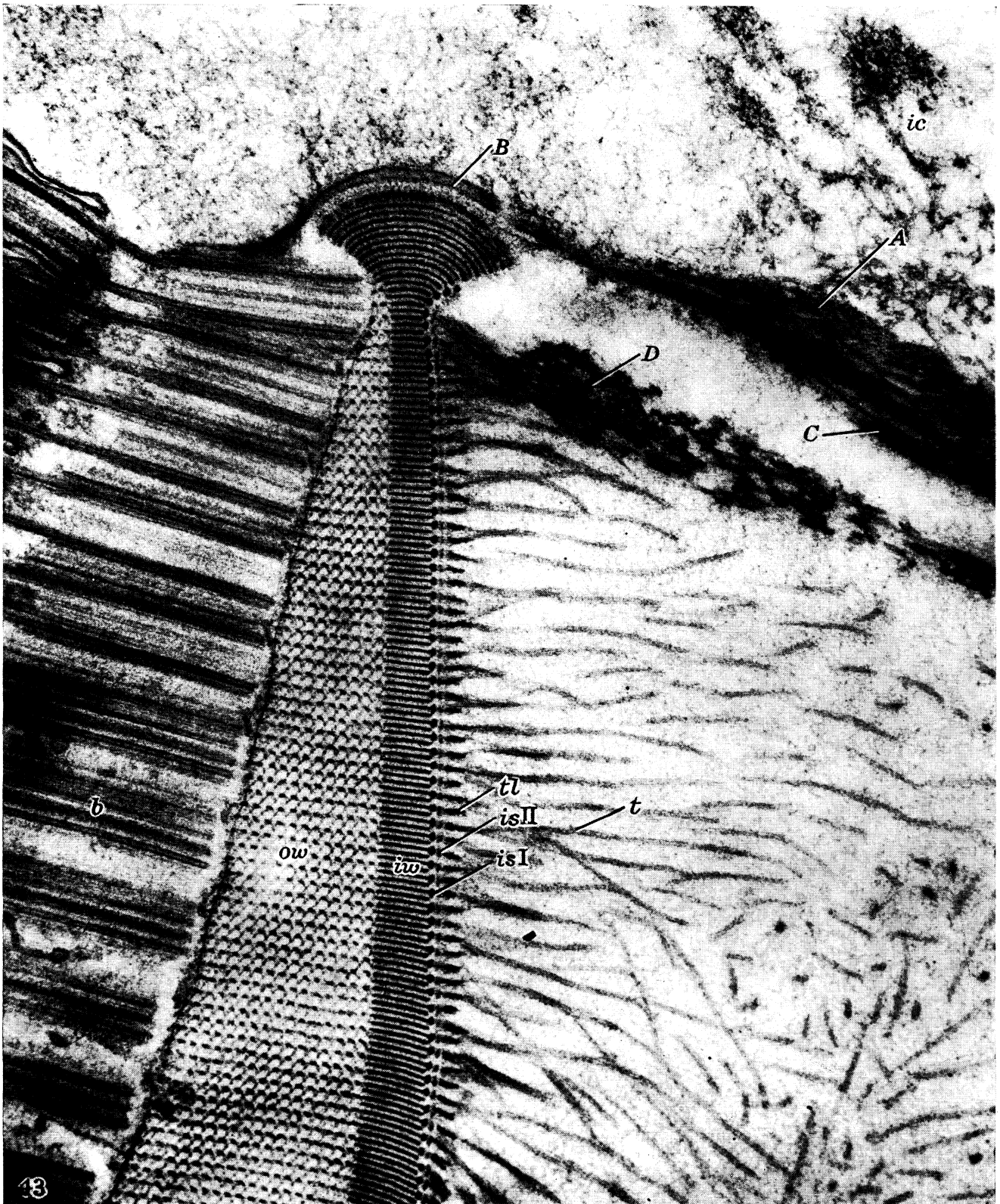
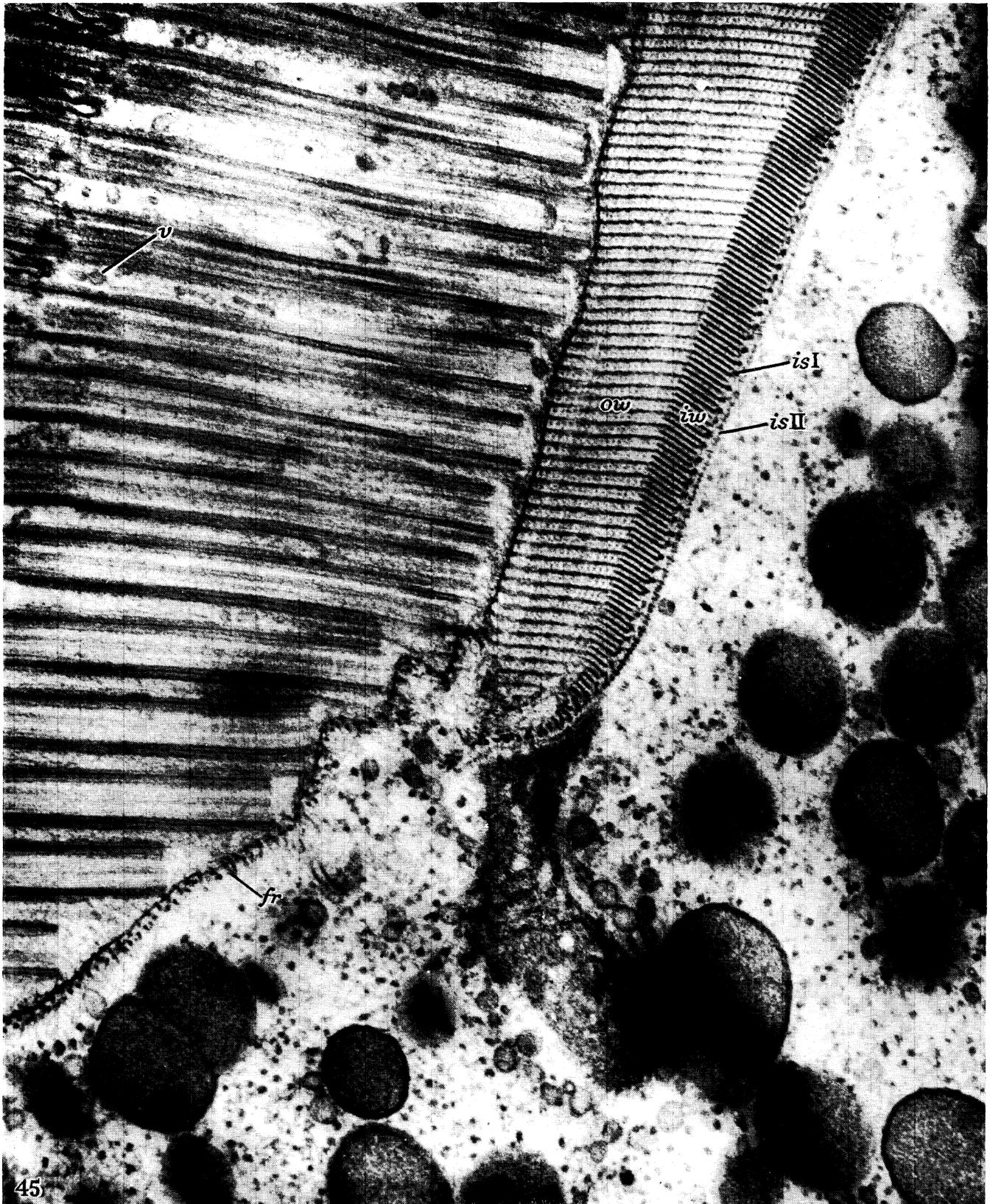


FIGURE 43. Enlargement from figure 42, showing the fine structure of the anterior end of the rostral tube. The various components of the tube and its surface layers are clearly displayed, as are the four structures (A, B, C, D) at its anterior end (compare figure 57) ($\times 55\,000$).

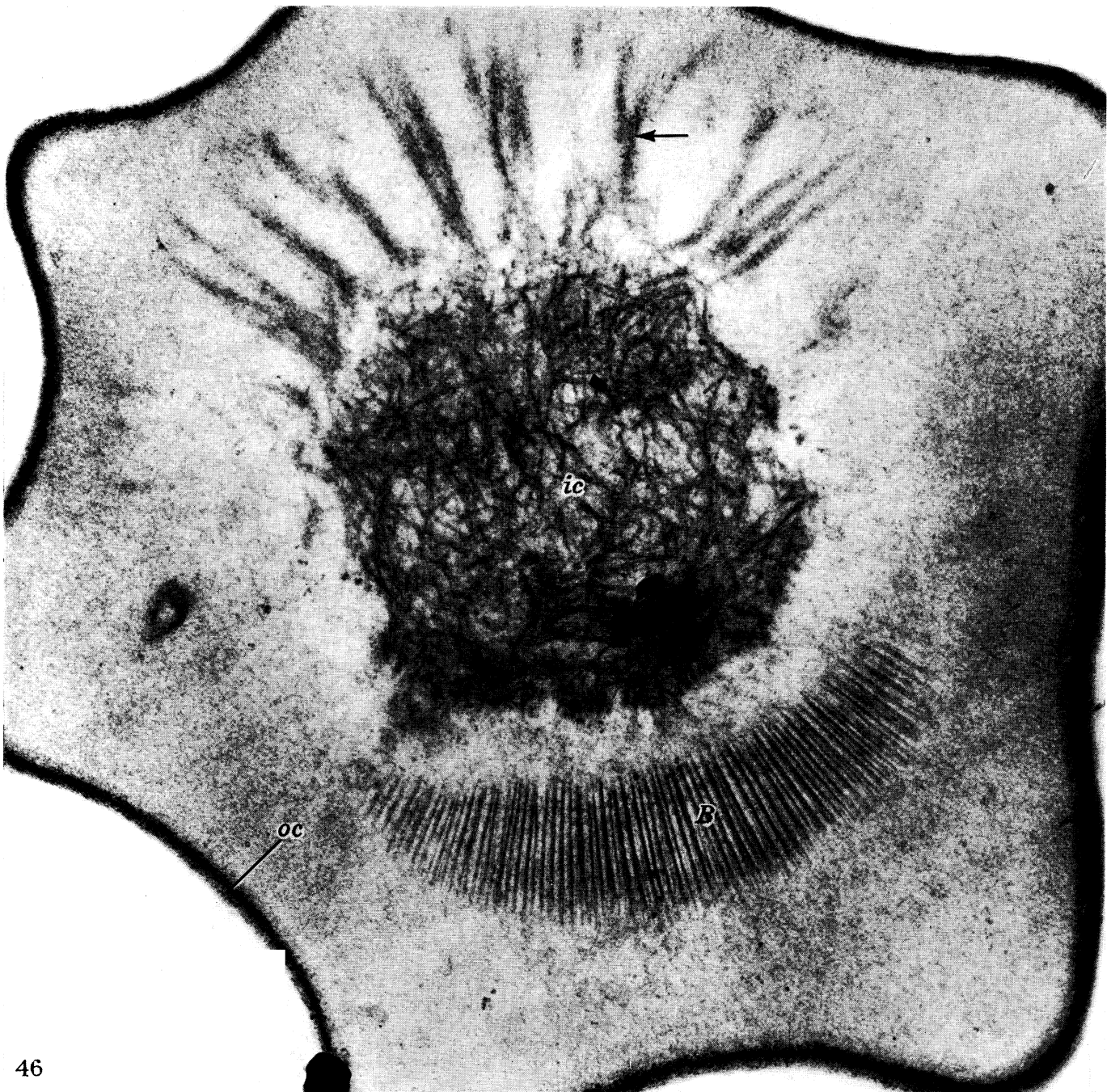


FIGURE 44. Continuation of figure 43, showing the structure of the rostral tube about halfway along its length. The micrograph shows in particular the transition of the tubule-bearing layer into the irregular strands of dense material which give rise to the centriolar bands. Inner surface layer I, made up of dense dots (representing transversely oriented fibres), becomes less well ordered posteriorly. Inner surface layer II is made up of separate elements anteriorly, apparently fusing further back into continuous fibres or membranes ($\times 55\,000$).



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FIGURE 45. Continuation of figure 44, showing the base of the rostral tube. The surface layers of the tube are clearly seen. Inner surface layer II has become a prominent cross-striated membrane, which stops at the base of the tube; the outer surface layer continues posterior to the tube in the form of striated fibrous ribbons running below the basal bodies. At the base of the rostral tube can be seen part of the annulus which connects with the centriolar bands. Note the vesicles between the distal ends of the basal bodies ($\times 55\,000$).



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FIGURES 46 to 52, PLATES 39 to 45, show a series of transverse sections of the rostrum of *Pseudotriconympha*, arranged in sequence and starting anteriorly. All the micrographs in this series, with the exception of figures 47 and 51, show sections from one organism.

FIGURE 46. Section through the tip of the rostrum, above the level of the flagella, passing through the base of the inner cap. The radiating structures at the bottom are the *B* fibres; the less well-defined ones at the top (arrow) are strands of inner cap material ($\times 25\,000$).

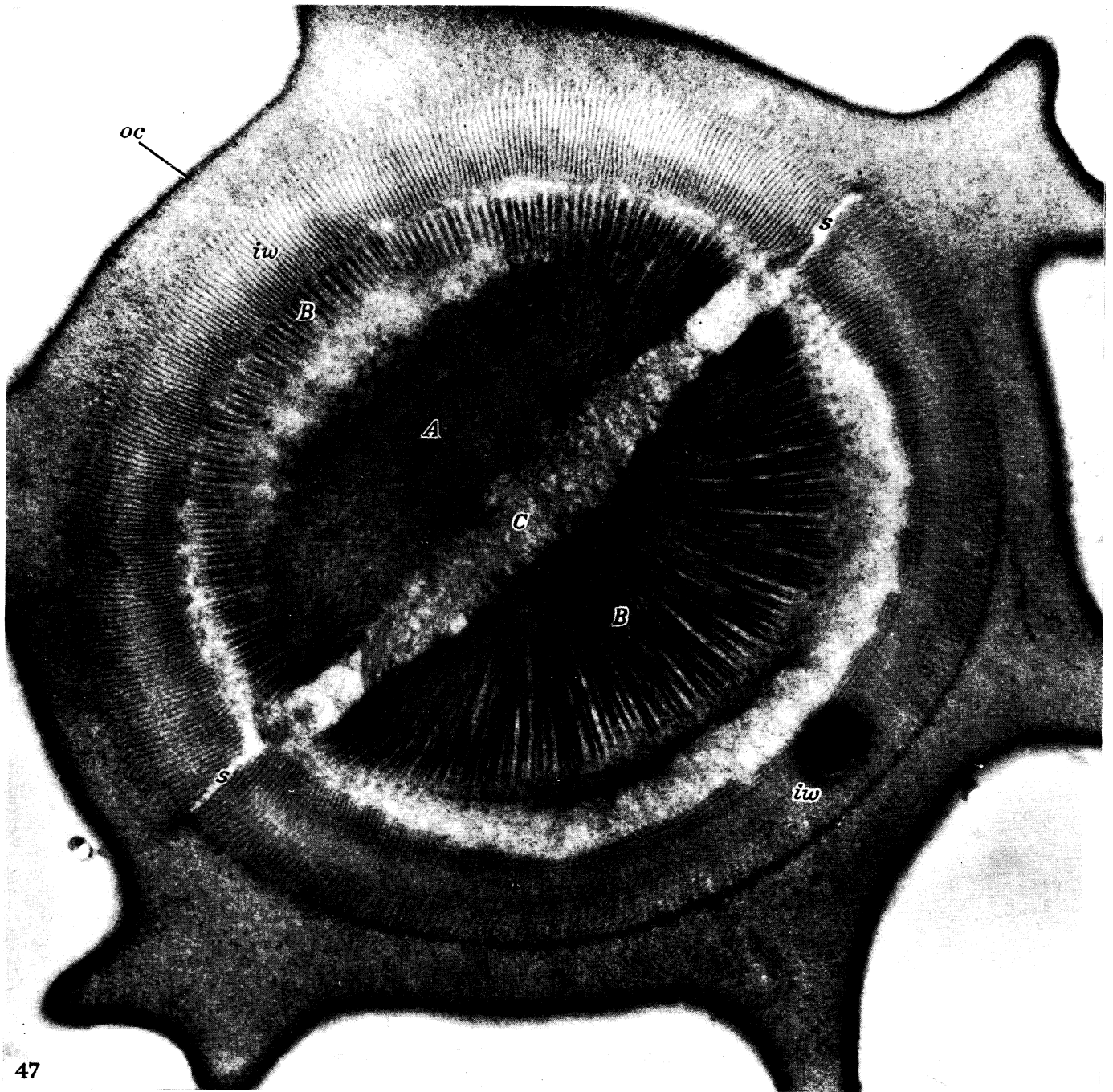


FIGURE 47. This shows three of the systems of fibres (*A*, *B* and *C*) lying under the base of the inner cap at the apex of the rostral tube. The section is slightly oblique, so that structures lying at a slightly more anterior level are seen in the upper left half of the picture than in the lower right half. Note the fine radial striations in the rim of the rostral tube ($\times 25\,000$).



FIGURE 48. The anterior end of the rostral tube, cut slightly obliquely and, like figure 47, showing more anterior structures in the upper, left half, where the section passes through the rim of the tube. In this half the system of coarse fibres (*D*) is visible. In the bottom, right half the polarized tubules (presumed spindle fibres) can be seen. The tubule-bearing layer is not distinct in this micrograph, but dense thickenings of it are present along the seams of the tube: these are the beginnings of the centriolar bands. Note the section through the rostral rod, and the two radial nets. In the rostral tube note the prominent radiating lamellae in the outer wall, and the dense, well-defined outer surface layer, made up of distinct strands ($\times 30\,000$).

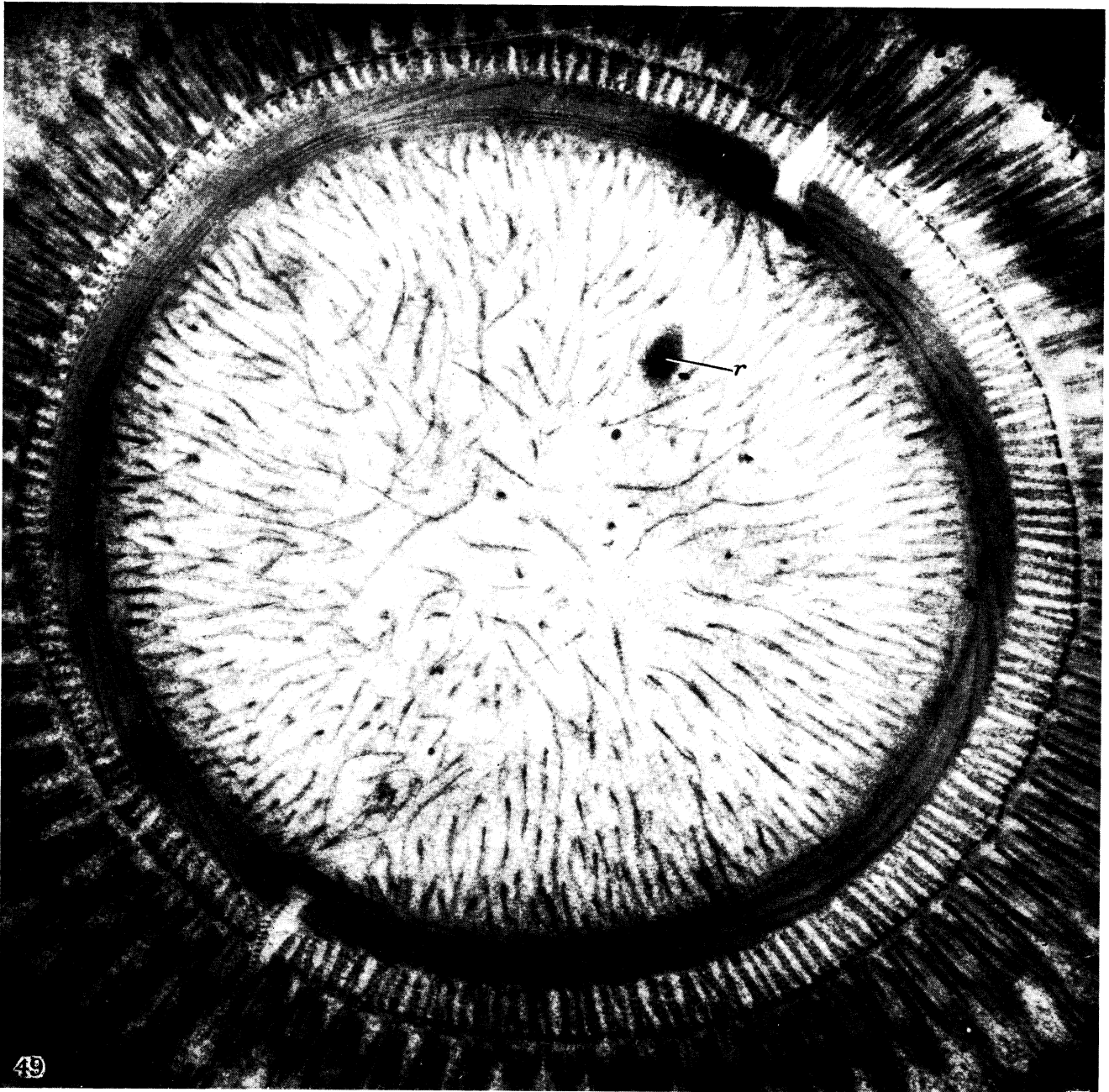


FIGURE 49. Section slightly posterior to that shown in figure 48, showing clearly the radially polarized tubules, occupying most of the lumen of the tube, the centriolar bands, rostral rod, and radial nets. Note also the material apparently connecting the proximal ends of the basal bodies to the outer surface layer ($\times 30\,000$).

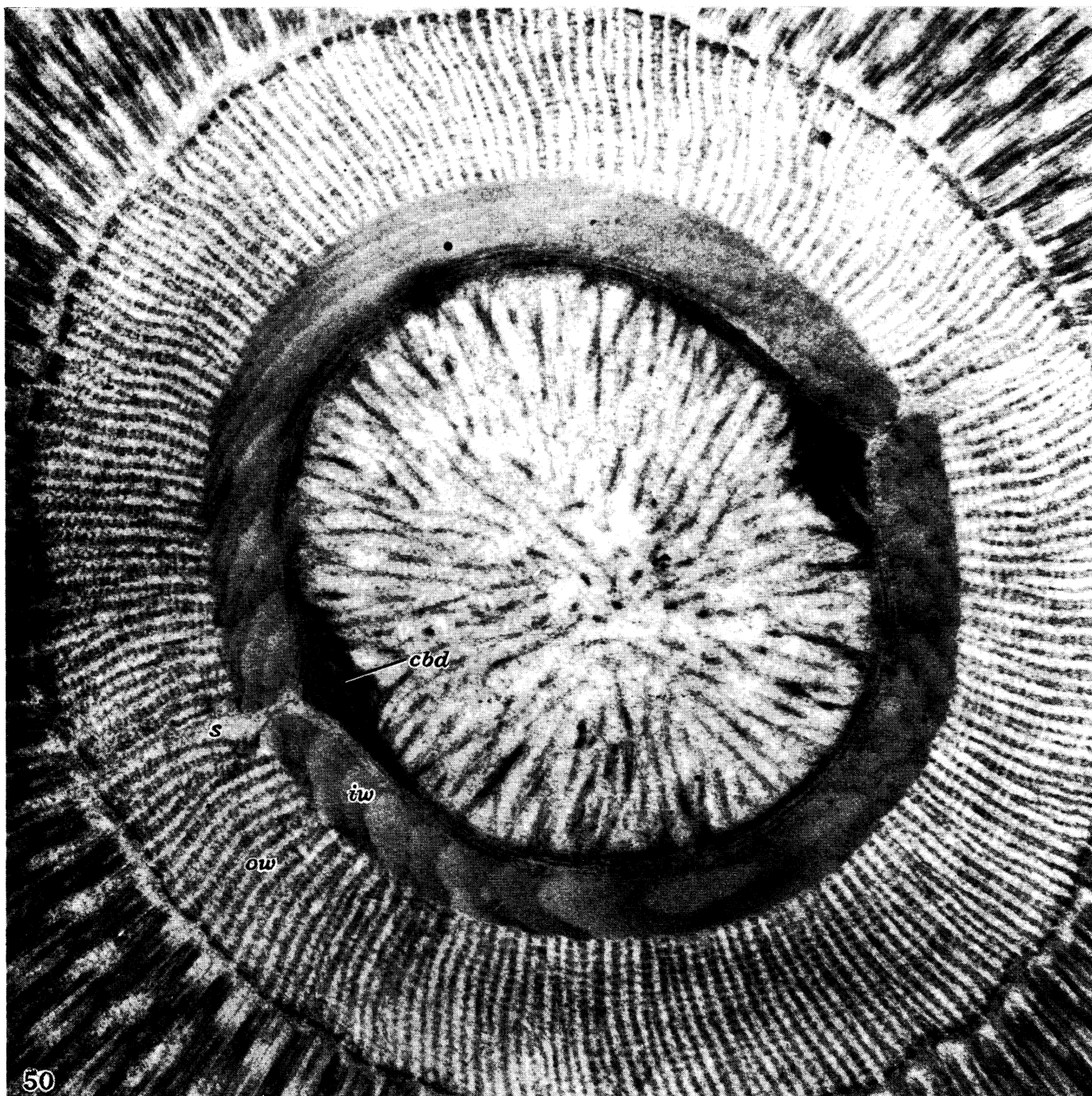


FIGURE 50. Section at the level at which the wide part of the rostral tube ends and the walls are thickest (compare figure 42). Radially polarized tubules still occupy most of the lumen of the tube. The tubule-bearing layer from which they arise is prominent, and its connexion with the centriolar bands is clear. Note the textures of the inner and outer walls of the tube ($\times 42\,000$).

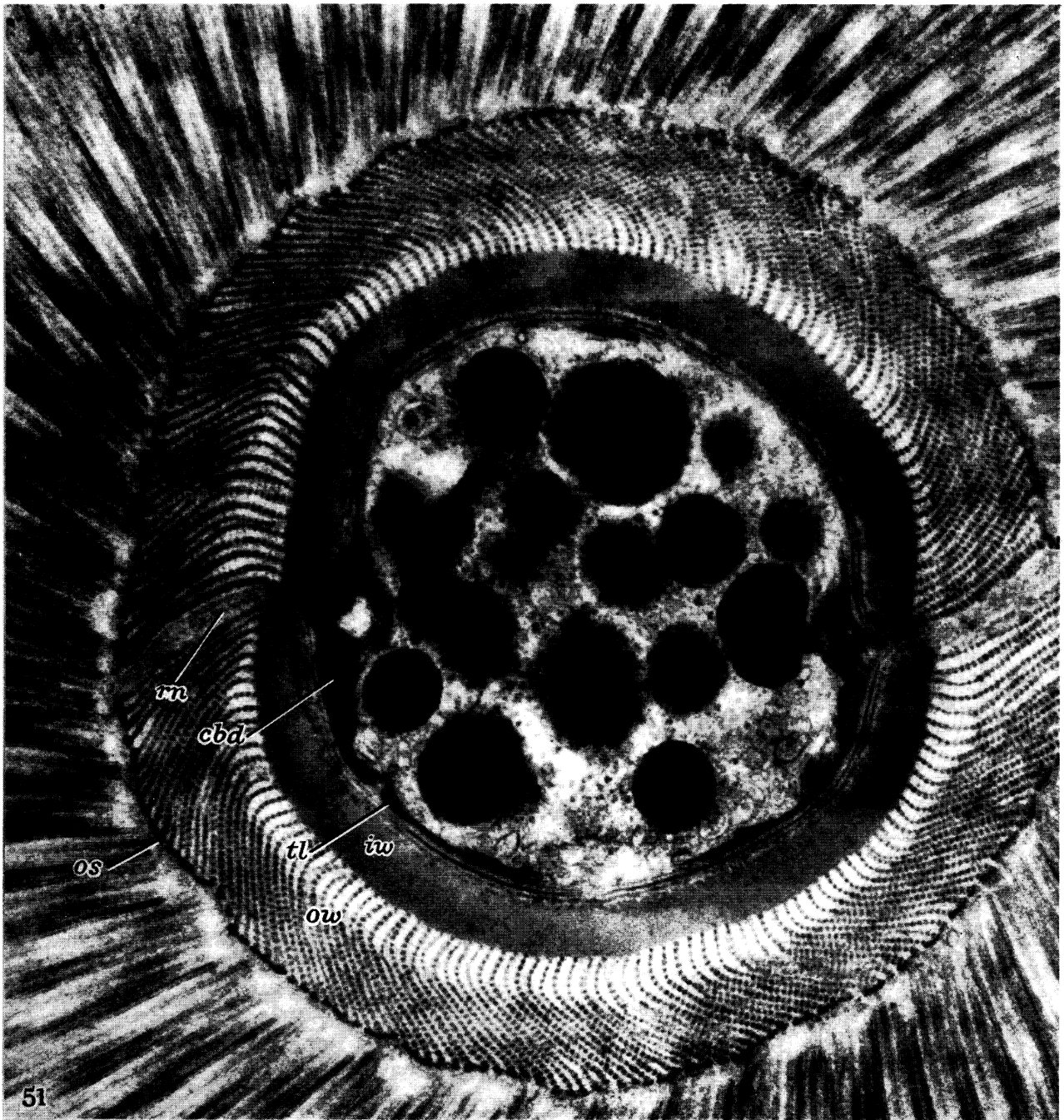


FIGURE 51. Section approximately two-thirds of the way along the rostral tube, showing the centriolar bands and their connexion to remnants of the tubule-bearing layer. Note the outer and the two inner surface layers ($\times 30\,000$).

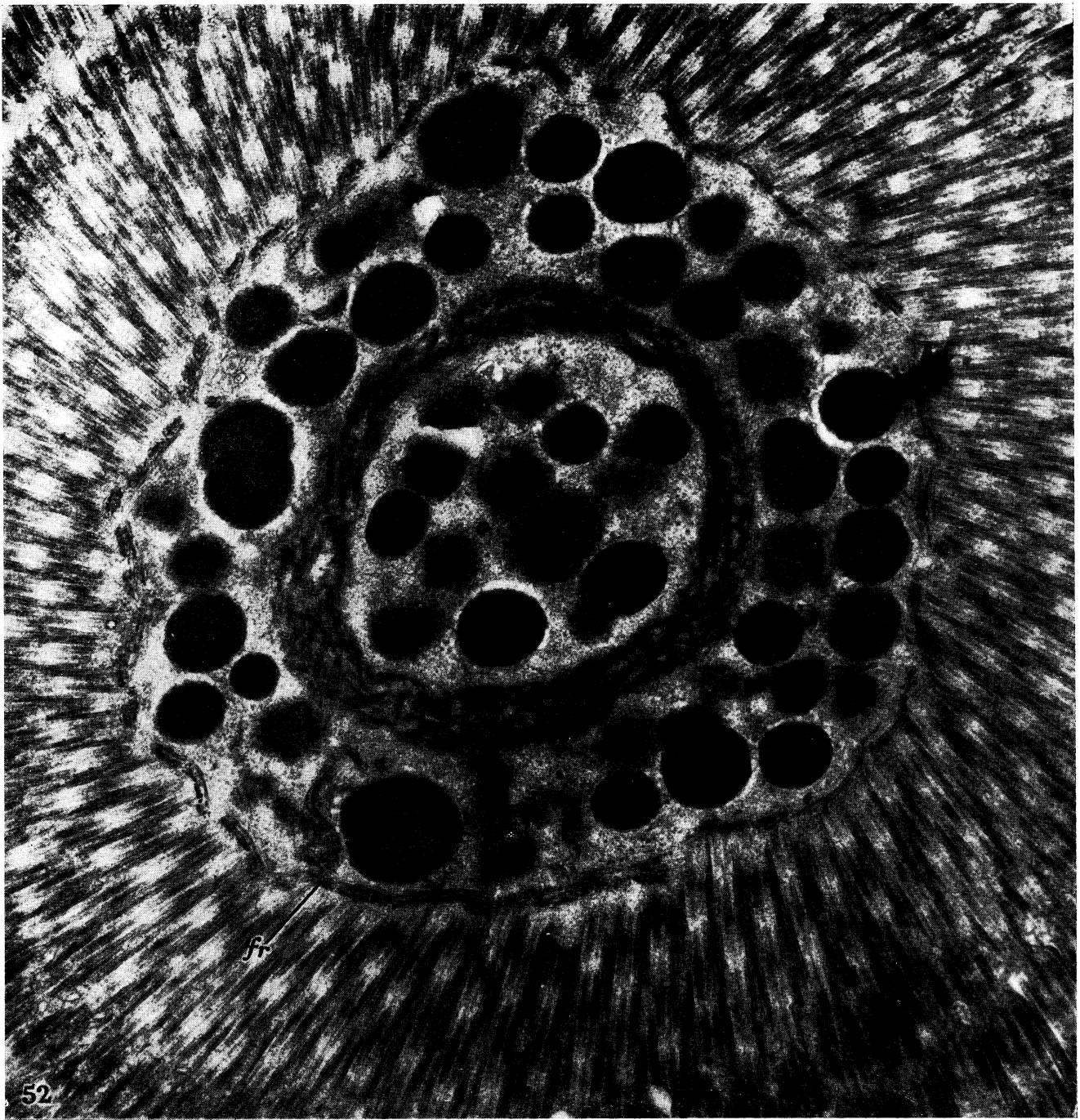


FIGURE 52. Section just below the base of the rostral tube showing the anulus which arises from the centriolar bands (compare figure 54). Note also the fibrous ribbons below the proximal ends of the basal bodies; the ribbons arise from the outer surface layer ($\times 30\,000$).

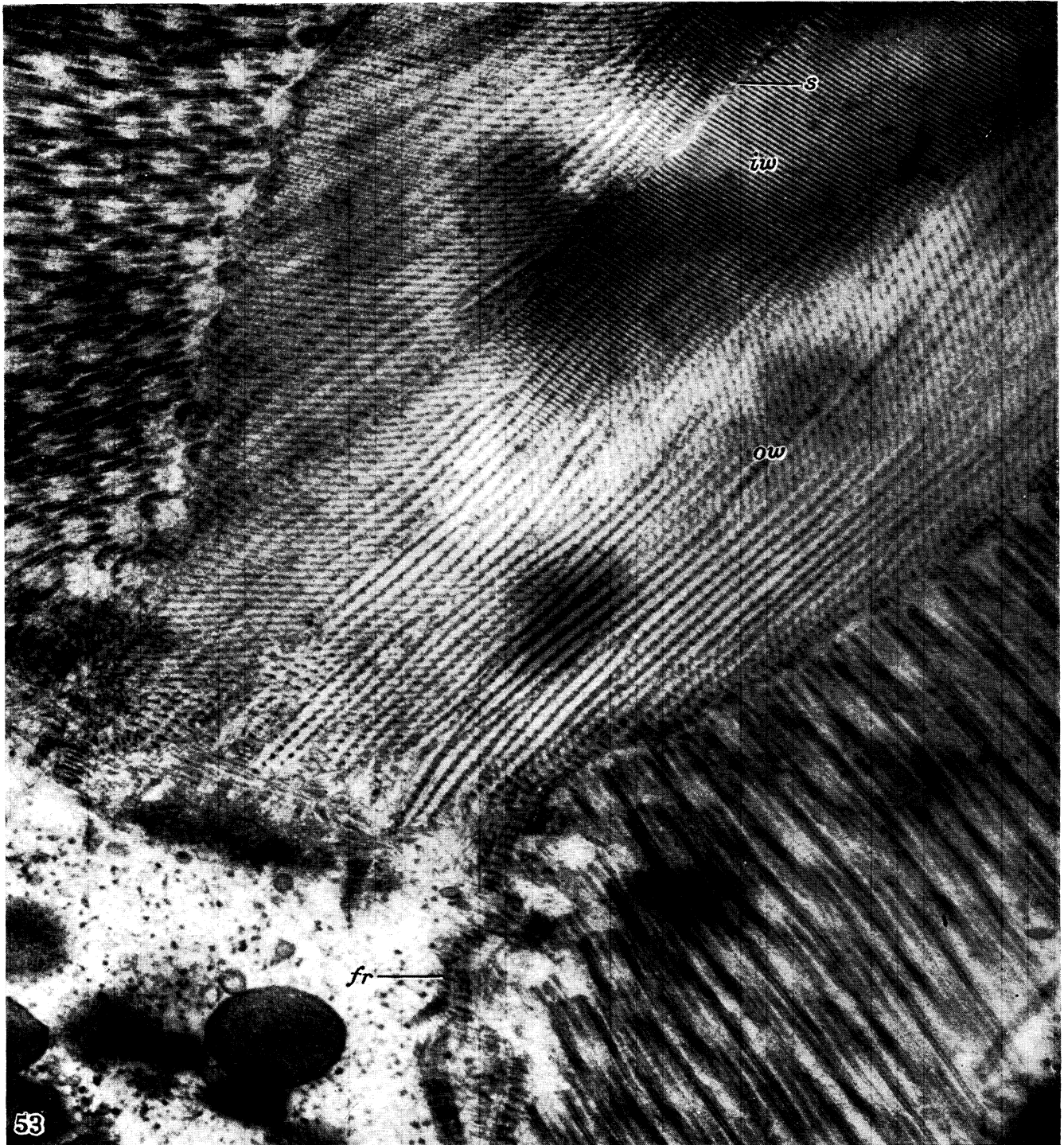


FIGURE 53. Longitudinal section at the base of the rostral tube, showing the inner and outer walls. Note the bifurcating lamellae in the latter. The origin of the cross-striated fibrous ribbons from the outer surface layer can be seen ($\times 42\,000$).



FIGURE 54. Longitudinal section at the base of the rostral tube, showing the annulus connected to one of the centriolar bands. Note also the outer surface layer and fibrous ribbons ($\times 42000$).

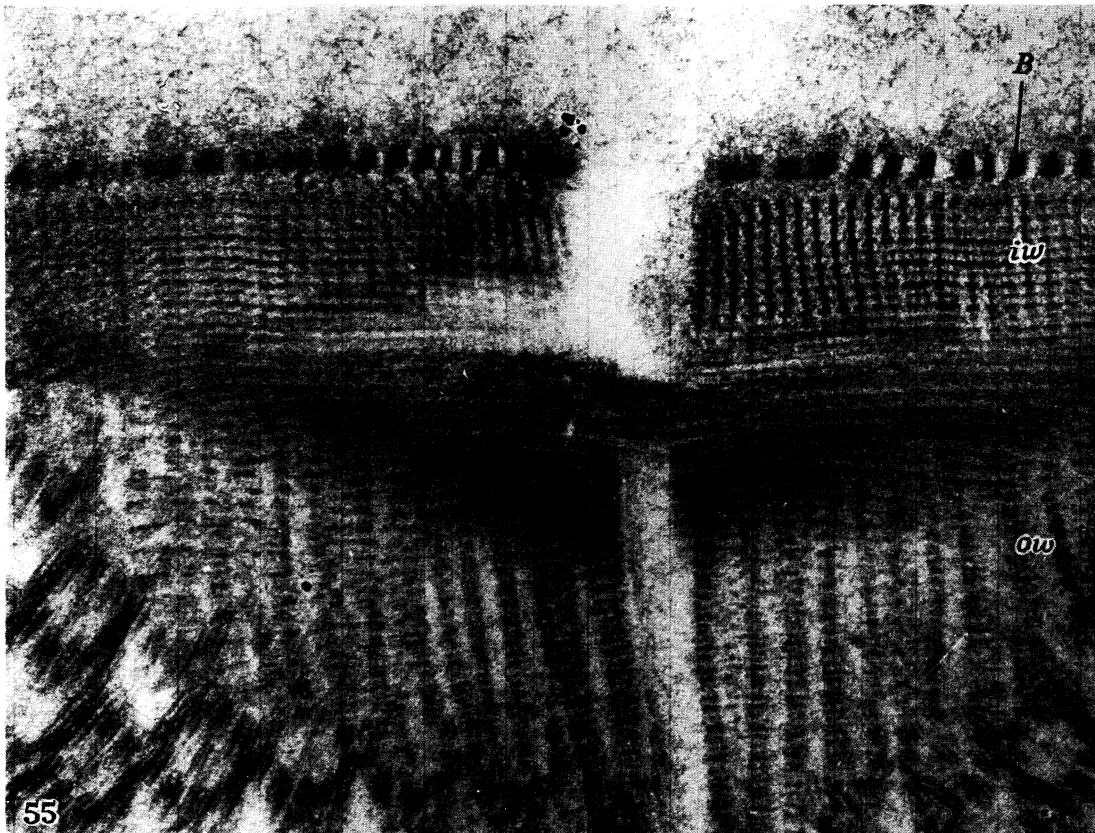


FIGURE 55. Longitudinal section at the anterior end of the rostral tube, to show particularly the *B* fibres, rectangular in section, running over the rim of the tube. Note also the cross-striations in the lamellae of the outer wall ($\times 65\,000$).

FIGURE 56. Longitudinal section at the anterior end of the rostral tube, showing the *B* fibres running over the rim, with the *A* fibrous bodies lying above them, nearer the centre. Note also the coarse *D* fibres, the tubules (presumed spindle fibres), and the section through the rostral rod, here seen to be circular in cross section ($\times 45\,000$).

The tube is bounded externally by a thin *outer surface layer*, which is apparently in the form of a continuous membrane anteriorly but divides further back into a number of separate, closely adjacent longitudinal ribbons (figure 48, plate 41; figure 50, plate 43). The outer surface layer has a periodic structure in longitudinal sections, with dense nodes occurring at intervals of 440 Å (figure 44, plate 37). Possibly these represent sections of transversely running fibres. Their spacing is the same as that of the striation of the outer wall of the tube and the two are exactly in register. The ribbons into which the surface layer divides continue posterior to the rostral tube, running close below the proximal ends of the basal bodies (figure 53, plate 46; figure 54, plate 47). Here they take on a better defined cross-striated appearance. They constitute the sinuous fibrous ribbons which have been described previously in connexion with the flagellar apparatus (Gibbons & Grimstone 1960).

A series of structures is found lining the inner surface of the tube. Some of these occur along its whole length, others are restricted to the wide anterior part. They are shown diagrammatically in figure 57.

The first of these structures will be termed *inner surface layer I*, and runs throughout the tube, lining the inner wall. This layer, probably like the inner surface layer in *Trichonympha*, is made up of a series of prominent transverse fibres, about 250 Å in diameter, which extend uninterruptedly around the inside of each half tube (figure 43, plate 36; figure 44, plate 37). In the anterior third of the tube the fibres are precisely located opposite alternate dense layers of the inner wall and appear as if they were marginal thickenings of those layers (figure 43). In the posterior part of the tube, however, they lose this precise location and are then seen to be separate structures (figure 44). This surface layer does not appear to continue beyond the base of the rostral tube (figure 45, plate 38), and it extends only about one-third of the way up the rim (figure 43).

Internal to this is a second layer, somewhat similar in appearance but rather less prominent, which will be called *inner surface layer II* (figures 43, 44, 45). Anteriorly this appears in longitudinal sections to be made up of a series of transversely running dense elements, similar to and located directly opposite the fibres of inner surface layer I, though less prominent than the latter (figure 43). In the middle region of the tube these elements seem to merge into a continuous layer, which becomes more prominent at the base of the tube and there takes on a distinctly cross-striated appearance (figure 45). This layer therefore appears to resemble the outer surface layer, and like that it seems to break up into a series of separate fibres which probably run back into the post-rostral cytoplasm.

There is yet another layer internal to inner surface layer II, this time made up of a series of transverse ridges or plates running continuously round the inside of each half tube (figures 42 and 43). The ridges are about 250 Å thick and they occur exactly in register with the dense components of the other surface layers. Examination of the micrographs leaves no doubt that they are separate elements and not denser regions in a continuous, banded structure. The inner margin of each ridge bears a row of delicate *tubules*, extending into the lumen of the rostral tube (figure 43, plate 36; figure 49, plate 42), and the ridges will therefore collectively be called the *tubule-bearing layer*. The tubules are about 250 Å in diameter and their substance merges continuously with that of the ridges bearing them, the junctions being distinguishable only by an alteration in density.

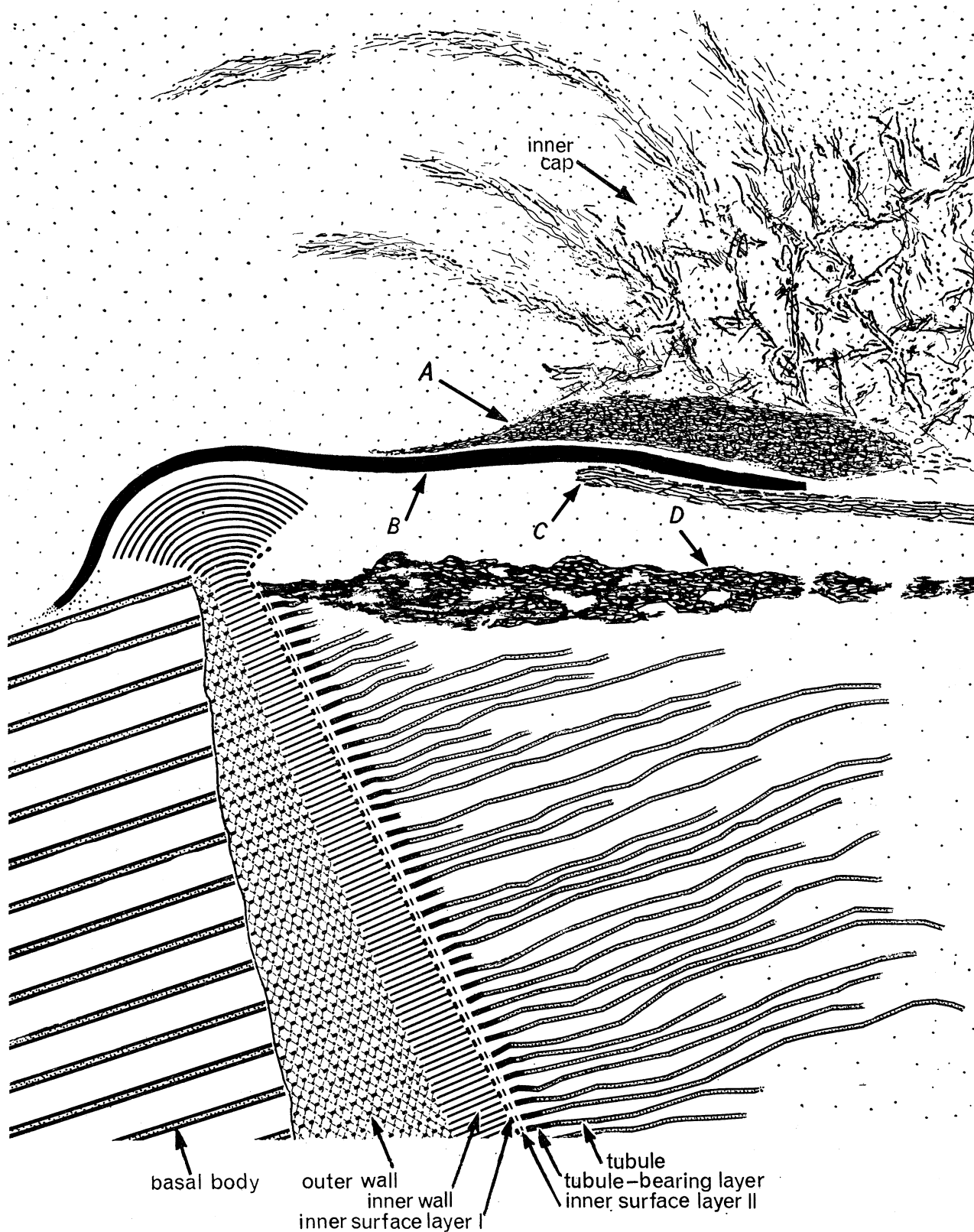


FIGURE 57. Diagram showing the structures associated with the inner cap and the anterior end of the rostral tube. Only one half of the inner cap and rostral tube are shown.

The tubules are restricted to the wide anterior part of the tube and extend out into the middle of the lumen (figure 41). They are always radially oriented. Anteriorly they run more or less transversely with respect to the axis of the tube, but posteriorly they are directed backwards. The tubules arising from each half tube are for the most part apparently restricted to the corresponding half of the lumen (figure 49, plate 42). It will be argued below (p. 236) that these tubules should be regarded as spindle fibres.

The tubule-bearing layer is lacking from the rim and extreme anterior end of the rostral tube (figure 43). Posteriorly, at the level where the lumen of the tube narrows into a straight cylinder and where the tubules stop, the layer undergoes a gradual transformation (figures 43 and 44), the ridges losing their well-defined shape and precise orientation and gradually becoming irregular, backwardly directed fibrous structures (figure 44). Around most of the circumference of the tube these fibres apparently extend back no further than the upper portion of the cylindrical part of the tube. Along the seams, however—that is, along the lines of junction of the two half tubes—they give rise to two thick bands of fibrous material which run back to the base of the tube (figure 50, plate 43). For reasons which will be presented in the Discussion these will be called *centriolar bands*. They are about 0.3 μm in diameter and have an irregular, sometimes coarsely reticulated structure. Just below the base of the tube they widen and give rise to a continuous ring of material with the same texture (figure 52, plate 45; figure 54, plate 47).

Apart from these surface structures the contents of the rostral tube call for little comment. Anteriorly the lumen of the tube is largely occupied by the tubules just described. Posteriorly, in the cylindrical part, there may be numerous large dense granules, similar to those found in *Trichonympha* (figure 52), as well as groups of small vesicles and scattered small granules which may be ribosomes (figure 44).

Inner cap and associated structures

Extremely elaborate structures occur at the anterior end of the rostral tube, embedded in or associated with the inner cap. The functions of these structures are for the most part unknown and they cannot readily be related either to the structures which have been described from light microscopy, or to the structures which are found in the same region in *Trichonympha*. All that can be done at present is to describe the main features of their morphology, in the hope that future studies of dividing cells, or of related organisms, may reveal their functional, developmental or evolutionary significance.

The *inner cap*, as in *Trichonympha*, is a more or less hemispherical structure lying over the anterior end of the rostral tube (figure 41, plate 34). It is relatively smaller and less compact than that of *Trichonympha* and the main part of it does not extend to the rim of the tube. It is made up of a rather coarse, very open reticulum of fibrous material and lacks sharp boundaries. Somewhat ill-defined strands of material, possibly finely fibrous, radiate from its upper surface into the amorphous substance which occupies the space beneath the outer cap (figure 42, plate 35; figure 46, plate 39).

Below the inner cap there are four distinct structures lying across the anterior end of the rostral tube. Since nothing is known about the significance of these structures they will be designated by the letters *A*, *B*, *C* and *D*.

The first of these (*A*) consists of two dense, finely fibrous bodies lying close against the

underside of the inner cap (figure 43, plate 36; figure 47, plate 40). These appear more or less semi-circular in transverse sections of the rostrum and one is associated with each half of the rostral tube. They are separated centrally by a gap along the diameter connecting the seams of the tube, and extend laterally to the margin of the inner cap. They do not make contact with the rostral tube itself. Possibly these bodies correspond to the so-called short centrioles described by Cleveland (1935, 1957) and Grassé & Hollande (1951), though there is nothing to suggest that they have a centriolar function.

Immediately below these structures there are two sets of transversely oriented fibres (*B*). These radiate from the centre of the lumen of the tube and extend beyond the periphery of the inner cap, running over the rim of the rostral tube to terminate near the distal ends of the most anterior basal bodies (figure 43, plate 36; figure 46, plate 39; figure 47, plate 40). Each set of fibres is associated with one of the half rostral tubes, and the two sets are separated by a gap along a diameter connecting the seams of the tube. The fibres are approximately rectangular in transverse section and 200 to 300 Å across (figure 55, plate 48).

Immediately below and overlapping *A* and *B*, there is a central, circular aggregate of finely fibrous material (*C*) (figures 43 and 47). This appears to have no connexion with the walls of the rostral tube.

Below the inner cap and the structures just described there is characteristically an empty region (figure 43). This is possibly a shrinkage artifact arising during preparation. Below it, in the most anterior part of the lumen of the rostral tube, there is a layer of dense, thick fibres (*D*), randomly arranged and forming a coarse reticulum extending across the tube (figure 48, plate 41). These fibres appear to arise from the inner wall of the tube, just below the rim. They lie anterior to and are distinct from the tubules previously described.

One final structure in this region remains to be described. This is a cylindrical rod, about 0.2 µm in diameter and 2 µm long, which originates from one of the seams of the tube, close to the rim, and runs in and down to about the centre of the lumen. It is amorphous in texture, moderately dense and not bounded by a membrane (figures 48 and 49; figure 56, plate 48). This will be termed the *rostral rod*. It is notable as being the only unpaired structure present in the rostral tube, but unlike the centriolar rod in *Trichonympha*, it shows no obvious influence on the polarization of the tubules in its vicinity.

No typical centriole has been detected anywhere in the rostrum of *Pseudotriconympha*, nor any flagellum which differs in orientation or location from the others. There is nothing in the non-dividing cell which closely resembles either the centriolar rod or crescentic bodies of *Trichonympha*.

Radial nets

Two radial nets are usually present, similar to those described in *Trichonympha*, originating from the inner surface layers of the half tubes and passing radially out through the seams of the tube and between the basal bodies of the rostrum (figure 48, plate 41; figure 49, plate 42). As in *Trichonympha*, they mark the plane along which the halves of the rostrum separate at division. It has not been ascertained how far back they extend, and it is not known precisely with which of the inner surface layers they connect. In a few cases more than one net appears to be present on each side (figure 51, plate 44).

Rostral flagella

The flagella of *Pseudotriconympha* were described in detail in a previous paper (Gibbons & Grimstone 1960). The only newly observed feature which need be mentioned here is the presence, as in *Trichonympha*, of small vesicles or tubules lying between the rows of basal bodies in the rostrum. These are invariably present and are concentrated near the distal ends of the basal bodies (figure 45, plate 38).

Outer cap

The outer cap is relatively more extensive than that of *Trichonympha*, its margins extending further back along the rostrum. In consequence of this some of the most anterior flagella of the rostrum fail to reach the exterior, since the grooves in which they lie are closed by the outer cap. The cap is normally dome-shaped in life but usually collapses and becomes wrinkled in fixed material (figure 41, plate 34). Its structure has not been studied in detail but it appears to be similar to that of the species of *Trichonympha* from *Zootermopsis*, consisting of an outer membrane, continuous with that of the general cell surface, with one or two layers of tubules beneath.

Koidzumi (1921) reports that the outer cap of *Pseudotriconympha grassi* can change shape. This has not been observed in the species studied here, but it is a property which would not be inconsistent with the presence of the tubules.

DISCUSSION

Centrioles, spindle fibres and mitosis

The centrioles of *Trichonympha* have been intensively studied by light microscopy by a number of workers and the present findings are, for the most part, not in serious conflict with the earlier observations. They do, however, add considerable detail to what was known previously, and they also reveal new structures, the existence of which was not before suspected.

The chief point at which the present findings are at variance with the most recent observations from light microscopy (Cleveland 1960 *a, b*) concerns the nature of the so-called 'short centriole' and the structure of the long one, here termed the centriolar rod. As already noted, the only structures which can be identified from electron microscopy as the supposed short centriole and the expanded end of the long one are the two crescentic bodies. These are not the distinct granules described by Cleveland, but differentiated extensions of the rostral tube itself. Further, there is no evidence from our observations that either of the crescentic bodies is in continuity with the centriolar rod. It is not known how the second centriolar rod originates at division, but if, like the first one, it is not connected to a crescentic body, then there would seem to be no justification for retaining the term 'short centriole' for the latter. Cleveland's conception of the life history of the centrioles of *Trichonympha* (see Cleveland 1957) would also require modification.

The most striking new findings in connexion with the centriolar apparatus of *Trichonympha*, reported in part previously (Grimstone 1961), are the observations on the anterior flagellum and apparently typical centriole (i.e. a basal-body-like structure which bears no flagellum or cilium). This appears to be the first case in which such an organelle has

been observed in a protozoon (with the possible exception of the ciliary basal bodies of certain ciliates, which play no part in mitosis but generate cilia at particular stages in the life cycle (Rouiller & Fauré-Fremiet 1958)). The function of the supposed typical centriole in *Trichonympha* is unknown and there is no information about its involvement in either mitosis or the formation of new flagella.

The anterior flagellum is also enigmatic, though a similar structure is possibly found in other complex flagellates, since Pitelka (1963) reports unpublished observations by Cook on the occurrence of one or more unusually oriented flagella in *Barbulanympha*. The presence of the anterior flagellum in *Trichonympha* is inconsistent with the apparent bilateral symmetry of the rest of the organism. Simpler flagellates, such as the Trichomonadidae, are, of course, typically quite asymmetrical; it may well be that the apparent bilateral symmetry of *Trichonympha* and other complex flagellates is a secondary state of affairs, and that the anterior flagellum is a relic of a more primitive stage of evolution.

Concerning the long basal body (or centriole) found in the rostral tube of a few individuals of *Trichonympha*, no interpretation can at present be offered, except that it possibly represents a morphogenetic 'mistake'—a basal body formed in, or transported to, the wrong location during development.

Pseudotriconympha has been less thoroughly studied by light microscopy than has *Trichonympha*, and previous observations are to some extent at variance with the present findings. In particular, we have not been able to find the supposed paired centrioles at the apex of the rostral tube, described by Grassé & Hollande (1951), though there are abundant structures there which could have formed the basis for their observation. Both Cleveland (1935) and Grassé & Hollande (1951) agree that in the non-dividing organism there is no persistent long centriole comparable to that of *Trichonympha*, and that the two long centrioles which grow out at mitosis appear to originate from the base of the rostral tube. The most likely candidates as progenitors of these centrioles appear to be the dense bands which lie along the seams of the lower half of the rostral tube. These are the only paired structures in this region, and they are similar in texture to the peripheral material of the centriolar rod of *Trichonympha*. For these reasons they have been termed centriolar bands. As described above, in the upper part of the rostral tube they merge with, and possibly arise from, the tubule-bearing layer. Since the latter has a similar texture and density it is not unreasonable to suggest that it, too, should be regarded as centriolar in nature. If this interpretation is accepted it becomes possible to identify the tubules which arise from the layer as spindle fibres, present throughout interphase. This hypothesis is supported by the fact that the diameter and general appearance of the tubules are markedly similar to those of spindle fibres in other cells (Roth & Daniels 1962; Harris 1962). Evidence is accumulating that spindle fibres not uncommonly persist through interphase in various types of cell, and that in some cases they may be identical with the microtubules commonly found as a cytoplasmic component (Slautterback 1963; Ledbetter & Porter 1963). For example, de Thé (1964) shows microtubules in a variety of interphase vertebrate cells, apparently indistinguishable from spindle fibres and in some instances clearly associated with pericentriolar material, while Gonatas & Robbins (1965) have demonstrated a similar relationship between spindle fibres and neurotubules in the retina of the chick embryo. The persistence of spindle material in interphase is quite possibly a

general phenomenon and in some organisms it may involve not merely the individual fibres (or tubules) but the mitotic spindle itself as an organized body. In the flagellate *Holomastigotoides*, for example, a small spindle is always present (Cleveland 1949*b*). In this light *Pseudotriconympha* can be regarded as one of a series of cell types illustrating different degrees of retention of spindle material. It is exceptional only in the abundance of the tubules and in their radial polarization with respect to peripherally disposed centriolar material. An at least partly analogous state of affairs is probably represented in *Trichonympha* by the backwardly directed fibres which arise from the peripheral material of the centriolar rod. The fibres here are more prominently cross-striated than typical spindle fibres and they have not been shown to be hollow, but their markedly polarized arrangement around centriolar material is very similar to the situation in *Pseudotriconympha*.

It may be noted that if, as seems probable, spindle fibres (or microtubules) possess some degree of rigidity, they may play a part in shaping the wide anterior end of the rostral tube in *Pseudotriconympha*, the inward growth of stiff fibres pushing the walls apart. Certainly the fibres occur abundantly only in that part of the tube.

A comparison of the centriolar apparatus and associated structures of *Trichonympha* and *Pseudotriconympha* shows that apparently very different structures may serve what are presumably similar ends. There appears to be nothing in *Pseudotriconympha* corresponding to the crescentic bodies, anterior flagellum or supposed typical centriole of *Trichonympha*, and nothing in *Trichonympha* corresponding, for example, to the complex fibre systems at the anterior end of the rostral tube in *Pseudotriconympha*. The common features seem to be first, that both organisms possess similar inner caps, and secondly, that there are general similarities in texture and density between the supposed centriolar material in *Pseudotriconympha* and the peripheral material around the centriolar rod in *Trichonympha*.

Comparison with the centrioles of metazoan cells is of interest chiefly because it shows that the supposedly typical, basal-body-like centriole of the latter is not essential for development of a polar spindle. Unpublished observations on the flagellates *Trichomonas termopsidis*, *Barbulanympha* sp., and *Joenina pulchella* indicate that a similar state of affairs holds there. In all these organisms, however, as well as in many metazoan cells (see de Thé 1964; Gonatas & Robbins 1965) dense, possibly fibrous material forms, or is associated with, the presumed centriolar structures, and it is to this that the spindle fibres appear to be attached. Conceivably it is the dense material, rather than the supposed centriole proper, which is the essential agent responsible for the orientation of spindle fibres.

Rostral tubes

The differences in shape and fine structure of the rostral tubes of *Trichonympha* and *Pseudotriconympha* provide a notable example of the way in which apparently comparable structures in these organisms differ in their detailed organization. It should be noted, however, that in spite of their overall differences in structure, the rostral tubes display the same major periodicity of about 440 Å in both genera.

The most striking observations in the present work are undoubtedly those on the rostral tube and associated structures in *Pseudotriconympha*, which possibly display a greater complexity of organization than any other subcellular structure so far described. Of particular interest is the fact that a number of components—the inner and outer walls

of the tube, and various structures on its inner surface—although differing widely in appearance, are all organized with a common periodicity. The pseudo-crystalline regularity of the walls of the rostral tube presumably reflects the highly ordered assembly of their protein subunits. Details of the intricacy with which protein subunits can be assembled have recently been elucidated in such relatively simple systems as collagen (Hodge & Schmitt 1960; Petruska & Hodge 1964) and aspartate transcarbamylase (Gerhart 1964), but at present it seems profitless to speculate in detail on the possible number and relationships of the subunits in a system of such greater overall complexity as the rostral tube. It would not be surprising, however, to find that the common periodicity of the various structures in the tube was determined by the periodicity of whichever structure formed first in development.

The most obvious function of the rostral tube is to serve as a relatively rigid support for the densely packed basal bodies of the rostral flagella. In *Trichonympha* the tube also gives rise to the parabasal filaments, which are the foci about which the parabasal bodies are organized (Grimstone 1959*a*). There is no evidence that the rostral tube is itself contractile or responsible for the bending movements of the rostrum that occur in both genera (see below).

Flagella

The most interesting questions concerning the rostral flagella of these organisms concern their coordination and the manner in which they seemingly produce the complex bending movements of the rostrum. In both genera the rostrum may be bent from side to side, sometimes through a large angle, and it may also be swung round in a circle. Kirby (1932) suggested that in *Trichonympha* these movements may be produced by appropriate co-ordinated activity of the flagella, temporary inactivity of those on one side resulting in bending in that direction if the flagella of the opposite side continue to undulate. This suggestion is consistent with our observations and, although it is difficult to prove that the movements are produced in this way, it is the most plausible mechanism which has been proposed. None of the other structures in the rostrum or immediately post-rostral region seems likely to be responsible.

If this is the case, the degree of coordination of the rostral flagella must be fairly high, and this seems a possible functional explanation for the complex networks of fine fibres connecting the basal bodies of the rostral flagella to the inner cap. There is, at any rate, apparently a correlation between the presence of these fibres and the ability to carry out highly coordinated flagellar movements, since we have not found the fine fibres in other regions of the body. Conceivably the inner cap acts as a coordinating centre. However, it must be acknowledged that an alternative possibility is that coordination is effected not by the fibres but by the membranous elements that run among the basal bodies of the rostral flagella; the arrangement of these membranes is, as already noted, somewhat reminiscent of the sarcoplasmic reticulum in striated muscle.

Generalities

Descriptions of structure are, in themselves, relatively unrewarding. Structure is static, and it becomes meaningful only when it is interpreted in dynamic terms, by consideration of its functional, or developmental, or evolutionary significance. It will be obvious from

the discussion so far that in these terms the significance of most of the structures which we have described is at present either unknown or a matter for speculation. Unfortunately, functional studies are difficult on complex anaerobic organisms such as these, which cannot as yet be cultured *in vitro* and which survive for only short periods outside their host. The study of their division and morphogenesis, at the fine-structural level, has only just begun, and with regard to evolutionary aspects of their structure, little can be said until a wider range of organisms has been studied. Dynamic interpretations of structure are therefore at present hardly feasible. It may perhaps be useful, however, to make certain general observations about the interpretation of submicroscopic structure in the light of our findings.

It is worth emphasizing, first, that the rules of submicroscopic morphology are far less well understood than those of grosser levels of anatomy. The present tendency is to try to find some function, usually described in biochemical terms, for each type of organelle, and to show how the structure of the organelle is related to that function. Such an approach is undoubtedly rewarding, and is perhaps particularly appropriate for the highly specialized cells of higher organisms, but it is not the only way to attempt to interpret cell structure, and other possibilities must be borne in mind.

One such possibility is that some submicroscopic structures are vestigial—the persistent relics of organelles which once served a function but no longer do so. It is difficult to evaluate the likelihood of this at present, for we know so little about how natural selection operates at the submicroscopic level of organization.

Another possibility is that certain morphogenetic processes in cells may necessitate the development of structures as intermediaries or initiators, which later persist although they serve no useful purpose. They would be analogous to scaffolding left intact at the end of a building operation. This is suggested, for example, by studies of the development of pollen grains, where Heslop-Harrison (1963) has shown that at an early stage cytoplasmic membranes come to lie under certain regions of the cell surface where the coat of the pollen grain will subsequently not become thickened. Comparable observations have been made in connexion with the development of cell-wall components in a variety of plant cells (see Heslop-Harrison 1963).

Apart from considerations such as these, however, a major difficulty in finding functional explanations for observed features of fine structure arises from the fact that little is known about the degree of adaptiveness demanded at this level, or about the extent to which alternative solutions to functional problems are possible (see Grimstone 1959*b*). At the more fundamental biochemical level Davis (1961) has adduced evidence which suggests that natural selection is rigorous, and that the common metabolic pathways, found in almost all organisms, are those which are most efficient. This is as might be expected. It is not clear, however, that this should invariably be the case at more complex levels. If it were, it would seem difficult to account for the immense diversity and complexity of form in, for example, the flagellates of termites, of which those described in this paper form but two examples. A naïve reaction to the intricacy of structure of an organism such as *Pseudotriconympha* is that the whole thing is unnecessarily complicated. Why is such elaboration needed, when far simpler organisms survive equally satisfactorily? Part of the answer to this no doubt lies in the fact pointed out by Pittendrigh (1958), that organisms

cannot escape the effects of mutation and recombination, which 'force novelty into the genetic message'. The organization of living systems may in consequence come to exceed the functionally adequate, in the sense that it may contain features which are not essential for survival. This, however, does not in itself explain why the termite flagellates should be so much more complex than most other protozoa. It seems possible that a special factor may operate here, namely, the symbiotic mode of life of these organisms. They live in an enclosed, highly stable, homogeneous environment, protected from predators, passed regularly to new hosts, and provided with unlimited food. In these circumstances the severity of selection may perhaps be less than that which operates elsewhere, and there may in consequence be a less rigorous elimination of genetic novelty. In other words, an undemanding environment may allow the deployment of a wider range of adaptive possibilities than is usually permitted. (A related effect of this mode of life, namely, the loss of sexuality, is discussed by Hawes (1963).) It is relevant in this connexion that the rumen ciliates, which live in a closely similar environment, are comparable in elaboration to the termite flagellates. In both cases, it is suggested, the complexity of these organisms is not necessary for survival, but it is permissible. If this argument is correct we are perhaps to some extent spared the necessity of seeking functional explanations for all the differences in structural detail which have been described. The variation in the outer caps in the different species of *Trichonympha*, for example, or in the rostral tubes of *Trichonympha* and *Pseudotriconympha*, are not necessarily indicative of subtle differences in functional organization: they may be merely possible alternative solutions to a relatively undemanding specification.

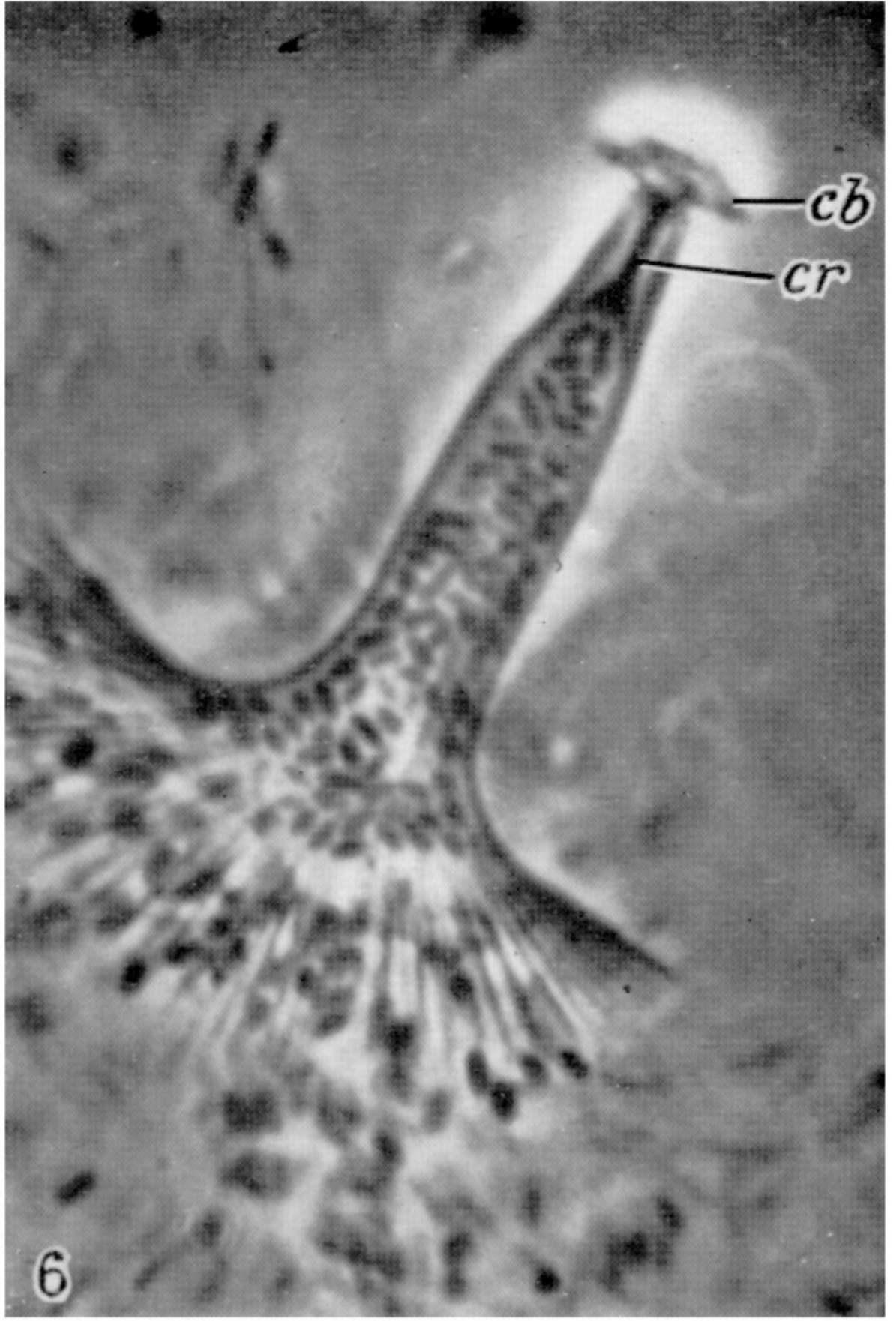
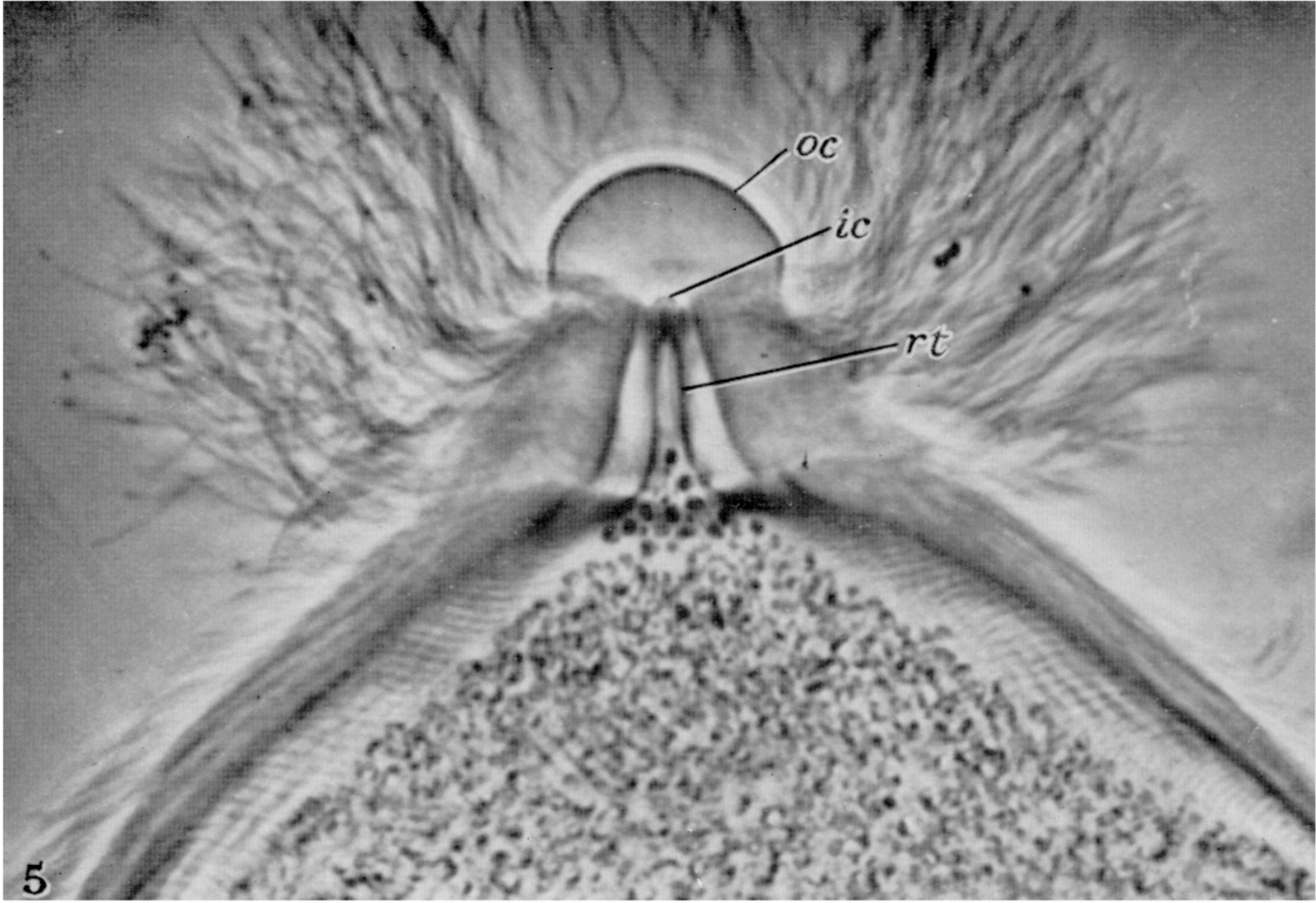
The elaboration and precision of organization found in these flagellates is unusual, but is not less important for that, since it demonstrates the degree of complexity to which subcellular morphology can attain. Although most cells do not display such elaboration of structure this does not necessarily mean that their organization is any the less complex, for complexity could well exist in a form not rendered apparent by currently available techniques. Indeed, there are already indications that this may be so: the complicated patterns of enzyme distribution demonstrated by Novikoff (see Novikoff, Essner, Goldfischer & Heus 1962), or the intricate assemblies of mitochondrial enzymes reviewed by Lehninger (1964), to give but two examples, both hint at a spatial organization of metabolic activities as complex as that manifest in the skeletal structures described here.

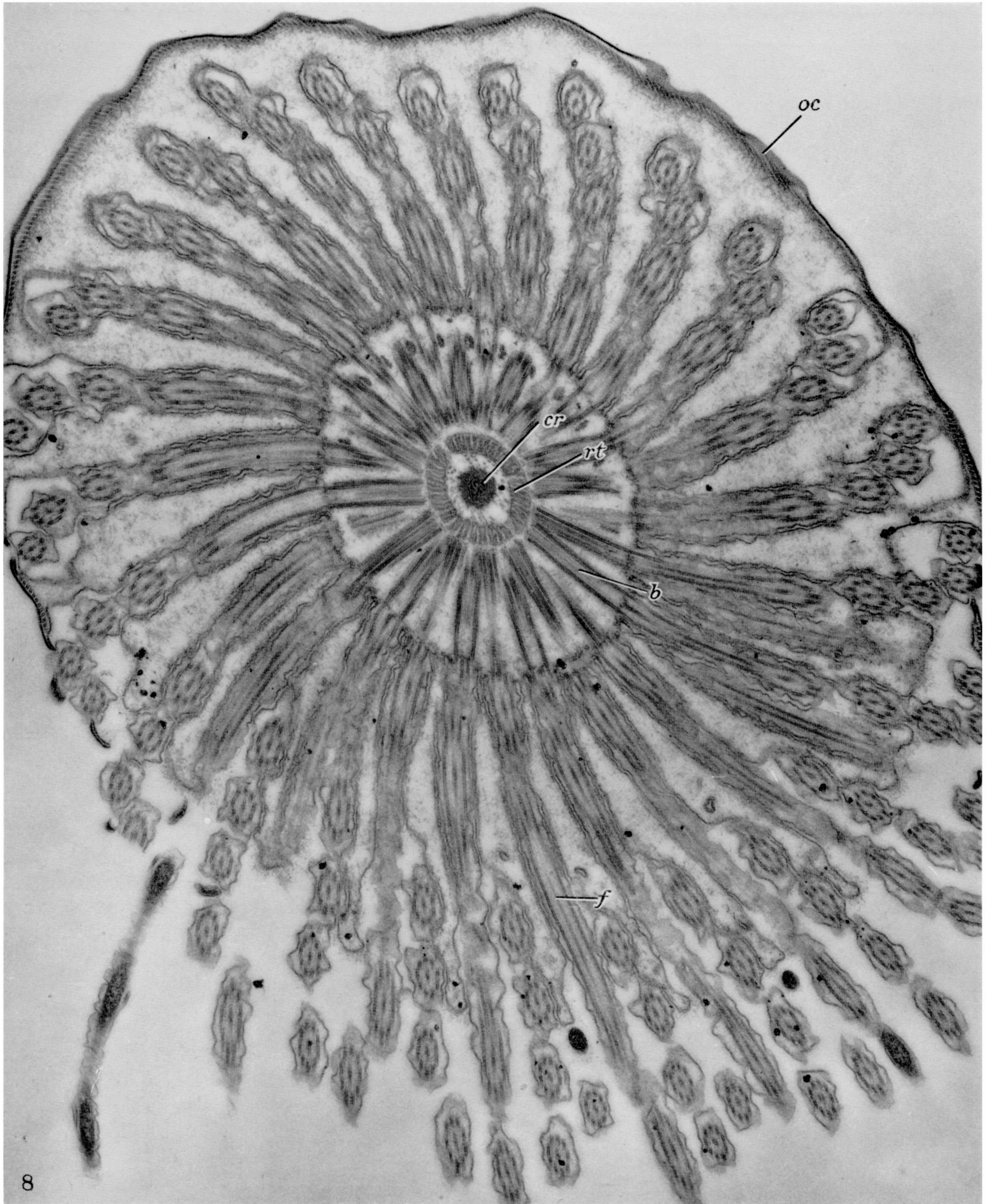
This work has been supported in part by grants from the Department of Scientific and Industrial Research and the United States Public Health Service.

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8

FIGURE 8. Transverse section of the rostrum of a species from *Cryptocercus*. The section is slightly oblique: at the top of the picture it passes through the outer cap, and at the bottom through the flagellar grooves. Note that with the exception of the rostral tube, most of the cytoplasm is occupied by flagella and their basal bodies. A whorled arrangement of dense lamellae is visible in the central region of the centriolar rod ($\times 25\,000$).

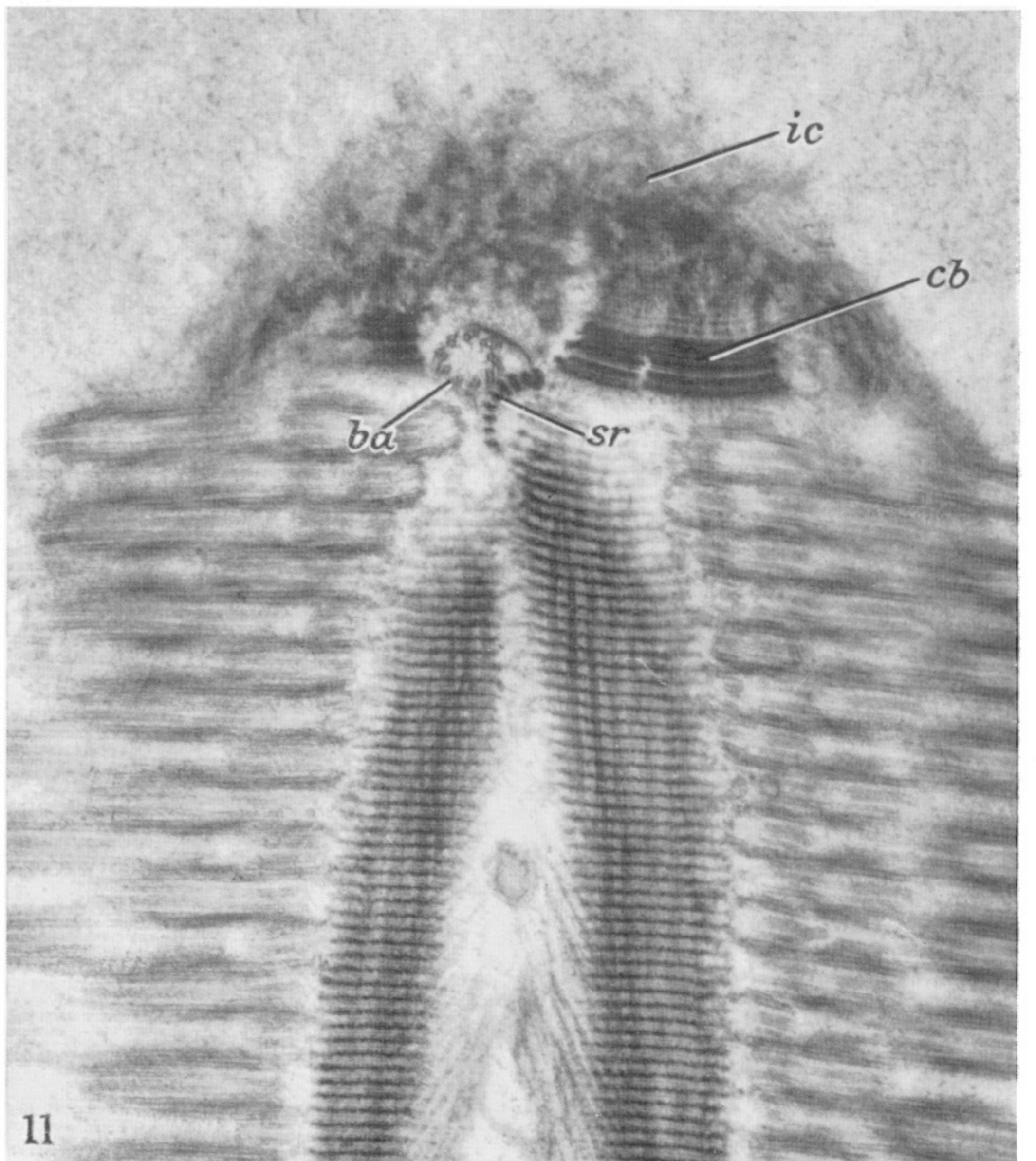
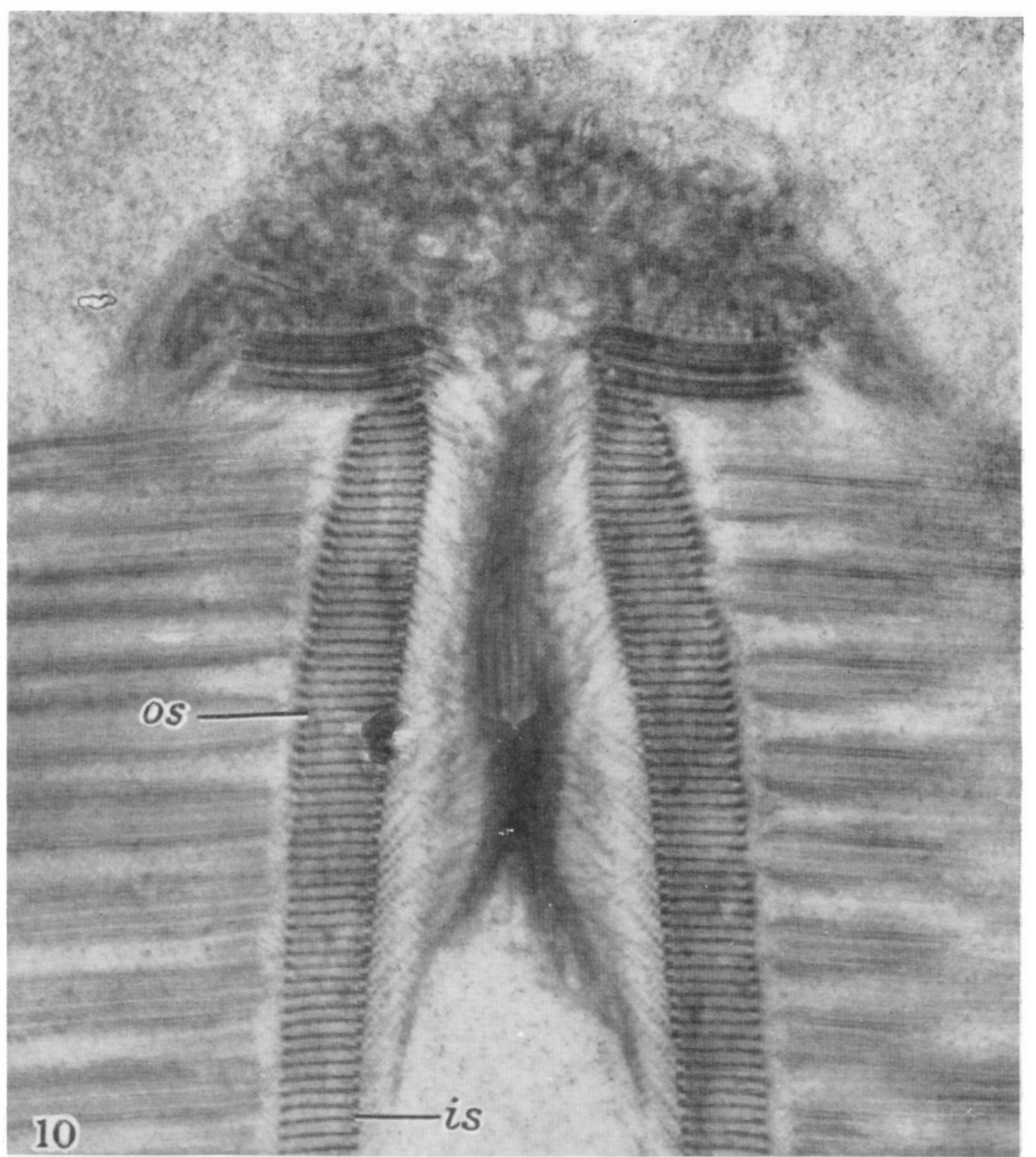
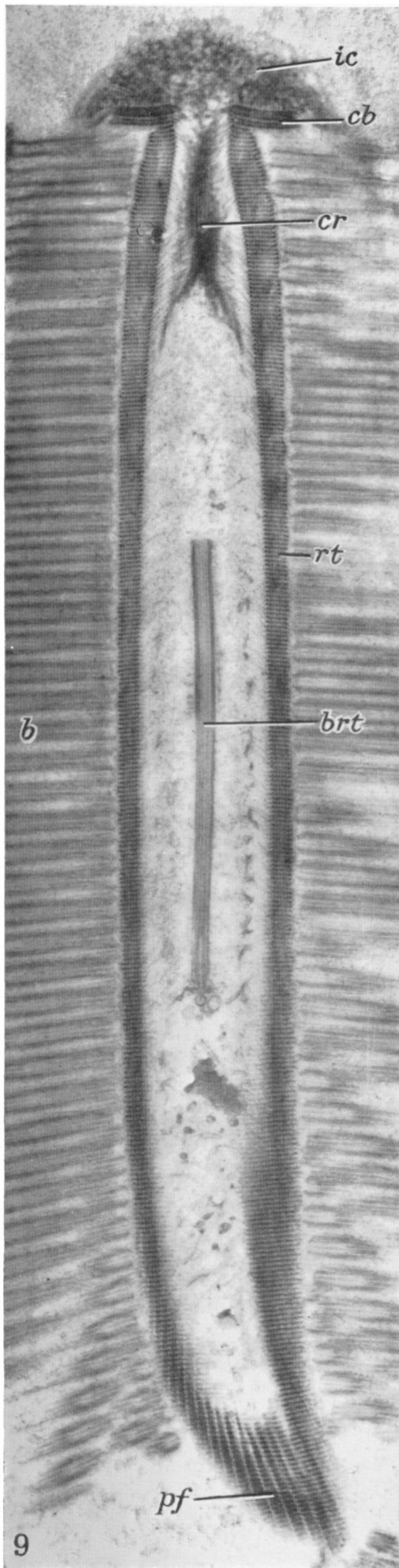
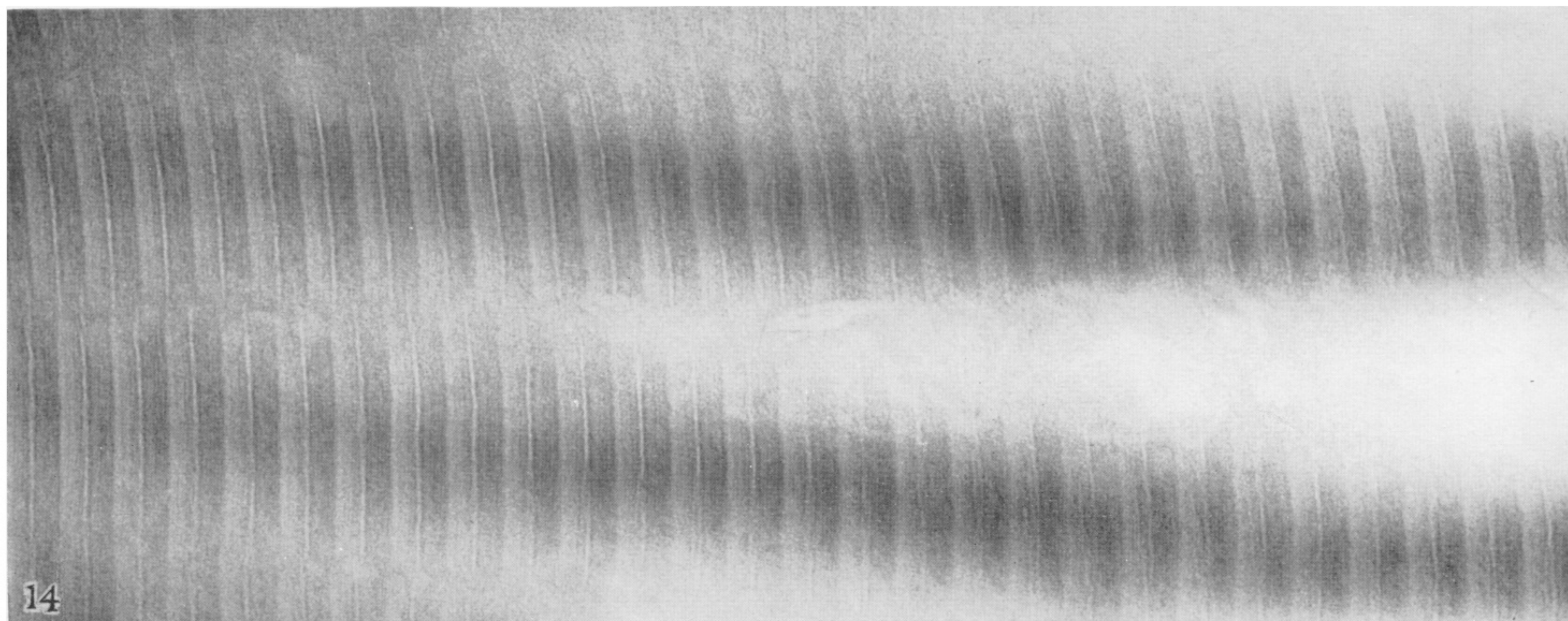
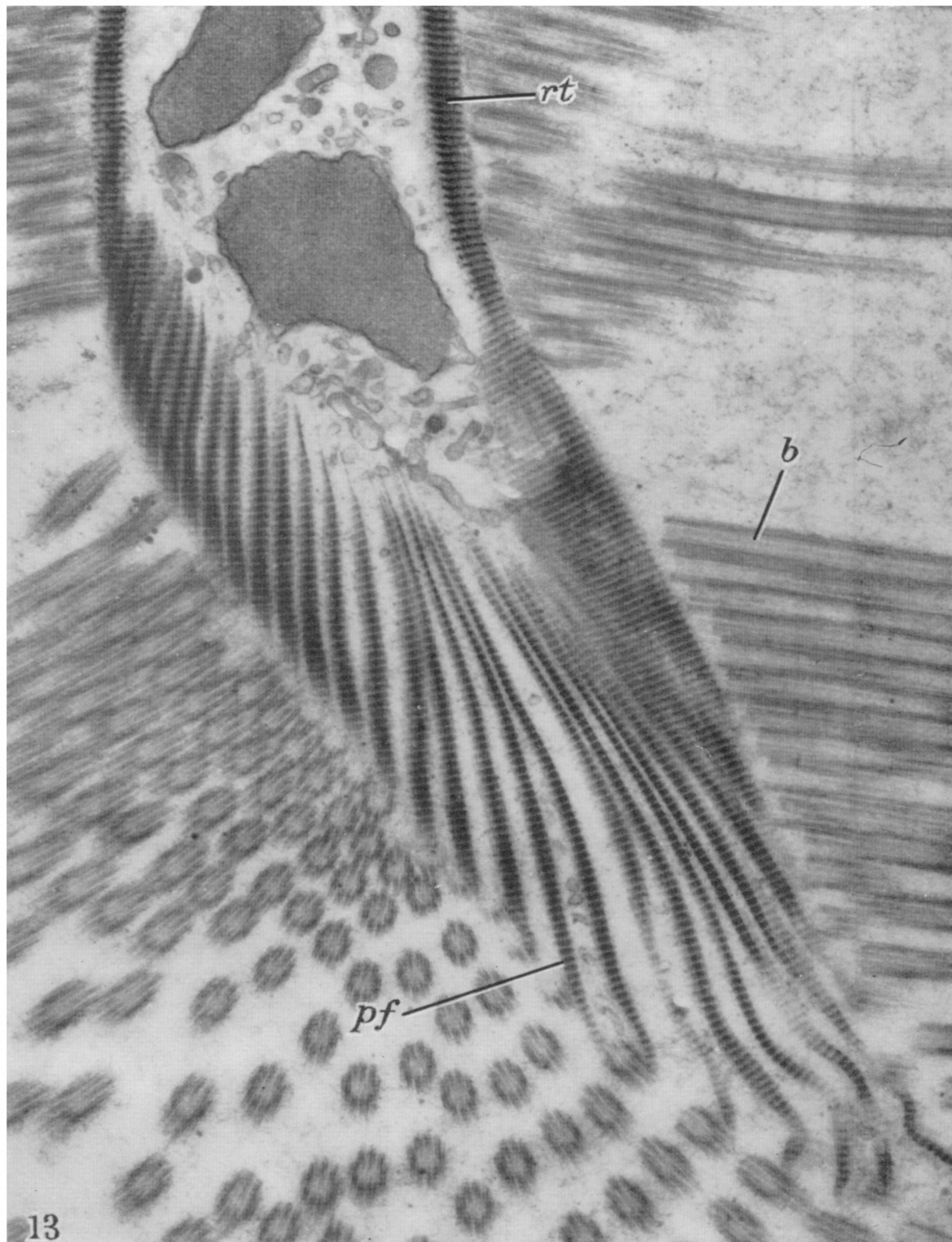
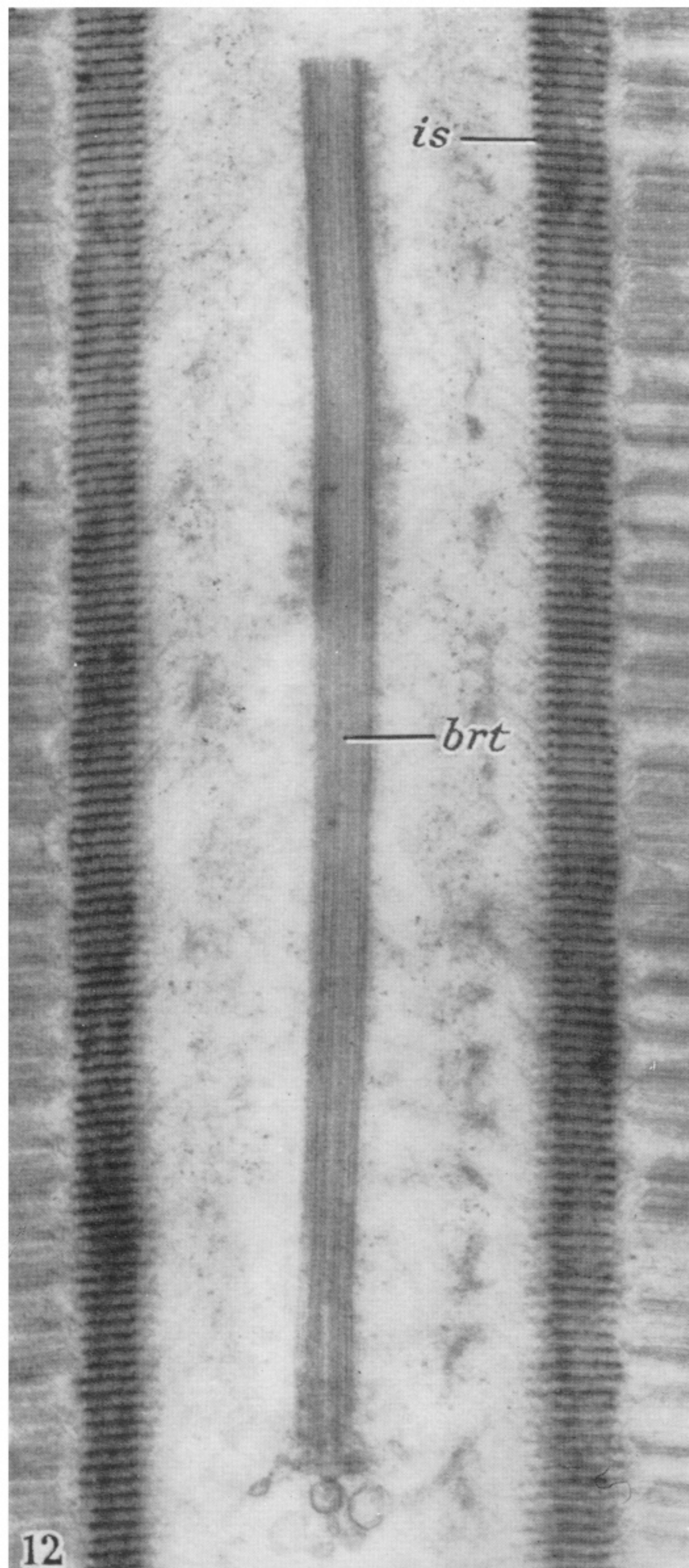
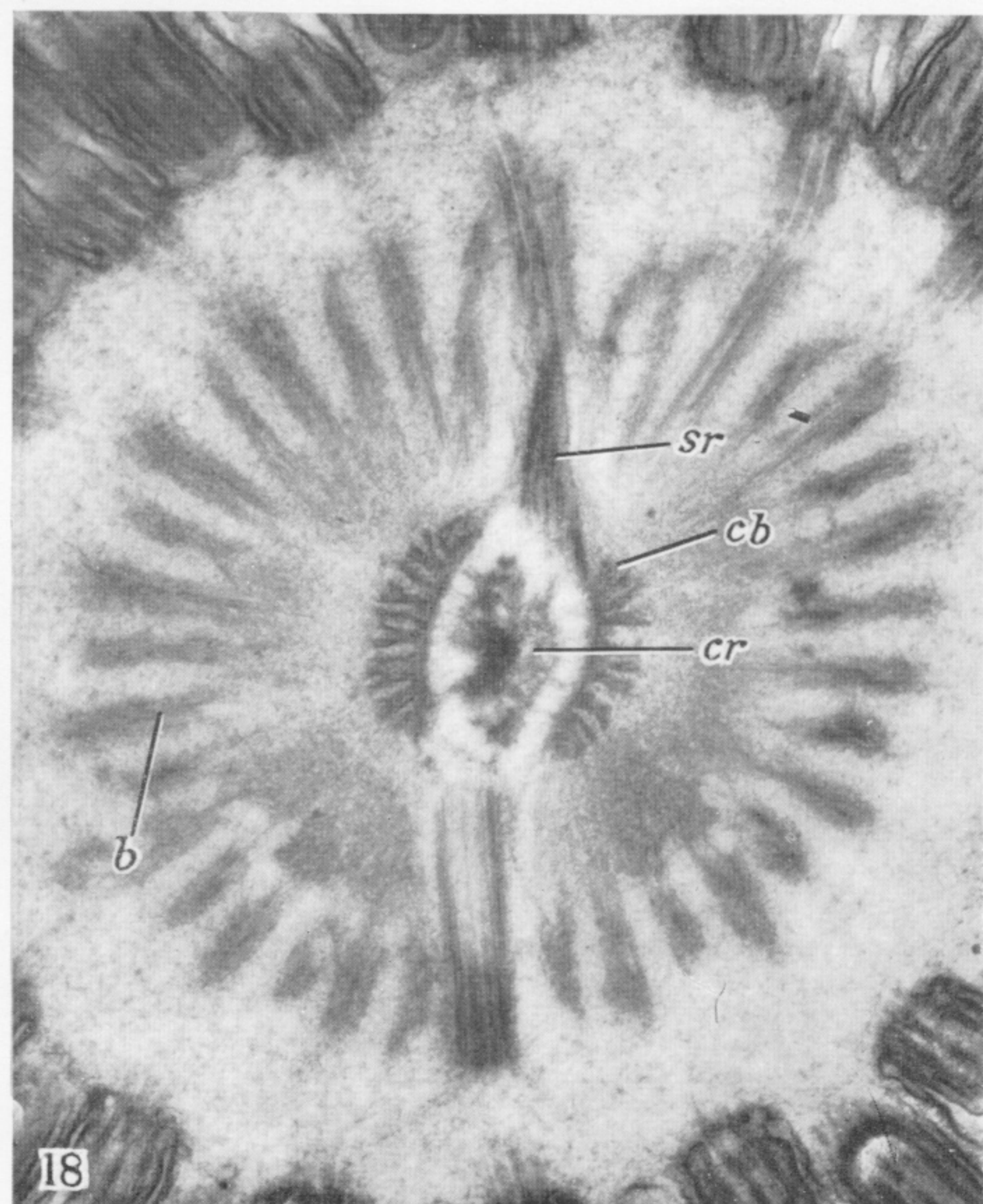
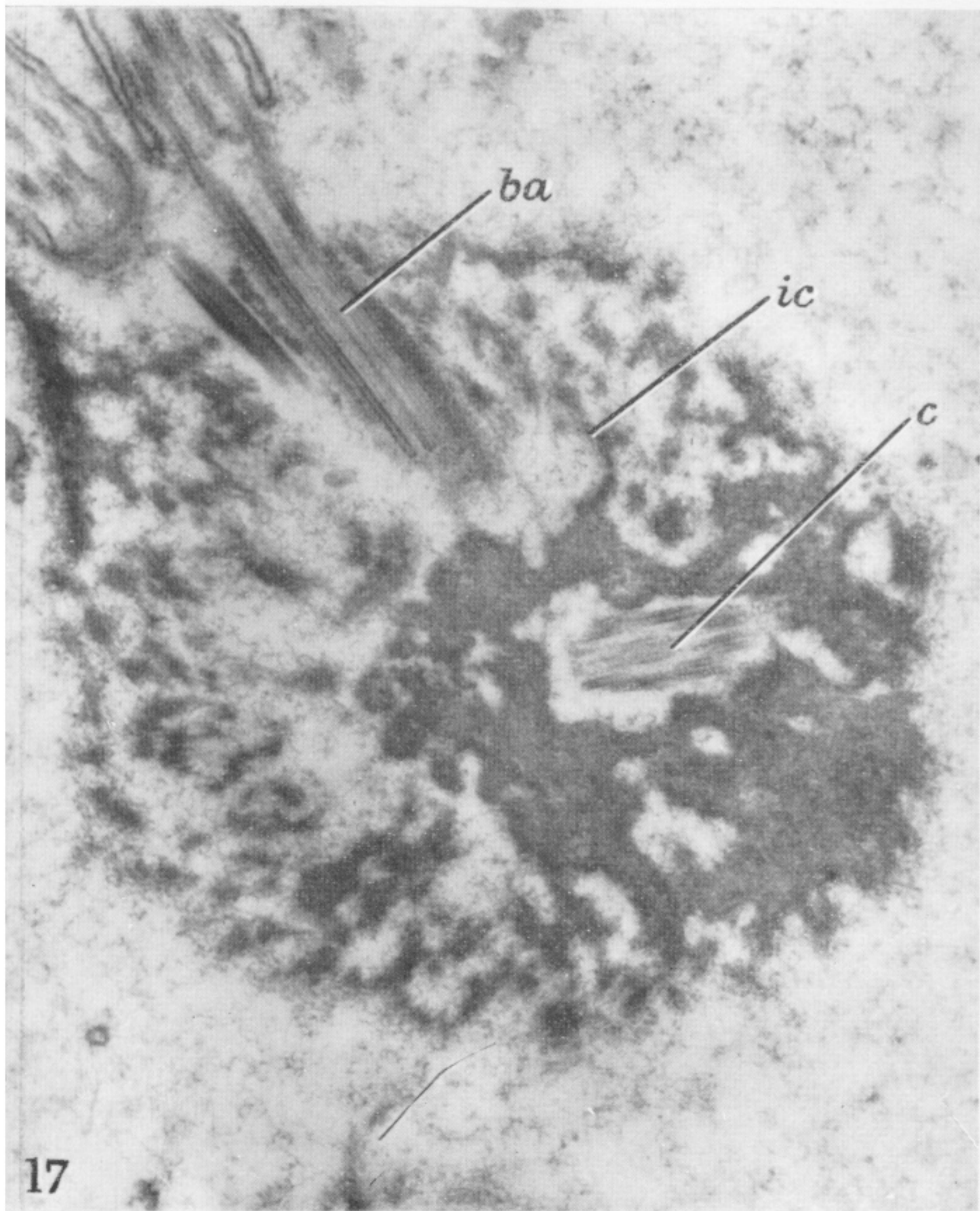
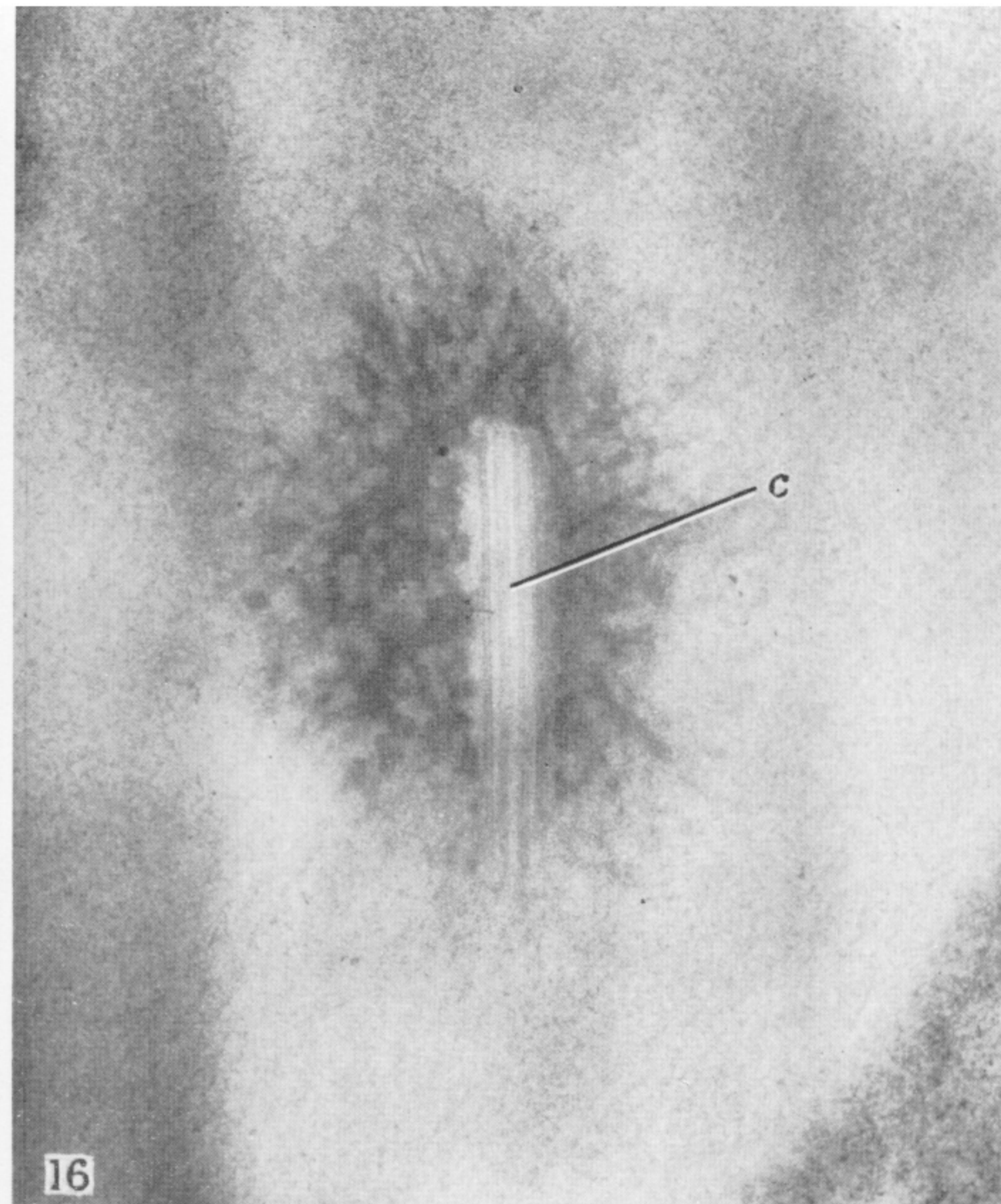
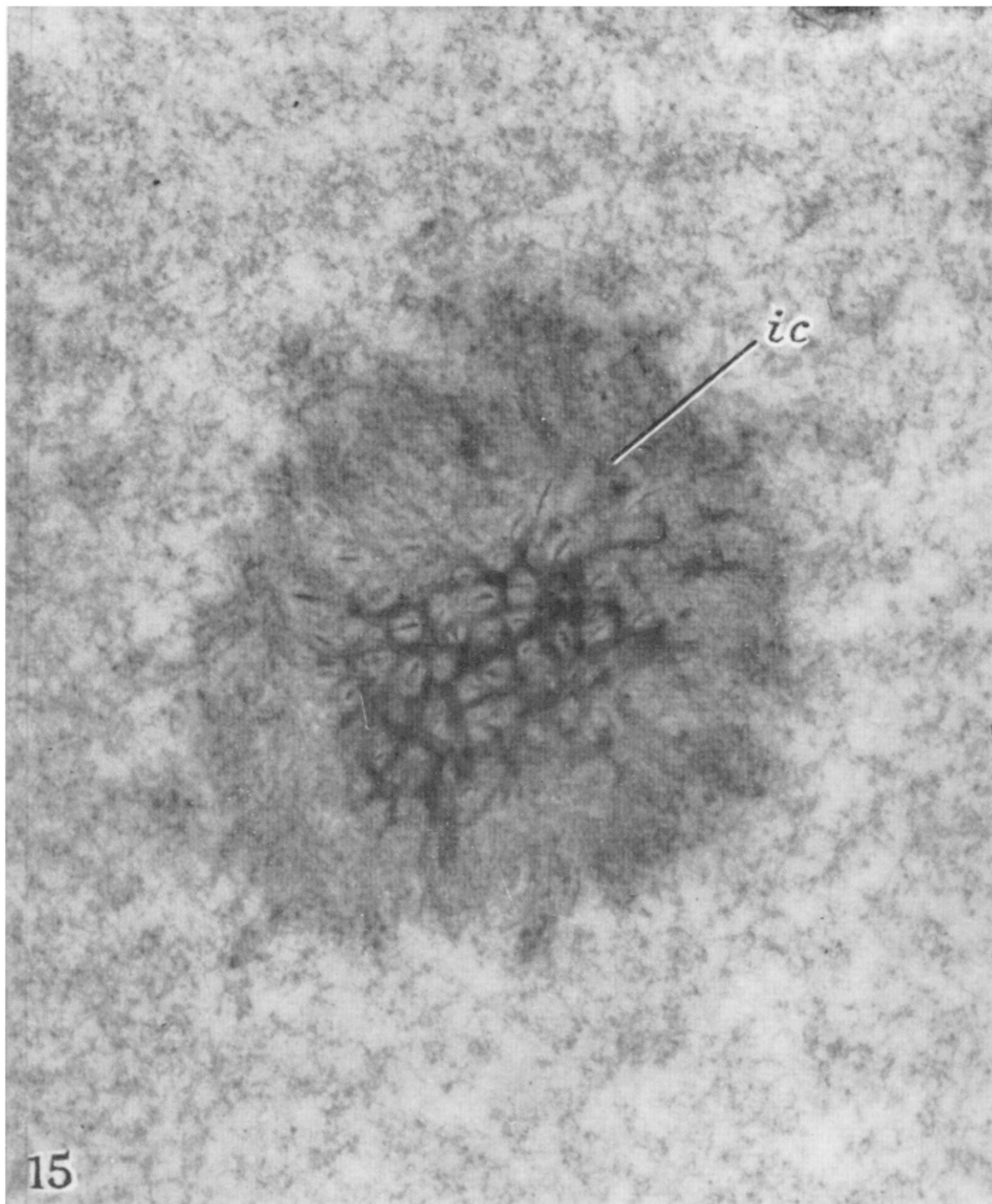


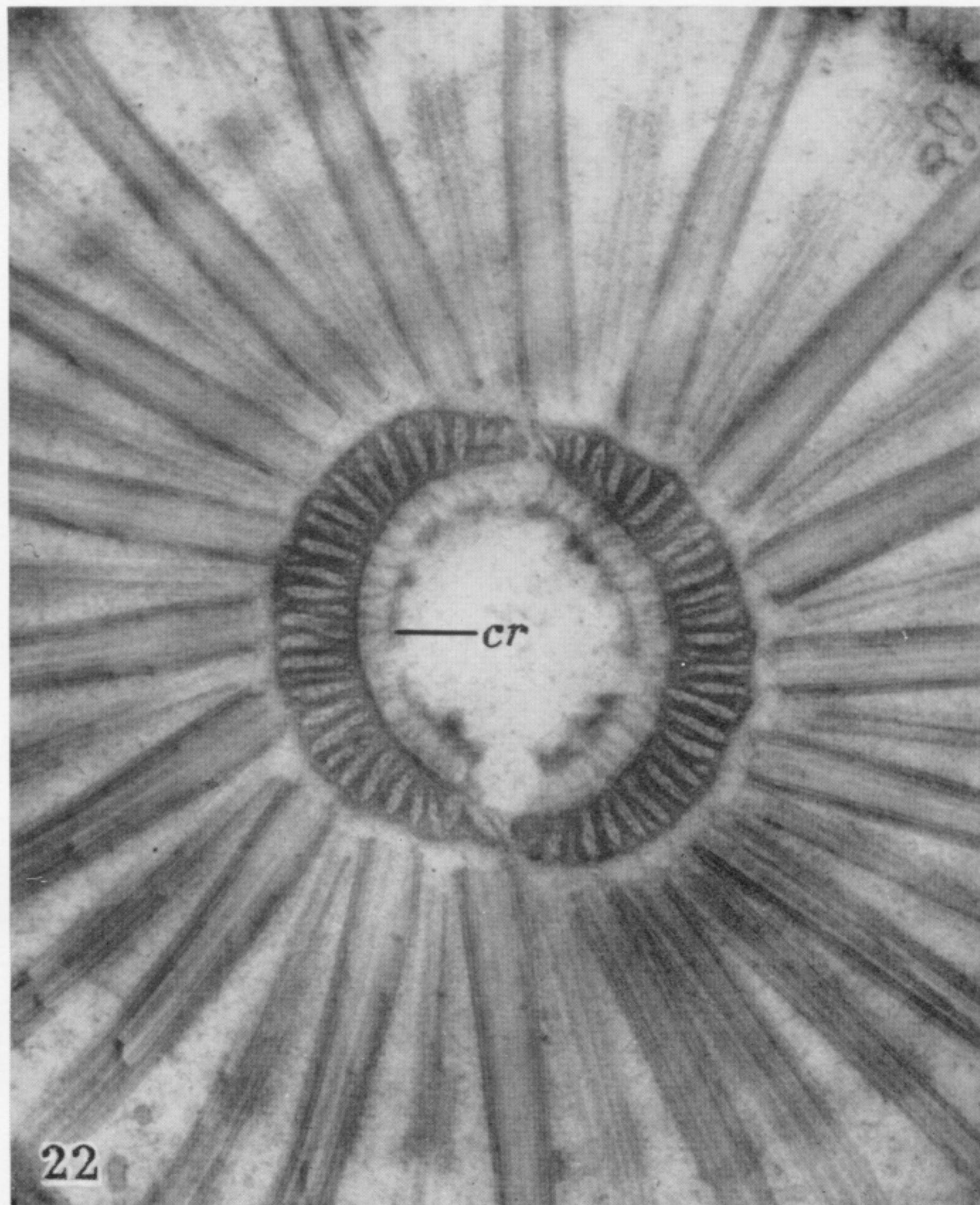
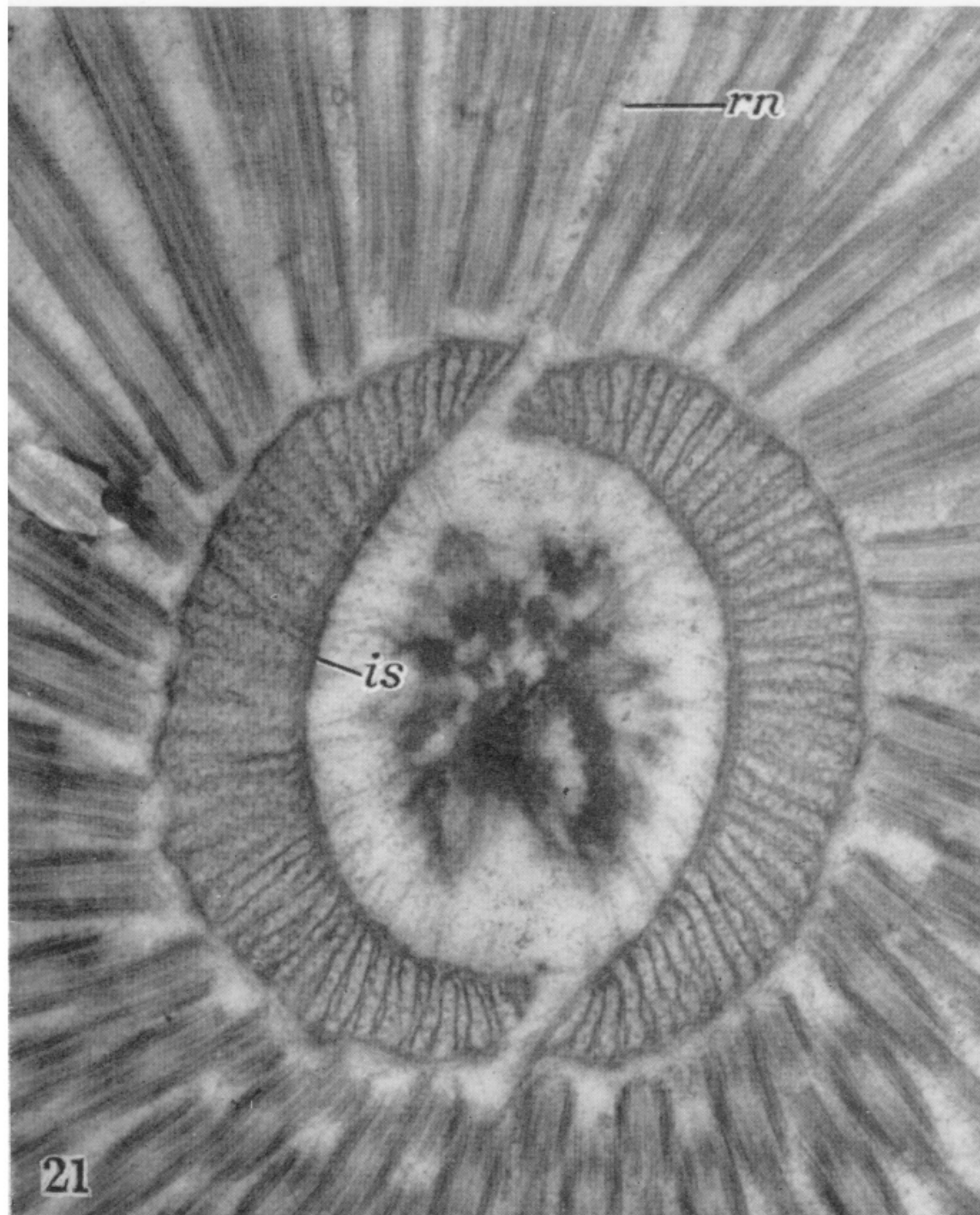
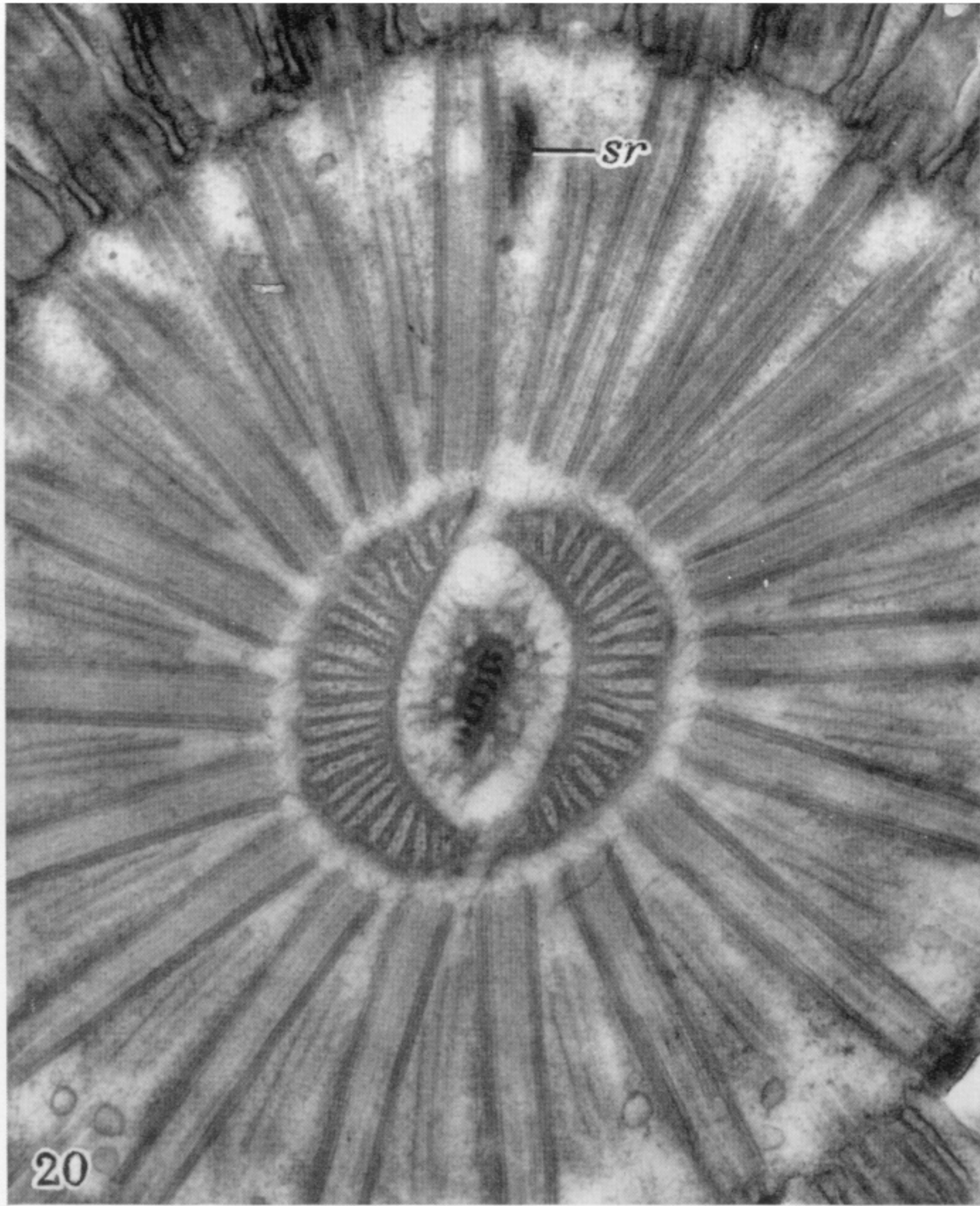
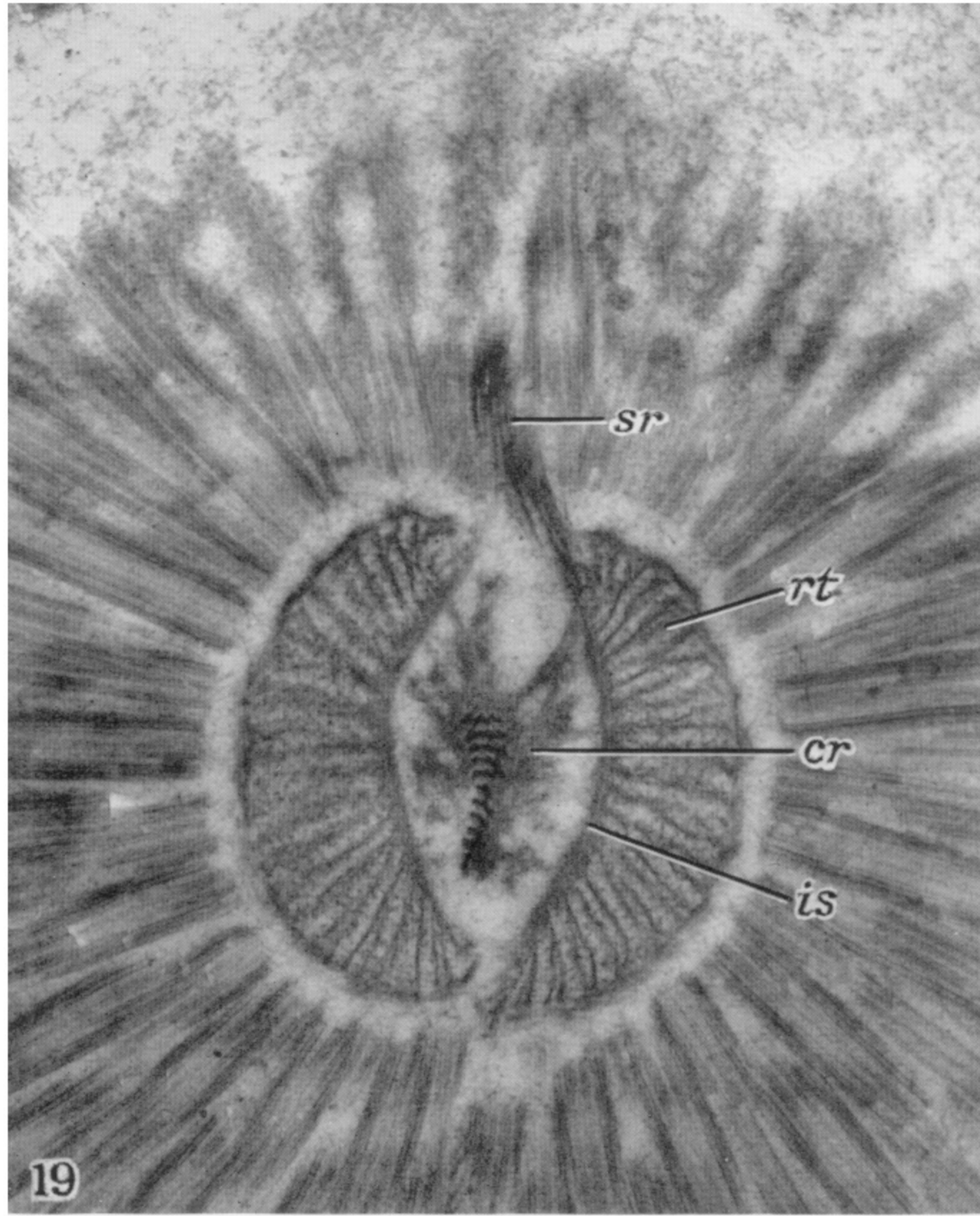
FIGURE 9. Longitudinal section showing the full length of the rostral tube. At the bottom of the picture the separate parabasal filaments can be seen. Note the rows of close-packed basal bodies, the inner cap, crescentic bodies, centriolar rod and the long basal-body-like structure in the rostral tube. This figure may be compared with figure 41, plate 34 ($\times 11\,000$).

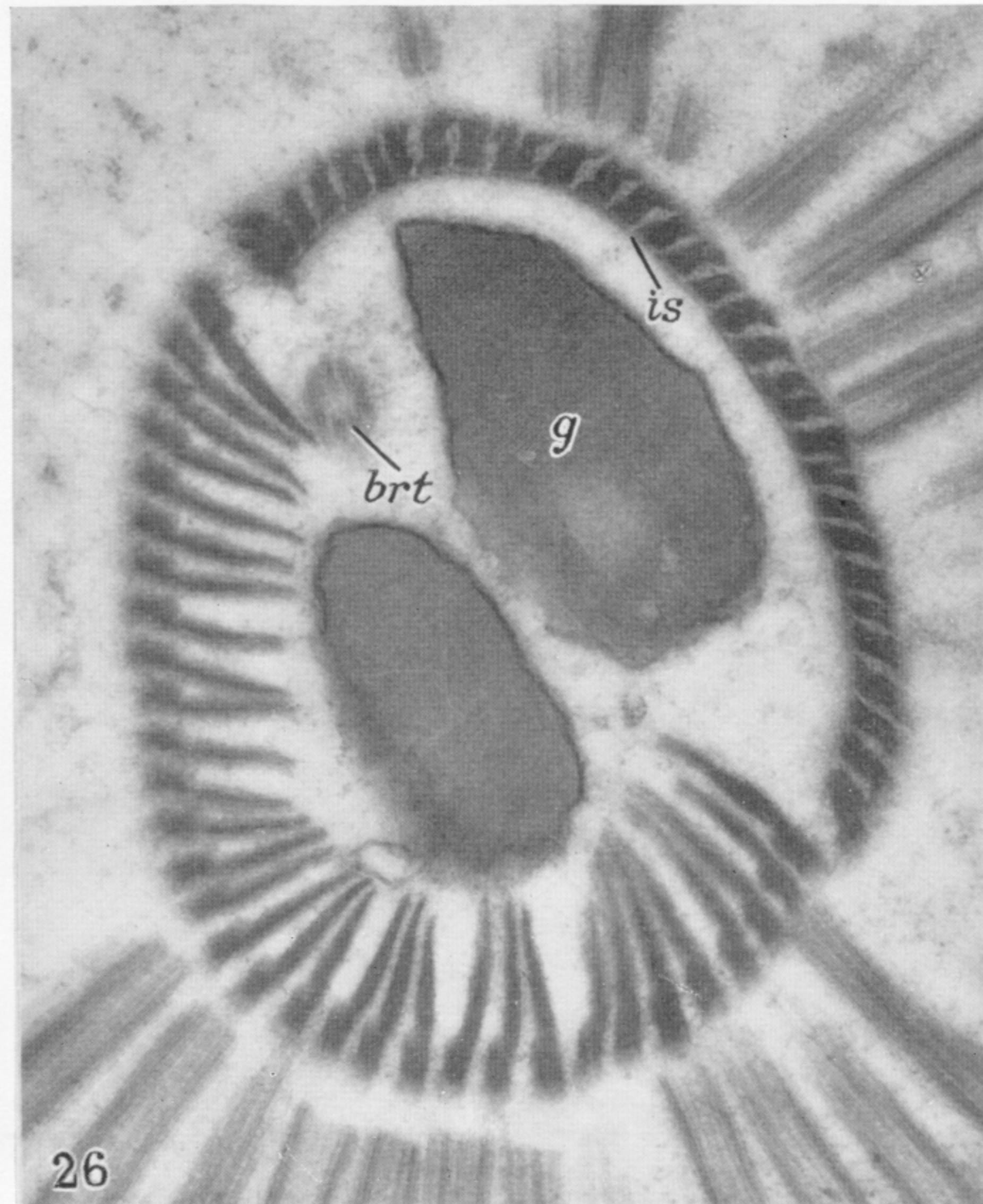
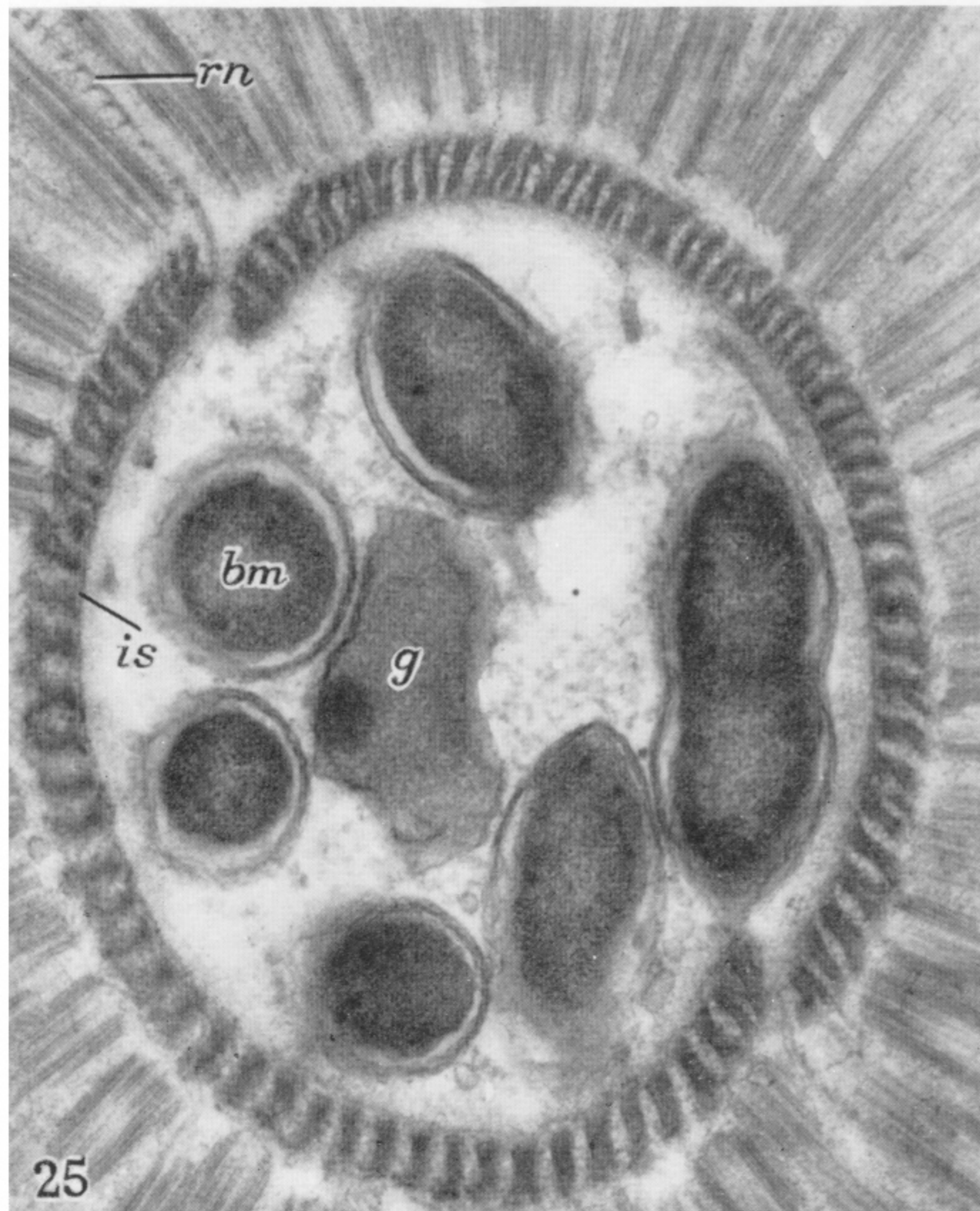
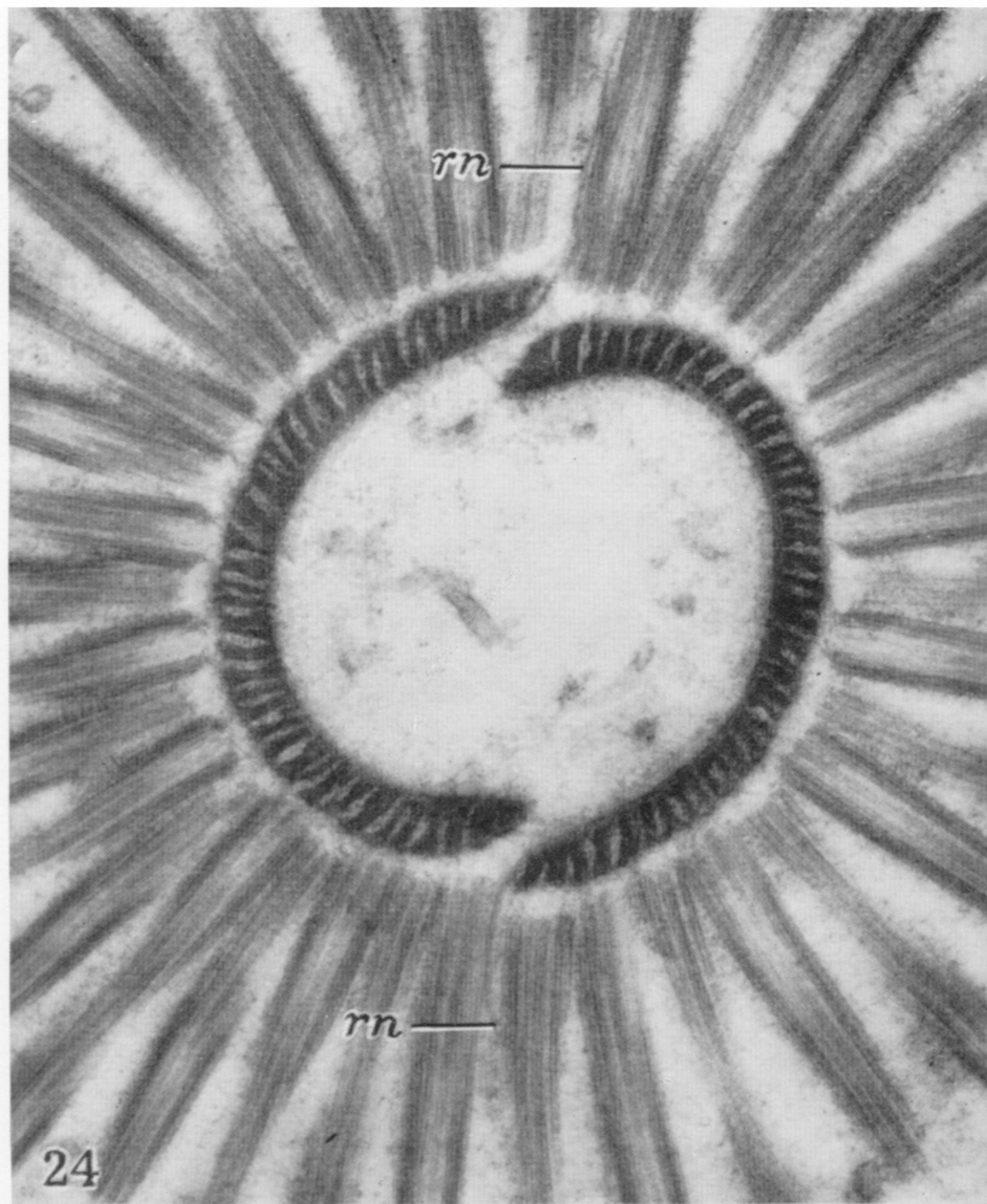
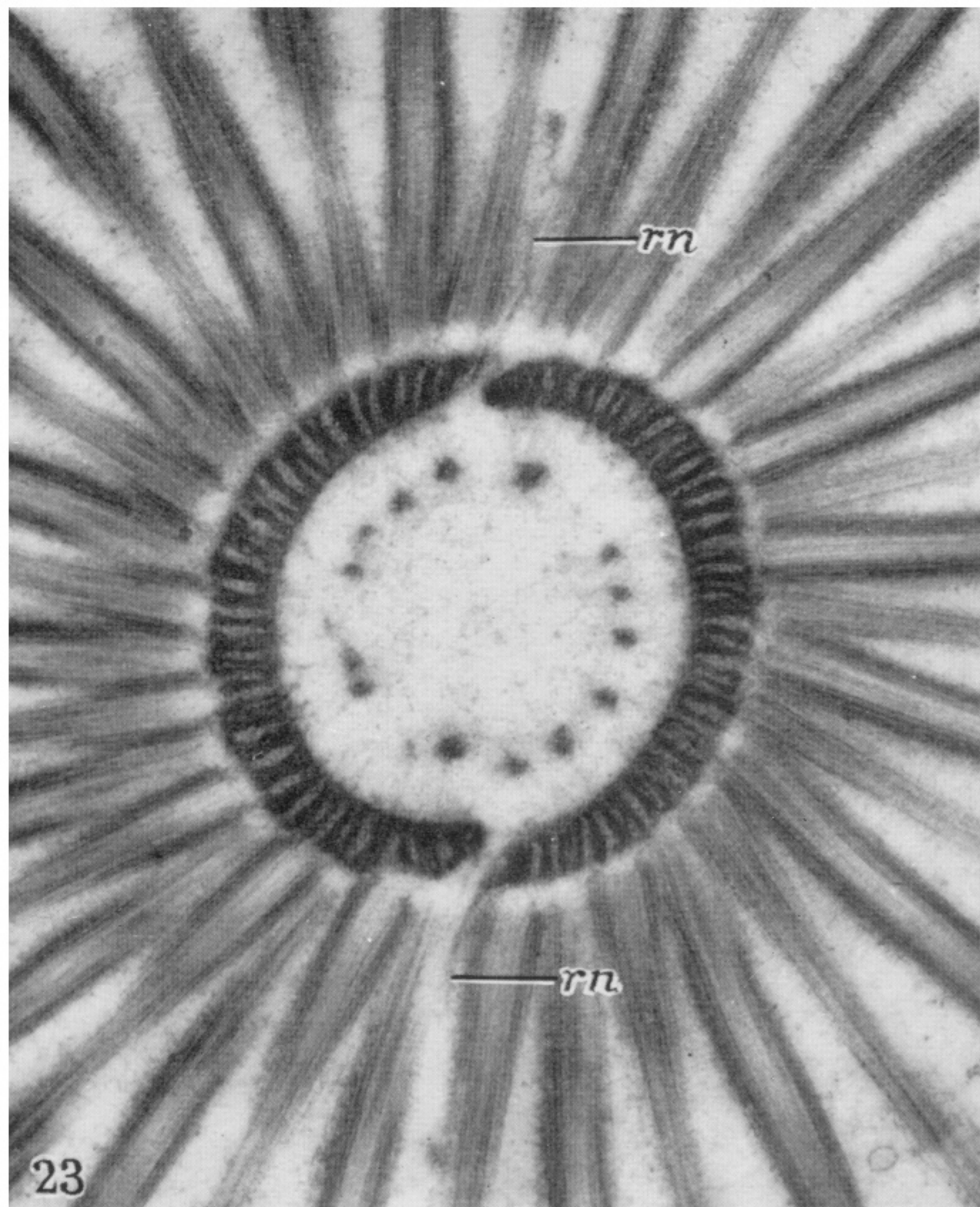
FIGURE 10. Enlargement from figure 9. The regularly arranged delicate fibres connecting the centriolar rod to the inner surface layer of the rostral tube are clear. Note the striated crescentic bodies, and the fine fibres running radially through the inner cap ($\times 30\,000$).

FIGURE 11. Serial section to that shown in figure 10. This shows the basal body of the anterior flagellum, lying between the two crescentic bodies, and also one of the striated ribbons ($\times 30\,000$).









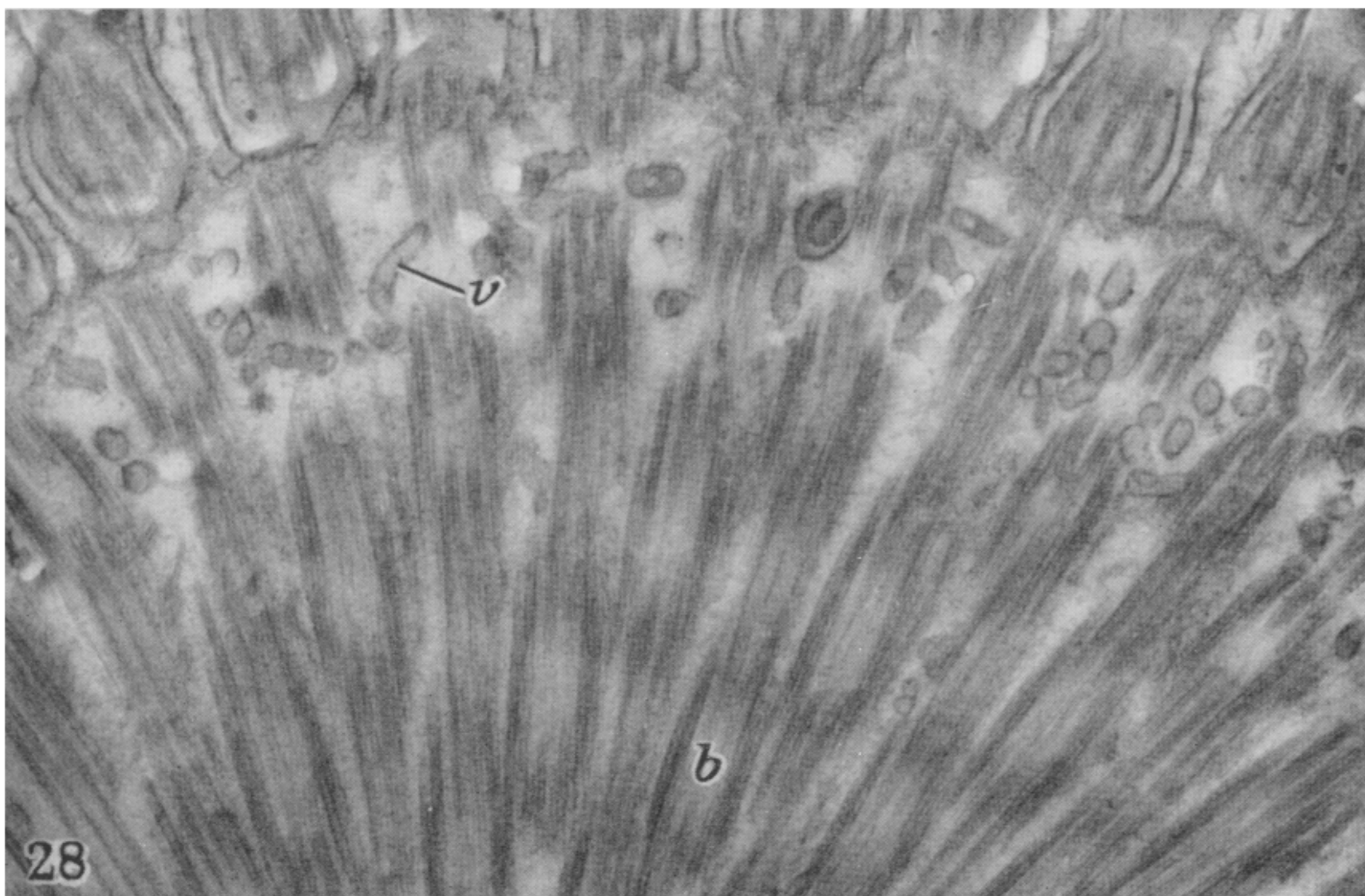
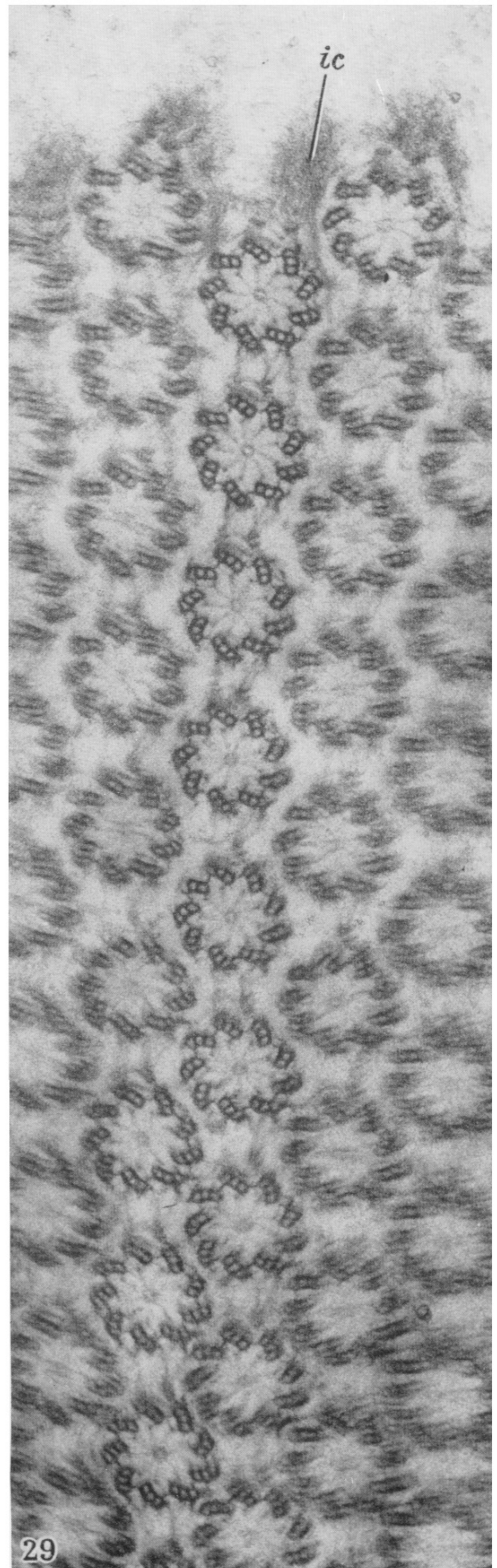
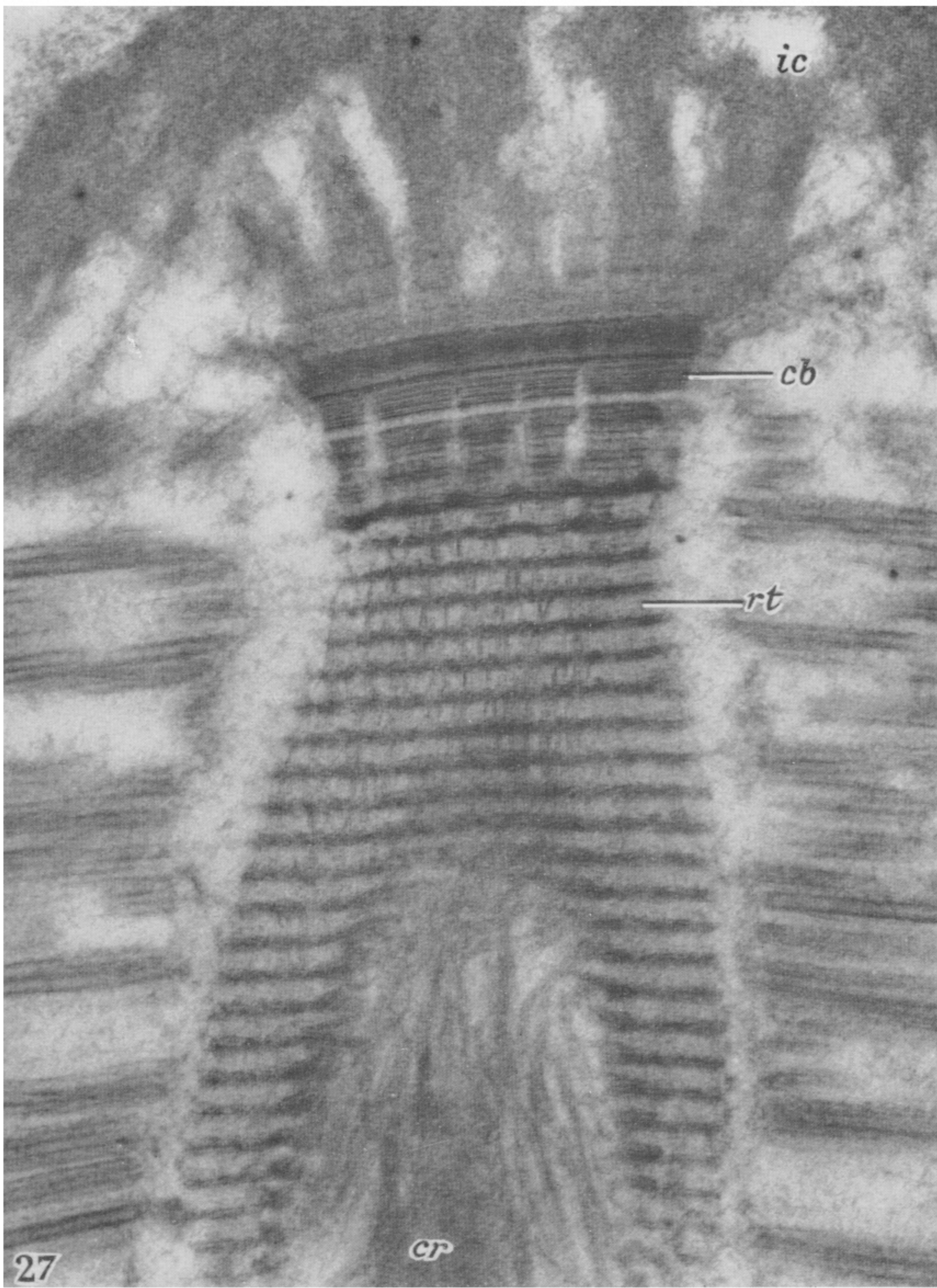


FIGURE 27. Longitudinal section of an organism from *Cryptocercus*, showing the anterior end of the rostral tube, which is continuous with one of the crescentic bodies. Note the complex striation of the latter, which is distinct from that of the rostral tube and extends faintly into the inner cap. At the bottom of the picture the fibres connecting the centriolar rod to the rostral tube can be seen ($\times 94\,000$).

FIGURE 28. Section showing the tubules and vesicles in the cytoplasm between the distal ends of the rostral basal bodies ($\times 36\,000$).

FIGURE 29. Section through five rows of basal bodies at the anterior end of the rostrum. Only in the central one are the basal bodies cut exactly transversely. The fine fibres between them are clear and the connexion of the fibres to the inner cap material at the top of the picture is evident ($\times 80\,000$).

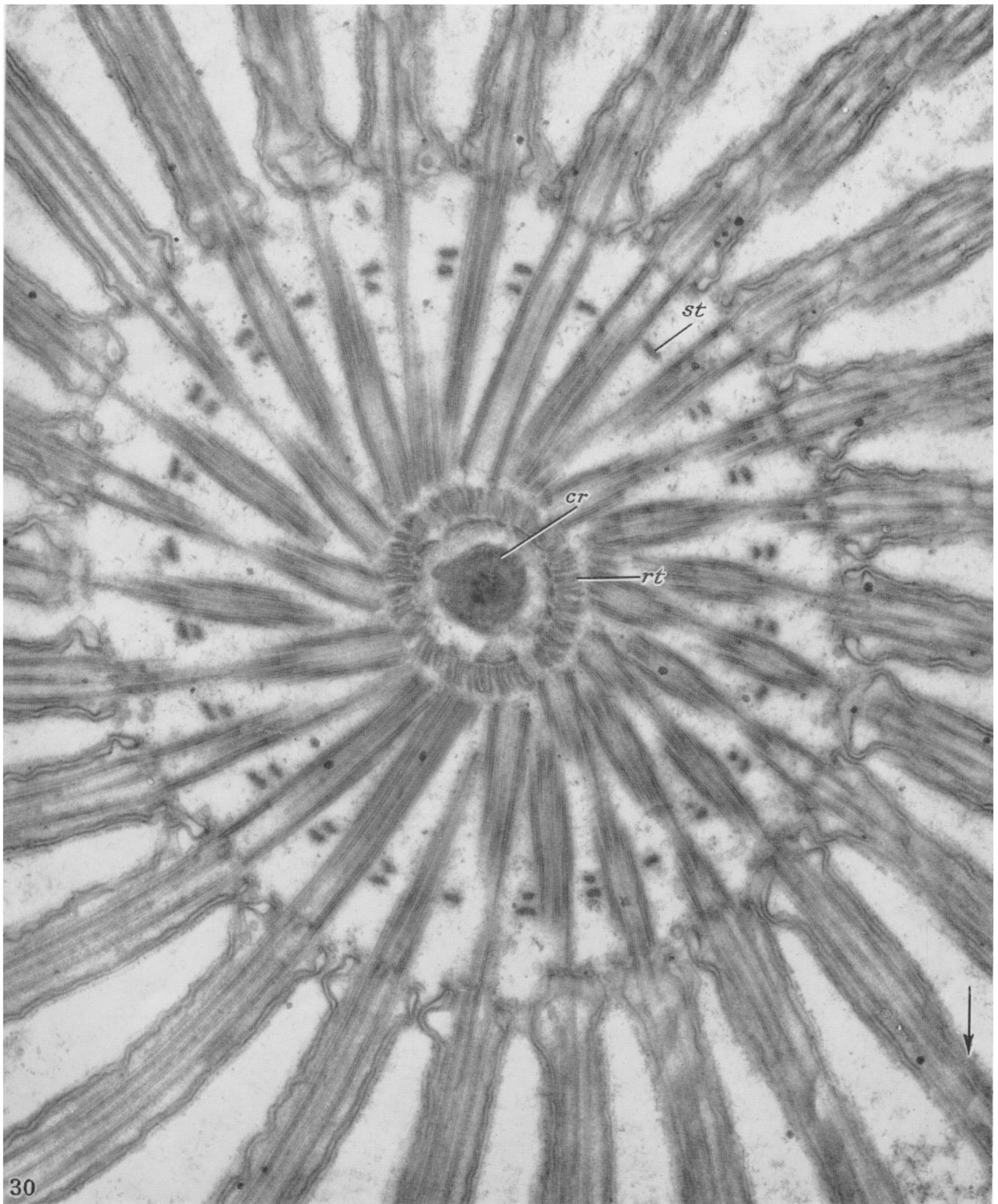
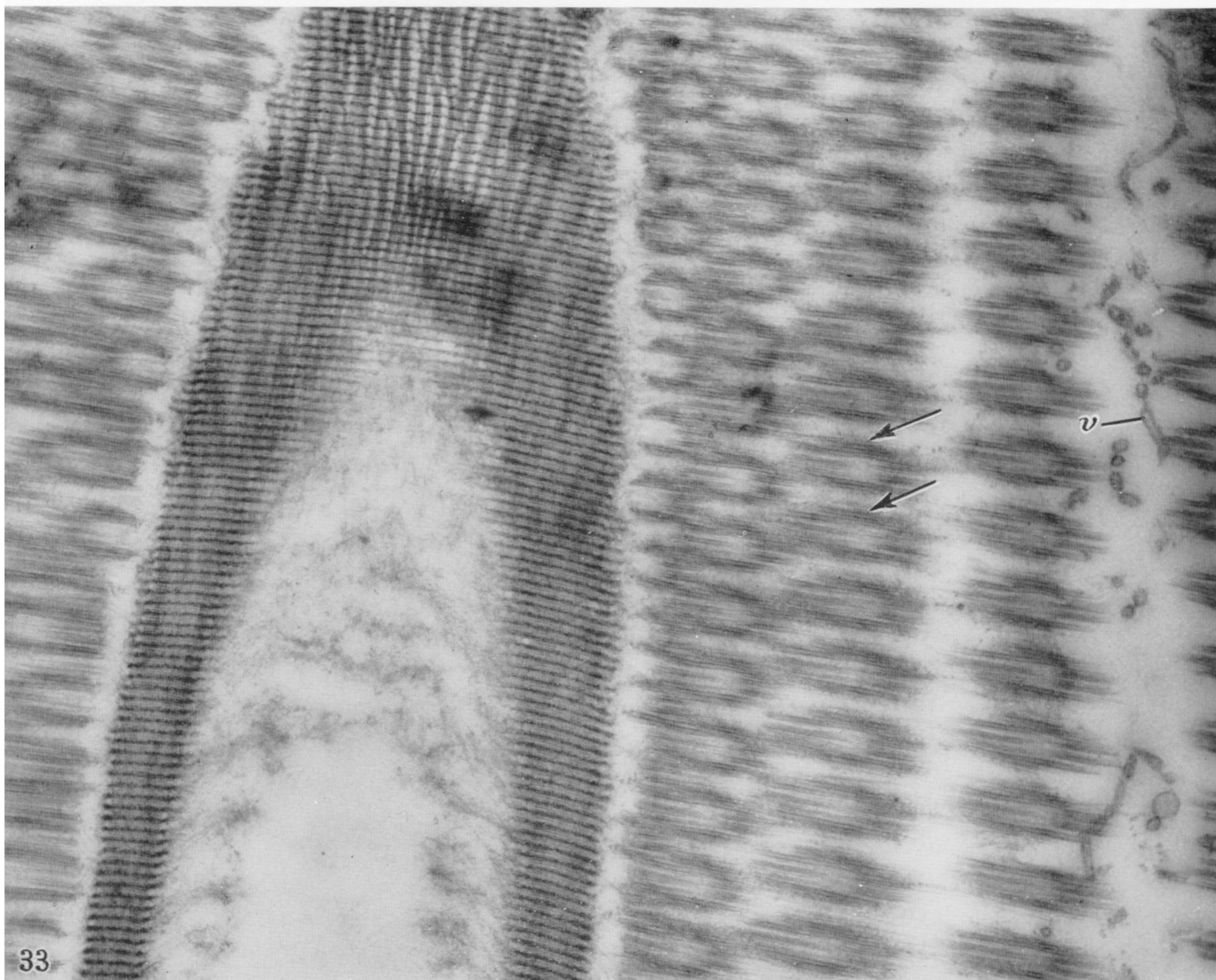
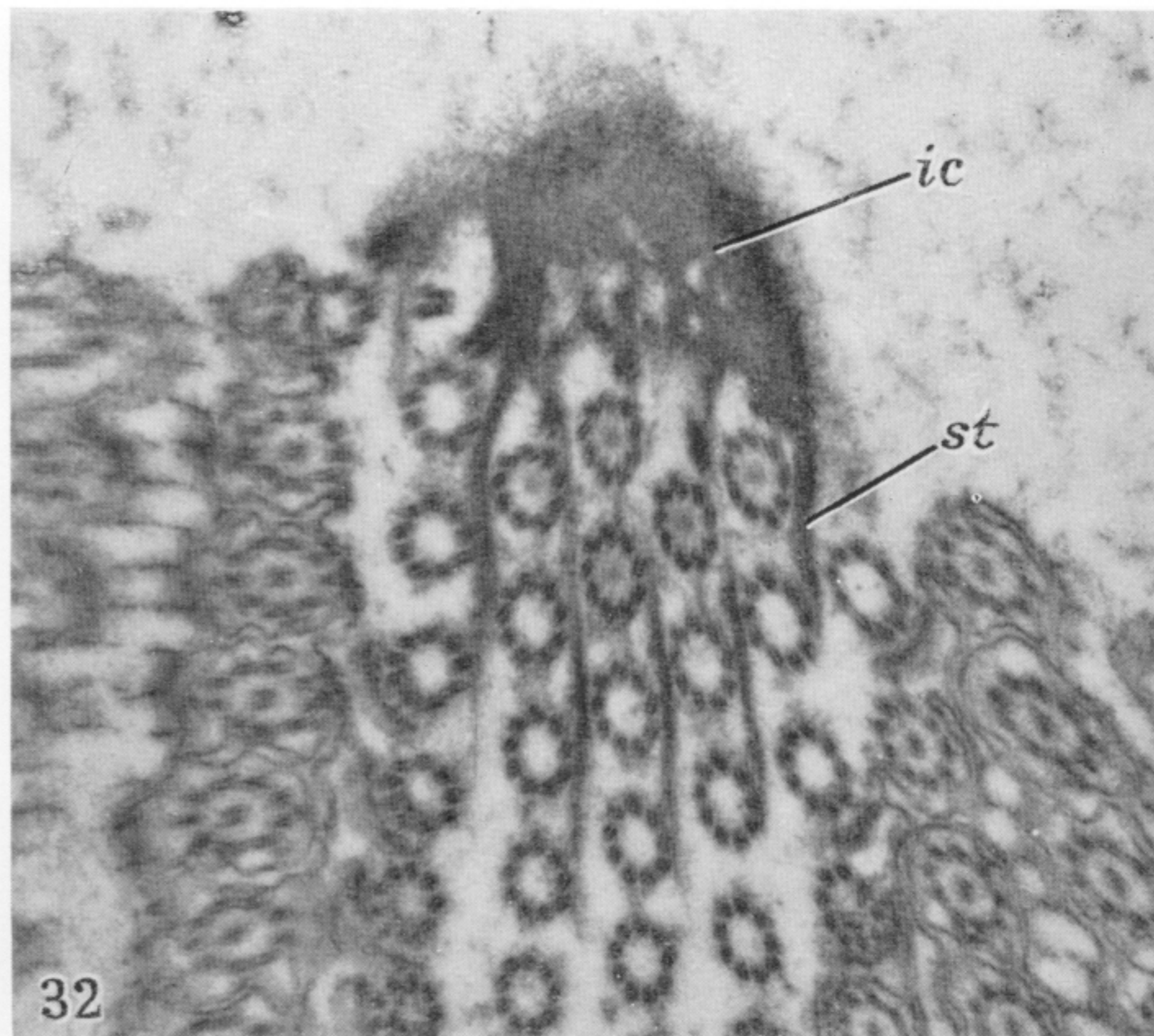
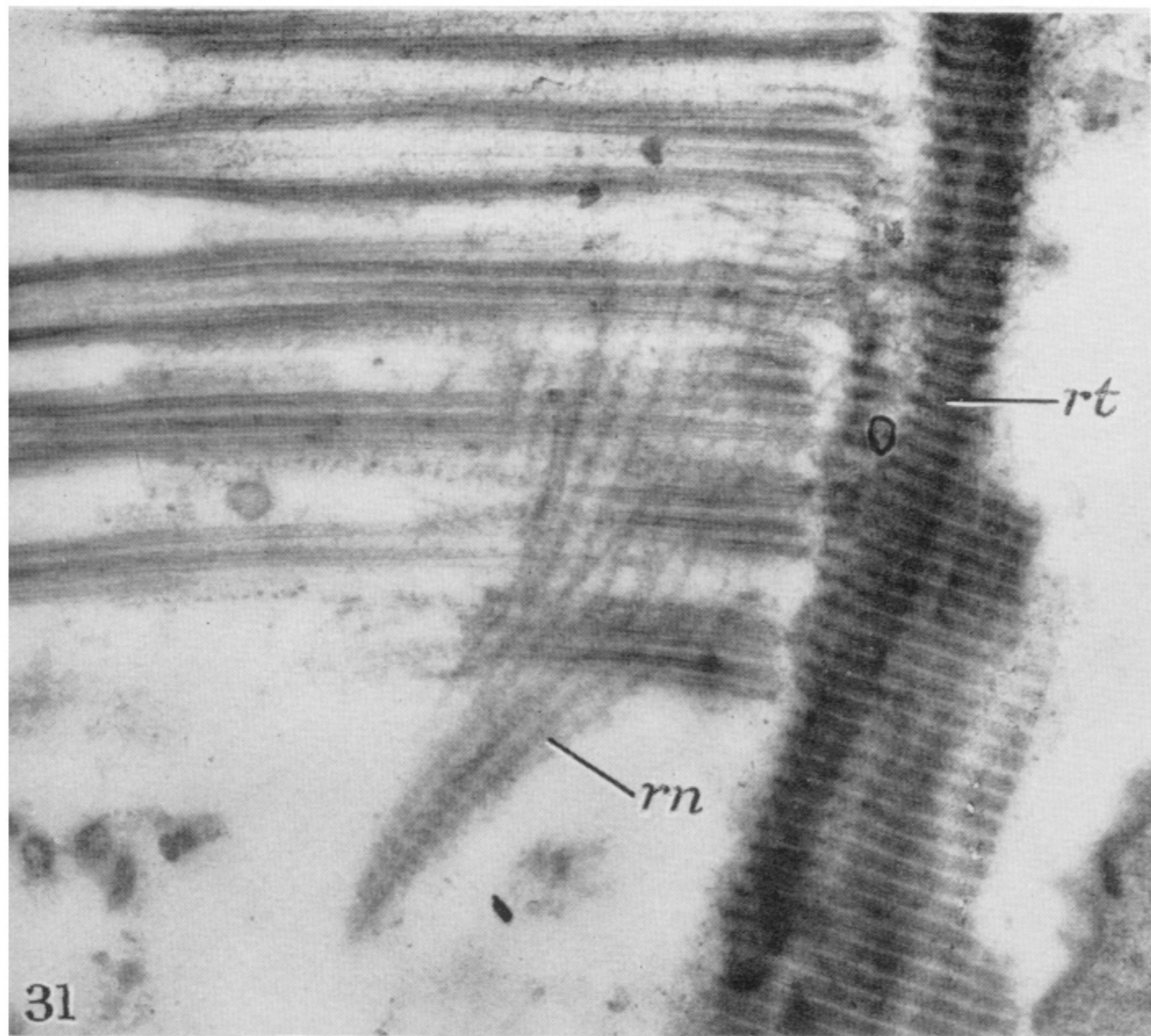
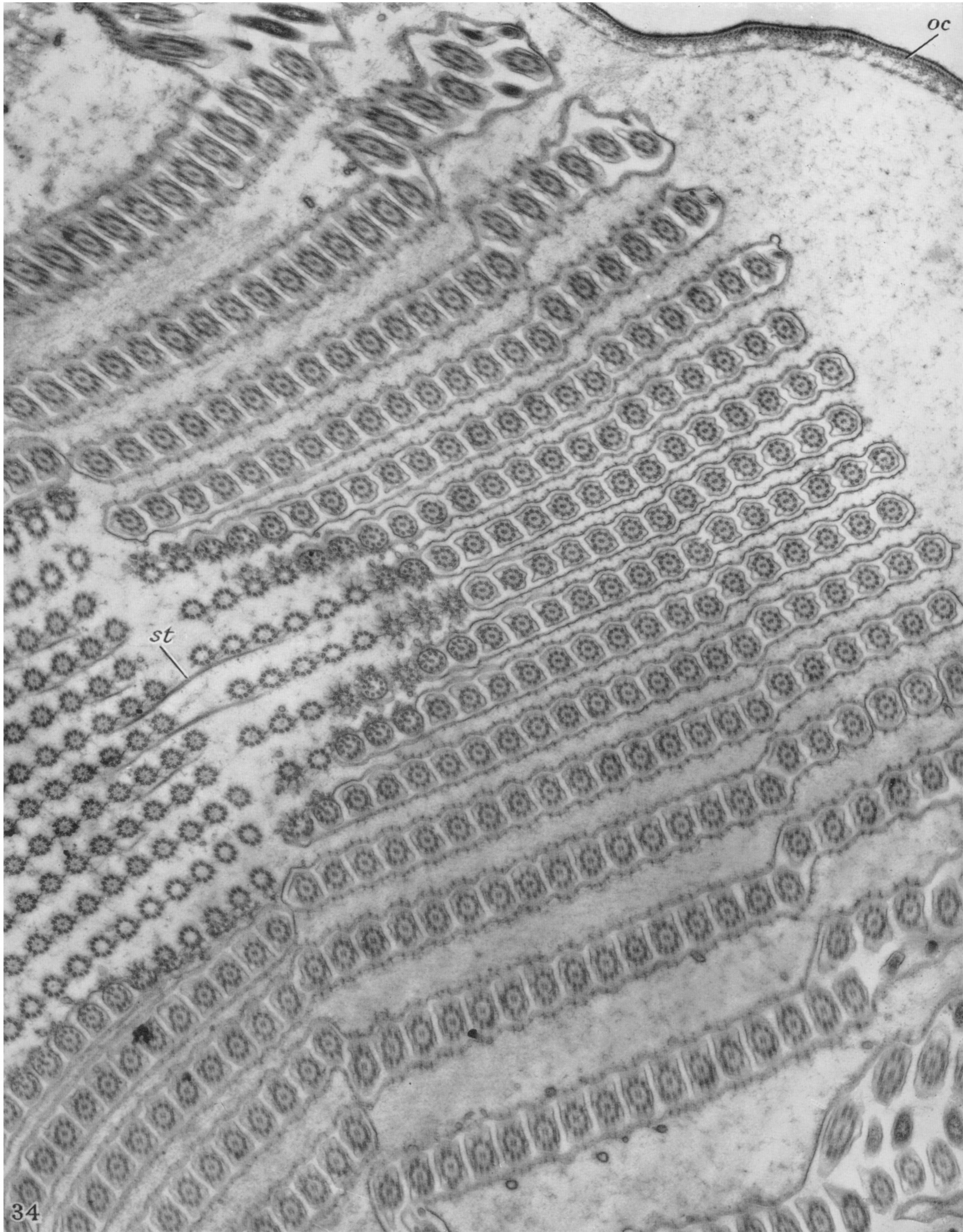
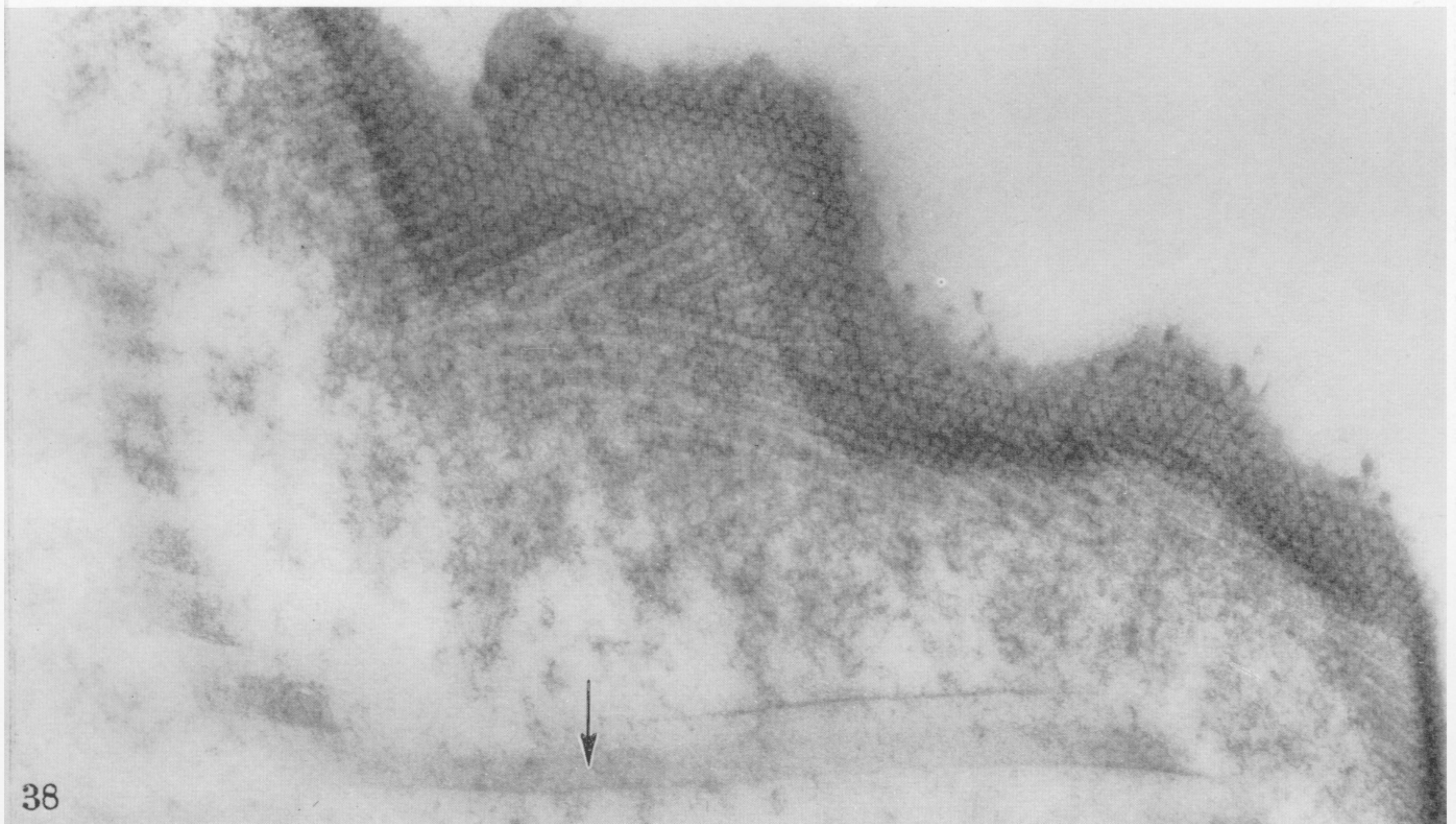
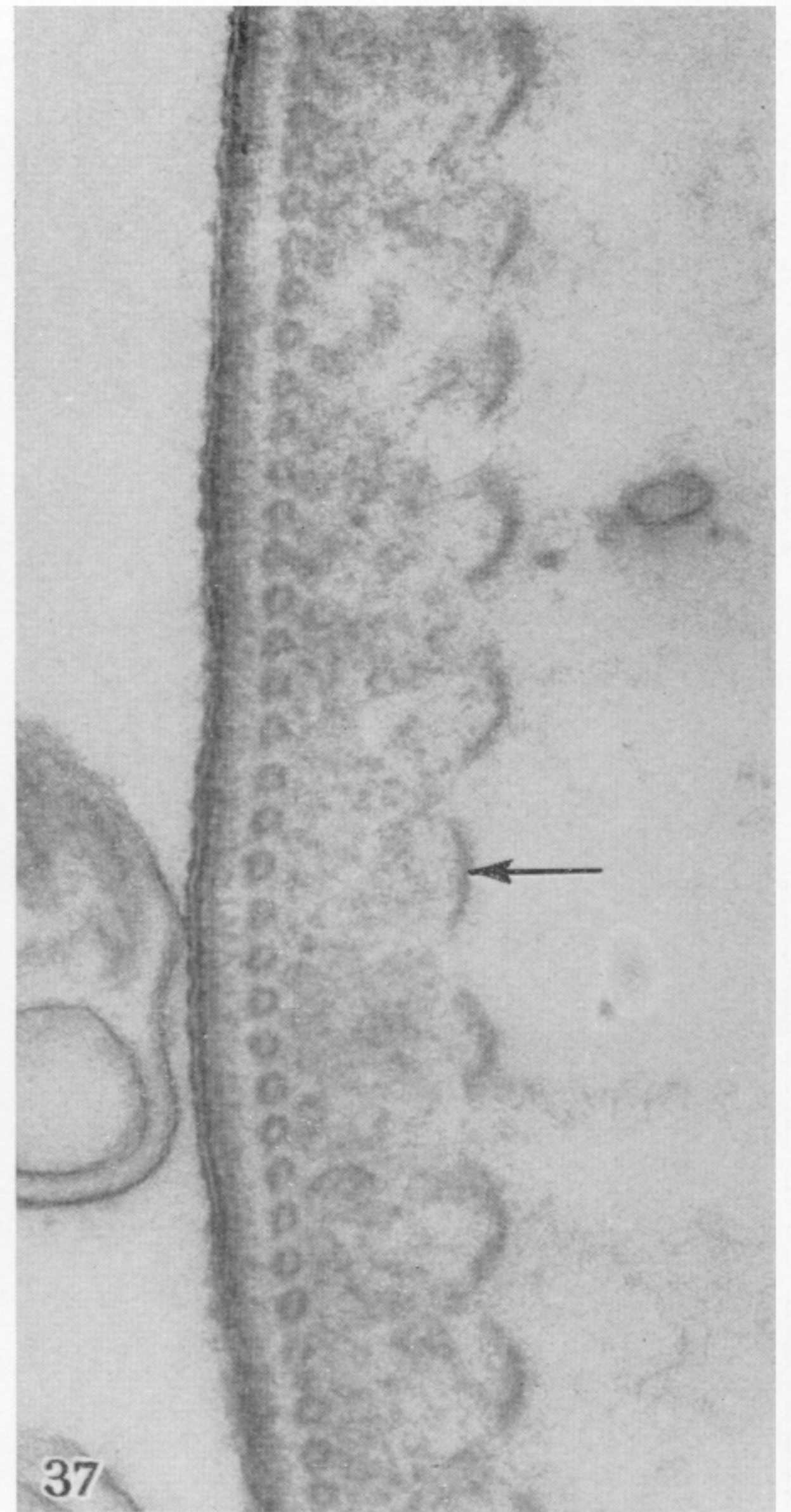
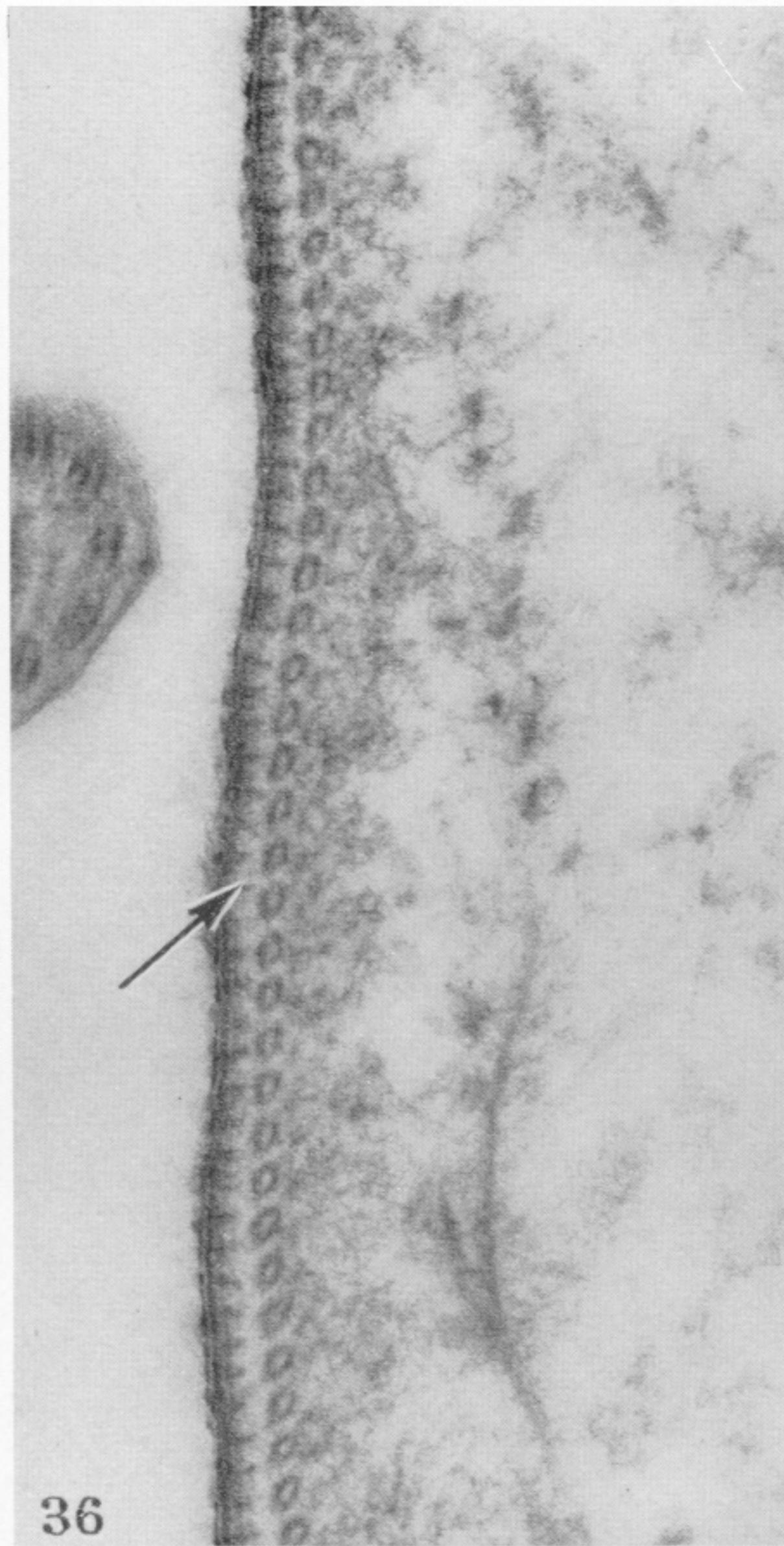
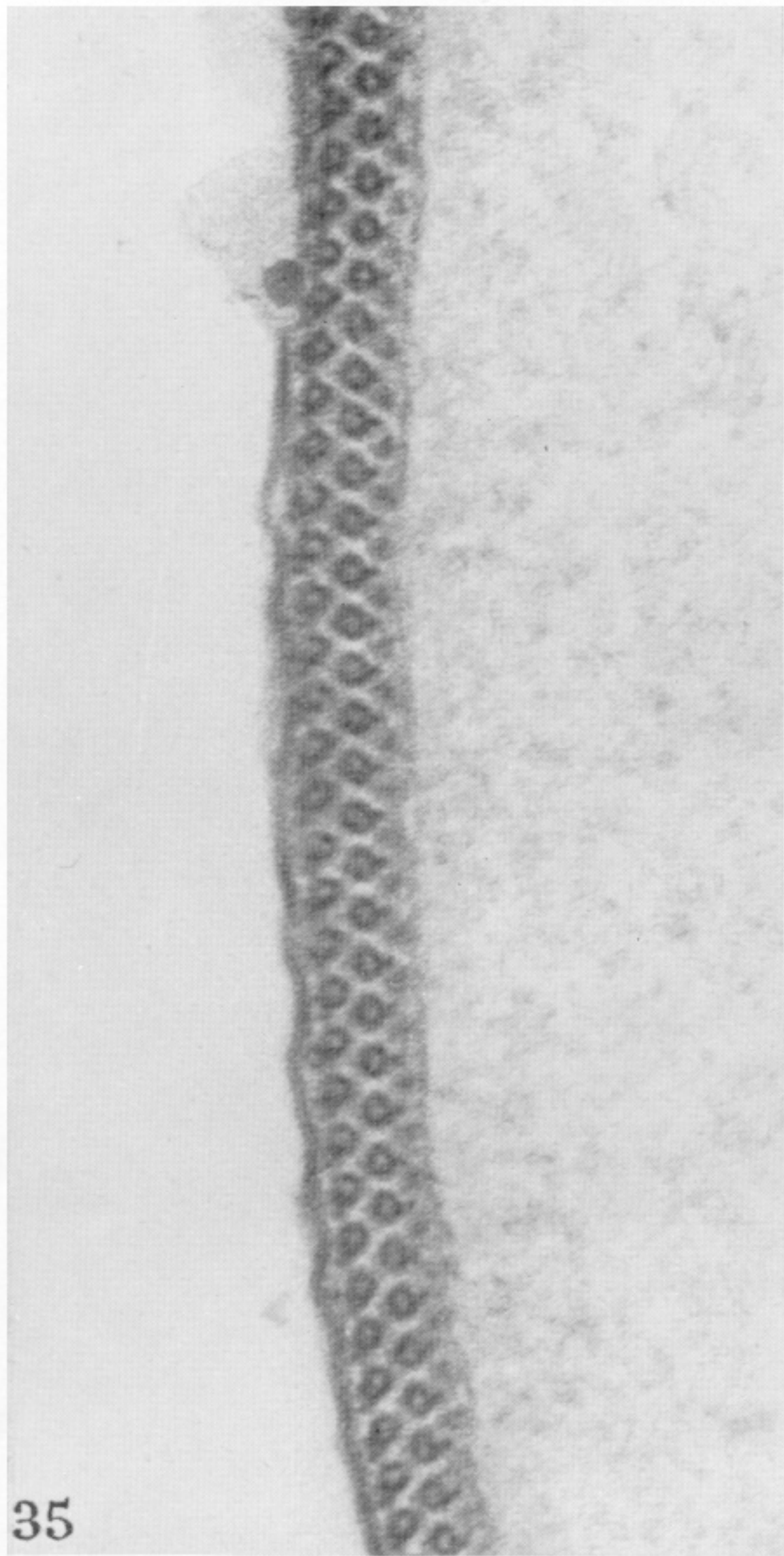


FIGURE 30. Transverse section of the rostrum of an organism from *Cryptocercus*, to show particularly the circle of dense strands running between the basal bodies of the flagella. These are the strands which originate from the margin of the inner cap (compare figure 32). One of the flagella (arrow) shows a prominent striation along one edge. The lumen of the rostral tube is largely occupied by the centriolar rod ($\times 44000$).







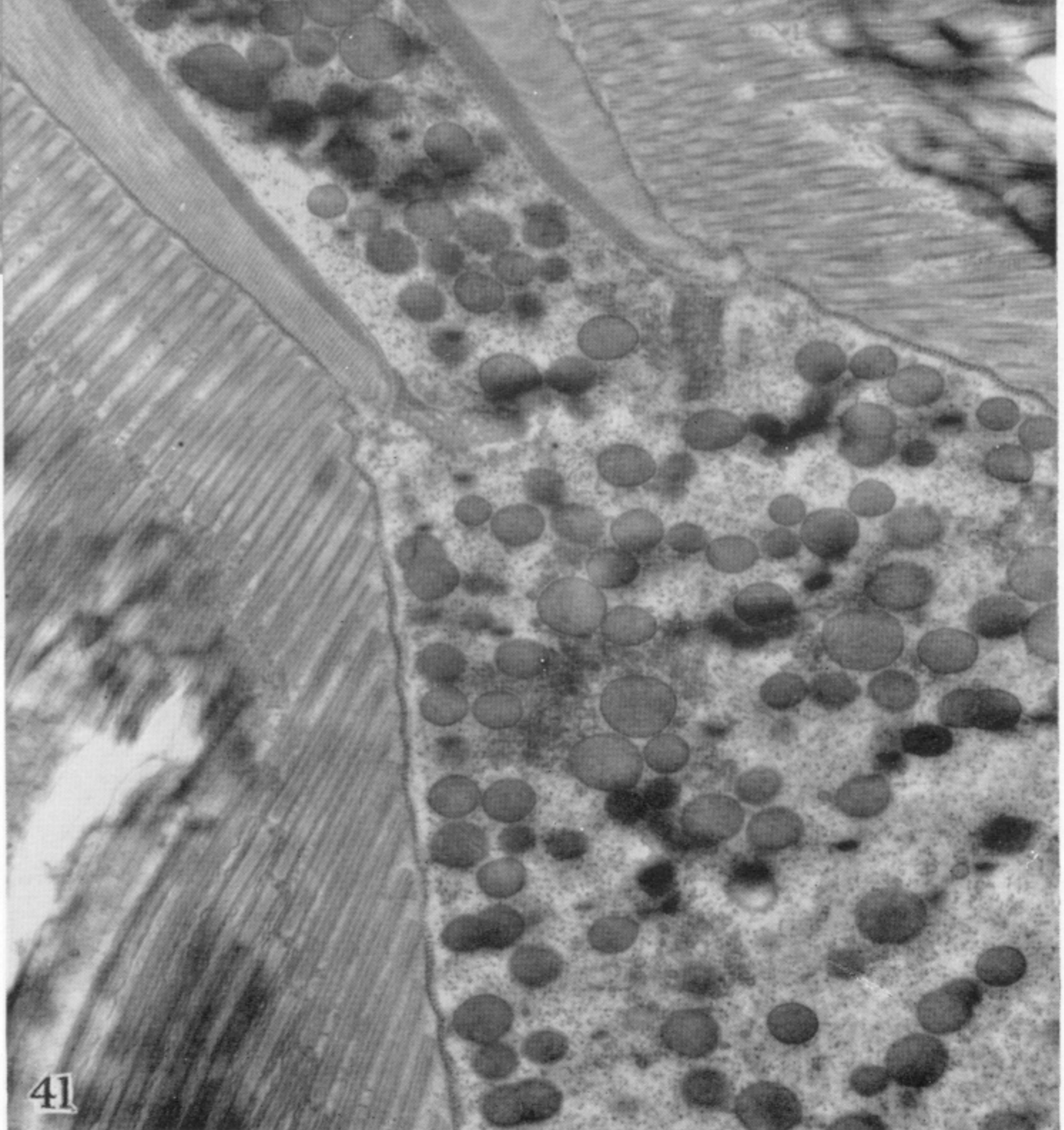




FIGURE 42. Enlargement from figure 41, showing the anterior end of the rostral tube. Note the inner and outer walls and the thickened rim. The general disposition of the inner cap and associated structures can be seen but details of fine structure are not clearly visible (see figure 43). In the lumen of the rostral tube note the restriction of the tubules to the wide anterior part ($\times 21\,000$).

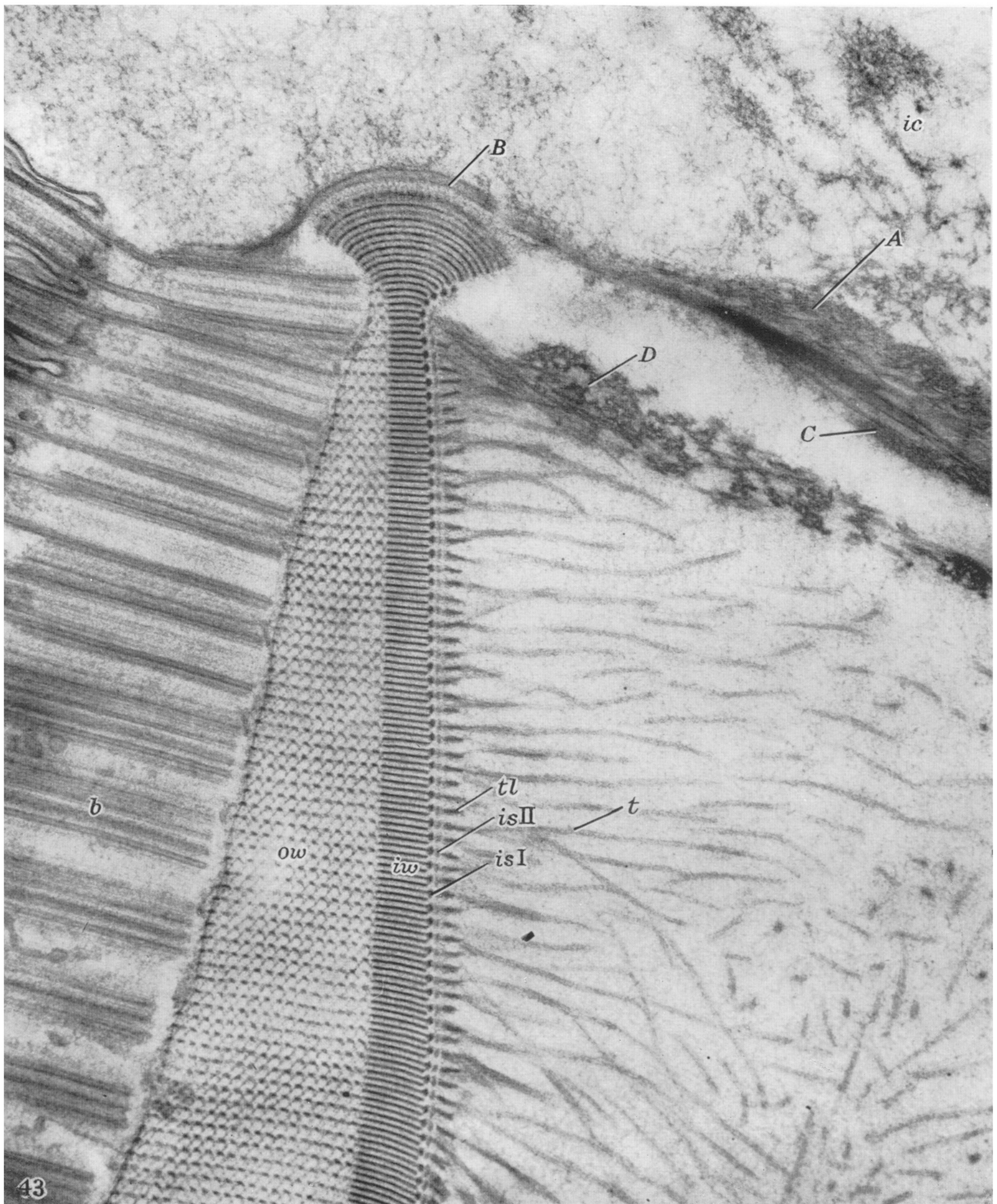
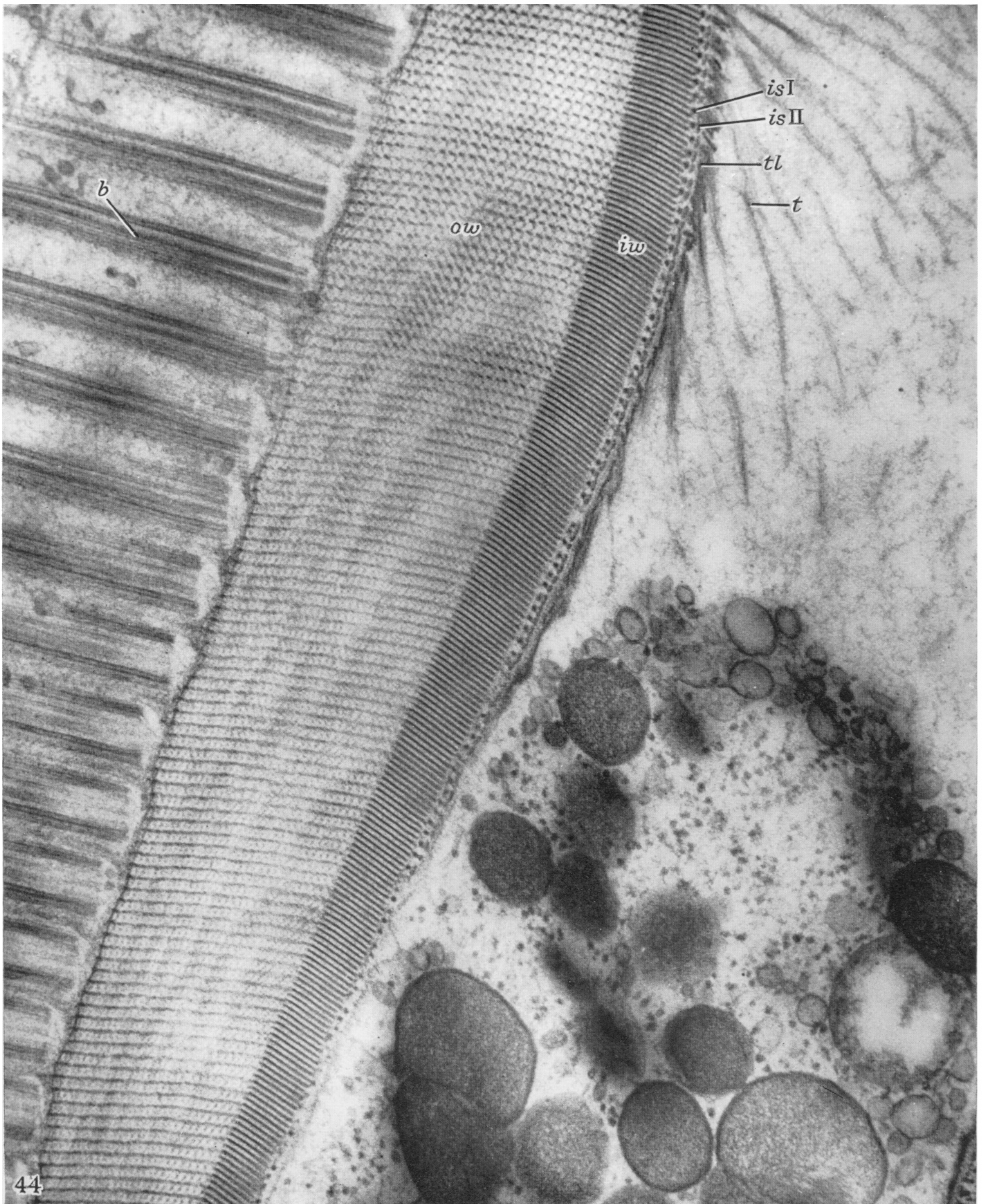
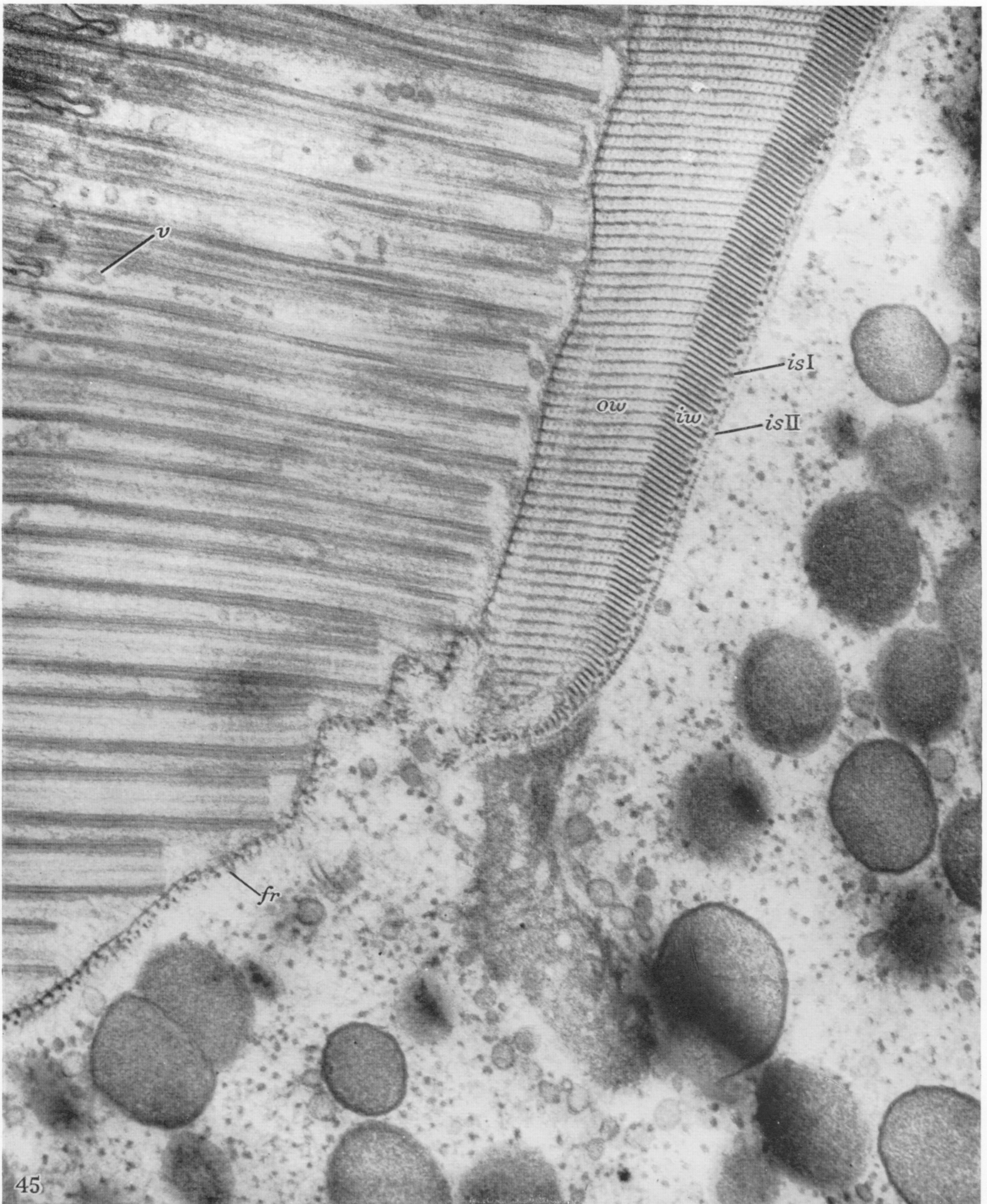


FIGURE 43. Enlargement from figure 42, showing the fine structure of the anterior end of the rostral tube. The various components of the tube and its surface layers are clearly displayed, as are the four structures (*A*, *B*, *C*, *D*) at its anterior end (compare figure 57) ($\times 55\,000$).



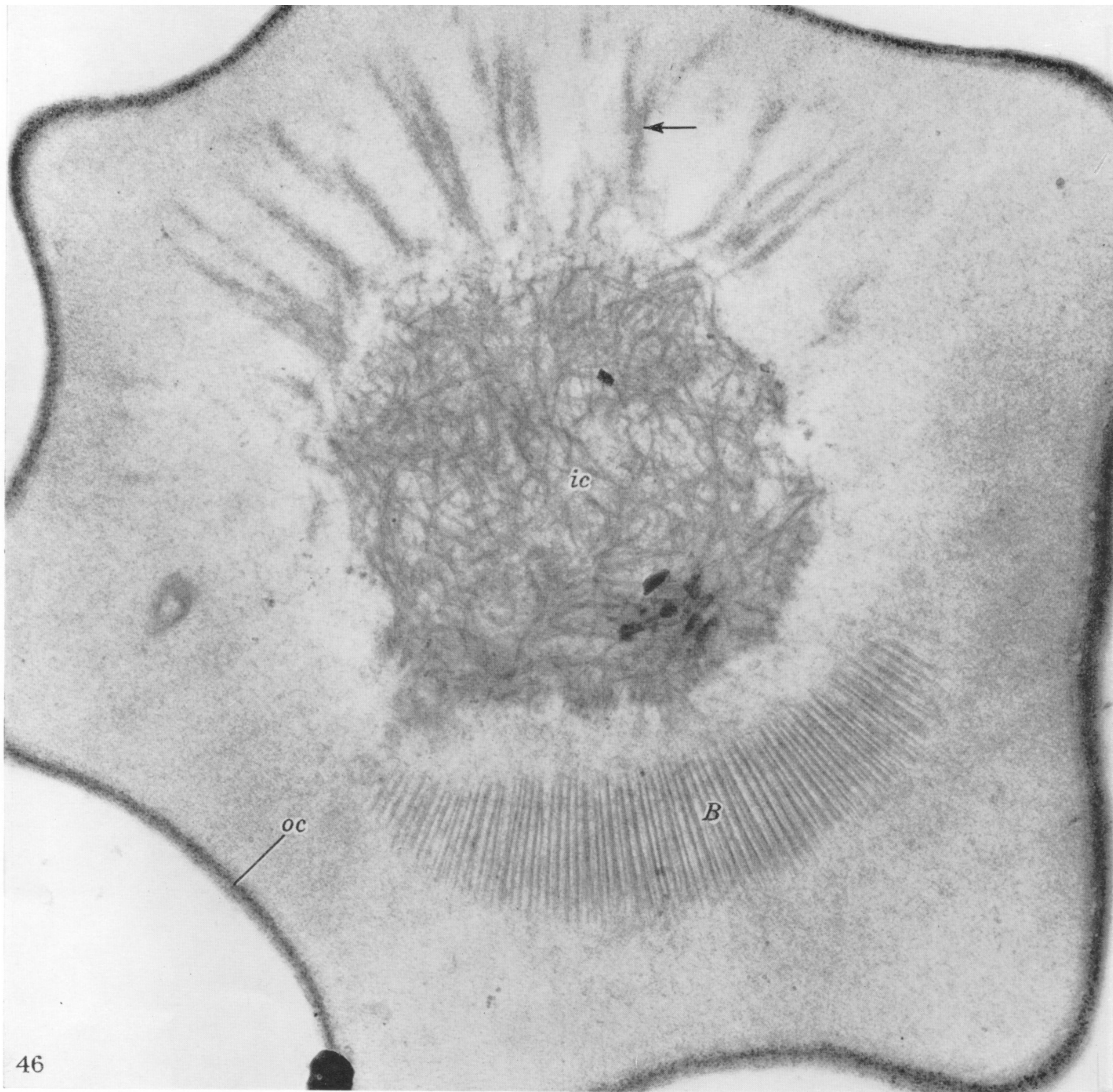
44

FIGURE 44. Continuation of figure 43, showing the structure of the rostral tube about halfway along its length. The micrograph shows in particular the transition of the tubule-bearing layer into the irregular strands of dense material which give rise to the centriolar bands. Inner surface layer I, made up of dense dots (representing transversely oriented fibres), becomes less well ordered posteriorly. Inner surface layer II is made up of separate elements anteriorly, apparently fusing further back into continuous fibres or membranes ($\times 55\,000$).



45

FIGURE 45. Continuation of figure 44, showing the base of the rostral tube. The surface layers of the tube are clearly seen. Inner surface layer II has become a prominent cross-striated membrane, which stops at the base of the tube; the outer surface layer continues posterior to the tube in the form of striated fibrous ribbons running below the basal bodies. At the base of the rostral tube can be seen part of the annulus which connects with the centriolar bands. Note the vesicles between the distal ends of the basal bodies ($\times 55000$).



FIGURES 46 to 52, PLATES 39 to 45, show a series of transverse sections of the rostrum of *Pseudotriconympha*, arranged in sequence and starting anteriorly. All the micrographs in this series, with the exception of figures 47 and 51, show sections from one organism.

FIGURE 46. Section through the tip of the rostrum, above the level of the flagella, passing through the base of the inner cap. The radiating structures at the bottom are the *B* fibres; the less well-defined ones at the top (arrow) are strands of inner cap material ($\times 25\,000$).

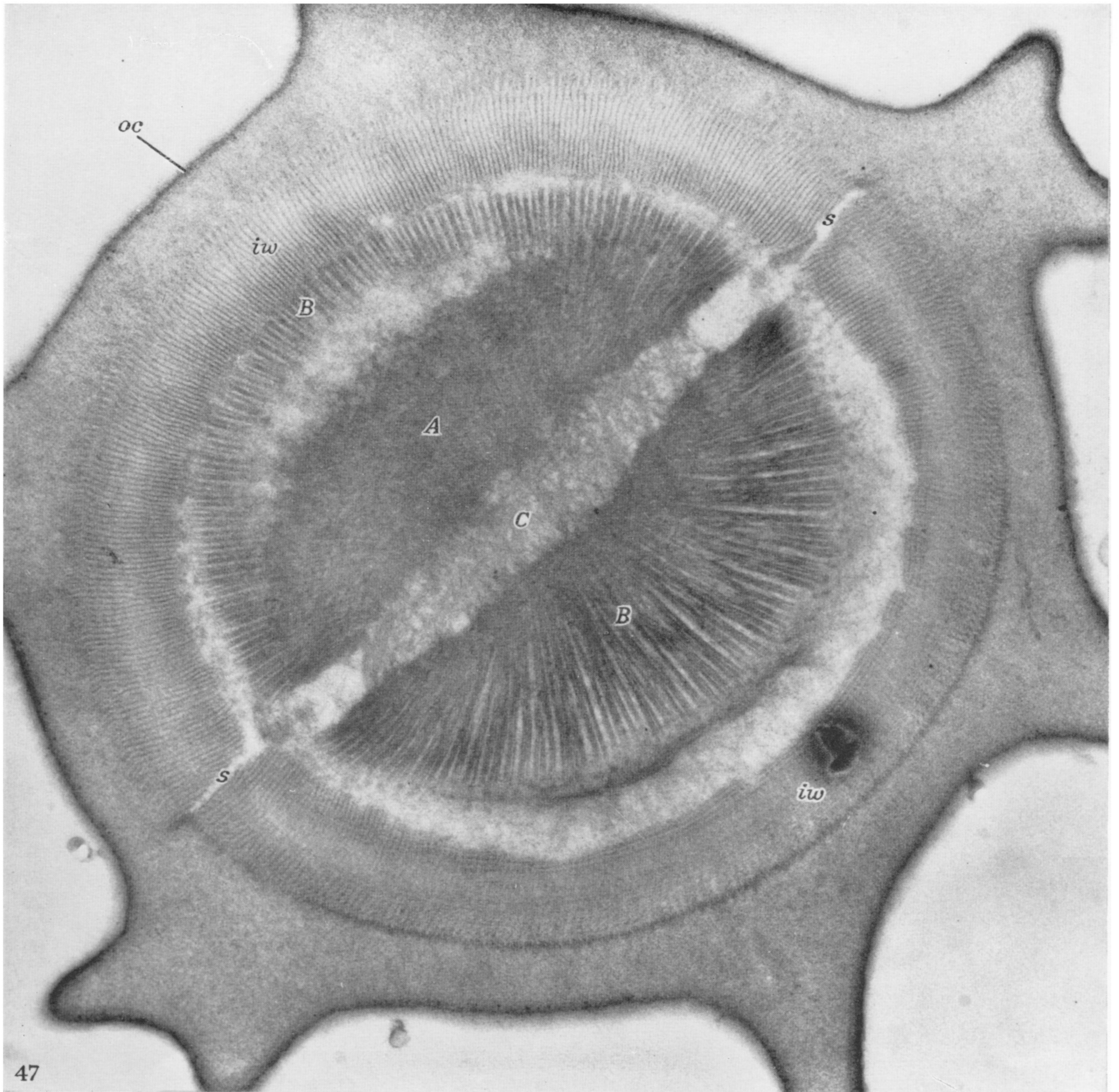


FIGURE 47. This shows three of the systems of fibres (*A*, *B* and *C*) lying under the base of the inner cap at the apex of the rostral tube. The section is slightly oblique, so that structures lying at a slightly more anterior level are seen in the upper left half of the picture than in the lower right half. Note the fine radial striations in the rim of the rostral tube ($\times 25\,000$).

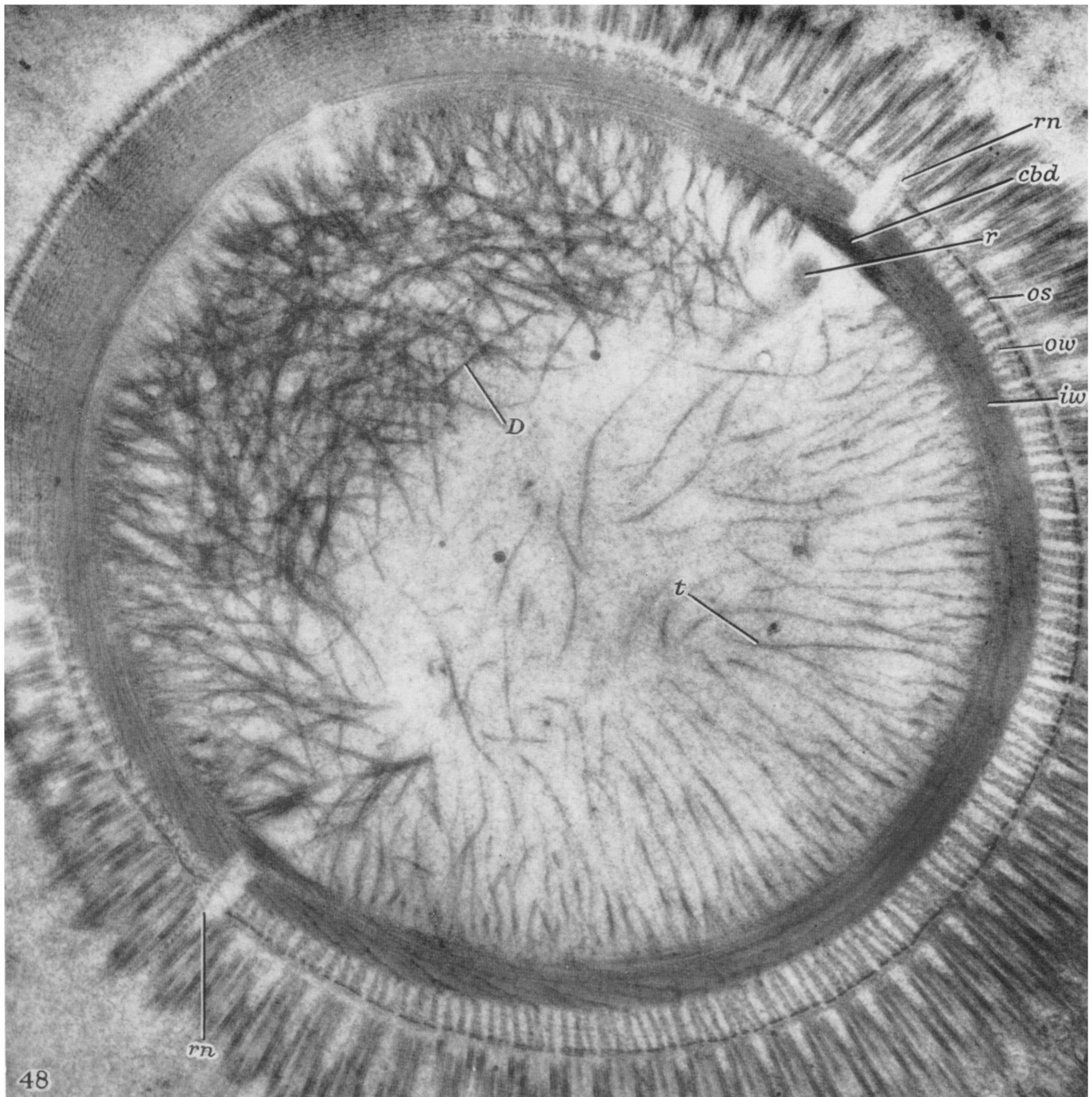


FIGURE 48. The anterior end of the rostral tube, cut slightly obliquely and, like figure 47, showing more anterior structures in the upper, left half, where the section passes through the rim of the tube. In this half the system of coarse fibres (*D*) is visible. In the bottom, right half the polarized tubules (presumed spindle fibres) can be seen. The tubule-bearing layer is not distinct in this micrograph, but dense thickenings of it are present along the seams of the tube: these are the beginnings of the centriolar bands. Note the section through the rostral rod, and the two radial nets. In the rostral tube note the prominent radiating lamellae in the outer wall, and the dense, well-defined outer surface layer, made up of distinct strands ($\times 30\,000$).

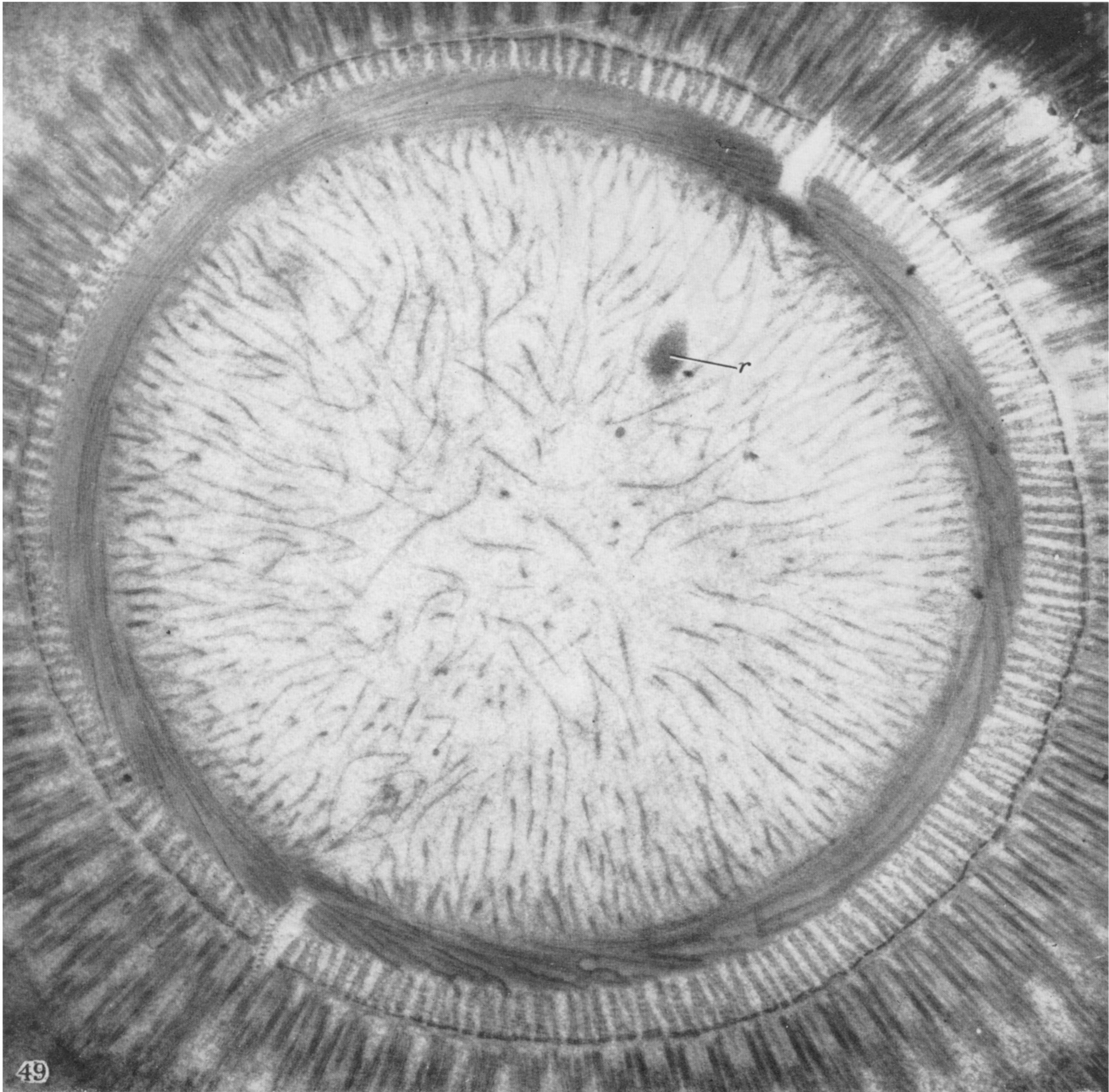


FIGURE 49. Section slightly posterior to that shown in figure 48, showing clearly the radially polarized tubules, occupying most of the lumen of the tube, the centriolar bands, rostral rod, and radial nets. Note also the material apparently connecting the proximal ends of the basal bodies to the outer surface layer ($\times 30\,000$).

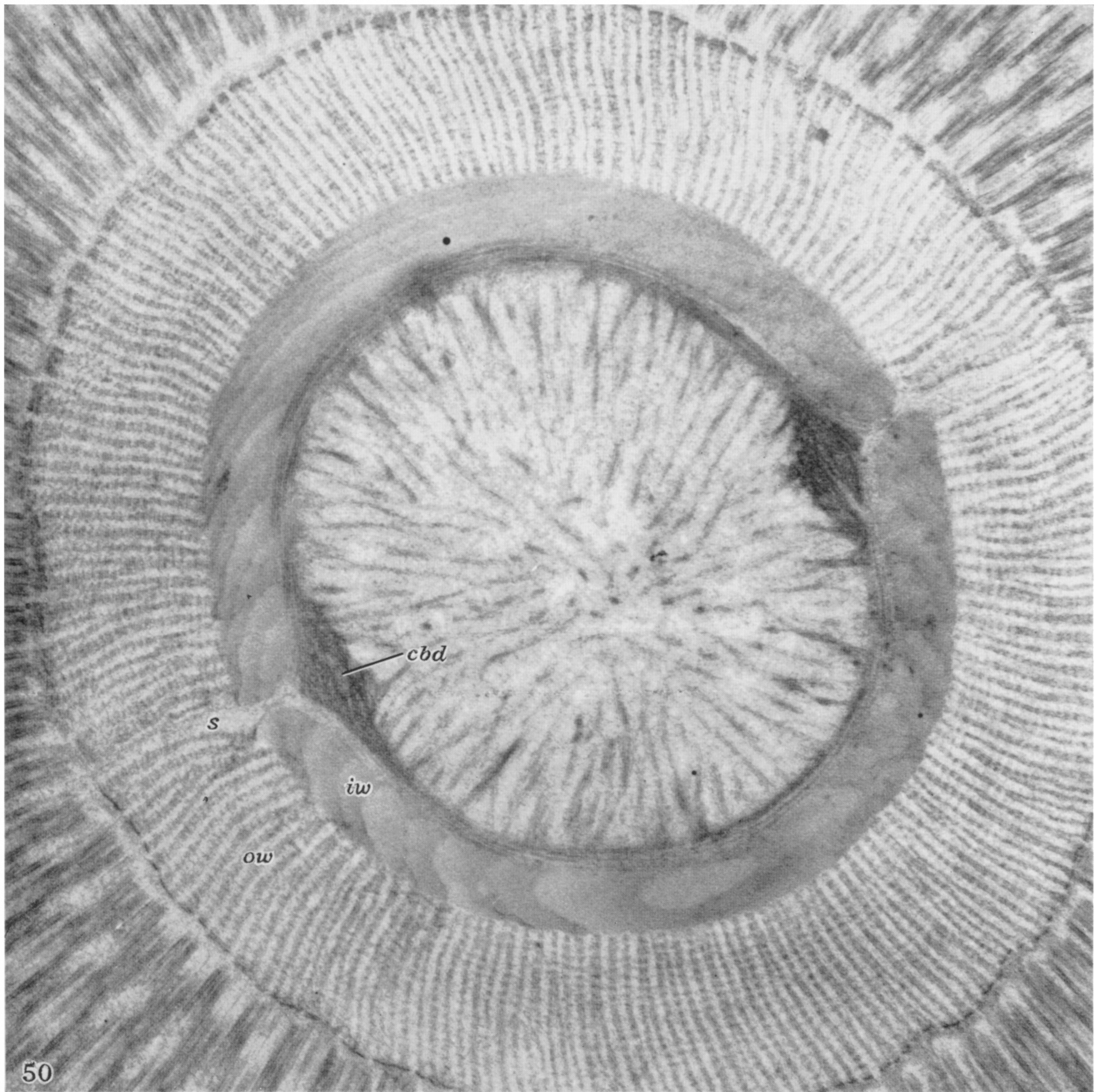
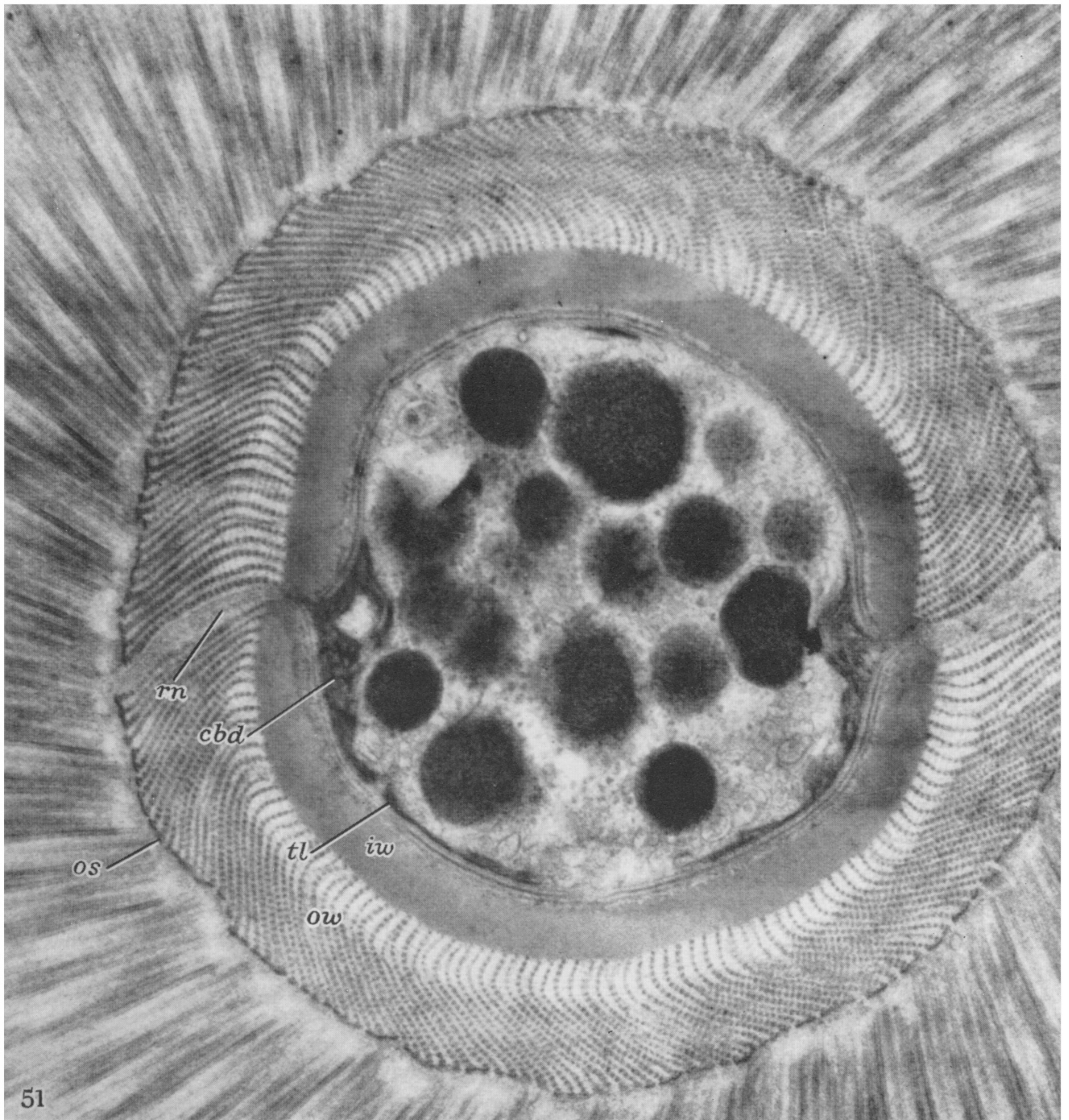
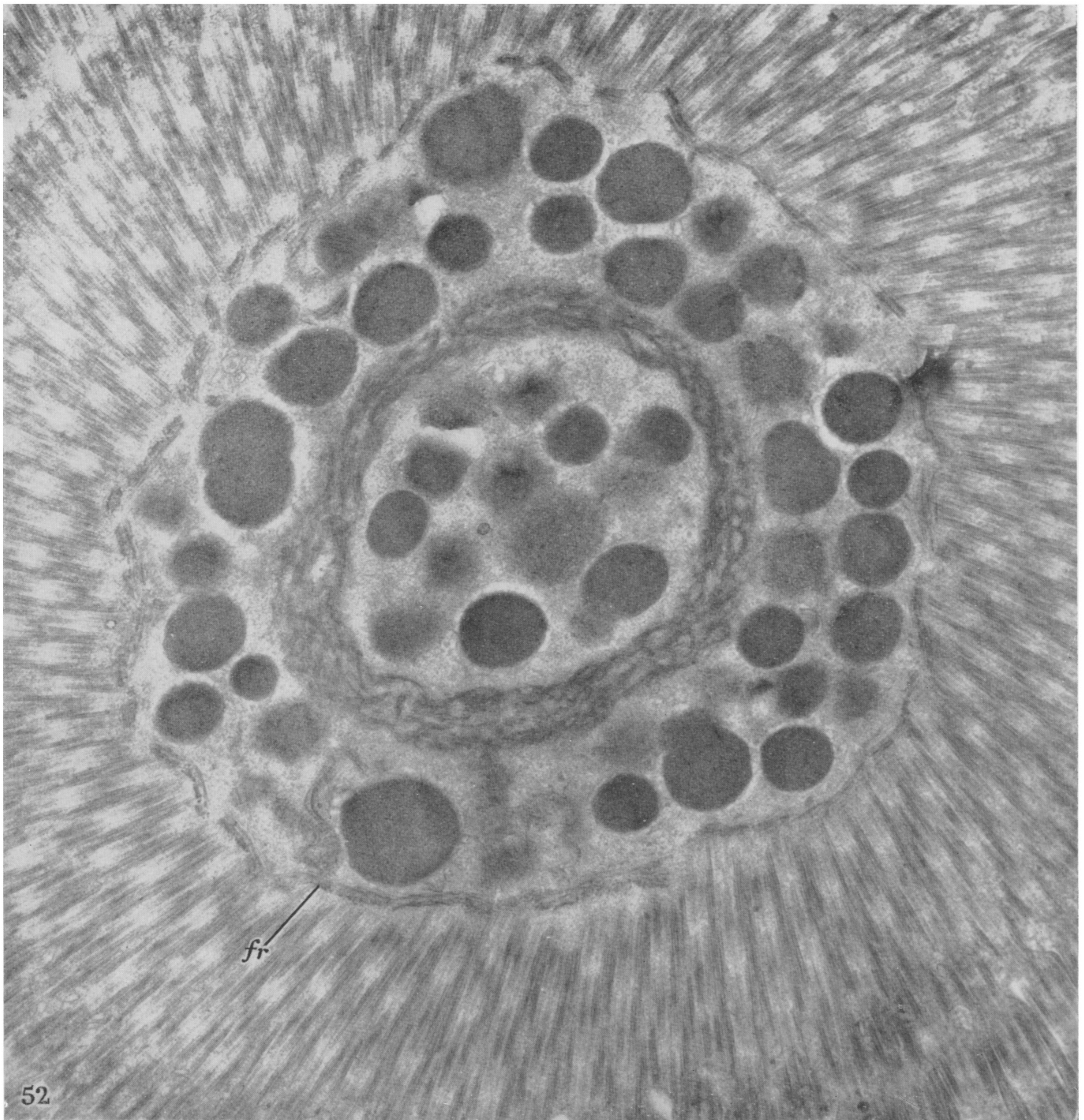


FIGURE 50. Section at the level at which the wide part of the rostral tube ends and the walls are thickest (compare figure 42). Radially polarized tubules still occupy most of the lumen of the tube. The tubule-bearing layer from which they arise is prominent, and its connexion with the centriolar bands is clear. Note the textures of the inner and outer walls of the tube ($\times 42000$).



51

FIGURE 51. Section approximately two-thirds of the way along the rostral tube, showing the centriolar bands and their connexion to remnants of the tubule-bearing layer. Note the outer and the two inner surface layers ($\times 30\,000$).



52

FIGURE 52. Section just below the base of the rostral tube showing the anulus which arises from the centriolar bands (compare figure 54). Note also the fibrous ribbons below the proximal ends of the basal bodies; the ribbons arise from the outer surface layer ($\times 30\,000$).

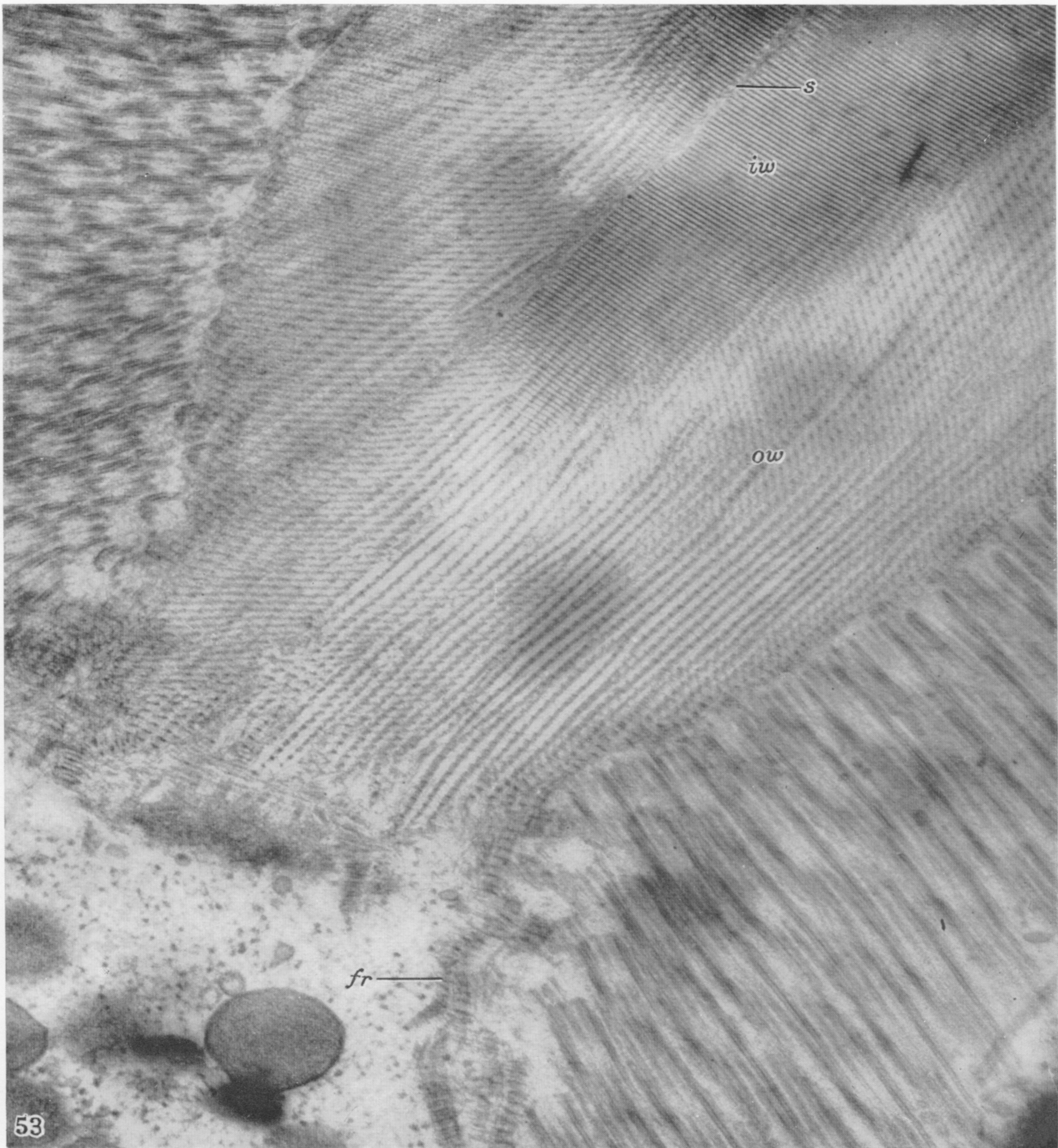


FIGURE 53. Longitudinal section at the base of the rostral tube, showing the inner and outer walls. Note the bifurcating lamellae in the latter. The origin of the cross-striated fibrous ribbons from the outer surface layer can be seen ($\times 42\,000$).



FIGURE 54. Longitudinal section at the base of the rostral tube, showing the annulus connected to one of the centriolar bands. Note also the outer surface layer and fibrous ribbons ($\times 42000$).

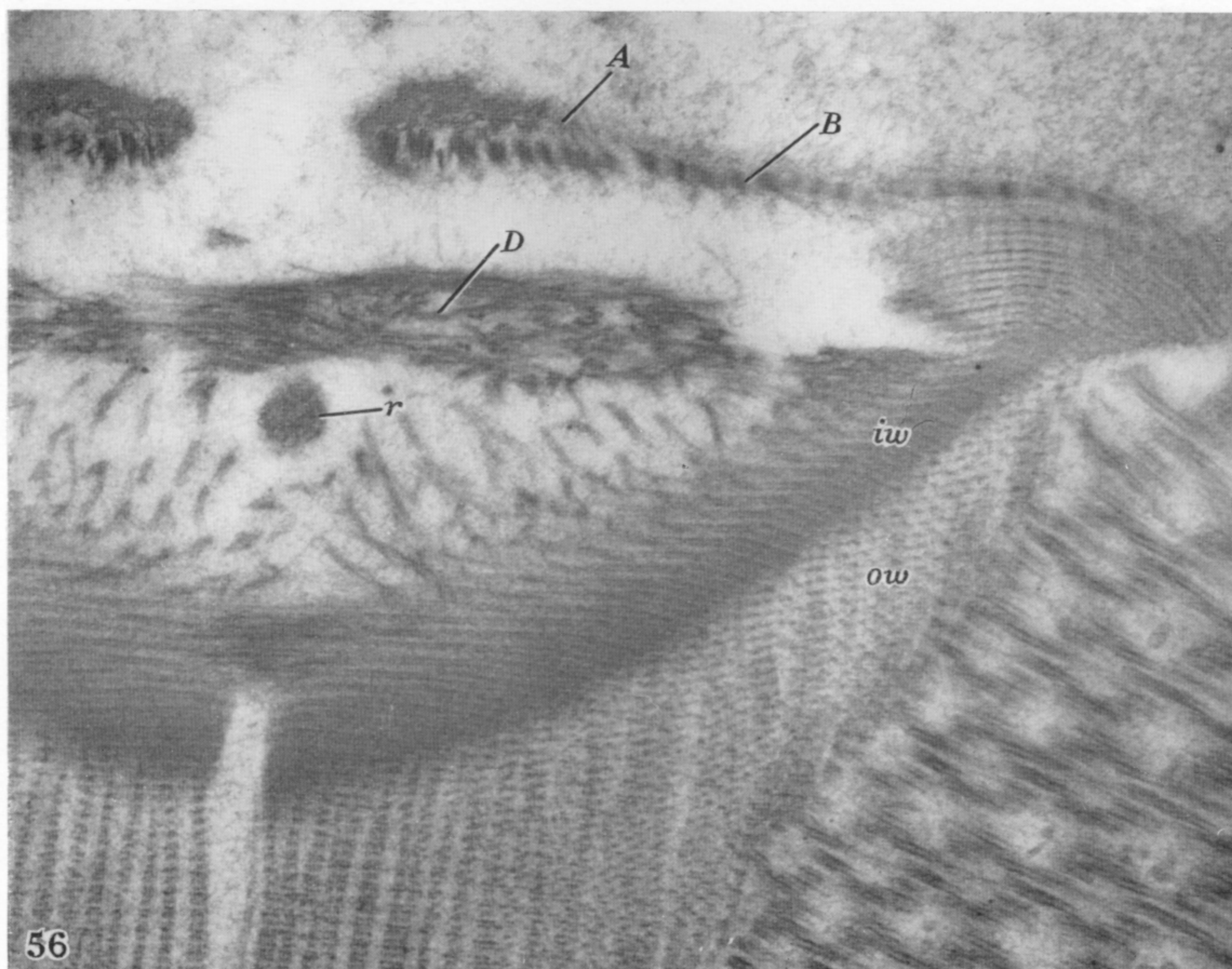
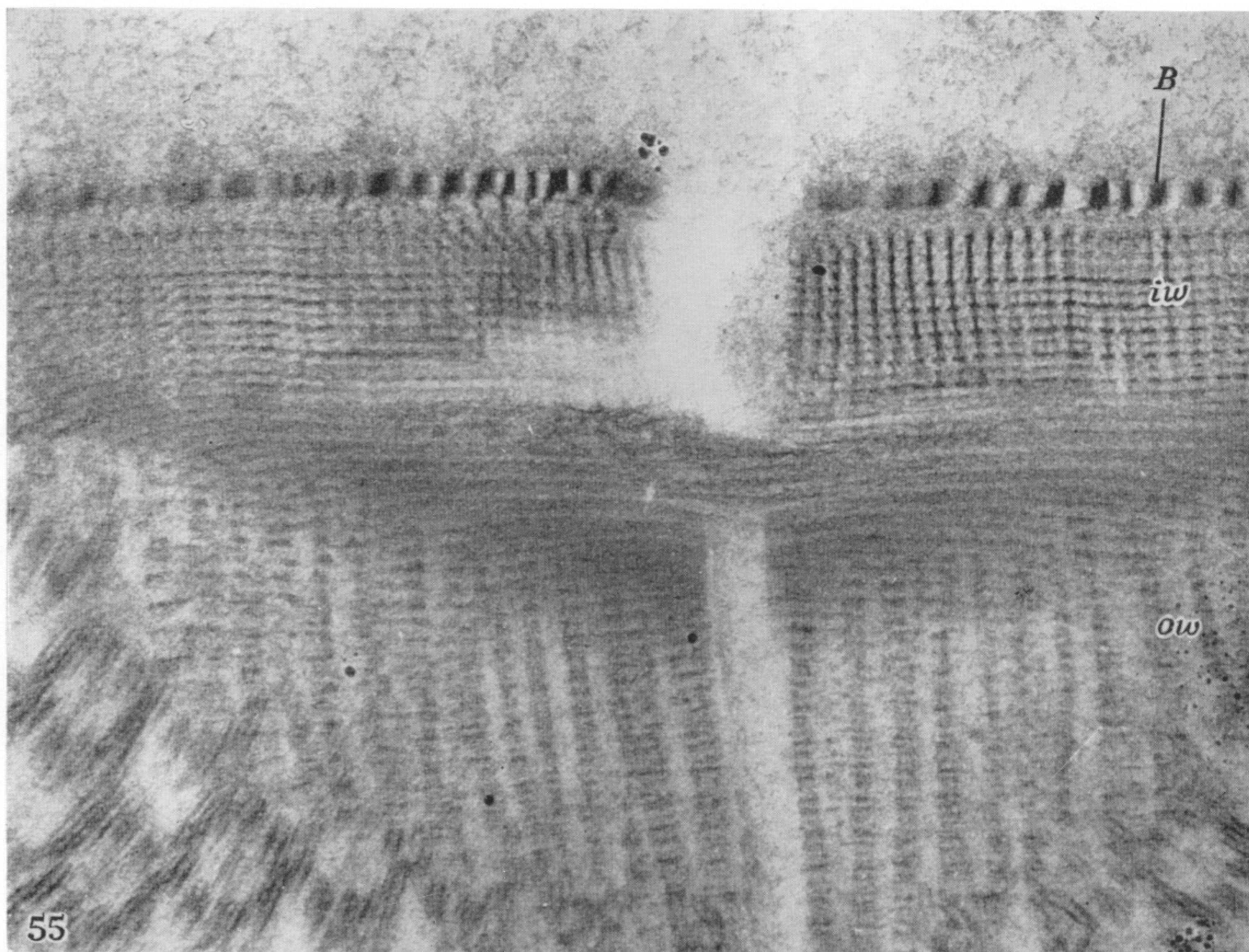


FIGURE 55. Longitudinal section at the anterior end of the rostral tube, to show particularly the *B* fibres, rectangular in section, running over the rim of the tube. Note also the cross-striations in the lamellae of the outer wall ($\times 65\,000$).

FIGURE 56. Longitudinal section at the anterior end of the rostral tube, showing the *B* fibres running over the rim, with the *A* fibrous bodies lying above them, nearer the centre. Note also the coarse *D* fibres, the tubules (presumed spindle fibres), and the section through the rostral rod, here seen to be circular in cross section ($\times 45\,000$).