ON THE STALKS OF CERTAIN PERITRICHS

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The stalks of peritrich protozoa have aroused great interest for many years and for many reasons. Some are contractile, some are not. This attribute of contractility, first observed by Leeuwenhoek in *Vorticella* and published in this journal in 1676, is one good reason for further studies of a structural, cytochemical and physiological character. This paper is mainly concerned with matters of fine structure which relate not only to the mechanism of contraction in those stalks that behave in this way, but also to wider problems of morphogenesis in ciliates.

The early literature of this subject is clouded with optical artifact and one of the first problems to be solved is the precise difference between non-contractile and contractile stalks. Seven families are now included in the suborder Sessilina of the Peritrichida and members of the Epistylididae and the Vorticellidae have been selected for this investigation. This choice has made possible a detailed comparative study of fine structure in the non-contractile stalks of the first group and the contractile ones of the second. All stalks possess longitudinally arranged structures. In the non-contractile stalks these structures are tubular in form and may be observed in the phase-contrast microscope. In the contractile stalks the longitudinal structures are of two main kinds, one of which is confined to the annulus and the other to an inner canal separated from the annulus by a membrane. The annular structures are tubular and numerous in *Carchesium* and *Zoothamnium* and transversely striated, while in *Vorticella* they are composed of unstriated fibres, few in number. The structure within the canal is the main feature that distinguishes the stalks of Vorticellidae from those of Epistylididae. It consists of a long bundle of closely packed fine fibrils and is to be identified with the stalk *spasmoneme* or *myoneme* of the older literature. Only one type of fibril has been observed in the spasmonemes and present facts are not consistent with the idea that they contract in the same way as muscles. The spasmoneme is protein in nature with positive indications of the presence of $-NH_2$, S—H, and S—S groups. The annular structure in the Vorticellidae and the tubular structures of Epistylididae have cytochemical affinities with the keratin group of proteins. Structurally, they grow out as the stalk develops from an assembly of organelles known collectively as the *scopula*. In the contractilia the spasmoneme passes into the zooid through a more or less central gap in the scopula and terminates in the form of a circular fan of fibrils on or close to the zooid pellicle.

The fine structure of the stalk tubules of the Epistylididae has been investigated in some detail, particularly for one species of *Epistylis*. In *Epistylis* and *Opercularia* the tubules are transversely

striated in a manner similar to that described for *Carchesium* and *Zoothamnium*. In *Campanella* each tubule consists of a loose helix of fibrils interlocked with those of neighbouring tubules.

The form of attachment of the tubules of *Epistylis* and *Opercularia* to the scopula organelles has been determined. Wherever the preparations were of sufficient quality a comparison has been made of scopula organelles and the corresponding and possibly homologous structures of normal cilia known as basal bodies or kinetosomes. There are similarities and, of course, differences. It seems justifiable to regard the scopula organelles as basal bodies modified in the course of the evolution of this Order for the purpose of contributing a degree of structural stability and rigidity to the stalks. No such obvious 'origin' for the spasmoneme has been found in the adult organisms. This illustrates the danger and perhaps sterility of attempts to link the genesis of one structure to another on purely morphological grounds. The role of the scopula organelles and in a wider context kinetosomes in the organization and possibly the synthesis of fibrous proteins is discussed.

Introduction

Mature ciliates of the Order Peritrichida take their name from the prominent anterior circular wreath of cilia which for many members of the group comprises the only body ciliation. The investigations to be described deal with sessile peritrichs of the families Epistylididae Kahl, 1933, and Vorticellidae Ehrenberg, 1838, in the suborder Sessilina Kahl, 1933, of the Peritrichida Stein, 1859, all of which possess a basal stalk or peduncle. It is these stalks that have stimulated a great deal of the past and present interest in the Order and which form the main subject of this paper. Detailed reference to much of the earlier work on the subject will be found in an article by Taylor (1941). Problems of characterization and classification have been summarized by Corliss (1961) and his nomenclature has been followed wherever possible. Preliminary accounts of some aspects of this work have already been given (Randall 1956, 1959*a*, *b*, *c*). It is not necessary here to refer extensively to the biology of peritrichs, but a few features of value to the general considerations of this paper will now be outlined.

Sessile peritrich ciliates are of two kinds, solitary or colonial. A mature solitary organism such as *Vorticella* (figure 54, plate 39) consists of a single zooid *Z* and its stalk *S*. It is the zooid that is ciliated anteriorly and in the buccal cavity; the zooid also contains the nucleus and other usual cytoplasmic elements such as mitochondria, membranes and vacuoles, in addition to the cortical infraciliary structures associated with the cilia already mentioned. The stalk may be attached distally to aquatic plants or detritus (as with *Vorticella*) or to aquatic animals (as with *Rhabdostyla*). Some so-called solitary peritrichs such as *Vorticella* are often found in considerable groups or stocks, although each organism is an independent entity. Others are more truly solitary and less suitable for an investigation in which substantial numbers of organisms are inevitably required.

Members of the other class of peritrichs are colonial and tree-like in character with a large number of zooids to the colony. Each zooid is attached to the tip of a branch of the stalk system. *Epistylis* (figure 1, plate 14), *Opercularia* (figure 24, plate 24) and *Carchesium* (figure 35, plate 30) are all of this type. The height and expanse of a colony may be several millimetres and the colony is readily visible to the naked eye.

A superficial picture of the origin and development of a peritrich stalk may be conveyed by a brief account of a typical life cycle. Peritrich ciliates reproduce either by conjugation, a complex sexual process involving a temporary union of two zooids for the purpose of exchange of nuclear material; or, asexually. Reference to the asexual

process is sufficient for our present purpose. In the stalked peritrichs this consists in the longitudinal fission of a zooid, one part of which remains attached to its stalk. The free-swimming individual, known as a telotroch, has no stalk at this stage of its existence, but possesses a posterior girdle or ring of cilia (see figure 17*a*, plate 22, for *Campanella* and figures 41, 42 and 43, plate 31, for *Carchesium*). Oral cilia are also visible in these illustrations; the entire oral apparatus is however retracted during this developmental stage. The telotroch is motile and develops its stalk after attachment to a substrate. This process has been followed in cine-films and has helped considerably in the correct identification of motile stages. Once this sessile state has been assumed, the posterior girdle regresses and disappears and the new stalk in a solitary type such as *Vorticella* grows out from the posterior or scopular region in a matter of a few hours. In any one group or stock of *Vorticella* the stalk lengths may vary from 50 to 4000 μ. The biology of species of *Zoothamnium*, a well-known colonial ciliate, has been studied very thoroughly by Wesenberg-Lund (1926) and Summers (1938*a*, *b*). A helpful review of the comparative morphology of species of *Vorticella* has been published by Noland & Finley (1931).

The stalks of peritrichs are fundamentally of two different kinds: non-contractile and contractile. The non-contractile stalks belong to members of the Epistylididae and the contractile ones to the Vorticellidae. Stalks of *Carchesium*, both extended or relaxed and withdrawn or contracted, may be seen in figures 35 and 37, plate 30. The time taken to contract lies between 10^{-1} and 10^{-2} s. The relaxation period is much longer and usually takes several seconds. Stalks are also possessed by some members of the Chonotricha and Suctoria, but these have not been investigated. These facts bring us to a brief outline of the main problems examined in this paper.

The overall complexity of protozoa makes it inevitable that one investigation such as this will raise quite as many questions as it answers. As a first step towards the understanding of the origin and behaviour of the stalks, the structures of a number of contractile forms have been determined. It this way it has been possible to decide at least morphologically what is responsible for contraction. Much of the earlier work cited by Taylor (1941) has postulated a more or less axial contractile organelle or spasmoneme. This turns out to be correct, but with present methods much greater overall certainty has been possible. New structural detail has been revealed in the spasmoneme itself, in the surrounding annulus or sheath, and in the junction between stalk and zooid.

The most significant recent contribution to the subject of peritrich stalks is that of Rouiller, Fauré-Fremiet & Gauchery (1956) in which the fine structure of stalks of *Campanella*, *Opercularia* and *Zoothamnium* was examined in the electron microscope and described for the first time. This work is a direct link with the much earlier paper by Fauré-Fremiet (1905) on stalk structure in the Vorticellidae in which the idea that the scopula is a collection of outward-facing cilia was first put forward.

In the developing peritrich the stalk, it will be remembered, grows out from the scopula; this process is accompanied or preceded by the regression of body cilia. A key question of general biological interest therefore concerns the structural origin of the stalk. Does the stalk originate from a system of internal scopula organelles hitherto insufficiently specified? Or is the developing stalk in any way associated with the regression of the posterior ring of cilia? This hypothesis at any rate can be disposed of immediately, since

telotrochs in the early stages of stalk development still retain the posterior girdle (figures 41 and 42, plate 31). If the first proposal is correct, what is the relationship of the scopula organelles to the basal bodies (*BB*) of normal cilia? We may also ask whether in the contractilia the spasmoneme continues into the zooid, thereby providing a means of contraction for this part of the organism. This paper goes some way towards answering these and other structural questions and also provides many comparative data for the Epistylididae and the Vorticellidae. The answer to the problem of the helical form of the contracted *Vorticella* stalks comes from a study both of the spasmoneme and of the annulus. The stalk structures of *Carchesium* and *Zoothamnium* prove to be very similar; but otherwise in no two genera are the stalks precisely alike either in organization or fine structure. The new features described in this paper appear to form, incidentally, an addition to the existing means of classification of the Sessilina which have perhaps tended to rely too much on organization of adoral ciliation. These matters, as well as those more in the main line of this investigation, will be discussed at the end of the paper. Some discussion of structure within the zooid is also necessary, particularly in relation to stalk origins, both axial and annular. Other features of the zooids will be examined in a separate paper.

Lack of sufficient material has prevented the exploitation of biochemical techniques to the extent originally hoped for, but cytochemical experiments show that the contractile element of the stalk is, not unexpectedly, essentially protein in nature.

One of the more exciting problems posed by the existence of contractility in protozoa is whether the mechanism that brings it about bears any similarity to the contractile processes in higher animals. Although we have made observations in this field, they are very limited in extent and have not enabled us to put forward any satisfactory hypothesis. Present indications are that the mechanism of contractility in peritrich stalks is different from that in higher organisms. It certainly appears to be more complex than is suggested by Hoffmann-Berling's (1958) studies of stalk preparations of *Vorticella gracilis*. Similar preparations of *Carchesium* have been found by us to behave differently and the whole question of contractility in these animals requires much more study. Further discussion at this stage would not serve any useful purpose.

Materials and methods

Organisms

Data have been obtained on species from the following genera:

Family Epistylididae Kahl, 1933

Genera: *Epistylis* Ehrenberg, *Opercularia* St. and *Campanella* Goldfuss

Family Vorticellidae Ehrenberg, 1838

Genera: *Carchesium* Ehrenberg, *Zoothamnium* Bory and *Vorticella* Linnaeus

Collections of the organisms were made from ponds throughout the milder months of the year. Since the object of this work was not directly concerned with problems of systematics, no precise identifications of species were attempted, but those examined were probably:

Epistylididae: *Epistylis plicatilis*, *Campanella umbellaria*, *Opercularia plicatilis*.

Vorticellidae: *Carchesium polypinum* and *Vorticella campanula*. The species of *Zoothamnium* was not identified.

The peritrichs were maintained in the laboratory in small glass dishes of filtered pond water supplemented by the addition of boiled wheat germ. The water in the dishes was aerated regularly and the room maintained at a temperature of about 10 °C. Under these conditions the organisms were maintained for a few weeks. Maintenance for longer periods was never successfully achieved.

Fixation for study in the electron microscope was carried out on groups of 40 to 50 individuals or members of a colony with the following reagents and schedules:

(i) 1% osmium tetroxide in fresh pond water; time of fixation from 2 to 10 min. at 4 °C; pH from 6·5 to 7·5.

(ii) 1% osmium tetroxide dissolved in Chalkley's medium; time of fixation from 2 to 10 min at 4 °C; pH from 6·5 to 7·5.

(iii) Palade fixative within the pH range 6·0 to 8·0; fixation at 4 °C for times ranging from 0·75 to 7·0 min.

(iv) 1% osmium tetroxide dissolved in normal Tyrode's solution; pH range 7·0 to 8·0, with a time of fixation of from 2 to 10 min at 4 °C.

After fixation the organisms were well washed in several changes of chilled and filtered pond water and then passed through a normal series of graded alcohols in each of which they remained for 5 min. Embedding was carried out in a mixture of 15 parts *n*-methyl methacrylate and 85 parts *n*-butyl methacrylate—or alternatively in 'Araldite', an epoxy resin. After polymerization according to well-known schedules, sections were cut from the prepared blocks (*a*) for light microscope studies at thicknesses varying from 2 to 5 μ and (*b*) in thin sections for electron microscope examination. The thin sections were picked up (in the normal way and after being allowed to flatten out) on standard carbon-coated copper specimen grids. After drying, the grids were placed section-side down on a fresh, saturated solution of uranyl acetate in 50% alcohol for 60 to 90 min, and then well rinsed in 50% ethyl alcohol. In the course of this work two electron microscopes have been used: a modified Metropolitan Vickers EM 3 and an RCA EMU 3 D.

In the study of contractility one is faced with a number of difficulties. As is well-known, all contractile protozoa contract violently on the application of fixative. In the past this phenomenon has been a serious hindrance to the proper examination of the organism and many authors have suggested a variety of empirical chemical means of avoiding the difficulty. In principle it is highly desirable to examine the organisms in two well-defined physiological states: a normally extended or 'relaxed' condition and in the 'contracted' one. It cannot lightly be assumed, however, that the chemically induced relaxation (which is maintained throughout and subsequent to fixation) is identical with the relaxed state of normal living organisms. Hoffmann-Berling (1958) has devised a technique for the relaxation of *isolated* stalks of *Vorticella* and the study of their contractility. This technique has also been used in the present morphological investigation, but with some misgivings and full awareness of its limitations. It is doubtful whether structure is well preserved after such treatment.

The non-contractile stalks

Epistylididae, Kahl.

Genus *Epistylis* Ehrenberg

The individual stalks of *Epistylis plicatilis* have for a long time been depicted in cross-section as bundles of fine tubules. This is particularly evident in Schröder's paper (1907). It is therefore of interest to see how far these early conclusions are in agreement with the findings of the present paper.

The overall height and expanse of a living colony of *Epistylis* may be as much as 0·3 cm. The more or less cylindrical stalks are about 15 μ diam. (figure 1, plate 14). Schröder's contention that each stalk is composed of a closely packed bundle of tubules is confirmed from the light microscope illustrations of figures 2, 3 and 4, plate 14. This conclusion has now been substantiated and extended by detailed electron microscope study of longitudinal and transverse sections.

Adjacent to the zooid the tubules are open-ended and fit closely over the scopula organelles; they are also rather variable in length (figure 5, lower portion, plate 14). Micrographs of near-longitudinal and near-transverse sections demonstrate that most of the tubules are in the form of long narrow cylinders with closed ends, although the manner of closure is somewhat obscure (figures 7 and 8, plate 15; figure 9, plate 16; figure 11, plate 17). They are thus superficially somewhat analogous to lengths of drawn-out closed capillaries. This view will be elaborated and qualified a little in due course. The tubular bundles are enclosed within the wall of the stalk (figure 10, plate 17) which is about 250 Å thick. This wall is seen at higher magnification (figures 6*a*, *b*, plate 15) to consist of four layers, *a*, *b*, *c* and *d*; values of the component layer thickness will now be given for the portion about 2 μ from the stalk-zooid junction, illustrated in figure 6*b*. The innermost layer *a* is about 33 Å thick and stains moderately well. The adjacent layer *b* is almost transparent and about 65 Å thick. This layer lies between *a* and *c*, which is the third and more densely staining layer about 50 Å thick. The outermost layer *d* is fibrous and rough in outline; it is about 110 Å thick and may be no more than an extension of *c*. The relationship of the stalk wall to the zooid pellicle will be referred to below.

Each of the stalk tubules immediately adjacent to the zooid is attached proximally to the base of the zooid through the peg-like insertion of a scopula organelle *SO* (figure 5, plate 14, and figure 14, plate 19) which will be described below. In the fixed state the tubules are about 0·3 μ in diameter, but substantial variations from this value have been observed.

In some stalks the outermost layers of tubules contain additional structural elements, often nine in number and probably fibrous. They are shown in figure 12, plate 18, in transverse section. Unfortunately no longitudinal sections of this region have been obtained. We are inclined to the view that these fibres are characteristic of the distal part of the stalk; they have not been observed in the middle regions in this conspicuous form, although traces can sometimes be seen (figure 13, plate 18). In a radial direction the thickness of these objects varies from 400 to 800 Å. In the perpendicular (or circumferential) direction they are about 1000 Å wide. Both dimensions and shape vary considerably and these values are given only for general guidance. It is also possible that the fibres are composite. The fibres are fairly uniform in density and do not possess the apparent

tubular twin form and characteristic appearance of the well-known internal fibrillar components of cilia.

That the tubules of the stalk have a more complex structure than we have yet described is evident in the near-transverse section of figure 11, plate 17, and the oblique, near-longitudinal section of figure 9, plate 16. The structures evident in these illustrations and in the longitudinal section of figure 8, plate 15, have led us to conclude that each tubule has the form of a banded stocking. As this description implies, there are two distinct components to the structure. The first and more obvious one is transverse to the long axis of the stalk and tubules and consists of fibrils generally about 180 Å in diameter, regularly spaced along the tubules at a distance of about 330 Å. These fibrils stain heavily and are conspicuous in all micrographs. They overlie the thin stocking and give a slightly ridged appearance to its surface, as may be seen in figure 7, plate 15. It will also be noticed in this figure that the transverse fibrils from neighbouring tubules are in register with each other and are joined together. From figures 7 and 8, plate 15, and figure 9, plate 16, it is clear that there is some degree of continuity between the surface of one tubule and another. This undoubtedly makes for geometrical complexity at the junction between one tubule and the next and may account for the greater amount of striation seen at these positions. These structures thus seem to be more complex than the term *closed tubules*, used on p. 64, suggests.

The longitudinal fibrils of the tubular stockings can be seen in figures 7 and 8, plate 15. Analysis suggests that, apart from the large transverse structures already referred to, the tubules are more or less sheet-like in character and that the longitudinal fibrils represent the smaller units from which the sheets are made. They are about 130 Å in diameter and are perhaps only visible when the continuity of the tubular stocking has been somewhat damaged in the course of preparation.

These then are the main features of the stalks of *Epistylis*. There is no evidence of any other structurally manifest components. Nor is it known what fluid, gel, or other matrix pervades the system.

It remains to describe, as well as the facts allow, the complex details of the structures at the junction between stalk and zooid. The chief feature of the junction, however, is the system of substantial structures already referred to as scopula organelles (*SO*) and somewhat similar in appearance to the basal bodies of normal cilia. This similarity is discussed on p. 76. The *SO* appear in two forms, *A* and *B*. In the more complex form *A* (figure 14, plate 19) there is both an upper cylindrical portion *C* ($0{\cdot}15\,\mu$ in diameter and $0{\cdot}5\,\mu$ long) and a lower, sac-like portion *S* ($0{\cdot}4\,\mu \times 1{\cdot}1\,\mu$). The cylinder *C* is fibrous in character, possibly closed at its base and definitely open at the upper end. In the less complex form *B*, usually observed in the inner portions of the junction, the upper cylindrical portion is apparently absent (figure 15, plate 20). The sacs *S* are formed from acute invaginations of the transverse membrane of the junction. In the *A* form the outer fibrils of the portion *C* fuse with the sacs below the main plane of the junction. The sacs are filled with fine fibrils 50 Å in diameter oriented roughly parallel to the stalk axis. In figure 14, plate 19, these fibrils extend beyond the base of the sac for a short distance. The base of the sac is striated perpendicular to its surface and may be composed of a group of pores through which the fibrils can pass. Similar fibrils are also attached to the uppermost part of *C*

and extend for long distances into the zooid. These fibrils are of two kinds. Those attached to the peripheral *SO* (as at *P*, figure 15, plate 20) are coarser and more deeply stained than those (as at *M*, same figure) attached to the inner *SO*. The *P* fibrils appear to fuse with a narrow ectoplasmic fibrillar sheath (0·5 μ thick) that underlies the whole pellicle (figure 16, plate 21). A possible function of these fibrils is discussed in the final section of the paper. Further reference to figure 14, plate 19, shows how closely the uppermost stalk tubules are intercalated with the sacs *S*. The longitudinal sections of the junction suggest quite strongly a structural differentiation between the central and peripheral parts. True transverse sections of this narrow region are required to confirm this.

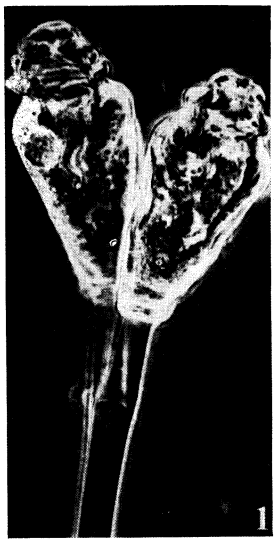
Attempts to obtain satisfactory X-ray diffraction diagrams from *Epistylis* stalks have so far been without success, but supplies of material have been limited. Debris and micro-organisms attached to the stalks have added to the difficulties of this particular problem. This difficulty was also experienced by Champetier in experiments on *Epistylis* and *Campanella* recorded by Fauré-Fremiet (1941).

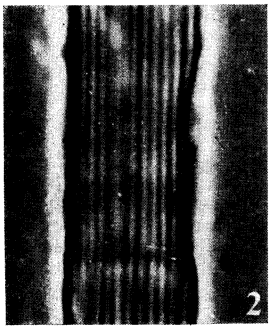
Genus *Campanella* Goldfuss

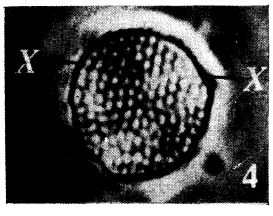
Campanella umbellaria Linnaeus

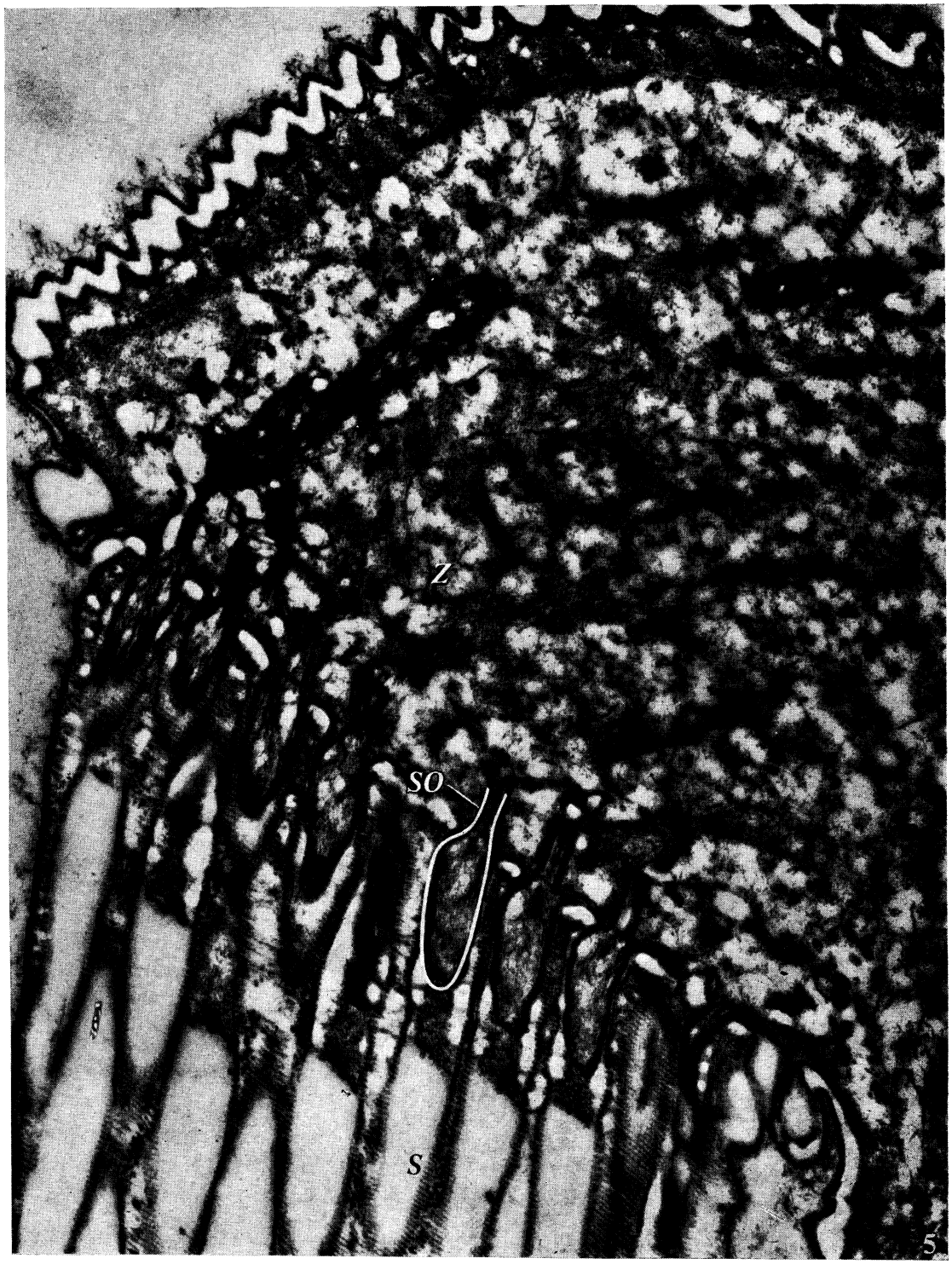
The stalks of this organism have not been examined in detail owing to lack of material, but sufficient is known for some results of interest to be included in this comparative study. The appearance of a single living zooid of *Campanella* and its stalk may be judged from figure 17*b*, plate 22. Members of this genus are readily distinguishable from those of *Epistylis* by differences in shape and size of zooid and in adoral structure. The stalk of *Campanella* is about 14 μ in diameter and, like that of *Epistylis* non-contractile. Studies in both the light and the electron microscope demonstrate that the stalk is differentiated into a peripheral region and a core. Figures 18, 19 and 20, plate 22, illustrate what may be seen optically and figure 21, plate 22, is a low-magnification electron micrograph of a transverse section which should be compared with the light micrograph of figure 18. The peripheral region of the stalk is filled with a circumscribing ring of about six layers of closely packed tubules which are in turn enclosed within the bounding membrane of the stalk (figure 21). These results are again in agreement with the early conclusions of Schröder (1907) and with the electron microscope results of Rouiller *et al.* (1956). The central core reveals no structural components. The tubular structures are somewhat polygonal in section, with at approximate 'diameter' of 0·3 to 0·5 μ. The tubules of *Campanella* are thus a good deal larger than those of *Epistylis*. The overall thickness of the annulus is about 2·5 μ. The more highly magnified micrographs shown in the transverse and longitudinal sections of figures 22 and 23, plate 23, suggest that the fibrils of neighbouring 'tubules' are interlocked, thus providing some rigidity to the outer shell of the stalk. No precise figure can be given for the fibril diameter which is somewhat variable in these preparations, but a value of 80 Å is a reasonable one from the results available. The outer membrane of the stalk is about 150 Å in diameter; no corresponding membrane separates the central canal from the peripheral tubules.

The internal components of the *Campanella* stalk are thus less well defined than those of *Epistylis*. It should also be noted that in longitudinal section (figure 23, plate 23) the stalk tubules have a somewhat lenticular shape and a length of about 2·5 μ. Continuity









Epistylis plicatilis

FIGURE 1. Small portion of living colony of *Epistylis plicatilis*: phase contrast (× 250).

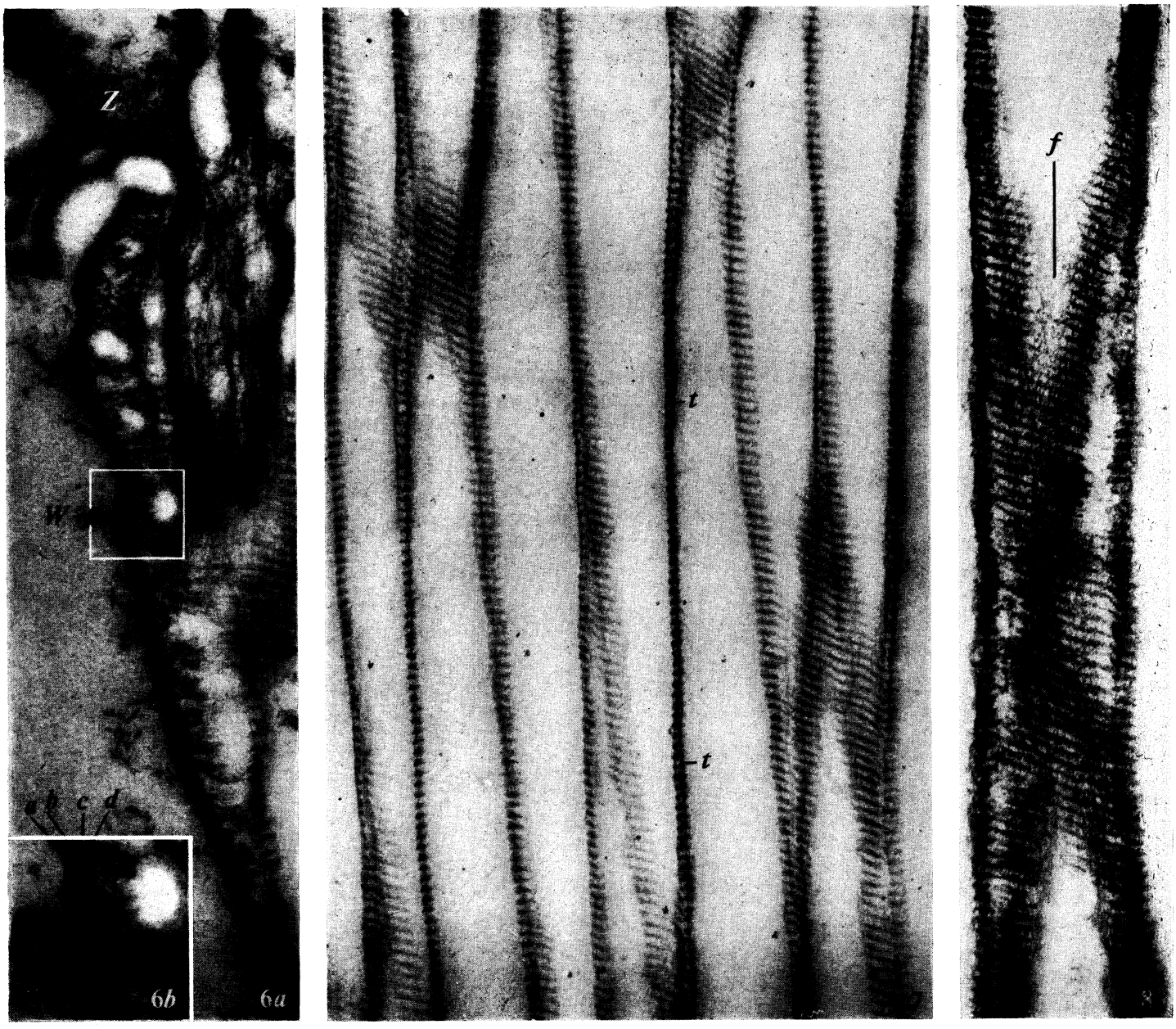
FIGURE 2. Photomicrograph of longitudinal section of single stalk indicating its probable tubular character confirmed in later figures: phase contrast (× 2750).

FIGURE 3. Photomicrograph of longitudinal section of bifurcated stalk as shown in figure 1. This illustrates the general continuity of the longitudinal structures through the junction: phase contrast (× 2750).

FIGURE 4. Photomicrograph of transverse section of single stalk. This confirms the tubular nature of the whole stalk inferred from figures 2 and 3: phase contrast (× 2750).

FIGURE 5. Low magnification electron micrograph of the junction between stalk *S* and zooid *Z*. Note the complex scopula organelles *SO* and their means of attachment to the stalk tubules. Comparison with figure 4 shows that this section was cut close to the surface, e.g. along *XX*, (× 21 520).

(*Facing p.* 66)



Epistylis plicatilis

FIGURE 6*a*. The stalk wall *W* and its attachment to the zooid *Z* (× 61 200).

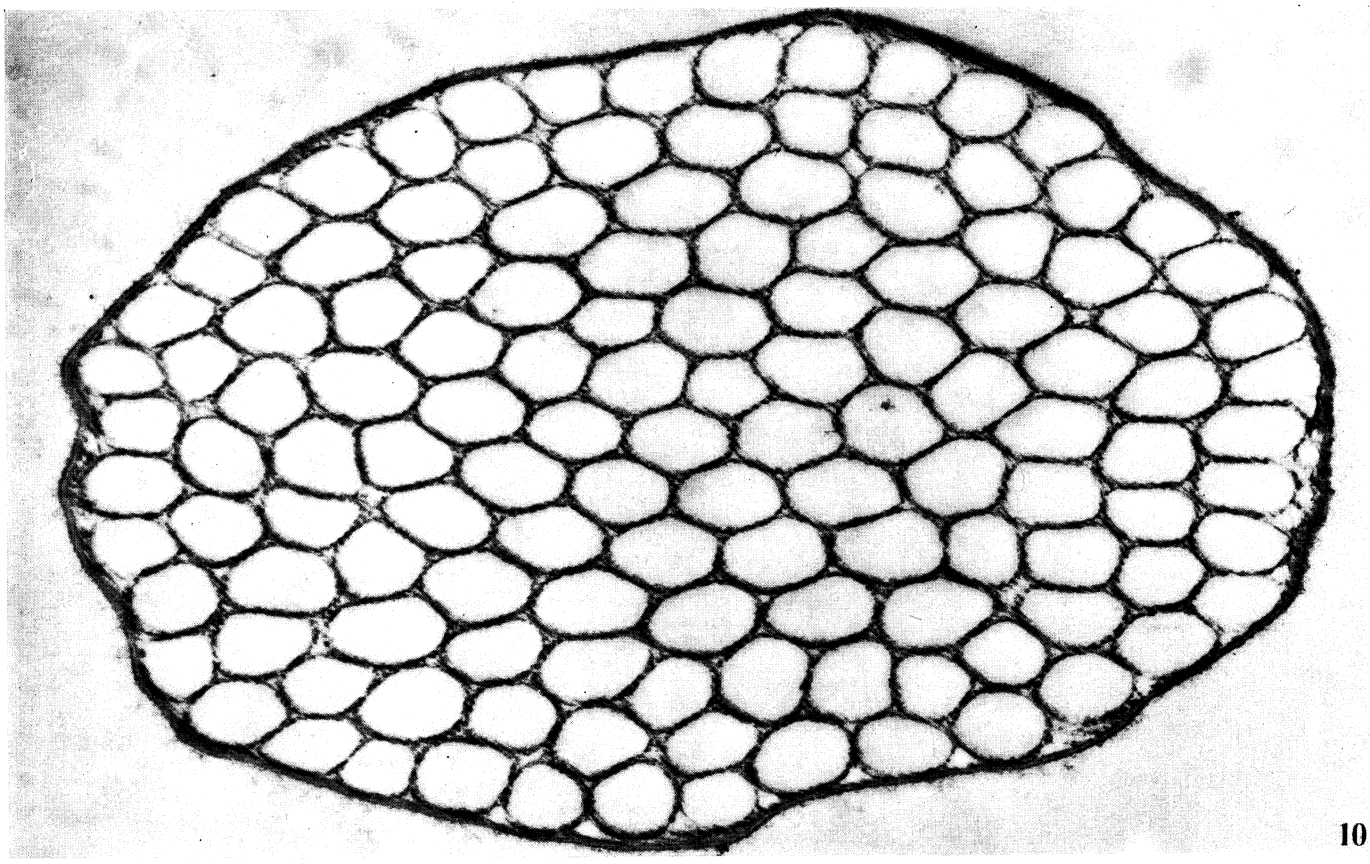
FIGURE 6*b*. Enlargement of portion of wall *W* marked in figure 6*a*. The four layers *a*, *b*, *c*, *d* can be more clearly distinguished (× 122 400).

FIGURE 7. Low magnification electron micrograph of longitudinal section of stalk. Note the striations of neighbouring tubules which are in register with each other (as along the junction *tt*) and join them together (× 39 600).

FIGURE 8. Higher magnification electron micrograph of a single stalk-tubule showing a large area of transverse striation and many much finer longitudinal fibrils as at *f* (× 61 200).

Epistylis plicatilis

FIGURE 9. This oblique near-longitudinal section of stalk tubules shows the complexity of structure at the junctions of neighbouring tubules (× 61 200).



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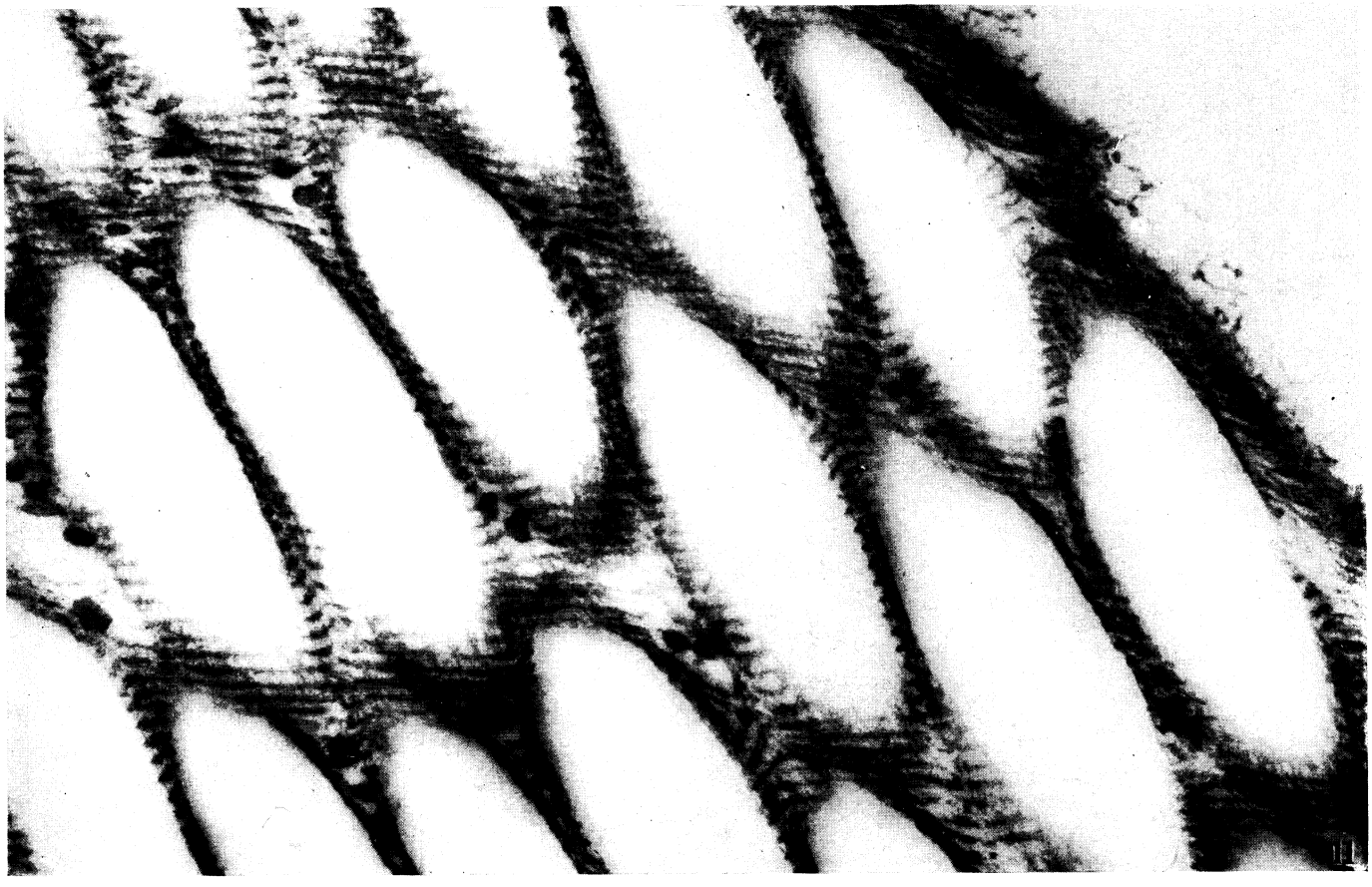
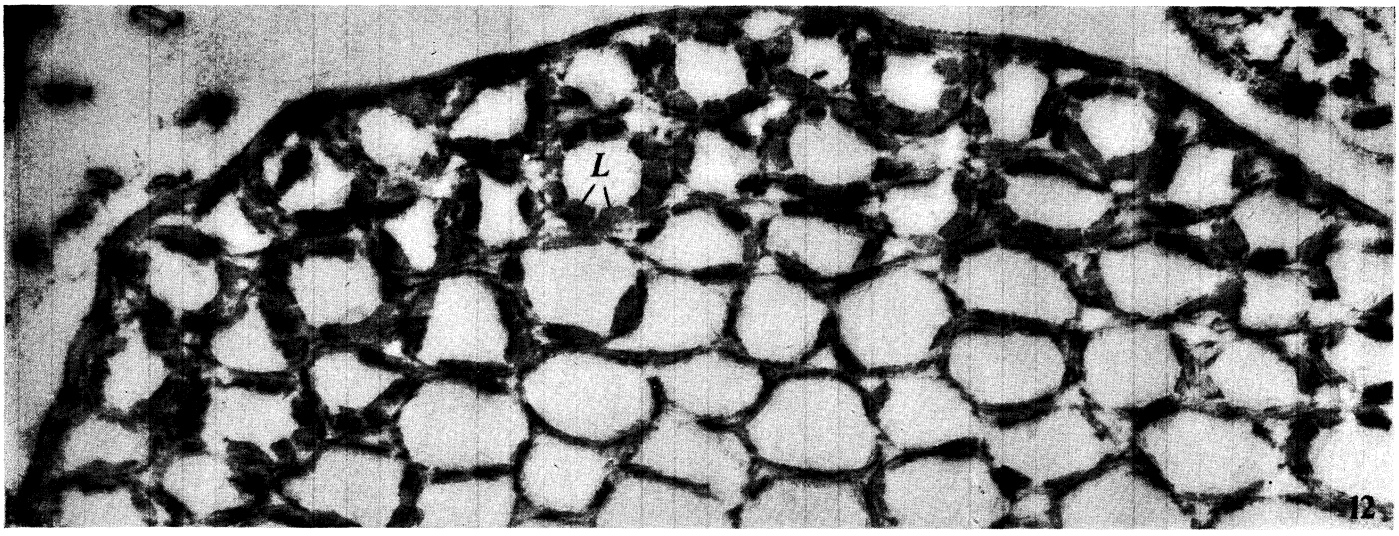
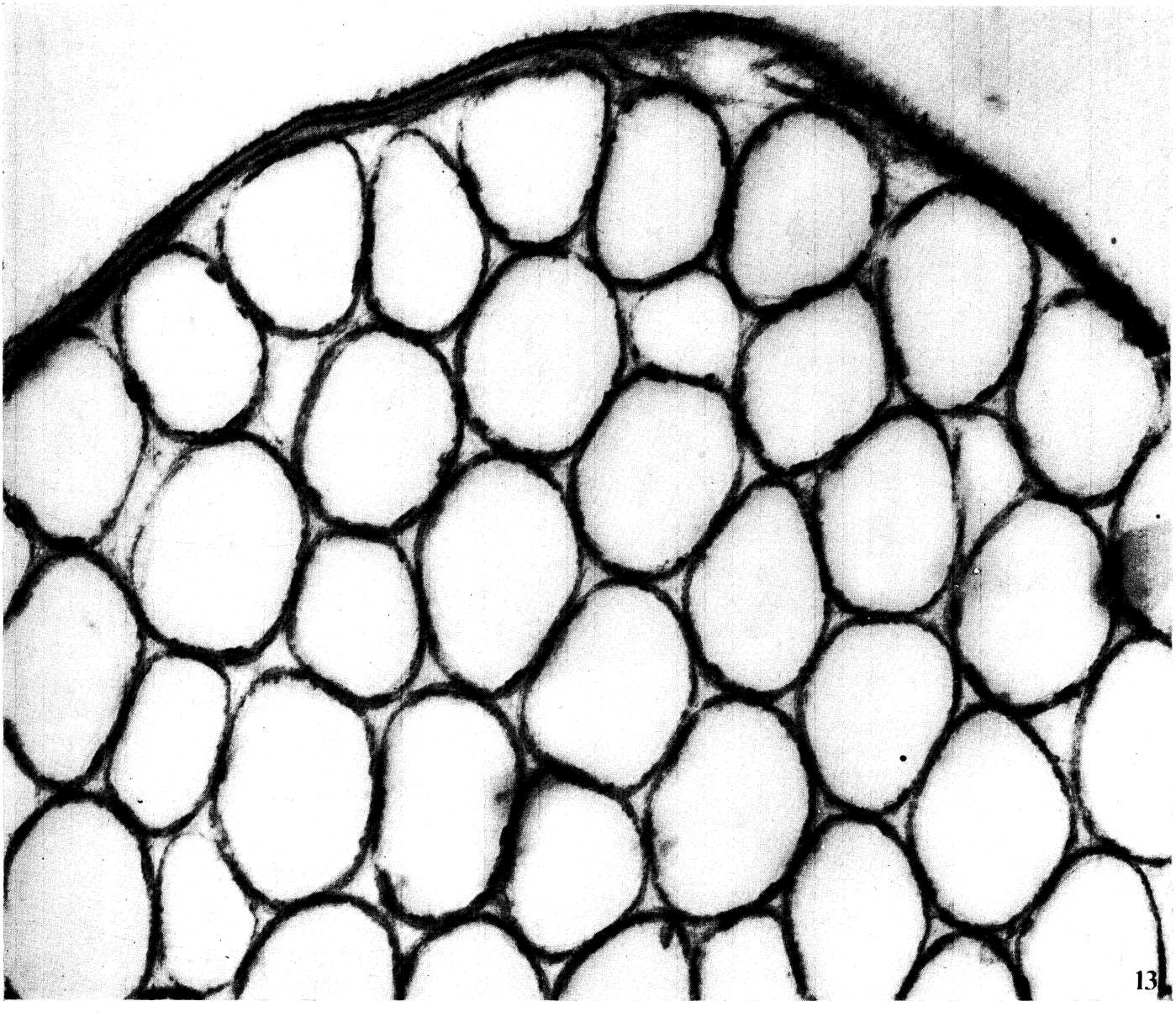


Epistylis plicatilis

FIGURE 10. Transverse section of stalk mid-way along its length. The tubules are close-packed and enclosed within a substantial wall (× 21 520).

FIGURE 11. Near-transverse section of stalk at higher magnification (× 61 200).





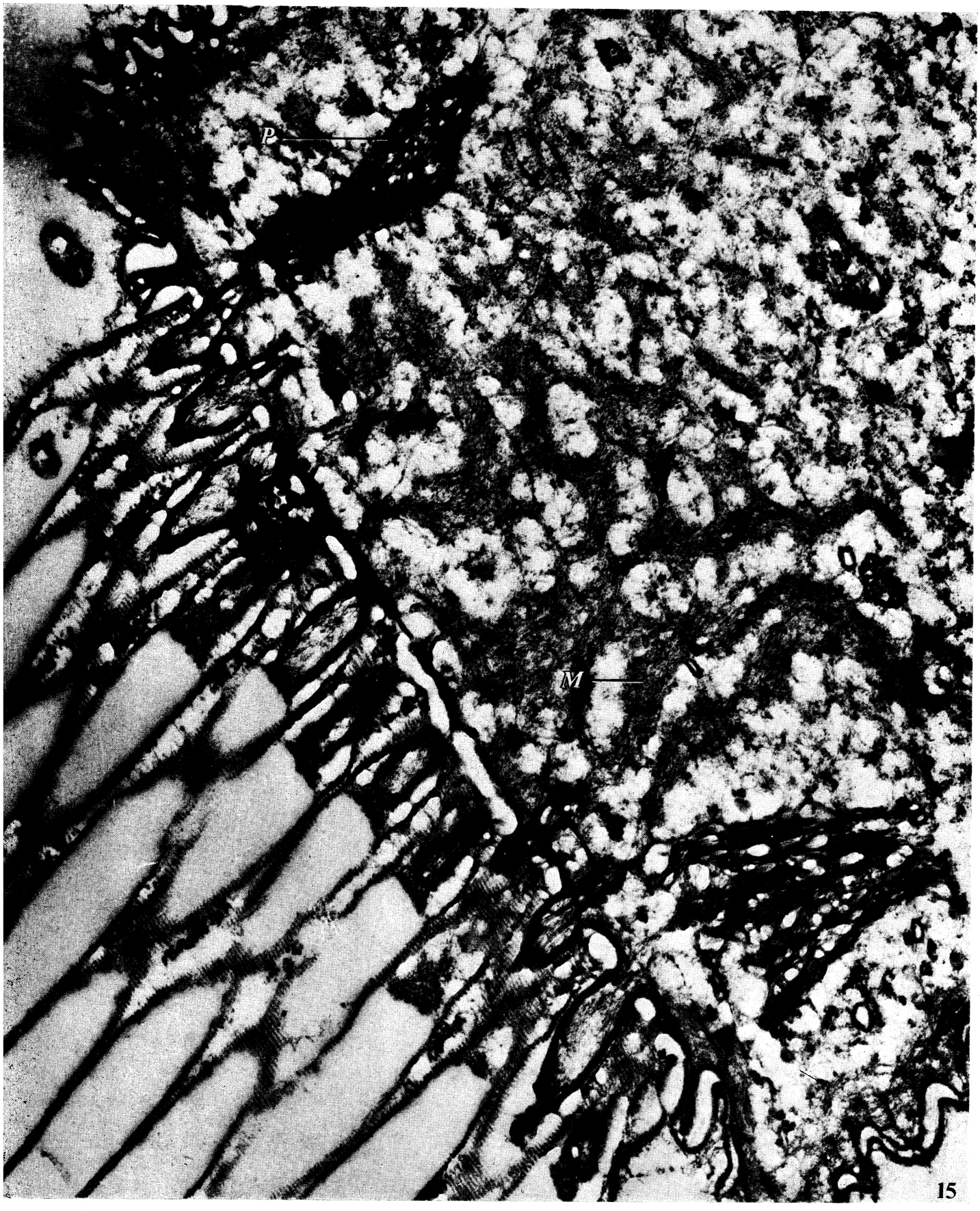
Epistylis plicatilis

FIGURE 12. View of section of part of a stalk cut from the distal region. Each tubule in the outermost layers contains a number of elements often lozenge-shaped in section, as at *L* (× 36000).

FIGURE 13. Remnants of the structures seen in figure 12 are present in this section from another region of the stalk. The complex wall of the stalk is also clearly shown (× 61200).

Epistylis plicatilis

FIGURE 14. Longitudinal section of scopula organelles, probably peripheral. They consist of an upper portion *C* in the form of a fibrous cylinder and a lower part or sac *S* depending into the stalk. The fibrils *ff* originating in the scopula organelles are eventually attached to the zooid wall and are probably concerned with zooid contraction. The stalk tubules (as at points *t*) fit closely over the sacs *S*. Fibrils *f* extend from these sacs into the stalk tubules possibly through the pore-like structures visible at *P*, *P* (× 114000).

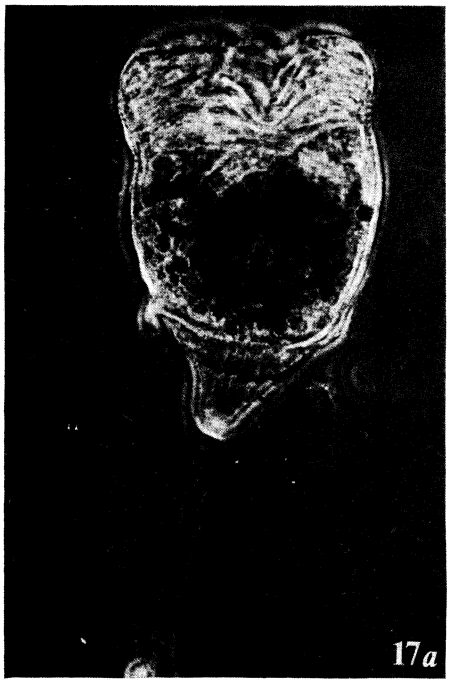


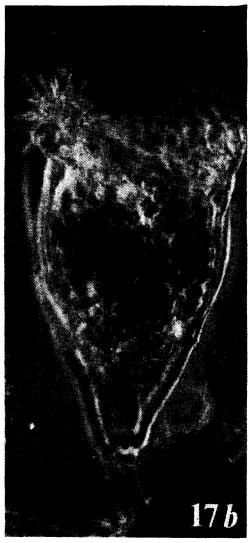
Epistylis plicatilis

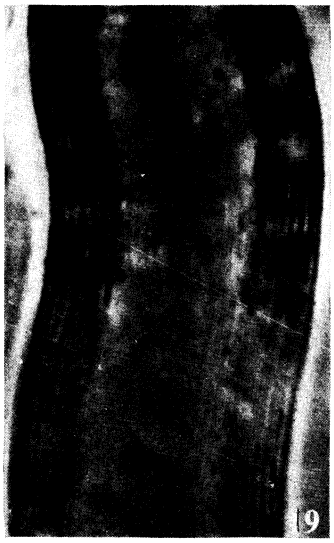
FIGURE 15. Longitudinal section through a stalk-zooid junction, probably cut through the middle of the stalk. The peripheral scopula organelles are seen to be different from the central ones (× 27 000). From the upper parts of the peripheral organelles long bundles of fibrils, *P*, extend into the zooid and terminate ultimately on or near its wall. Shorter bundles, *M*, are attached to the more central scopula organelles.

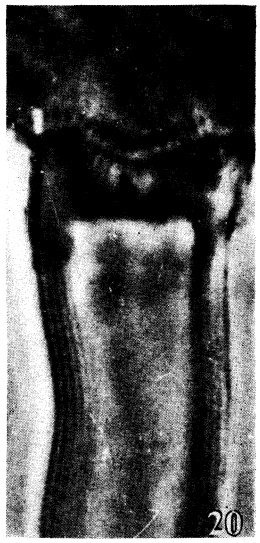
Epistylis plicatilis

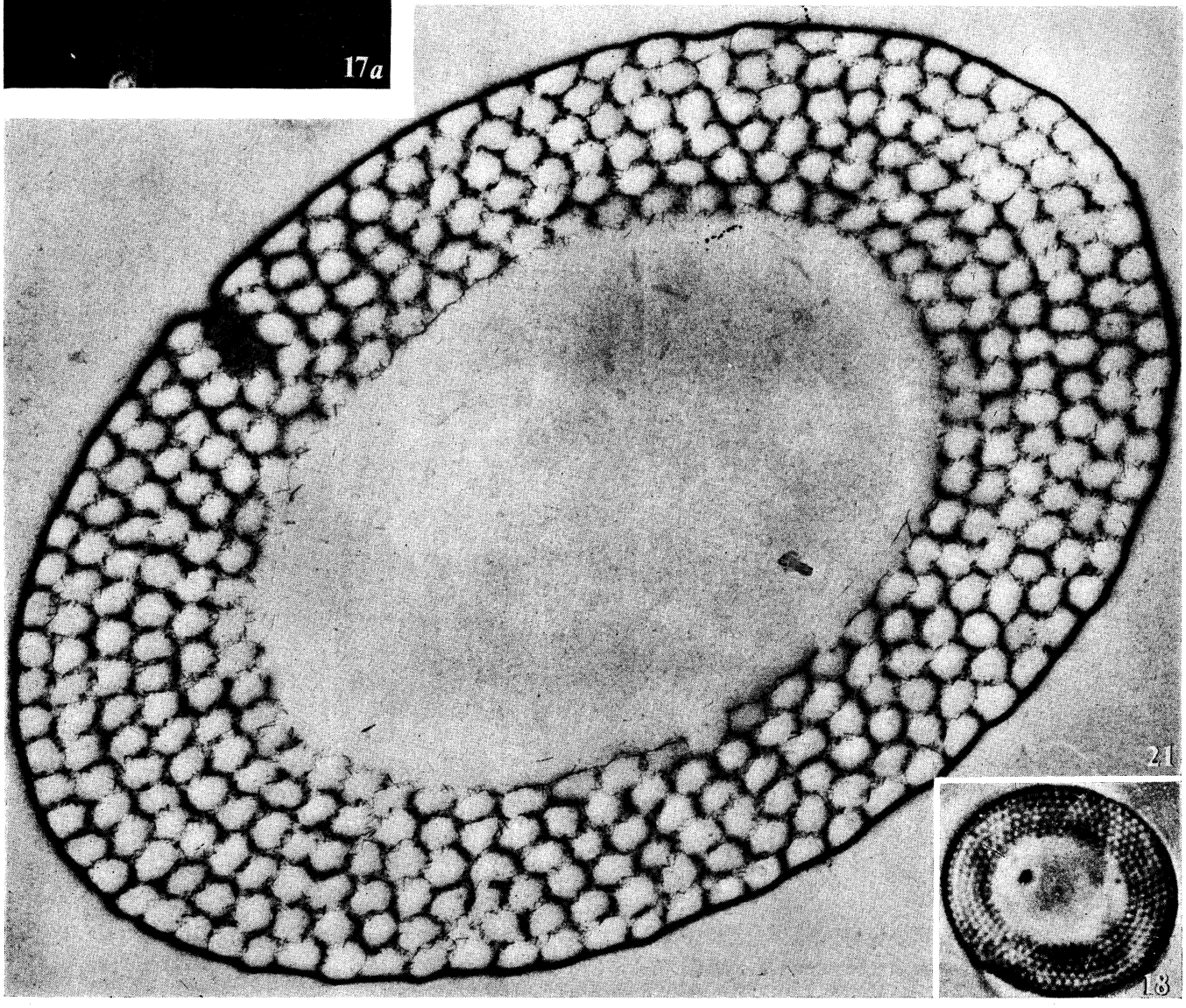
FIGURE 16. View of the crenellated wall *cw* of the fixed zooid with its ectoplasmic layer *ee*. The fibrillar bundle *PP* originating in the peripheral scopula organelles appears to fuse with this layer in the region *FF* (× 76500). Mitochondria such as mit. are commonly observed in the cytoplasm.











Campanella umbellaria

FIGURE 17*a*. Developmental stage with young stalk. The ciliary girdle of the free-swimming telotroch is still apparent (×320).

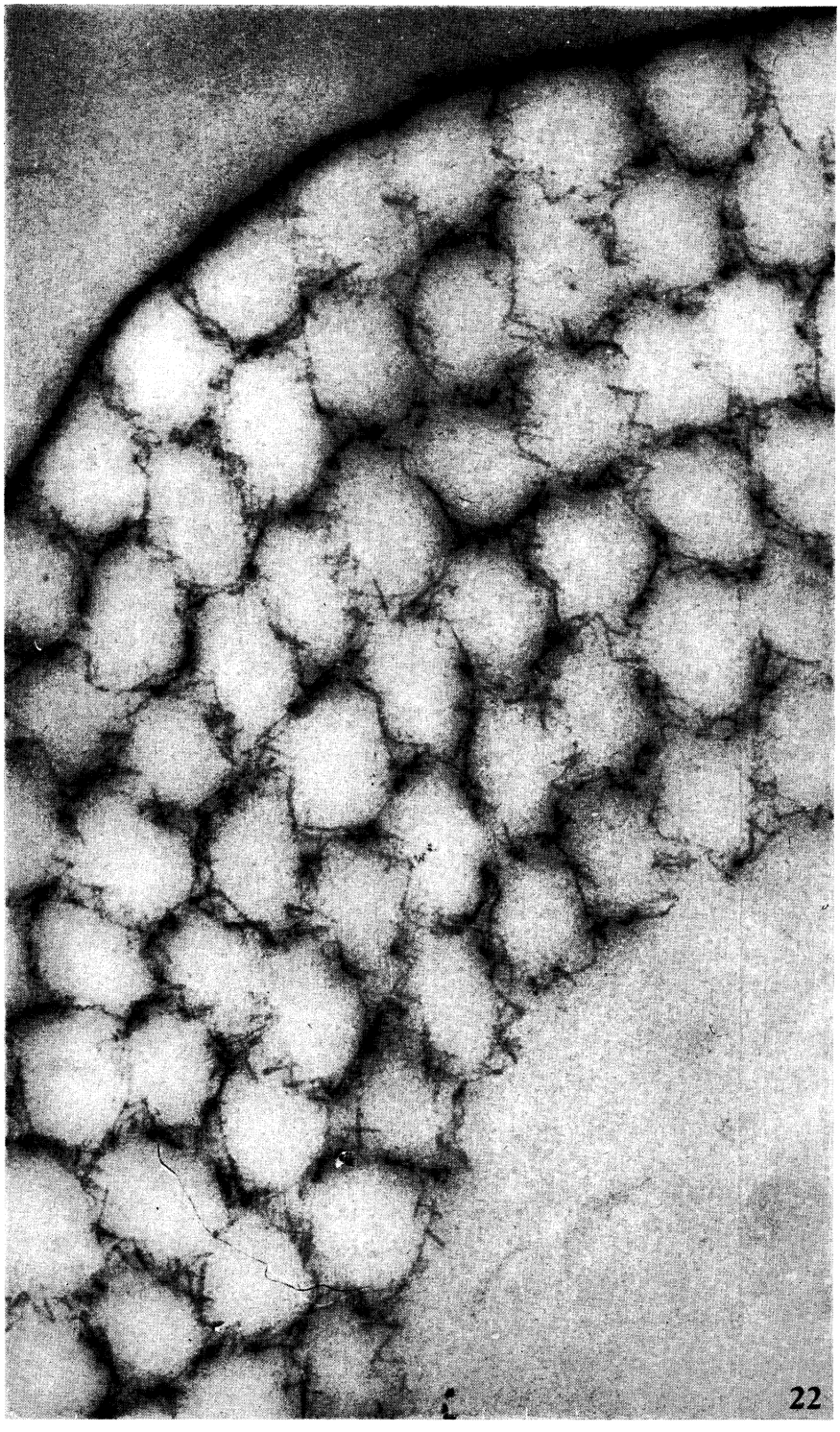
FIGURE 17*b*. Adult specimen to be compared with figure 17*a* (×300).

FIGURE 18. Photomicrograph of *Campanella* stalk that shows characteristic tubular structure with central structureless canal (×2750).

FIGURE 19. Longitudinal section of stalk seen in light microscope and consistent with figure 18 (×2750).

FIGURE 20. Longitudinal section of stalk and its junction with the zooid. At the junction the stalk tubules appear to extend over the whole section. At a distance of about 2 μ from the junction the inner tubules cease abruptly. Note the scopula organelles (×2750).

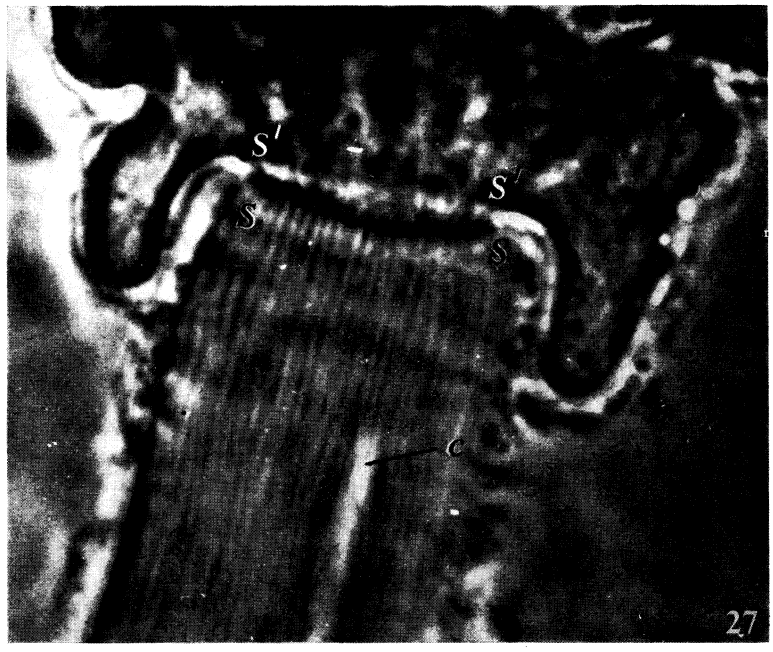
FIGURE 21. Low magnification electron micrograph of transverse section of stalk (×13600).

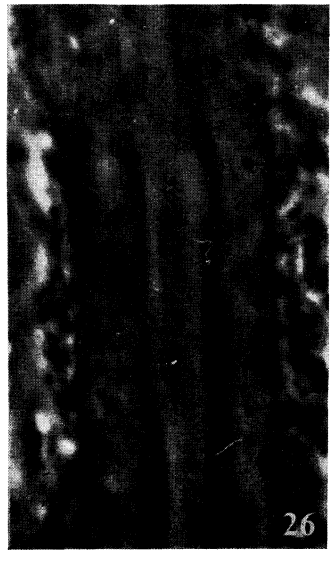


Campanella umbellaria

FIGURE 22. This transverse section of a sector of the stalk shows six peripheral rings of fibrous tubules and a canal in the centre of the stalk. The tubules are closely packed, devoid of structure rather polygonal in outline and of linear dimensions 0·3 to 0·5 μ (× 42000).

FIGURE 23. Longitudinal section of fibrous tubules indicating as in figure 22 how one tubule is interlocked with its neighbours (× 60000).





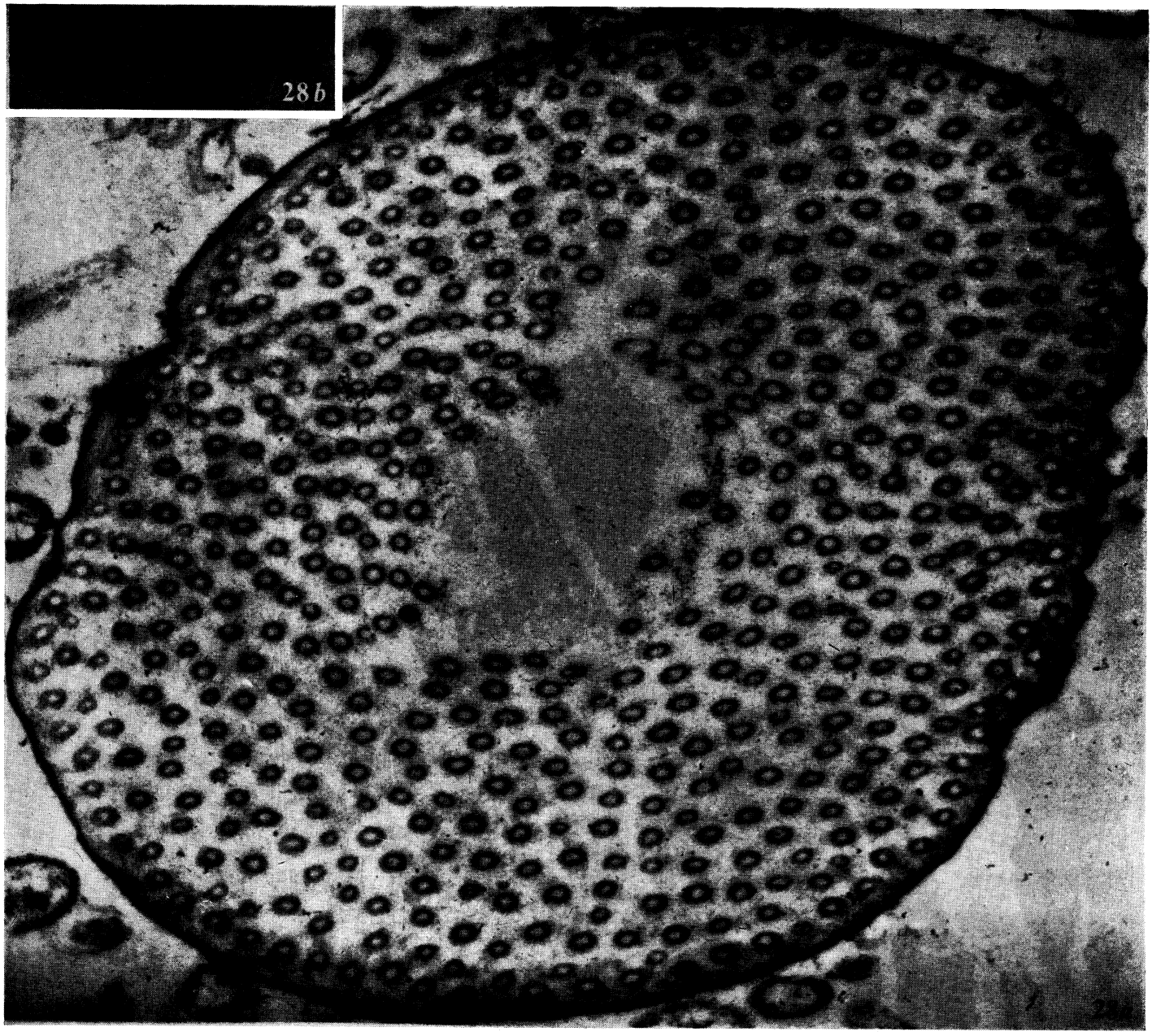
Opercularia plicatilis

FIGURE 24. Small living colony showing its tree-like character and the form of the individual zooid (× 112).

FIGURE 25. Photomicrograph of transverse section of stalk which shows the individual tubules. The more or less central canal is very irregular in this illustration and this may in part be artifact. See also figures 26 and 27 (× 2750).

FIGURE 26. Photomicrograph of longitudinal section of stalk. The central canal is prominent (× 2750).

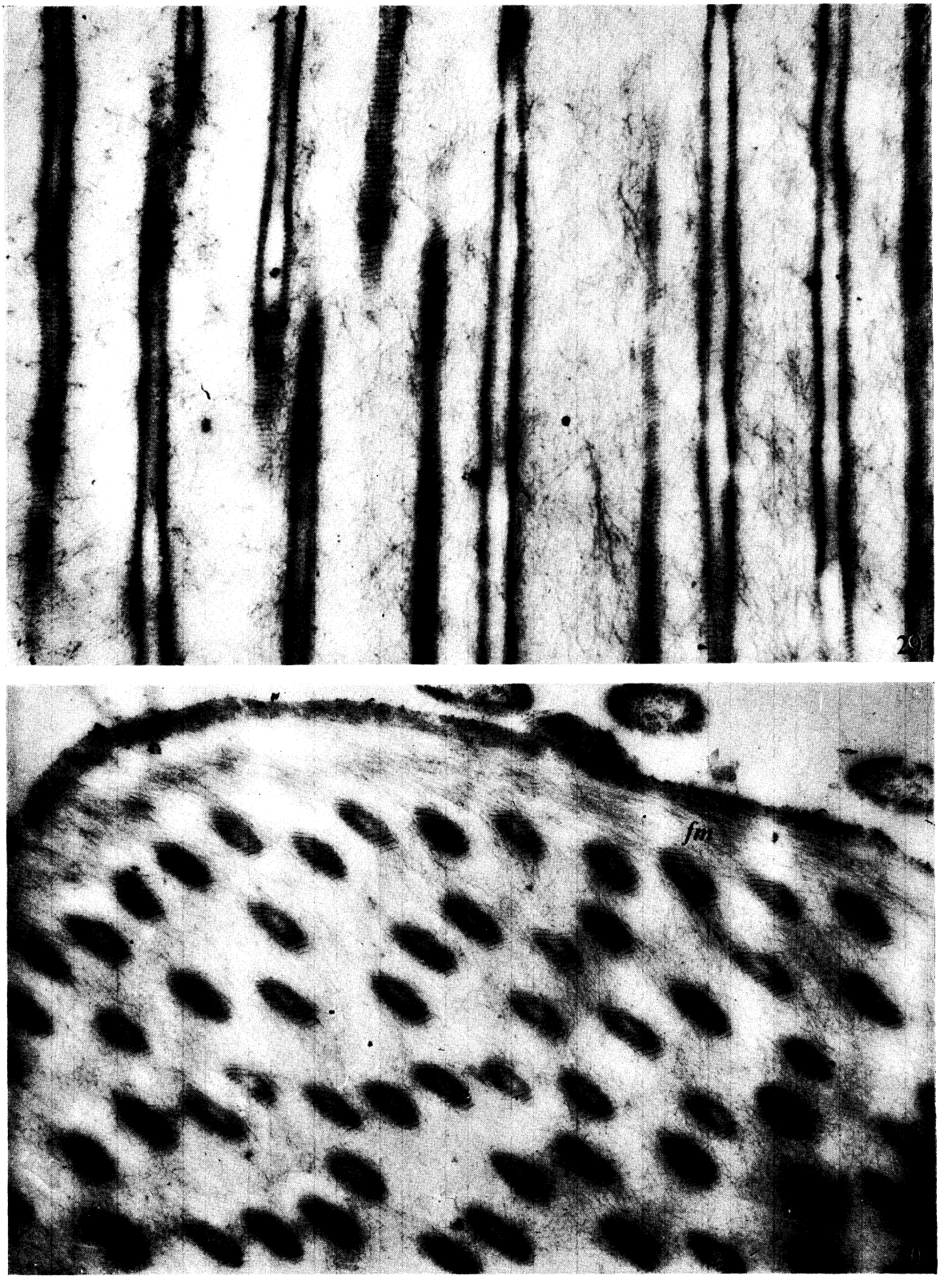
FIGURE 27. Photomicrograph of longitudinal section of junction between stalk and zooid. The central canal *c* does not appear to start at the junction (× 2750).



Opercularia plicatilis

FIGURE 28*a*. Low magnification electron micrograph of transverse section of stalk showing the many tubules and central canal (cf. figure 27). No membrane separates the canal from the annulus (× 13 600).

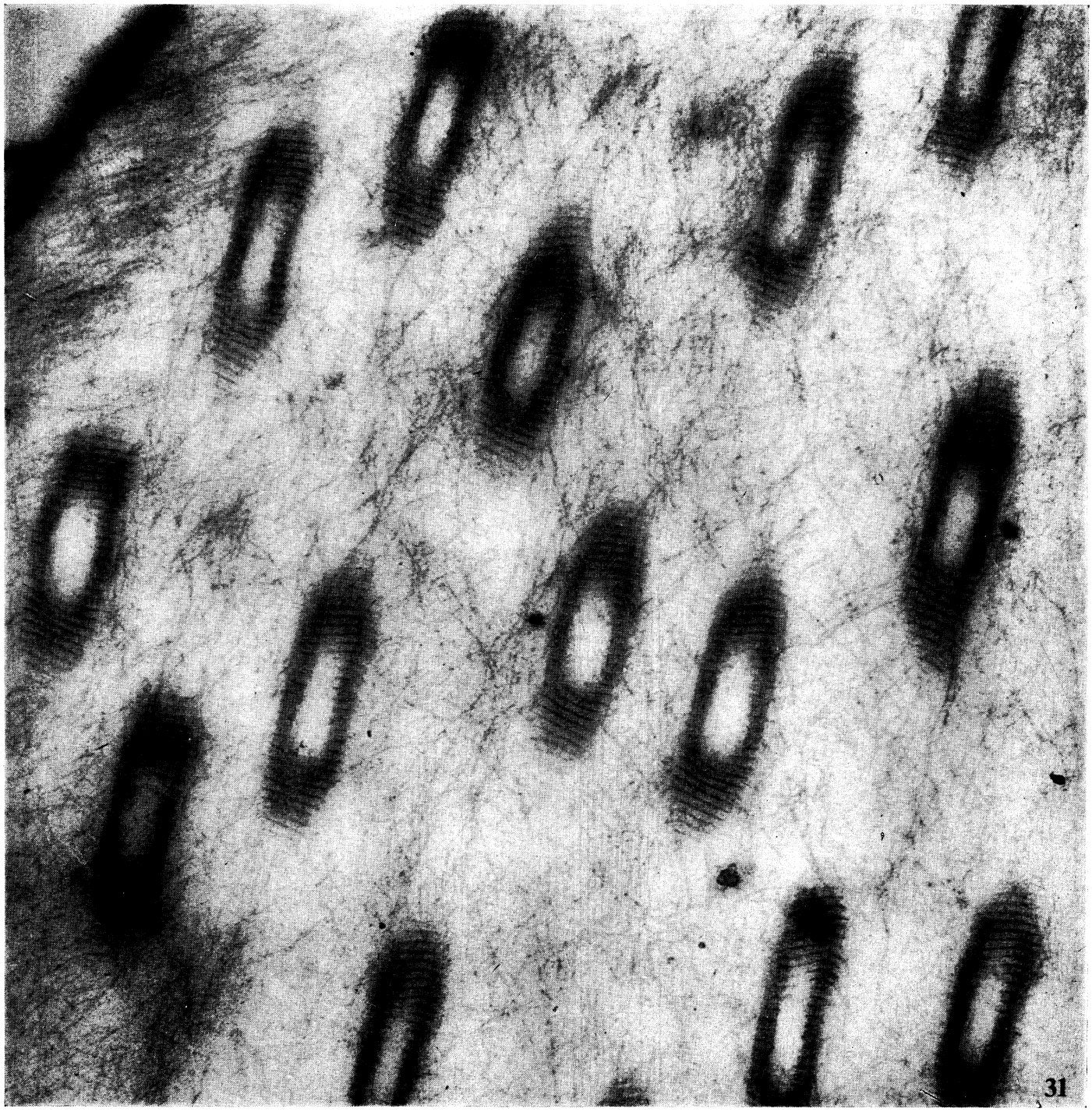
FIGURE 28*b*. Transverse section of two tubules at higher magnification indicating probable fibrous nature of the wall (× 27 200).



Opercularia plicatilis

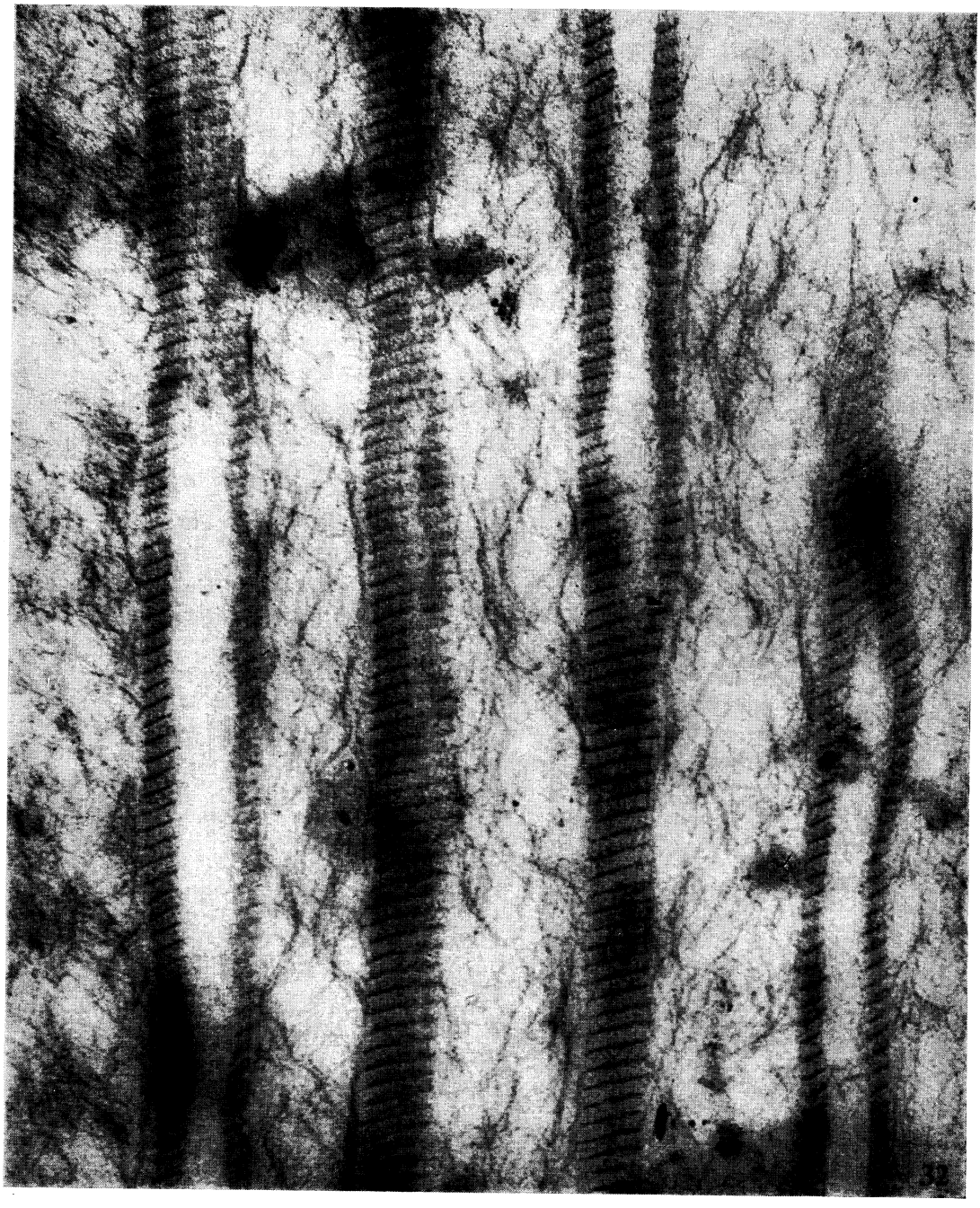
FIGURE 29. The stalk tubules in longitudinal section showing transverse striations (period 400 Å) and fibrillar matrix (× 27 200).

FIGURE 30. Oblique section of the stalk in which the striations of figure 29 are also visible as well as the attachment of the fibrillar matrix *fm* to the wall (× 27 200).



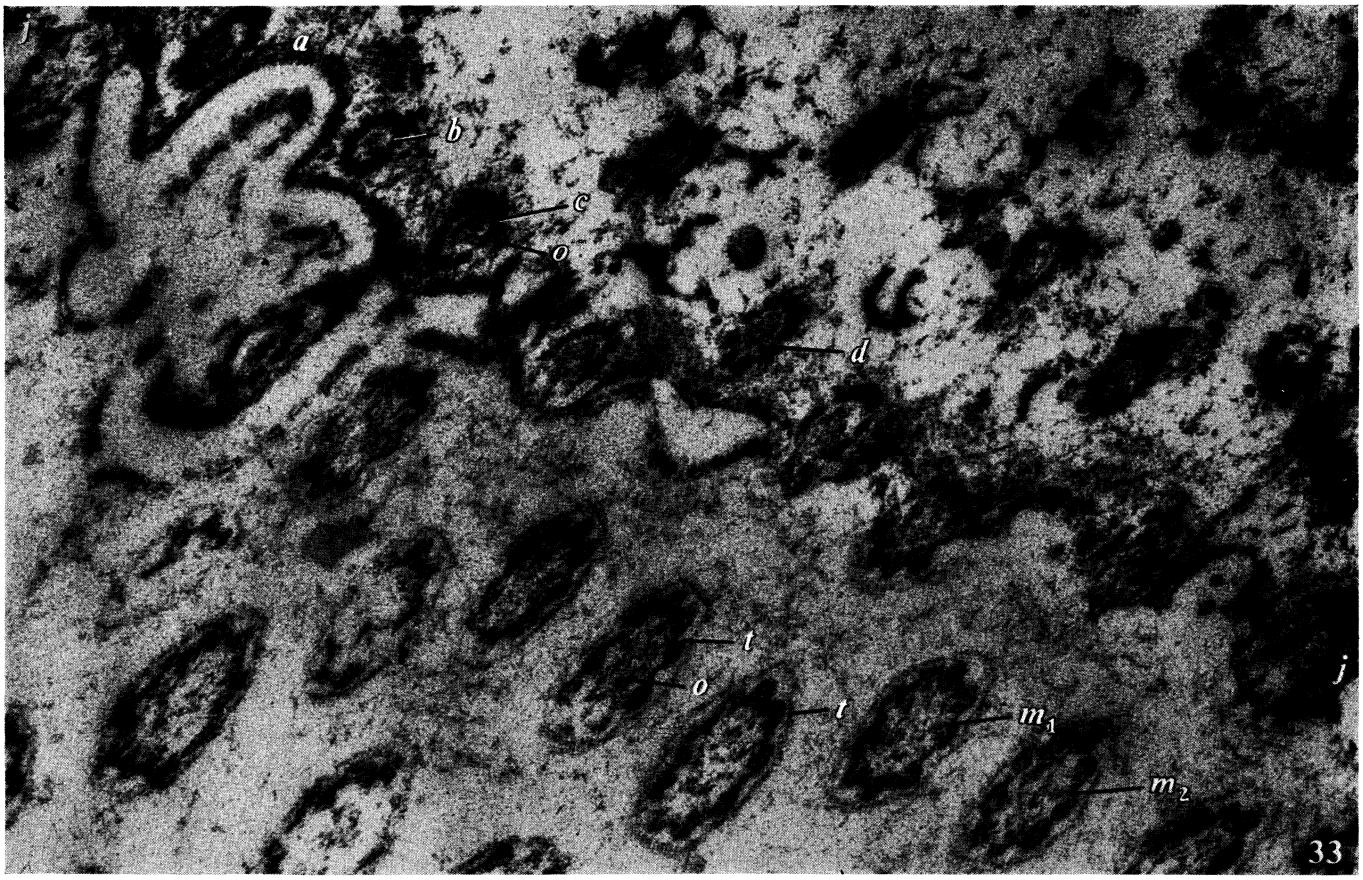
Opercularia plicatilis

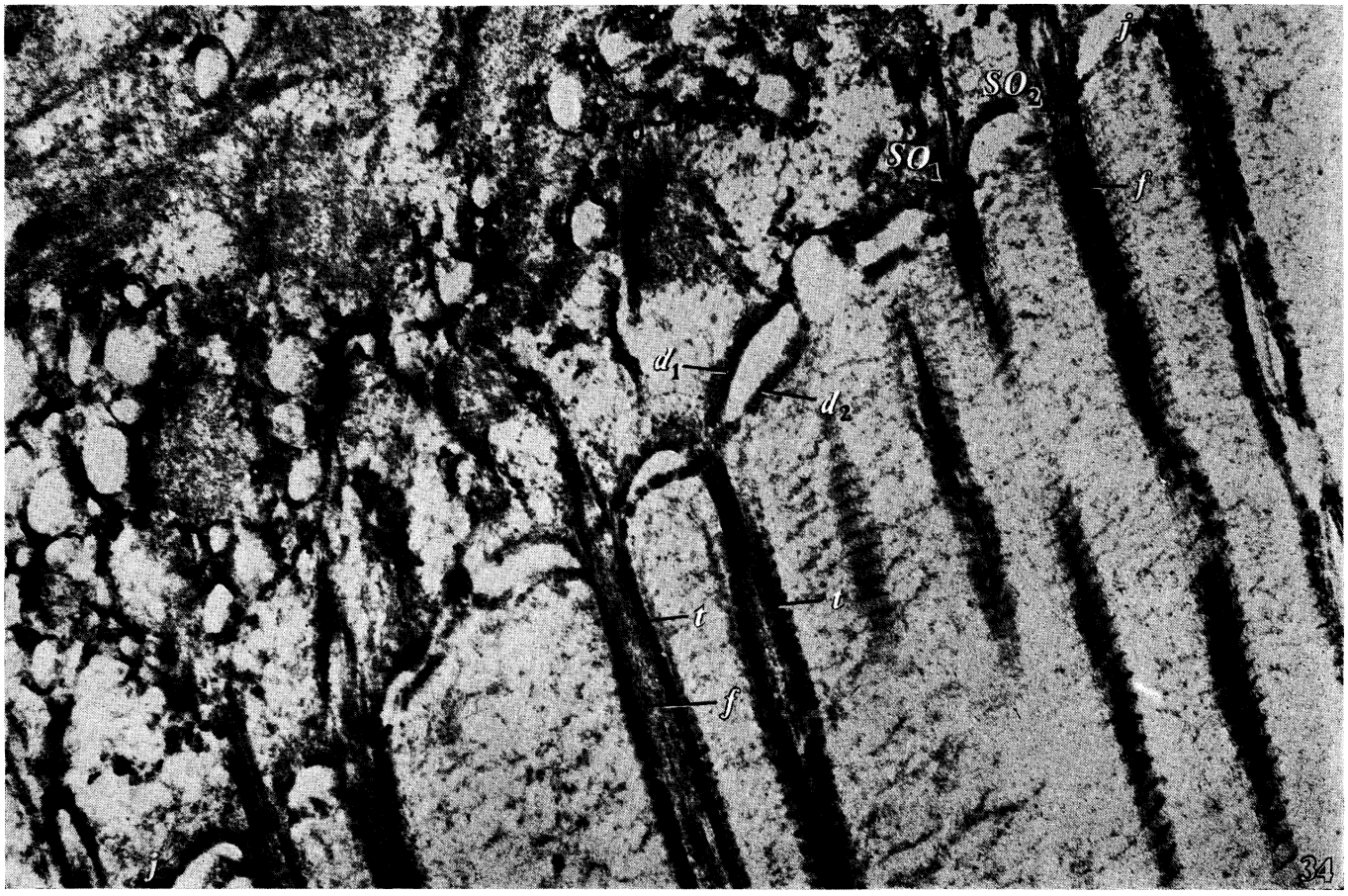
FIGURE 31. This section shows (*a*) striations of tubules each about 60 Å wide and 400 Å apart, (*b*) matrix fibrils which are not striated, and (*c*) component longitudinal fibrils of the tubules (× 55 500).



Opercularia plicatilis

FIGURE 32. Demonstrates the regularity of the transverse striations over considerable lengths of tubule and the unstriated nature of the matrix (× 66 600).

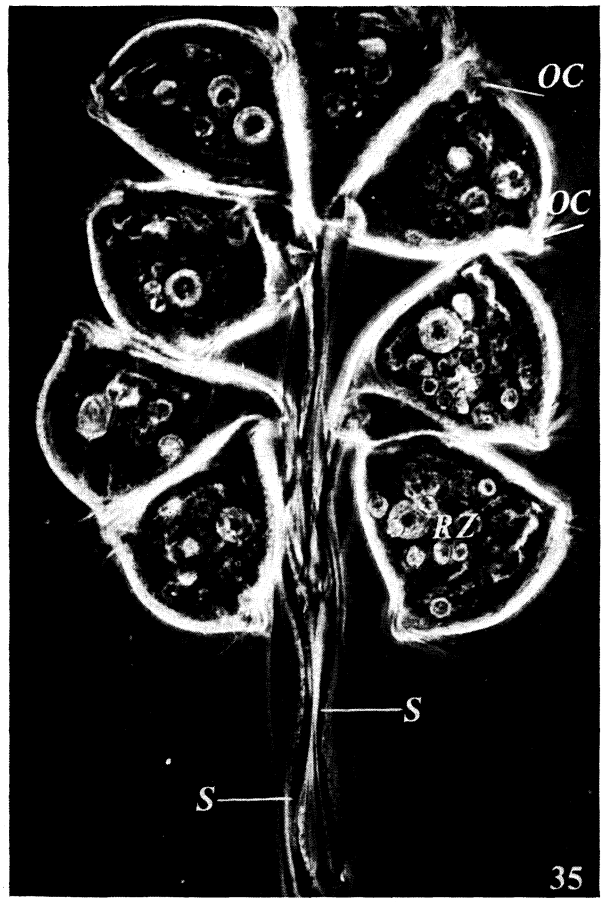


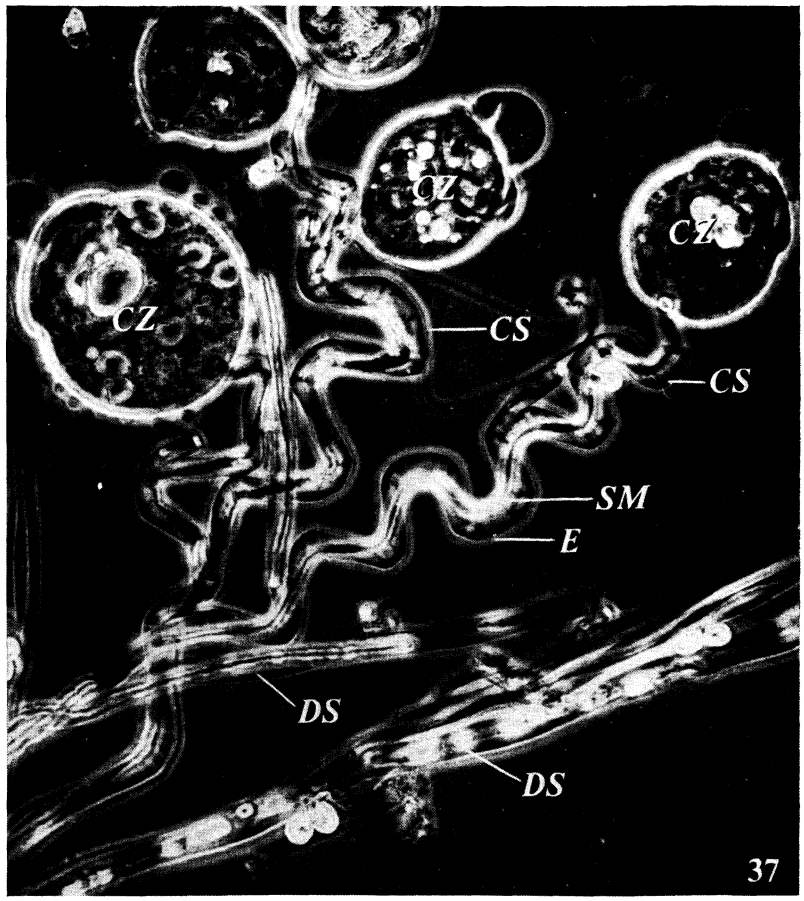


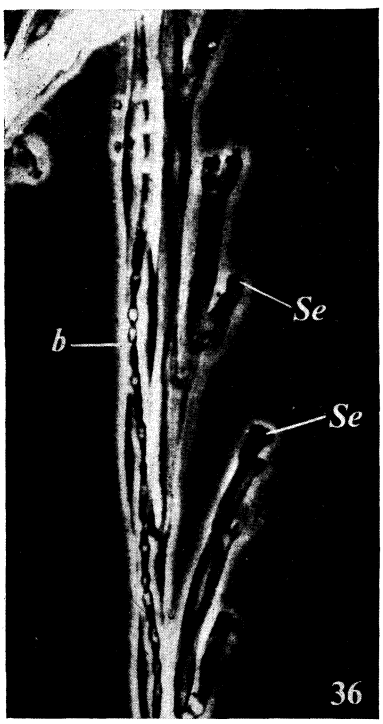
Opercularia plicatilis

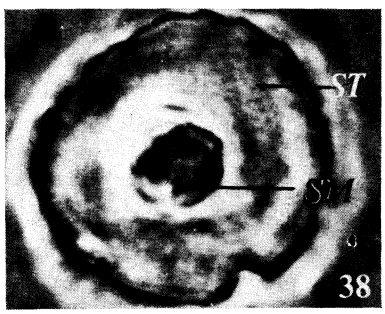
FIGURE 33. Oblique section of the junction *jj* between stalk and zooid. Parts of scopula organelles lying within the zooid are visible at *a*, *b*, *c* and *d*, and are structurally analogous to basal bodies. The component fibres are not enveloped by a membrane above the junction but lower parts of the *SO* as at *t*, *t* are. Material, possibly fibrous, is also visible within the fibrous peripheries of the *SO*, as at *O*, both above and below the junction plane *jj*. The main fibrils of the *SO* below the junction appear to be surrounded by a scalloped membrane (m_1, m_2) and are probably attached to it (× 44400).

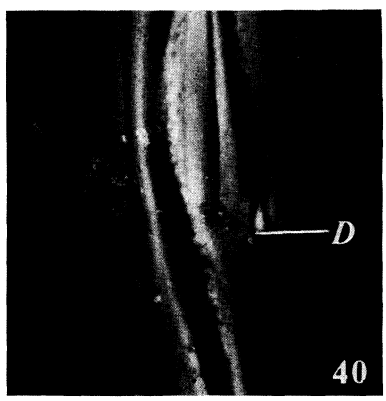
FIGURE 34. This longitudinal section through the junction *jj* shows the duplex nature of the junction membrane (d_1, d_2) and the fibrous interior of the *SO* as at *f*, *f*. The stalk tubules are recognizable at *t*, *t* by their striations and fit closely over the elongated bases of the scopula organelles. This manner of attachment is similar to that adopted in *Epistylis* (cf. figure 14, plate 19 (× 44400).

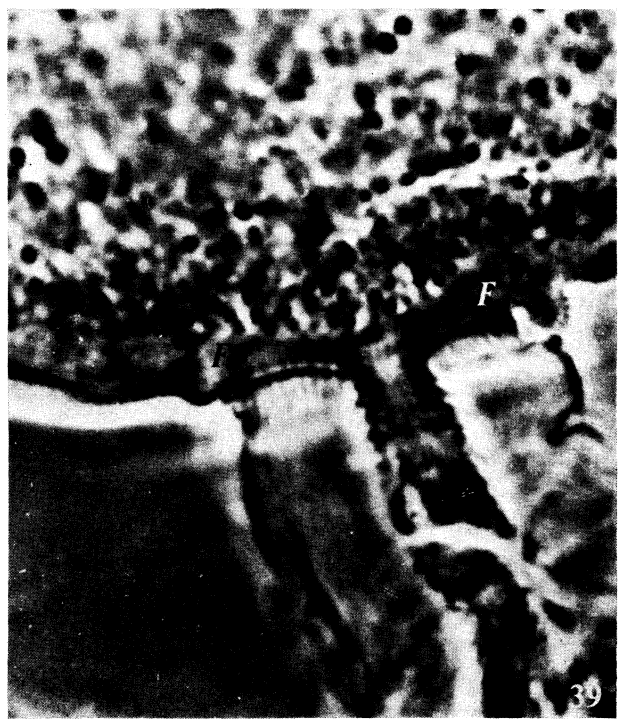












Carchesium polypinum

FIGURE 35. View of portion of living colony with the stalks extended or relaxed. The spasmonemes of individual stalks can be seen at points *S*, *S*, and the oral cilia at *OC* (×380).

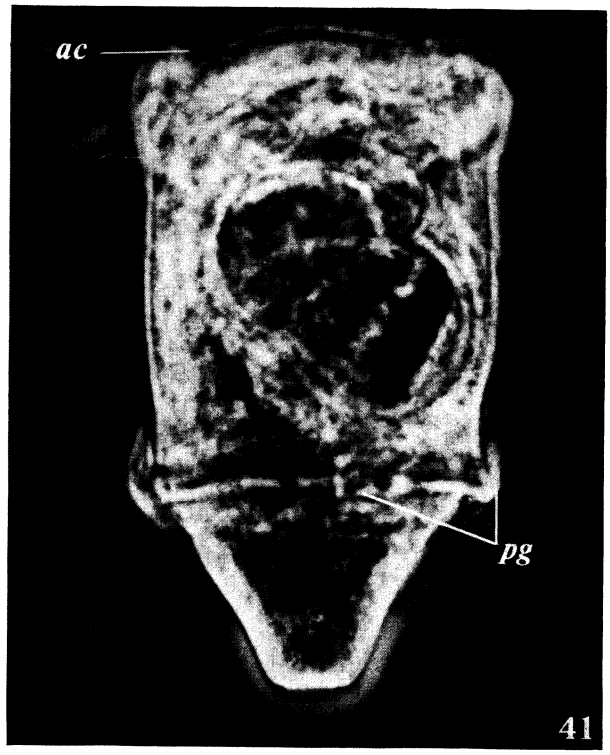
FIGURE 36. Portion of living colony that has lost its zooids. The exposed ends of the stalks are visible at *Se*. The stalks so deprived are always extended and the spasmoneme is often beaded as at *b*.

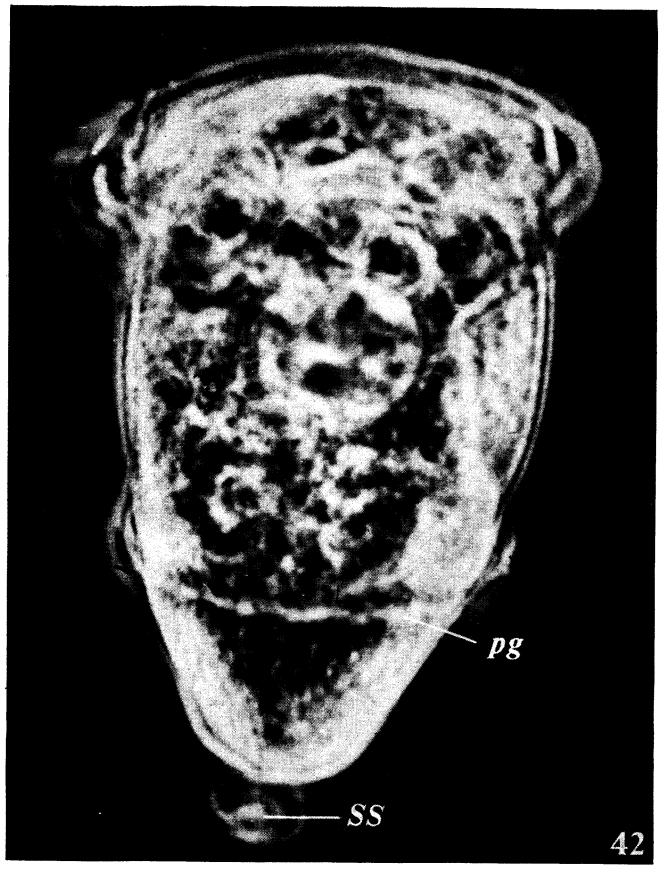
FIGURE 37. Photograph of living colony in contraction. Note the convoluted contracted stalks *CS* and that the spasmoneme *SM* takes the shortest path within the envelope *E* of the stalk. The zooids *CZ* have all changed shape and are quite different from the 'relaxed' zooids *RZ* of figure 35. The oral cilia are also withdrawn (×208).

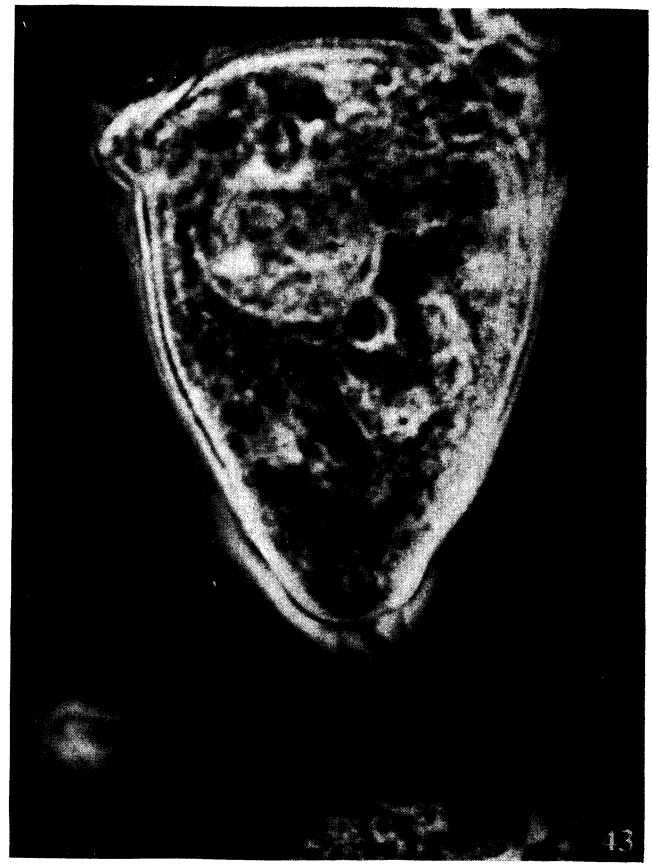
FIGURE 38. Photomicrograph of transverse section in which both the internal spasmoneme *SM* and the annular stalk tubules *ST* can be seen (×2750).

FIGURE 39. Longitudinal section of stalk and scopula showing spasmoneme and individual scopula organelles. The spasmoneme is seen to fan out in to the zooid at *F* and *F*: photomicrograph (×2750).

FIGURE 40. Enlarged photograph of stalk with spasmoneme discontinuity *D* at bifurcation (×950).





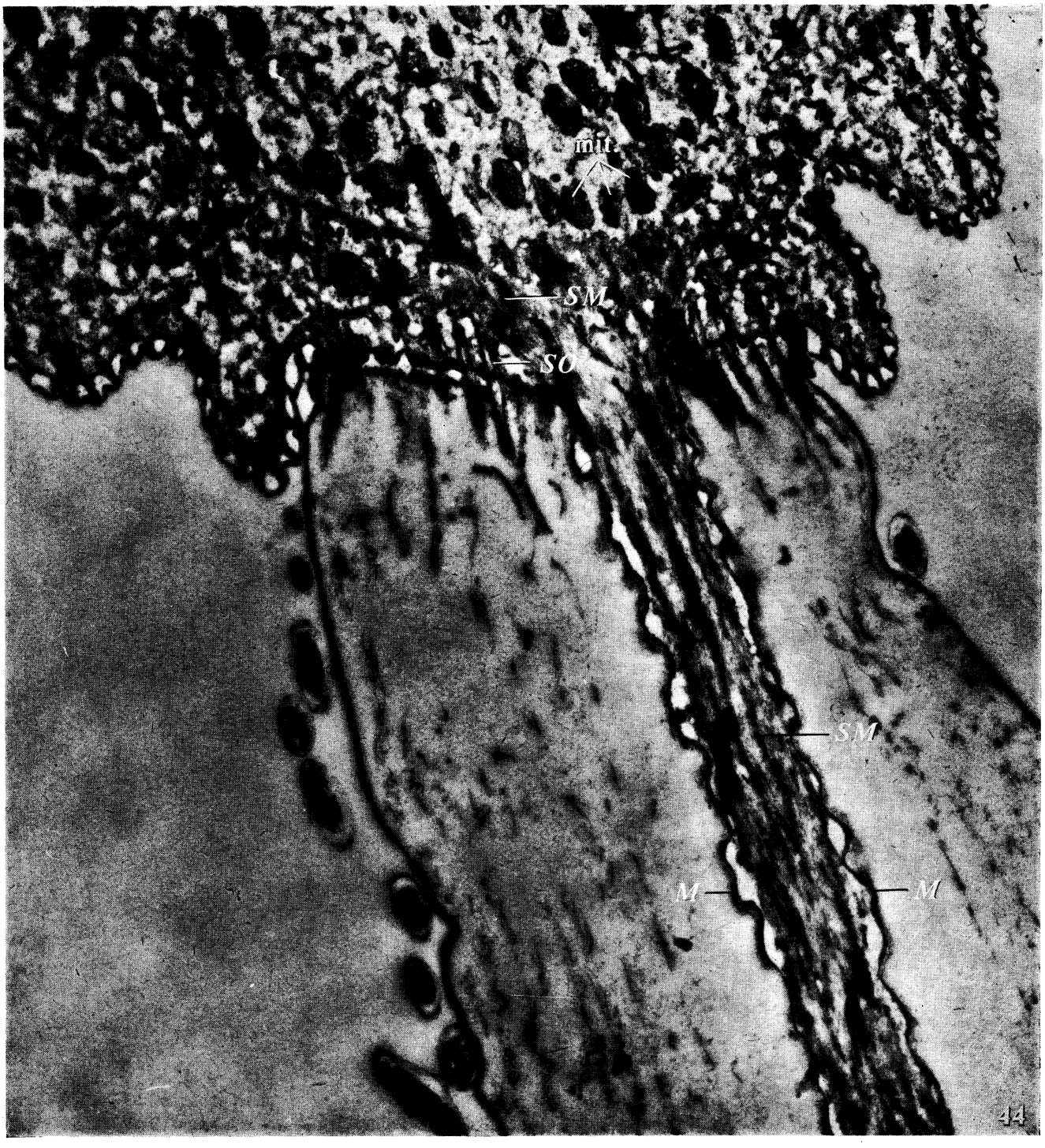


Carchesium polypinum in development

FIGURE 41. Free-swimming telotroch with apical cilia at *ac* and posterior girdle at *pg* (× 950).

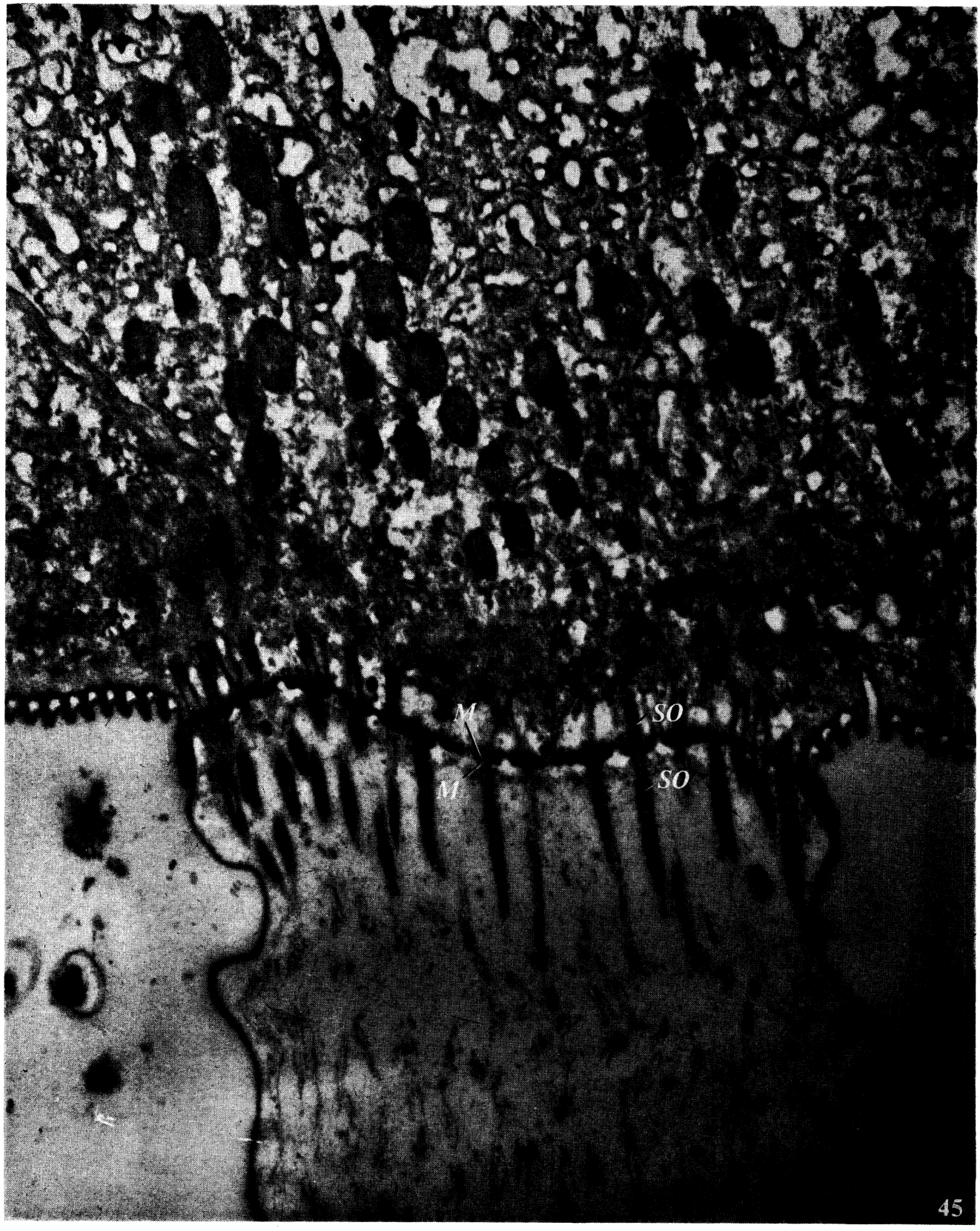
FIGURE 42. Telotroch with very short stalk *SS* attached. The posterior girdle of cilia *pg* is still present and has not yet regressed obviously (× 950).

FIGURE 43. Young adult with longer stalk. The posterior girdle has disappeared and the overall shape of the zooid has changed (× 950).



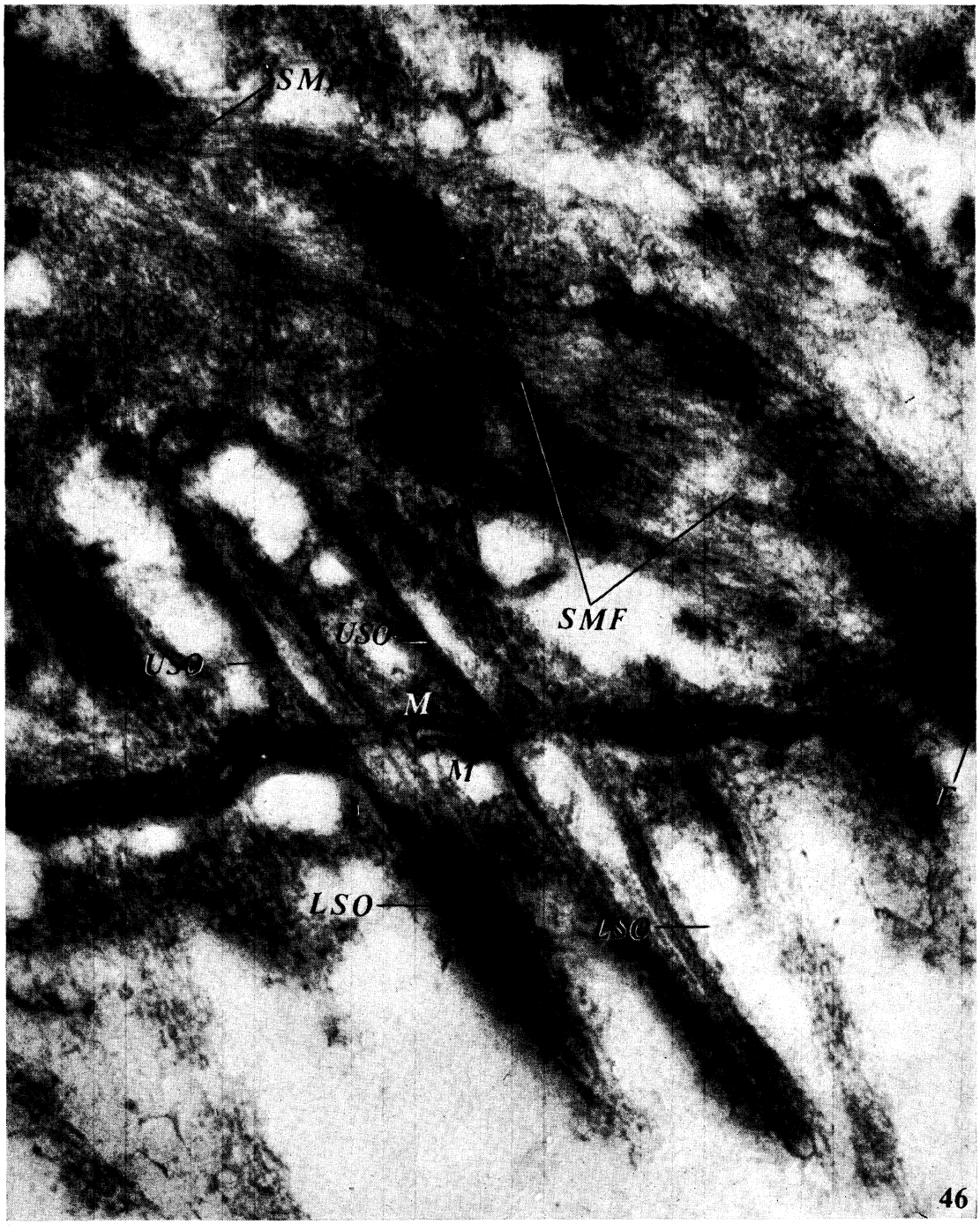
Carchesium polypinum

FIGURE 44. Longitudinal axial section of stalk, scopula and part of zooid. The spasmoneme *SM* is visible within its membrane *M* and can be seen to pass into the zooid. Scopula organelles *SO* and mitochondria (mit.) are also visible (× 13 600).



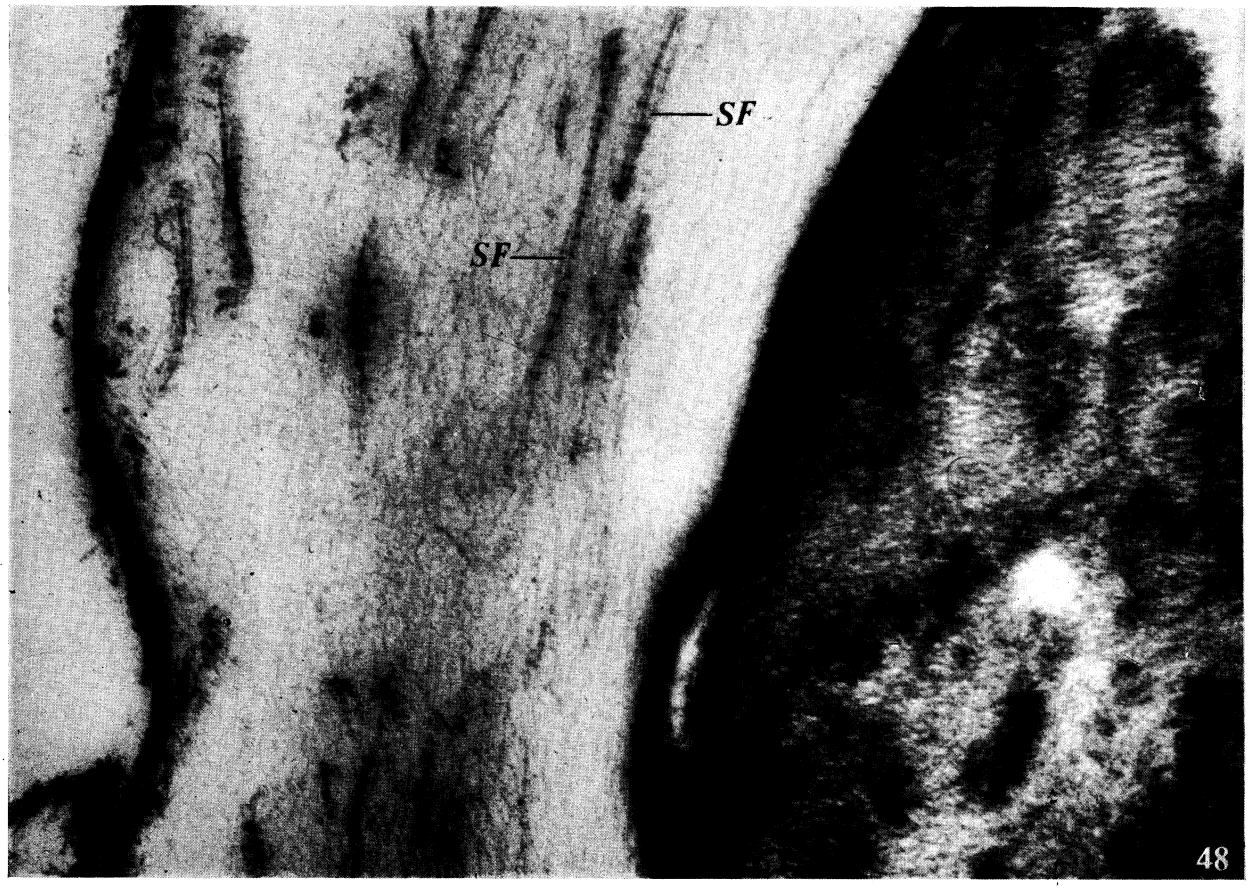
Carchesium polypinum

FIGURE 45. Off-axis longitudinal section in which the spasmoneme is not visible because the section is cut closer to the surface of the stalk. This micrograph is particularly useful in demonstrating the specialized form of scopula membrane *MM* and scopula organelles *SO* (×28000).



Carchesium polypinum

FIGURE 46. Higher magnification electron micrograph of part of scopula and zooid. The upper (*USO*) and lower (*LSO*) parts of two scopula organelles are shown and the complex scopula membrane can be seen at *MM*. This micrograph shows part of the spasmoneme emerging from the stalk into the zooid at *E*. The spasmoneme fibrils *SMF* spread out fan-wise to the upper left hand corner of the plate (× 90 800).



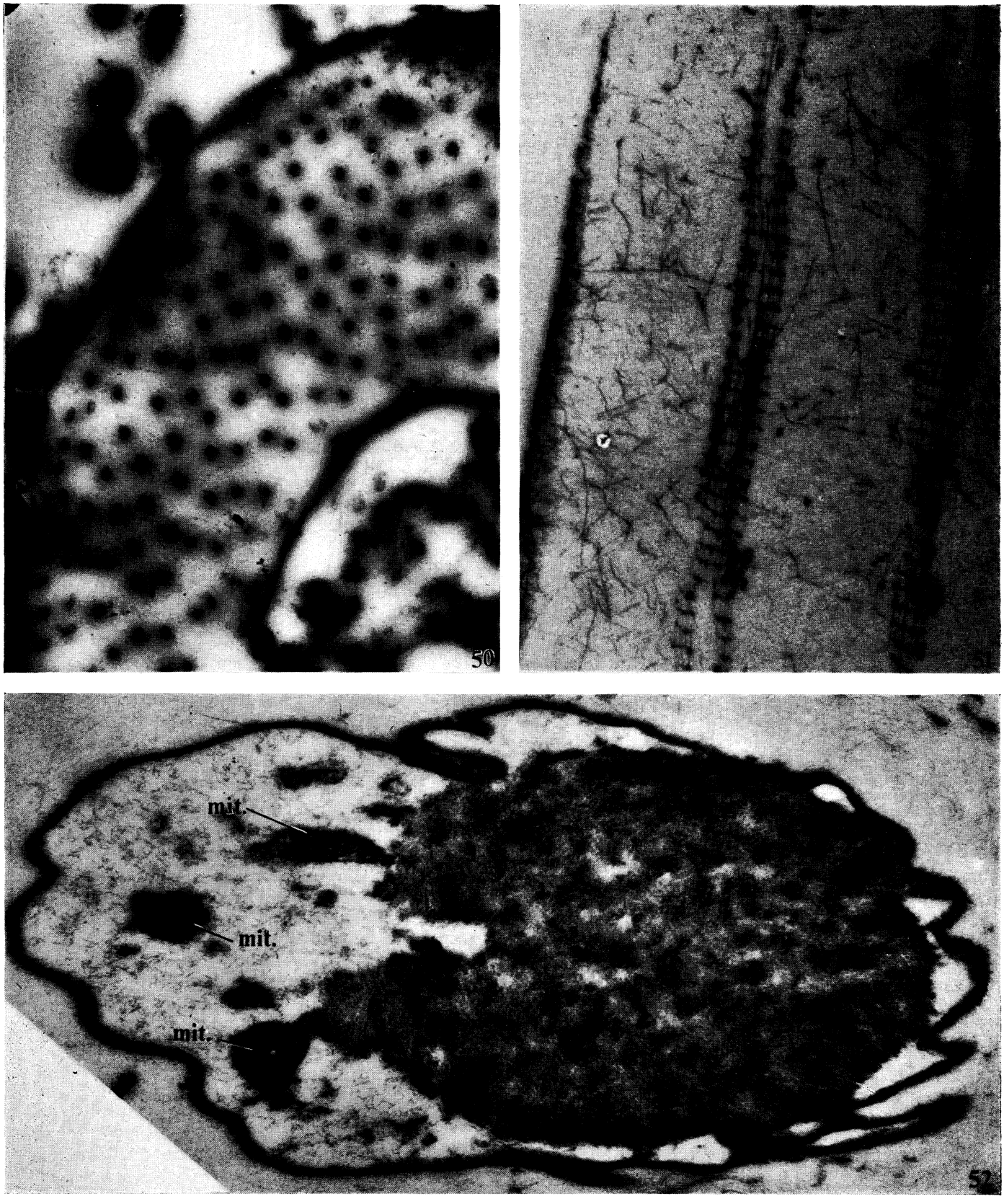
Carchesium polypinum

FIGURE 47. Longitudinal section of part of stalk: individual fibrils of the spasmoneme *SM* are visible (× 27 200). The irregular membrane of the spasmoneme canal is seen at *M*, *M*.

FIGURE 48. Oblique section of stalk. Note individual fibrils of spasmoneme and striated fibrils *SF* of the annular tubules (× 58 000).

Carchesium polypinum

FIGURE 49. Longitudinal section of annular region of stalk filled with short lengths of the striated tubules. The matrix contains finer fibrils (× 36000).

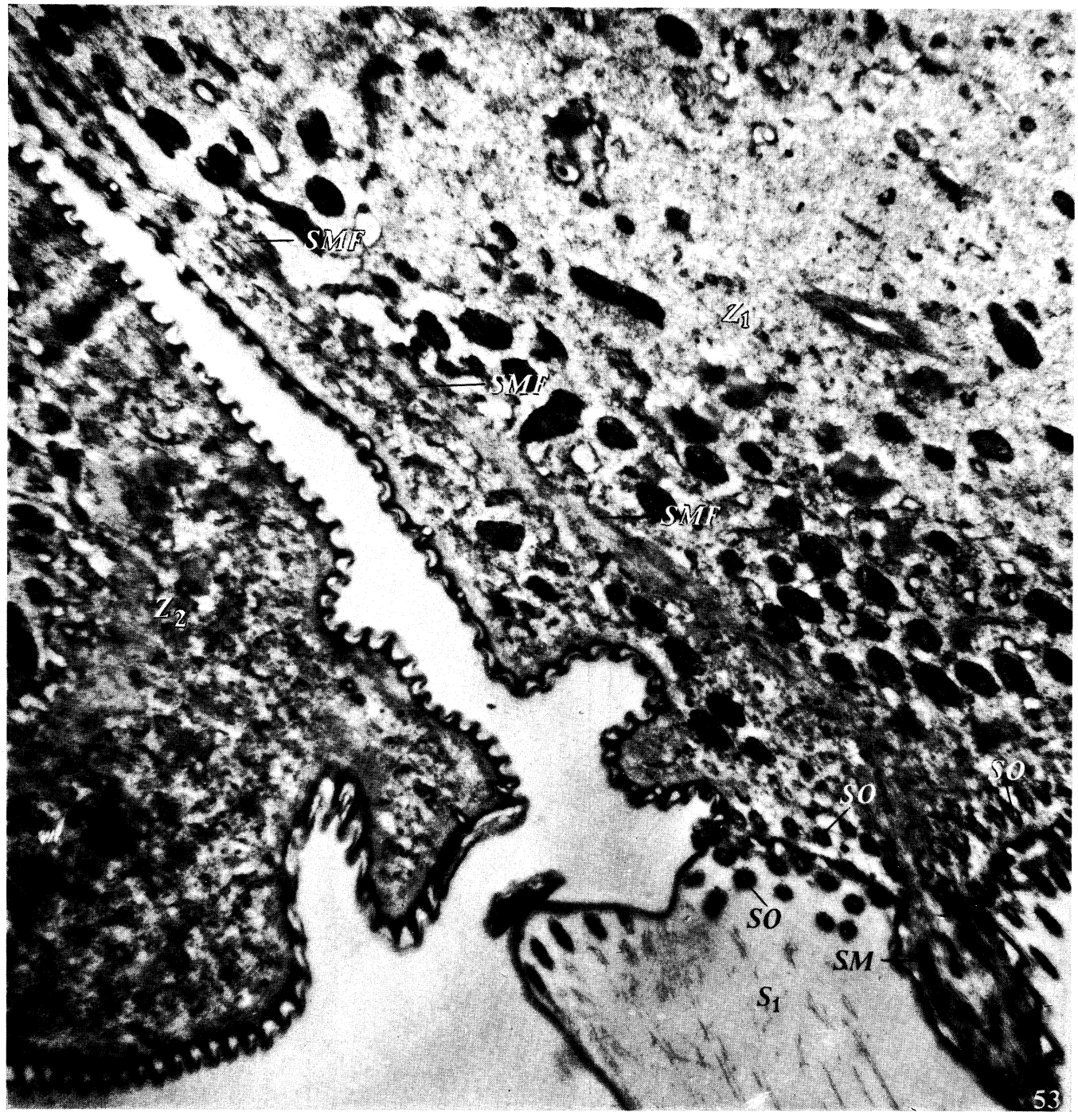


Carchesium polypinum

FIGURE 50. Transverse section of sector of stalk, showing chiefly the annular tubules (× 10000).

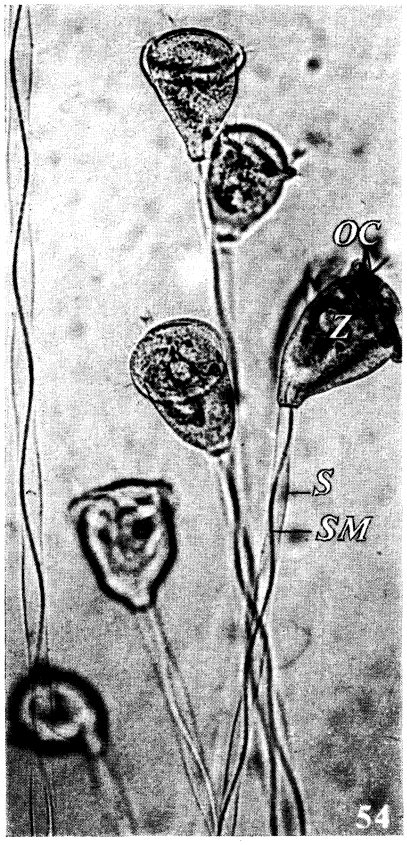
FIGURE 51. Longitudinal view at higher magnification of individual tubules. The striations and fine component fibrils are clearly visible (× 60000).

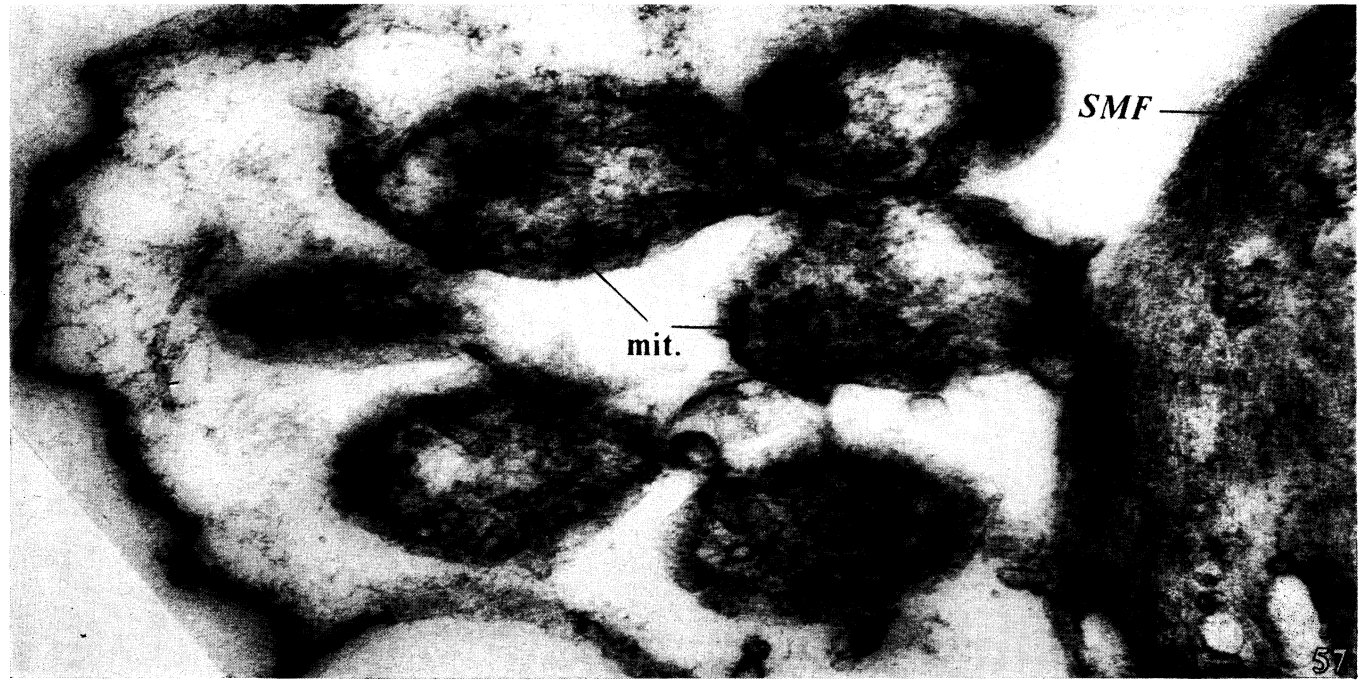
FIGURE 52. Transverse section of stalk core in which can be seen mitochondria (mit.) (× 27200).



Carchesium polypinum

FIGURE 53. Oblique section through part of a zooid Z_1 and its stalk S_1. Another zooid Z_2 shows at the left of the plate. Almost transverse sections of scopula organelles at points *SO* shows them to contain component peripheral fibrils. The spasmoneme *SM* passes into the zooid and its fibrils may be seen to extend to points *SMF* (× 44400).





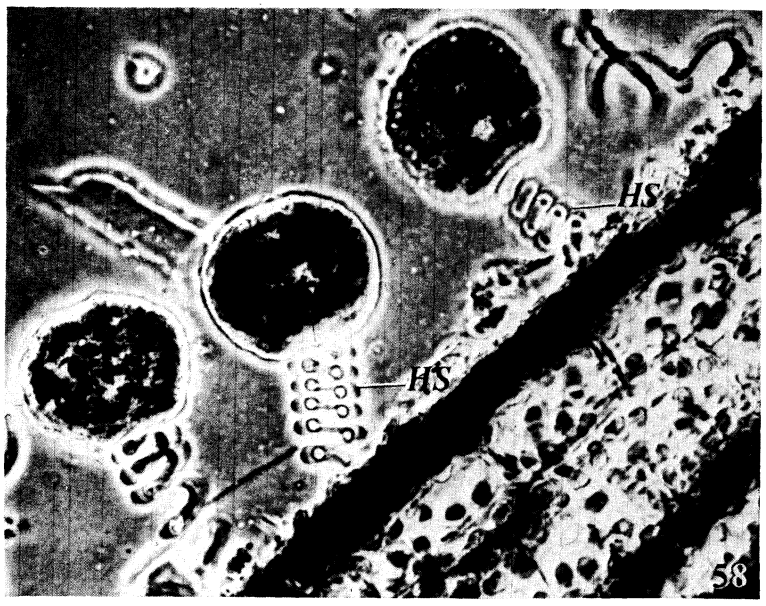
Vorticella campanula

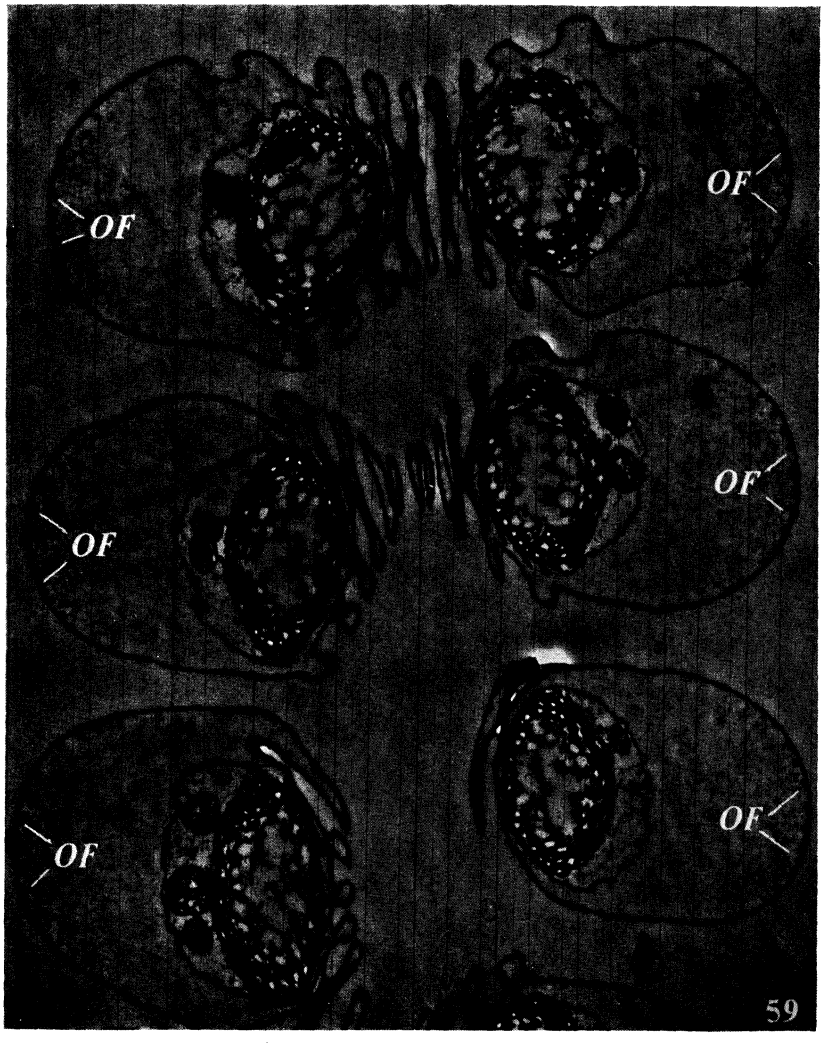
FIGURE 54. Living group of individuals. Note the zooid *Z* with the oral wreath of cilia *OC* and the curved (helical) path of the spasmoneme in the stalk *S* (× 400).

FIGURE 55. Photomicrograph: transverse section of stalk. Spasmoneme *SM* and outer fibres *OF*, cf. figure 56 (× 2750).

FIGURE 56. Electron micrograph low magnification. Transverse section of stalk with poorly fixed spasmoneme *SM*, outer fibres *OF* and matrix fibrils *mf* (× 30000).

FIGURE 57. Oblique section of stalk core with individual fibrils *SMF* of spasmoneme resolved. The stalk canal contains mitochondria (mit.) (× 76400).

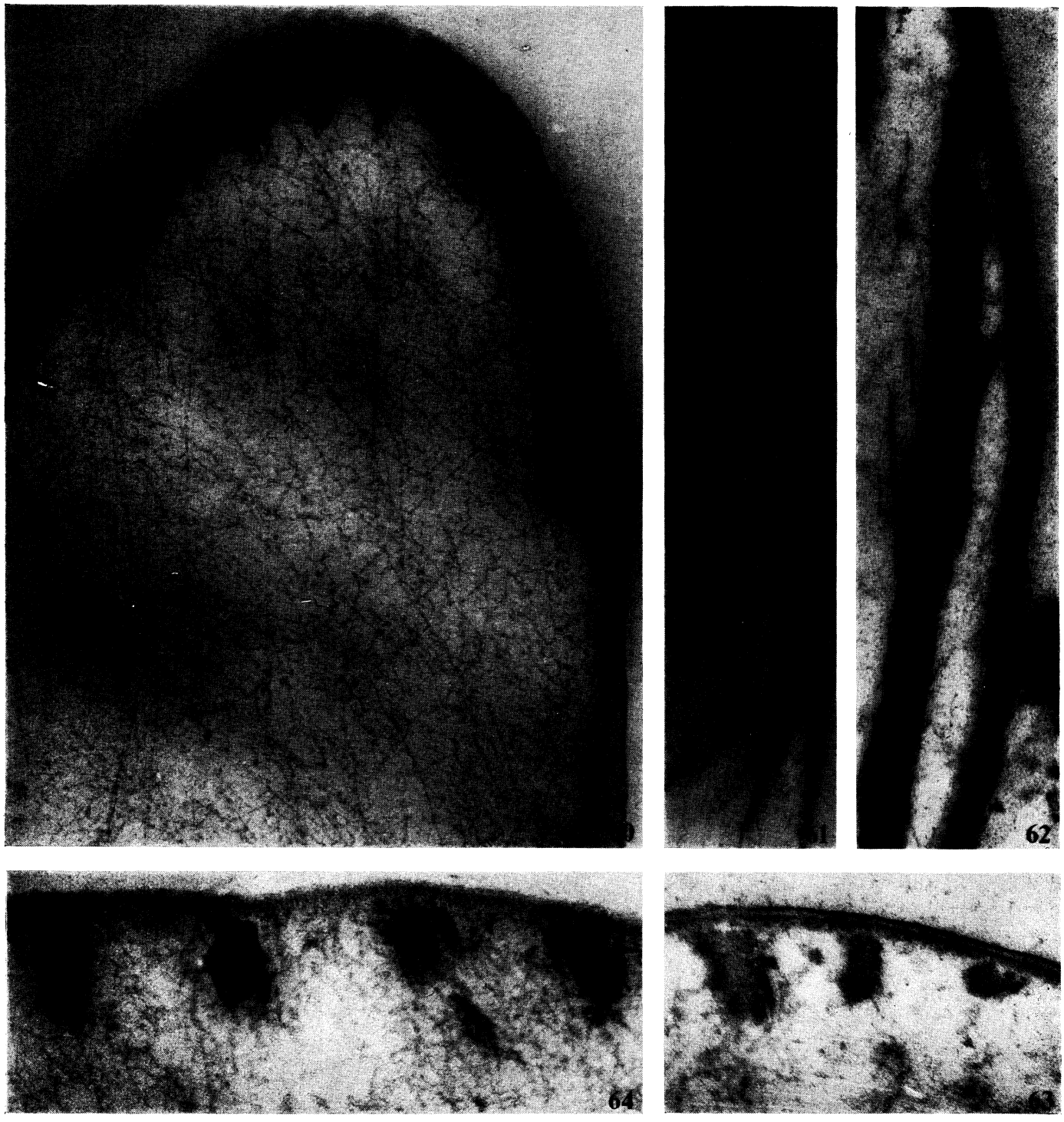




Vorticella campanula

FIGURE 58. Fixed organisms in section to show the helical character of the contracted stalks, *HS* (× 350).

FIGURE 59. Low-power electron micrograph. Longitudinal section of stalk through several turns of contracted helix. The *OF* fibrils are always on the outer rims of the coils (× 6320).



Vorticella campanula

FIGURE 60. Slightly oblique section of outer part of stalk. The *OF* fibres are composite and the matrix fibrils beaded (× 46 200).

FIGURE 61. Oblique section. Composite *OF* fibres (× 36 000).

FIGURE 62. Almost longitudinal section of one *OF* fibre (× 58 000).

FIGURE 63. Almost transverse section of three *OF* fibres. This sections also shows the triple layer character of the stalk wall (× 70 200).

FIGURE 64. Somewhat oblique section of *OF* fibres and of stalk wall which is finely striated (× 108 000).

of 'surface' between one tubule and the next may also exist here as in *Epistylis*, but the nature of the lenticules makes this interpretation less sure. No electron micrographs on the structure of the stalk-zooid junction are yet available, but the longitudinal section shown in the photomicrograph of figure 20, plate 22 gives useful preliminary information. Comparison of figure 20 with figure 21 shows that the section illustrated in the former must pass in part at least through the central cavity. Figure 20 thus indicates that the scopula and its immediately adjacent stalk tubules cover the whole section. The inner tubules are short and terminate about 2·5 μ below the junction of the zooid and stalk.

Genus *Opercularia* Stein

The species examined appeared in a mixed assembly of peritrichs and was probably *O. plicatilis* Stokes (figure 24, plate 24). The detailed structure of the stalks is interesting and differs from that of *Epistylis* and *Campanella* The fixed stalks of *Opercularia* are roughly circular in section with a diameter of 13 μ. The stalk is enclosed in an outer membrane, which appears moderately dense in the electron microscope preparations (figure 28*a*, plate 25). This membrane is not obviously fibrous and is of variable thickness, often about 500 Å. Photomicrographs of transverse and longitudinal sections of the stalk are given in figures 25, 26 and 27, plate 24. Corresponding low-magnification electron micrographs appear in figure 28*a*, plate 25 and figure 29, plate 26. An oblique section of the stalk is shown in figure 30, plate 26. From figure 28*a*, plate 25, and figure 25, plate 24, it seems that the central irregularly shaped canal contains no discernible structures, but is surrounded by an assembly of hundreds of long tubules some 1500 Å in diameter and well separated from each other at distances varying from 0·5 μ to well over 1 μ. Figure 28*b* suggests that the wall of each tubule is fibrous but this may only be true of sections taken moderately close to the scopula.

Some stalks are elliptical in cross-section; in these there is a thicker assembly of tubules in the direction of the major axis of the section than in the perpendicular direction. As in *Campanella*, there is no membrane to separate the axial canal from the peripheral portions of the stalk. The tubules show very marked and narrow transverse striations, each about 60 Å wide, at approximately equal intervals of 400 Å (figure 29, plate 26, and figure 32, plate 28). These striations are also to be seen in figure 30, plate 26. Longitudinally the tubules are composed of still finer fibrils, perhaps not more than 30 Å in diameter. The interstitial space between the tubules also contains large numbers of fine fibrils which appear (figure 31, plate 27) to be very similar to those of the tubules. These free fibrils are also visible in the oblique section of figure 31, which also demonstrates the attachment of some of the fibrils to the stalk membrane. The free fibrils are not striated.

Some useful information has been obtained about the structure of the scopula organelles of *Opercularia* and of the mode of attachment of the stalk tubules, and is contained essentially in figures 33 and 34, plate 29. Figure 33 is of an oblique section through the junction of stalk and zooid and figure 34 gives a longitudinal view. The junction runs along the line *jj* in both illustrations and appears to be formed of a looped double membrane with the *SO* situated at the nodal positions, e.g. SO_1 and SO_2 figure 34. The upper parts of the *SO*—i.e. those parts lying within the zooid and above the line *jj* of figure 33—are clearly structurally homologous with basal bodies (e.g. at *a*, *b*, *c* and *d*). The outer boundary of

the *SO* at *a*, *b*, *c* and *d* is composed of nine pairs of fibrils and these surround some rather ill-defined axial material, which is probably fibrillar. Similar material (*f*, figure 34) below the junction is clearly fibrillar. The nine pairs of fibrils visible in each scopula organelle of figure 33 are not surrounded by a membrane. Below the plane of junction *jj* the structure of the *SO* is different. The nine pairs of fibrils are still apparent but less distinct and almost certainly surrounded by and perhaps attached to a membrane (m_1, m_2, figure 33) which is distinctively scalloped in outline. The internal material also visible in these *SO* appears rather more dispersed. It extends into the stalk tubules for a distance of about $2\,\mu$ and is never visible in transverse sections taken lower down the stalk. Both figures 33 and 34 show that the striated tubules visible at positions marked *t* are attached to the scopula organelles in the manner of a closely fitting sheath. The tubule striations are visible at *tt* in the section of figure 34 as series of linear dots. The lower part of the *SO* of *Opercularia* shows none of the sac-like organization visible in the corresponding structures of *Epistylis*. In this sense the scopula of *Opercularia* appears to be less highly differentiated. Apart from this the stalk tubules are attached to the scopula in precisely the same way.

The contractile stalks

Vorticellidae Ehrenberg, 1838

All the non-contractile stalks examined in this paper contain longitudinally arranged structures partly or wholly filling the cross-section of the stalk. In the contractile stalks of the Vorticellidae there is in addition to peripheral longitudinal structures (fibres or tubules) a new and significant component: an inner core of much finer fibrils enclosed within a membrane. This new stalk organelle occupies and more or less fills a space similar to the central canal observed in many of the Epistylididae.

Genus *Carchesium* Ehrenberg

The organism examined (probably *Carchesium polypinum* Linnaeus) is found in colonies, the overall height of which may reach two to three millimetres. A small part of one colony is illustrated in figure 35, plate 30. The stalks are here extended or relaxed. This should be compared with figure 37 in which the stalks are withdrawn or contracted. Examination of figures 35, 38 and 40, plate 30, confirms the view that each branch stalk of *Carchesium* has its own independent and discontinuous spasmoneme. This discontinuity is consistent with the fact that each zooid and its attendant stalk exercises an independent contraction. In *Zoothamnium* the spasmoneme is continuous and the colony contracts as a whole. Any component stalk of a living colony of *Carchesium* that has lost is zooid is apparently incapable of contraction and remains stiffly extended (figure 36, plate 30). The length of a living zooid of *Carchesium* is about $115\,\mu$ and the diameter of a main stalk about $15\,\mu$. The diameter of the spasmoneme is about $4\,\mu$. Very considerable shrinkage occurs on fixation and rough comparative measurements for the fixed components are: zooid $38\,\mu$; stalk diameter $6\,\mu$; spasmoneme $1{\cdot}8\,\mu$. Various attempts to reduce or eliminate this artifact have failed.

In contraction the spasmoneme takes the shortest possible path within the core or canal of the stalk (figure 37, plate 30). At maximum contraction the spasmoneme is about 80% of the axial length of the canal in which it lies. There seems to be complete correlation

between the possession of a spasmoneme and the ability of the stalk to contract. Further evidence obtained from subsidiary experiments on contractility not described in this paper confirms this view.

During the course of visual study of the colonies many telotrochs of *Carchesium* have also been observed and their development recorded by cine-camera. Three photographs from a series have been selected (figures 41, 42 and 43, plate 31) to show: (*a*) the free-swimming telotroch which possesses both a posterior girdle of cilia and an anterior oral group (figure 41); (*b*) a telotroch shortly after it has settled on the substrate (figure 42); the continuing presence of the posterior girdle, together with a very short stalk, are the features to be noted in this figure; (*c*) a young adult with short stalk and no posterior girdle. No branching has yet occurred. The shape of the zooid is distinctly different and in the form of an inverted rounded cone. In the telotrochs of figures 41 and 42 the anterior (and longer) part is almost cylindrical and the posterior part conical.

The fine structure of the stalks of *Carchesium* will now be described. Figures 44 and 45, plates 32 and 33, show the upper part of a stalk and its junction with a zooid in longitudinal section (cf. figure 39, light microscope). The stalk is bounded by a membrane about 350 Å thick. This appears to be continuous with the outer membrane of the zooid pellicle. The stalk is separated from the zooid by a complex scopula which is differentiated into central and annular portions (figure 44, plate 32). The annular portion is characterized by a double membrane (*MM*) which is pierced by a number of scopula organelles (*SO*). Attached to the posterior parts of the *SO* are tubular fibres (*OF*) to be described below. There are usually several hundred *OF* fibres in each annulus. Although good transverse sections through the scopula have not yet been obtained, it is reasonable to deduce that each scopula contains the same number of *SO*, viz. several hundred, as the stalk annulus contains *OF* fibres. The *SO* structure follows a now rather familiar pattern; it is probable that further study will bring out additional features. The *SO* project into the zooid for a distance of almost 0·5 μ; they are cylindrical and fibrous (figure 46, plate 34). The uppermost portion of the *SO* cylinder is open-ended and the lower part at the junction closed by one double and one single membrane (figure 45, plate 33). Below the junction the cylinder extends about 1 μ before differentiation into the annular *OF* fibres already referred to. The central part of the junction contains no membrane or *SO* and is presumed to be a more or less circular vent through which the fibrous material of the core passes before ultimately joining up with the zooid pellicle (figure 46, plate 34, and figure 44, plate 32). In higher magnification micrographs of the core (figures 47 and 48, plate 35) the individual fibrils are about 160 Å in diameter. These fibrils once inside the zooid splay outwards towards the mid-zooid region of the pellicle, to which they are probably attached (figure 39, plate 30; figure 46, plate 34; and figure 53, plate 38). The scopula membrane is continuous with that of the core and is complex. Where it is part of the structure of the junction two well-stained layers, each double, have been observed (figure 46, plate 34). As a sheath for the core it has only been observed as a single double layer of the sandwich type. The outer and inner parts of the sandwich are about 75 Å thick and the overall thickness of this sheath about 250 Å. In the space between the contracted core and its surrounding membrane mitochondria have been observed (figure 52, plate 37). (See also *Vorticella* below.) In figure 45, plate 33, many mitochondria may be seen in the lower

part of the zooid. Photographs of living material indicate that the core does not extend the full length of the main stalk but terminates some 30 to 40 μ from the substrate. Figure 50, plate 37, is a transverse section of part of a stalk and indicates the tubular character of the longitudinal fibres, of which there are several hundred. All structural components of these annular fibres appear to lie in the peripheral sheath or wall seen in the transverse section of figure 50. The fibre as a whole is some 1400 Å in diameter and is transversely striated at intervals ranging from 300 to 700 Å (figure 49, plate 36; and figure 51, plate 37). The wall of each *OF* fibre is composed of longitudinally arranged fine fibrils each about 60 Å in diameter. Similar fibrils are also randomly dispersed throughout the annular matrix. The period of the striation on the fibres averages something like 400 Å and the width of the striation itself is about 120 Å. It is difficult from such micrographs as figure 49 to estimate the length of the *OF* fibres. If the fibres are not straight parts may lie out of the plane of section and thus give the appearance of breaks seen in the figure.

Genus *Zoothamnium* Bory

Apart from the well-known continuity of the spasmoneme, no important differences of either structure or organization have been noticed between the stalks of this genus and that of *Carchesium*.

Genus *Vorticella* Linnaeus

The species examined was probably *V. campanula* Ehrbg. Although members of this genus often occur in groups or stocks they are not colonial and each animal is independently attached to a substrate by the distal end of its stalk. The zooid is well-shaped with the widest portion uppermost and is attached at its apex to the proximal end of the stalk (figure 54, plate 39). In the adult organism ciliation is confined to the adoral zone and the buccal cavity. The posterior girdle of cilia is characteristic of developing telotrochs and not of adult animals. The spasmoneme of *Vorticella* stalks is visible in the light microscope (figures 54, 55) and follows a drawn-out helical path within the outer membrane of the stalk. The stalk length is very variable and may be as much as 4 mm or as little as 0·5 mm.

Although reasonable quality has been attained in the fixation of the zooids, good fixation of the spasmonemes has eluded us. From figure 57, plate 39, it is inferred that the core is composed of thin fibrils about 50 Å in diameter. This illustration also demonstrates the presence of mitochondria within the core, the outer membrane of which is also visible at the left of the illustration. The annulus contains many short, unattached beaded fibrils; the beads are about 80 Å in diameter and the threads that join them together rather less than half this size. In *Carchesium* there is a uniform annular distribution of several hundred longitudinally arranged striated tubular fibres (*OF*). In *Vorticella* there is no such uniform distribution of *OF* fibres. Instead there is a limited number (usually between 15 and 20) of substantial and asymmetrically arranged fibres attached to the inside of the external membrane of the stalk (figures 55 and 56, plate 39; figures 60, 61 and 64, plate 41). The *OF* fibres are not single but composite, of irregular outline (e.g. figures 62 and 63, plate 41) and roughly 1000 Å thick. They originate, like the corresponding ones of other members of the Peritrichida, in the scopula. The fibres are confined entirely to one side of the stalk membrane in a crescent-like array and are about 0·35 μ apart in fixed-and-embedded

specimens. The spasmoneme always lies on the opposite side of the stalk (figures 55 and 56, plate 39).

One further observation should be recorded which has a bearing on the explanation of the well-known coiling of the stalk of *Vorticella* when in contractile spasm. If longitudinal sections of the coiled stalk (figure 58, plate 40) are examined in the electron microscope of the *OF* fibres always lie on the *outside* rim of the coil (figure 59, plate 40).

The *OF* fibrils of the *Vorticella* stalk are far fewer in number than those of other peritrichs examined. The number of longitudinal sections so far obtained that display stalk-to-zooid junctions is limited. It is, however, clear that the feature observed in other organisms of this Order is also shown by *Vorticella*. The *OF* fibres start from cylindrical bodies about 3300 Å long and 660 Å in diameter at the base of the zooid. The *OF* fibres penetrate a double membrane before passing into the stalk.

Although observations are incomplete, there is evidence to show that the material of the core does not terminate abruptly at the stalk-zooid junction. On the contrary it appears to continue into the zooid, as in *Carchesium*, in the form of a thin shell or fan which ultimately terminates very close to the zooid pellicle at a level roughly half way between mouth and junction.

Cytochemical observations

Some limited cytochemical observations have given useful indications about the biochemical nature of the chief portions of the non-contractile stalks of *Campanella* and the contractile ones of *Carchesium*. For this purpose two fixatives have been used: (*a*) a modified Carnoy consisting of 5% acetic acid and 60% ethyl alcohol, and (*b*) a 10% formaldehyde solution in 0·75% sodium chloride. Dehydration procedure consisted of the usual sequence of graded alcohols; specimens were embedded in paraffin wax and observations with various reagents made on 4 μ sections.

Tests for protein

The dinitrofluorobenzene test stained the annulus of the *Carchesium* stalk strongly and that of *Campanella* moderately. The presence of tyrosine, histidine, $-NH_2$ or $-SH_2$ groups may thus be inferred in this part of the stalk. All parts of the *Carchesium* stalk were stained by fast green used at a pH of 3·7 (Alfert's test); *Campanella* stalks were not stained at this pH although there was some coloration at pH 7·1. At this value the *Carchesium* spasmoneme stained more strongly than the annulus. This stalk was not stained with fast green at the higher pH value of 8, and none appeared after either deamination or after extraction with trichloroacetic acid. Tests for the presence of arginine were made on formalin-fixed stalks of *Carchesium* and of *Campanella*, but positive results were not obtained.

The ferricyanide procedure devised by Chèvremont & Frédéric (1943) was used in a search for the presence of S—S and —SH groups. *Campanella* stalks gave no reaction. The *Carchesium* spasmoneme stained strongly and the outer part of the stalk moderately. From the results of appropriate blocking and reduction tests it has been concluded that both the spasmoneme and the annular fibres contain a large proportion of S—S and —SH groups. Tests for nucleic acids using methyl green and pyronin did not give positive results. No staining was observed when the periodic acid-Schiff (*PAS*) reagent was used in either aqueous or alcoholic solution to test for the presence of polysaccharide in the stalks.

At pH 4·2 toluidine blue stains the outer part of both *Carchesium* and *Campanella* stalks blue, and thus indicates the presence of basiphilia. At the lower pH values of 1 and 2 the annulus of the *Carchesium* stalk was stained green; this is a consequence of the presence of strongly acid groups which were shown not to be polyphosphates. Tests for the presence of free lipids, free phospholipids and oil droplets, using Sudan black, oil red O and acid haematein, did not give positive results. Treatment with trichloroacetic acid indicates the presence of some very tightly bound phospholipid in the outer part of the *Carchesium* stalk. No free fatty material could be detected.

To summarize these observations, it seems that both contractile and non-contractile stalks are essentially protein in nature and both contain tyrosine, histidine, $—NH_2$ or $—SH_2$ groups. The *Carchesium* spasmoneme contains a higher proportion of S—S and —SH groups than does the annulus. The protein of the *Campanella* stalk probably belongs to the keratin group and is similar to that of the *OF* fibres of *Carchesium*. The strongly acidic groups found in the outer part of the *Carchesium* stalks are not explained by the presence of polyphosphates.

Discussion

In this paper the structural and, to a lesser degree, cytochemical characteristics of a number of peritrich stalks have been investigated. We shall summarize here some of the main features that have been elucidated or confirmed and discuss more thoroughly aspects of the work considered to be important.

Morphology of the spasmoneme

The properties of the stalks as well as other distinctive characteristics divide the organisms investigated into the two well-known families Vorticellidae and Epistylididae. The stalks of the Vorticellidae contract; those of the Epistylididae do not. Morphologically the stalks of the contractilia are distinguished by the presence of an internal spasmoneme of very fine fibrils. The spasmoneme is enclosed within a membrane which is probably a continuation of that which partially separates the stalk from the zooid and which is associated with the scopula. Diagrammatic representations of the mid-region of the stalks have been drawn approximately to scale in figures 65*a* to *e*. Similar drawings of the scopula regions, with the exception of that of *Campanella*, are given in figures 66*a* to *d*.

It is impossible in discussion of the stalks to avoid entirely the properties or structures of the zooids. For example, in the Vorticellidae the contractile spasmoneme does not terminate at the junction between stalk and zooid. It continues into the zooid through a gap in the scopula; the fibrils diverge into a cone-like array and eventually terminate either on the zooid pellicle or on its closely associated ectoplasmic layer. This has been illustrated in figure 46, plate 34, and figure 53, plate 38, for *Carchesium*. These and other micrographs are the basis for the more comprehensive diagrammatic interpretation of figures 66*c* and *d*. Observation of living organisms has made it clear that when a stalk contracts the zooid also undergoes a marked axial shortening; this is accompanied by withdrawal of the oral cilia into the oral cavity. A headless stalk is normally fully extended (*Carchesium*, figure 36, plate 30). This does not necessarily mean that the stimulus to contraction can be made effectual only through the zooid; it may be that the end of the headless stalk becomes sealed and thus impervious to an external stimulus.

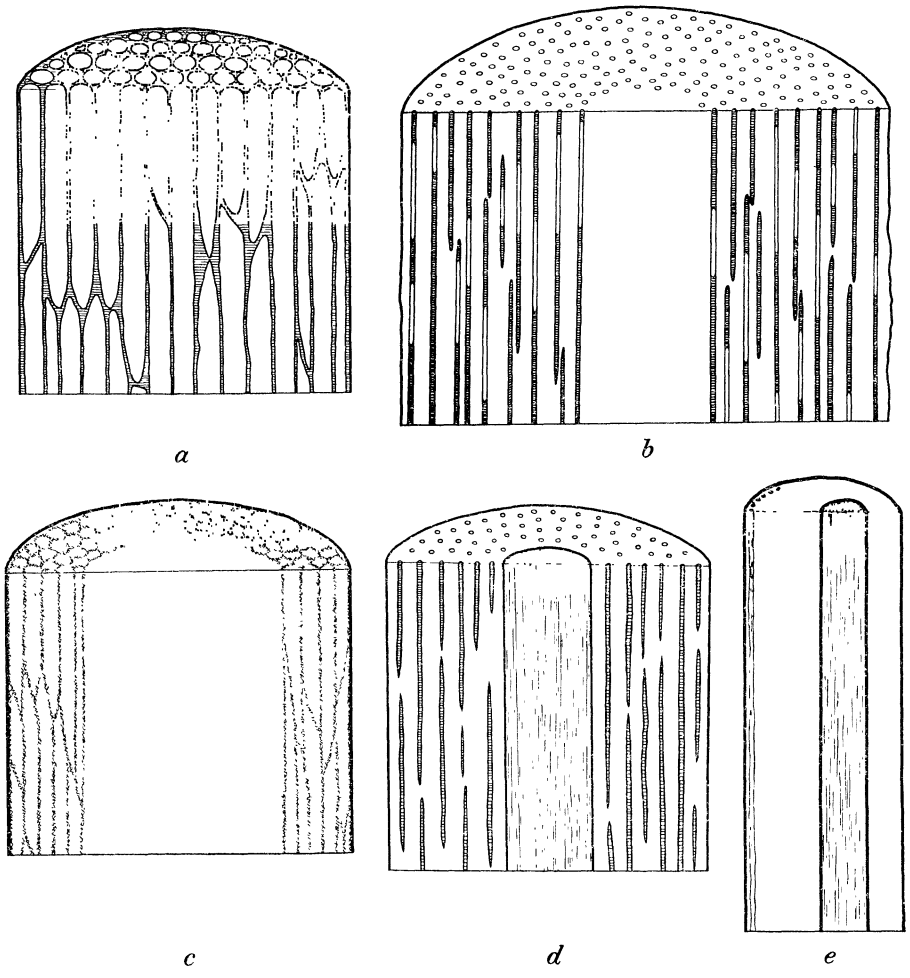


FIGURE 65. View of mid-regions of non-contractile and contractile stalks; to scale. (*a*) *Epistylis*, (*b*) *Opercularia*, (*c*) *Campanella*, (*d*) *Carchesium*, (*e*) *Vorticella*.

The fact that the spasmoneme in Vorticellidae forms part of the zooid and the stalk is circumstantial evidence in favour of the simultaneous retraction of the one and contraction of the other. The property of zooid retraction—a convenient word for something easy to see and difficult to measure—is not however confined to the Vorticelliade. It can be seen quite easily, for example, in *Campanella* and in *Epistylis*. The zooids of *Epistylis* contain fibrils that have their origin in the scopula and are directed outwards towards the pellicle and its ectoplasmic layer (figure 16, plate 21, and figure 66*a*). While in this family there is no proof that these fibrils are the instrument of retraction, it seems very reasonable to infer this function from the facts available. It is interesting to observe that the motile telotrochs also exhibit a similar contractile character; as we shall see, this is only one of the many reasons pointing to the importance of further investigation of this developmental phase in the life cycle of the Sessilina.

The scopula

The junction of zooid and stalk is an important feature in both Vorticellidae and Epistylididae. Functionally, it is the region of the animal from which the stalk begins to grow when the motile telotroch settles on a substrate. Structurally, it contains the scopula

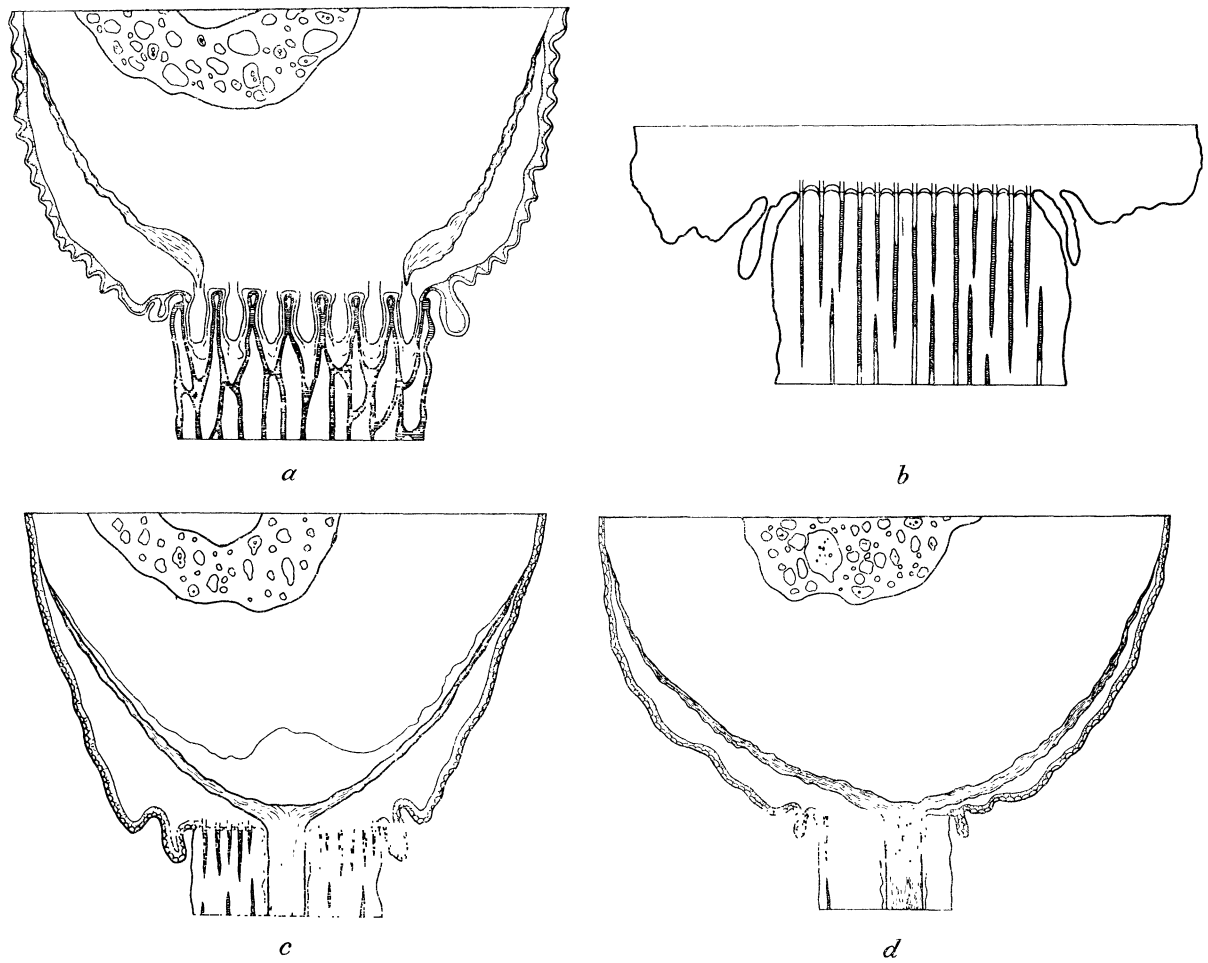


FIGURE 66. The scopula and associated regions in: (*a*) *Epistylis*, (*b*) *Opercularia*, (*c*) *Carchesium* and (*d*) *Vorticella*.

organelles (*SO*) which exhibit marked similarities with the basal bodies (*BB*) of the normal cilia of protozoa and those of ciliated tissues of higher animals. Some similarity also exists between the structures of both these types of organelle and those of centrioles (Bessis *et al.* 1958; Burgos & Fawcett 1955, 1956). Longitudinally the scopula extends over a narrow region and this has so far prevented the preparation of good transverse sections passing exactly through the junction. For this reason the evidence on which the following conclusions are based, although extensive, is incomplete.

We shall first make some broad comparisons between the scopulas of the Epistylididae and Vorticellidae. The genus *Epistylis* is the only one yet examined in which the scopula with its numerous organelles (*SO*) appears to cover the whole cross-section of the junction. The *SO* are not, however, uniformly alike over the whole section (figure 15, plate 20, and figure 66*a*), nor are their fibrous attachments within the zooid and within the stalk the same (figure 12, plate 18).

In *Campanella* optical evidence (figure 20, plate 22) suggests that the stalk canal to be seen in figures 18, 21 and 22, begins a short distance ($\sim 2{\cdot}5\,\mu$) below the scopula. If this is correct, it seems likely that the scopula itself covers the whole cross-section of the

junction, as in *Epistylis*. However, the stalk is formed during the course of development of the adult organism. In consequence the position of the stalk adjacent to the scopula is the last to be secreted. It is possible in these circumstances that the scopula in the earlier stages of stalk formation begins as a ring-like assembly of organelles which are later redistributed uniformly over the junction.

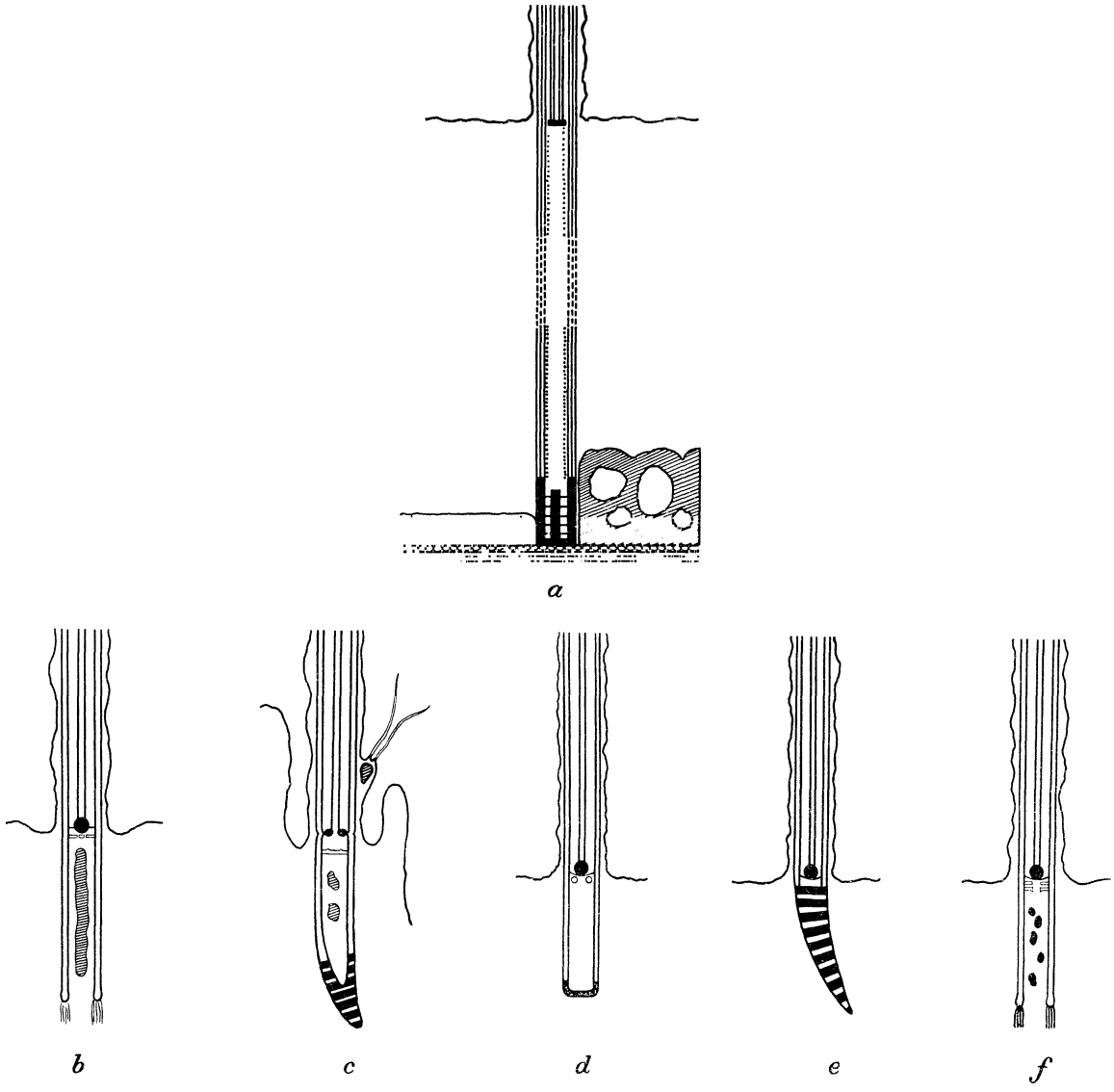


FIGURE 67. Diagrammatic illustrations of basal bodies from a variety of plant and animal sources. (*a*) *Zamia*, (*b*) *Tetrahymena*, (*c*) *Plagiomonas*, (*d*) *Stentor*, (*e*) *Gallus*, (*f*) *Vorticella*.

The results for *Opercularia* (figure 27, plate 24) indicate that the canal is continuous throughout the whole length of the stalk. It is, however, very irregular in section (e.g. figure 25, plate 24) and narrows most noticeably as it approaches the scopula. It is therefore possible that the *SO* cover the junction plane and that the canal is a result of closer packing of stalk tubules over the greater part of the stalk length.

In the Vorticellidae the scopula is peripheral. In *Carchesium* it has the form of an annulus of several hundred uniformly distributed *SO*. In *Vorticella* the number of *SO* is small

(apparently variable) and about 15 to 20. The distribution of stalk fibres suggests that the *SO* are arranged in a crescent adjacent to part of the outer membranes of the stalk.

Two important matters relating to the scopula remain for discussion: first, an examination of the structural basis underlying the broad similarity between basal bodies (*BB*) of ordinary cilia and the scopula organelles, *SO*. Structural similarities are also known to exist between both these types of organelle and those of centrioles. (leucocytes (Bessis *et al.* 1958); spermatozoa (Burgos & Fawcett 1955, 1956)). For purpose of comparison the detailed structures of *BB* from a variety of plant and animal sources have been drawn in longitudinal section in figures 67*a*, to *f*. These are to be compared with the scopula organelles similarly presented in figures 68*a* to *d*.

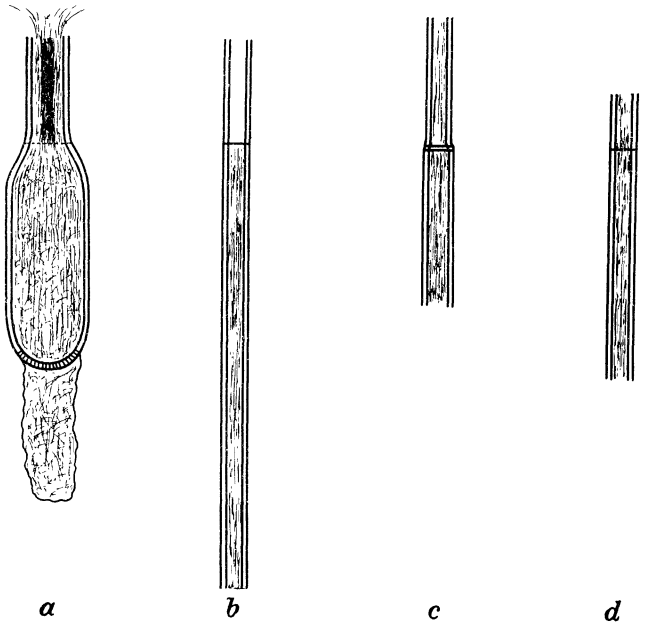


FIGURE 68. Diagrammatic representation of scopular organelles from contractile and non-contractile peritrich stalks. (*a*) *Epistylis*, (*b*) *Opercularia*, (*c*) *Carchesium*, (*d*) *Vorticella*.

It would be unwise to draw too strongly on the structural similarities of the scopula organelles to the basal bodies of cilia, but attention is drawn to the following features of each class of structure.

(i) Both *SO* and *BB* are essentially cylindrical structures of similar length and diameter. *BB* are usually bounded by nine fibres. Some *SO* are similarly constructed (*Opercularia*, *Carchesium*, *Vorticella*) but not all (*Epistylis*).

(ii) Both *BB* and *SO* are polar. *BB* have anterior cilia and posterior roots. Some *SO* have bundles of anterior fibrils attached (e.g. *Epistylis*); all have posterior fibres (viz. the *OF* stalk fibres). The attachment may be precise and continuous with the *SO* structure (*Vorticella*) or more complex (*Epistylis*).

(iii) The *OF* fibres of peritrich stalks seem most directly comparable with the well-known roots attached to the *BB* of many cilia. In *Opercularia* a structure apparently exactly the same as that of a normal cilium extends into the stalk. At first sight this would seem to be in agreement with Fauré-Fremiet's (1905) contention that the scopula is equivalent to a collection of cilia facing outwards into the stalk. This is the view adopted by

Rouiller *et al.* in their 1956 paper. The higher resolution electron micrographs presented here show, however, that the situation is more complex. First of all the structure analogous to that of a normal cilium extends only a short distance into the stalk (e.g. figure 34, plate 29). Secondly, the *OF* fibres have been shown in both *Opercularia* and *Epistylis* to be attached to the emergent *SO* in the form of a striated sheath or tubule (figure 5, plate 14, and figure 34, plate 29). It is the characteristic striations of the *OF* fibres that make them seem more comparable with ciliary roots (cf. Fawcett & Porter 1954) than with normal external cilia. In view of the greater complexity of *SO*, such comparisons are perhaps in any case not very profitable.

(iv) The bundles of anterior fibrils attached to some *SO* (e.g. *Epistylis*) have no precise form and do not resemble cilia proper in any obvious way. These fibrils are quite distinct from the nine peripheral fibrils characteristic of cilia proper and of many *BB* and *SO* (see (i) above). It is inferred that the anterior fibrils referred to here are associated with the function of zooid retraction in the Epistylididae.

(v) Neither ciliary roots nor posterior *OF* fibres originating at or near *SO* are contractile. Some are striated.

(vi) The only useful function of *OF* fibres would seem to be to give strength and some rigidity to the stalks.

A satisfactory analysis of the origin and development of the *SO* cannot be provided at present. For this to be attempted much more information is needed about the development of the adult organism from the telotroch. The problem of the origin of the *SO* is particularly interesting. In the telotroch stage there is no obvious sign of scopula development. This begins only when the organism becomes sessile and the almost equatorial ciliary girdle (e.g. figure 17 *a*, plate 22, *Campanella*) starts to regress. The important question is whether there is more than a temporal connexion between these two events. Scopula organelles could arise *de novo* or develop from pre-existing centres by some mechanism at present unknown. Alternatively, they could develop from the basically similar *BB* of the ciliary girdle. Such a process would necessitate migration of the *BB* over something like half the axial length of the organism. In its simplest form this rather unlikely hypothesis also requires the ciliary girdle to have the correct number of *BB* for the formation of the scopula. Any substantial difference between these two numbers could only be accounted for by rather artificial *ad hoc* reasoning. The problem of the origin of the scopula therefore remains unsolved; but the probabilities are in favour of its development from pre-existing centres. It is clear that further and more detailed study of telotrochs would be valuable.

Important considerations of a rather different kind relate to the precise function of a scopula organelle. Earlier in this discussion the broad similarity between *SO* and *BB* of normal cilia was noted. Almost certainly the questions now to be asked will be solved more readily by a study of *BB* rather than *SO*; but the questions and their answers are equally significant in both fields of investigation.

First of all it seems reasonably certain that *BB* are organizers. A number of flagellated organisms are known to regenerate their flagella when these motile structures have been artificially removed (*Peranema*, Chen (1950); *Chlamydomonas*, Papazian, personal communication). There seems no reason to doubt that a similar function may be ascribed to the *SO*. What is quite unknown at present is whether the proteins associated with *BB* (cilia and

their roots) and *SO* (*OF* stalk fibres and zooid fibrils) are synthesized at these sites or elsewhere in the cell. The problems of *SO* and *BB* are essentially the very large ones of protein synthesis and morphogenesis. The following brief remarks and queries may be of value in placing the immediate subject in its wider perspective.

(*a*) The smallness of the *SO* and *BB* makes it unlikely that an answer to the question of the site of ciliary and stalk protein synthesis will be got from cytochemical experiments.

(*b*) *SO* and *BB* form a precise and recognizable pattern in the organisms with which they are associated. In an animal or cell where anatomy is at present assessed in terms of fine structure virtually at the macromolecular level what physico-chemical principles determine the precision of position and overall pattern of these organelles?

(*c*) Given the facts of position, there are also those of multiplicity of function and organization. *BB* and *SO* are adequately endowed biochemically to organize and perhaps to synthesize at least two proteins.

(*d*) As in all problems of morphogenesis, the processes under discussion are active for a limited period of time during the course of normal development or artificially induced regeneration.

(*e*) It is natural to relate the processes of synthesis (and possibly those of organization) of the fibrous structures of *SO* and *BB* to nuclear intervention and control; although no direct proof of this has yet been obtained, fragments of nucleus are known to be necessary to regeneration in *Stentor* (Tartar 1961) and *Bursaria* (Schmähl 1926).

The association of the scopula organelles and of basal bodies in particular with the genesis of fibrous structures is a natural consequence of known biological facts. The outgrowth of the stalk from the scopula during maturation of the organism provides a useful body of evidence for peritrichs. And the full development of oral structures in ciliates from kinetosomal anlage during division provides similar data for cilia.

It is therefore natural but possibly naïve to seek for some similar organizers for the spasmonemes of Vorticellidae stalks. Our evidence for the continuity of the spasmoneme through a gap in the scopula and into the zooid is based on longitudinal sections only and therefore incomplete. So far there has never been any hint of scopula involvement in the spasmoneme of the adult. Further studies of post-telotroch development would be most valuable in advancing our knowledge of this question. For the present the possibility that the scopula initially covers the whole function of the contractilia cannot be excluded, since we do not know the structure of the junction in development.

It is necessary to point out that to seek such structurally visible organizers may well be a sterile enterprise. The non-contractile stalk structures and scopula organelles are clearly associated; but this is no reason for assuming that the spasmoneme has a similar structural origin.

Stalk structure and classification of the Peritrichida

It is not the purpose of this paper to enter into this problem in any detail, but rather to point out the value of the additional information provided by electron microscope investigation. The structures of the middle regions of all stalks examined have been drawn approximately to scale in figures 65*a* to *e* and summarize many of the facts set out earlier in the paper. The genus *Epistylis* appears to be alone in having a stalk completely filled with striated tubular fibres. *Opercularia* also has striated tubules, but they do not fill the whole cross-section. This genus possesses a rather irregular central core. Neither in this

genus nor in that of *Campanella*, in which the core is better developed, is there a membrane between it and the peripheral fibres. These fibres are in *Campanella* quite different in general structure from those of *Epistylis* and *Opercularia* and have not been observed in this form in any of the other organisms examined.

In the Vorticillidae the contractile core is fully developed and separated from the annulus by a membrane. The outer fibres of *Carchesium* and *Zoothamnium* are similar to each other and also striated like those in the genus *Epistylis*. The canal and spasmoneme of *Vorticella* are not central in the contracted state and the *OF* fibres are few and appear in transverse sections to lie diametrically opposite to the canal and its spasmoneme.

We are indebted to Dr John O. Corliss of the University of Illinois for helpful discussion; to Mr H. R. Munden for supervision and maintenance of the electron microscopes; to Dr J. Chayen for advice on cytochemical procedures; to Mr J. P. G. Williams for cine-photographs and some preliminary physiological observations; to Miss Claire B. Campbell for general assistance and in particular for many of the photomicrographs; and to Miss F. Ticehurst and Mr R. L. Jones for the preparation of photographic prints from the original plates. Dr R. Barton has kindly allowed us to use the drawing of the basal body from the spermatozoon of *Zamia* used in figure 67*a*. We are specially grateful to the Wellcome Trust for making available to us through the Medical Research Council an RCA electron microscope; and to the Rockefeller Foundation for continued support.

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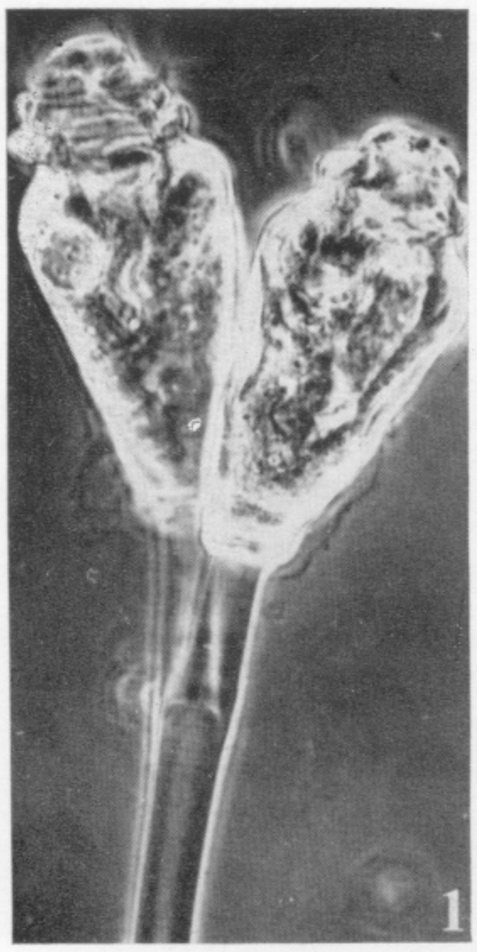
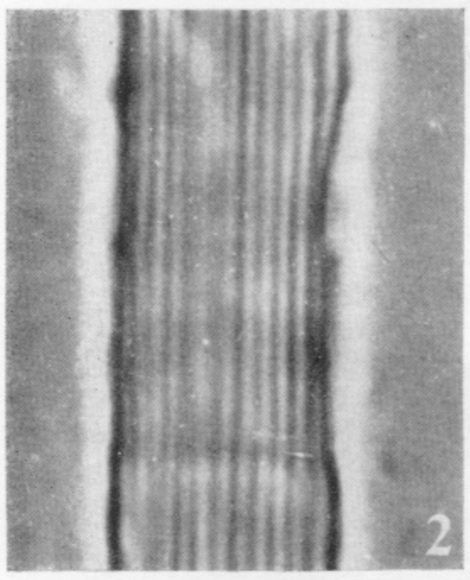
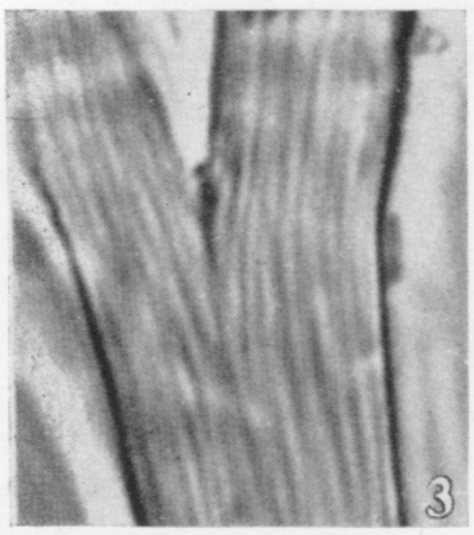
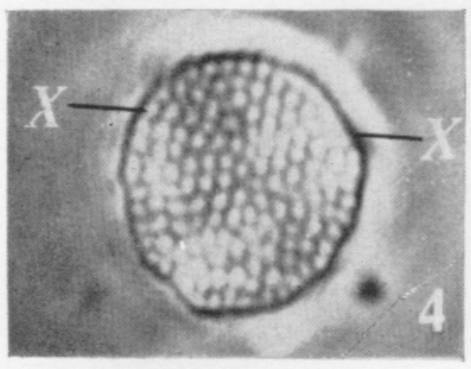
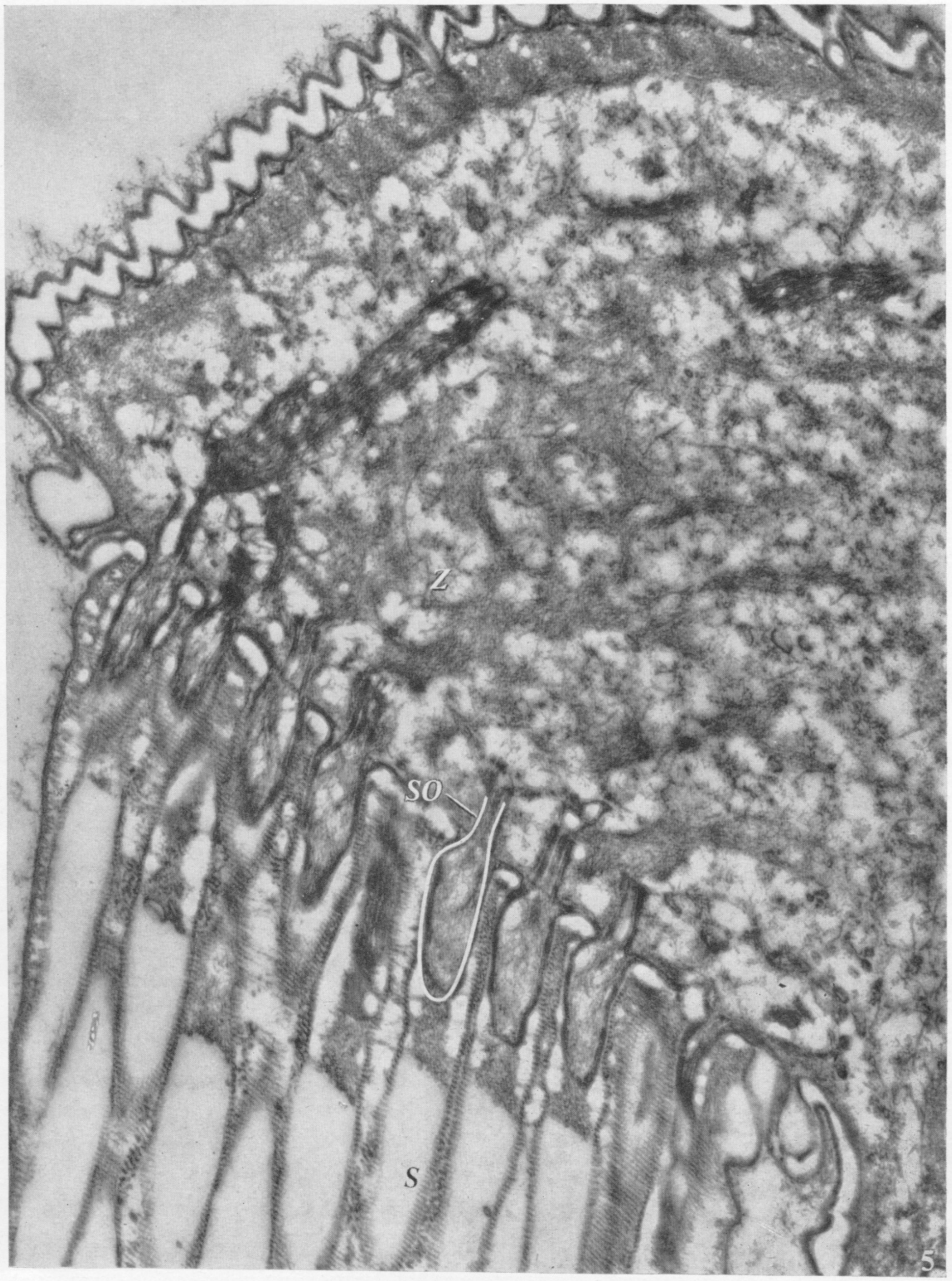










Epistylis plicatilis

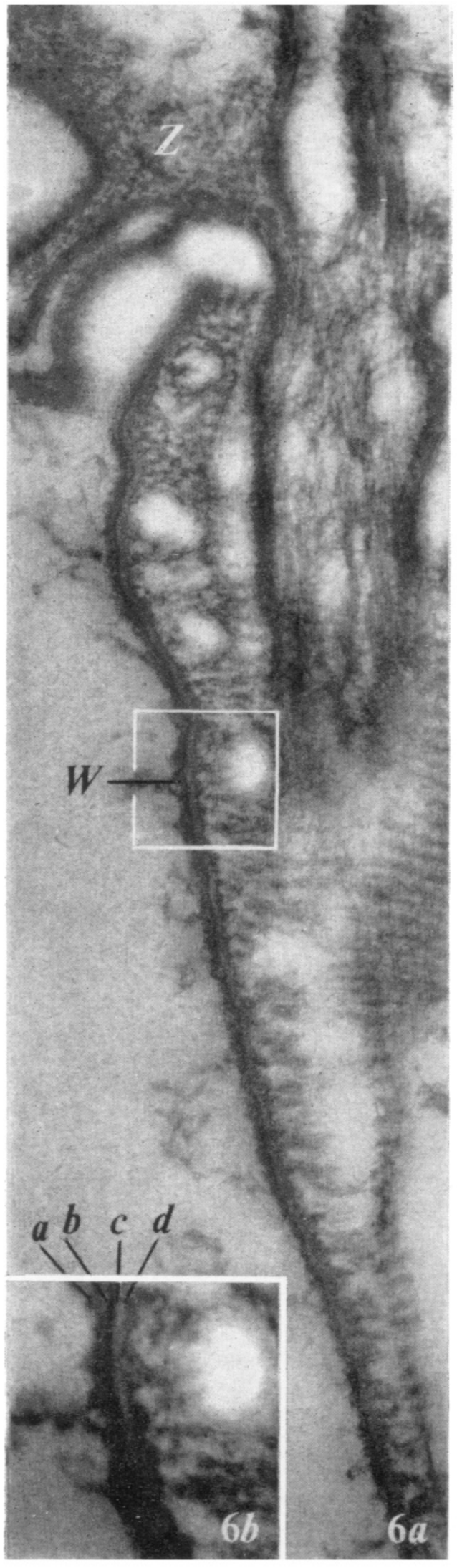
FIGURE 1. Small portion of living colony of *Epistylis plicatilis*: phase contrast (× 250).

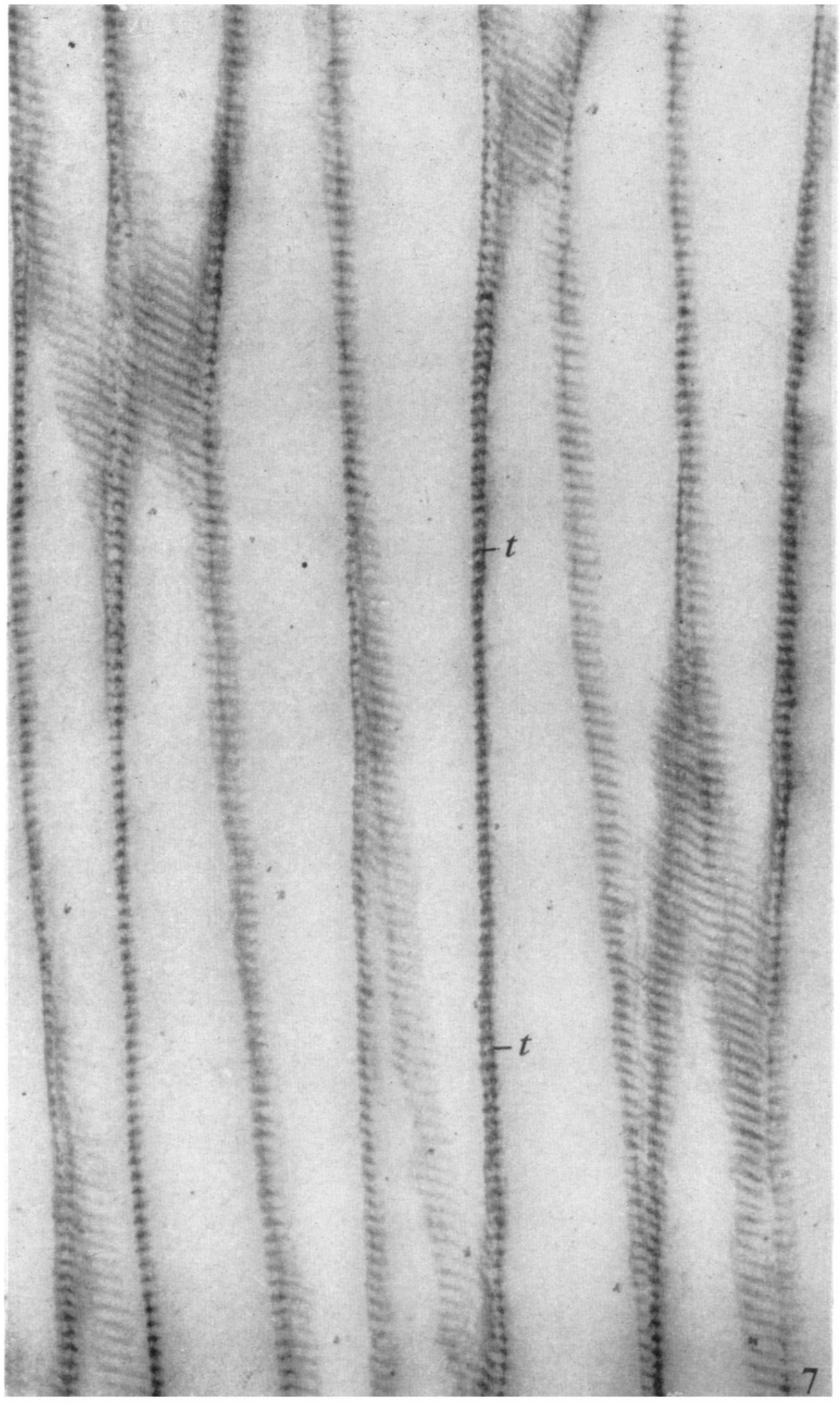
FIGURE 2. Photomicrograph of longitudinal section of single stalk indicating its probable tubular character confirmed in later figures: phase contrast (× 2750).

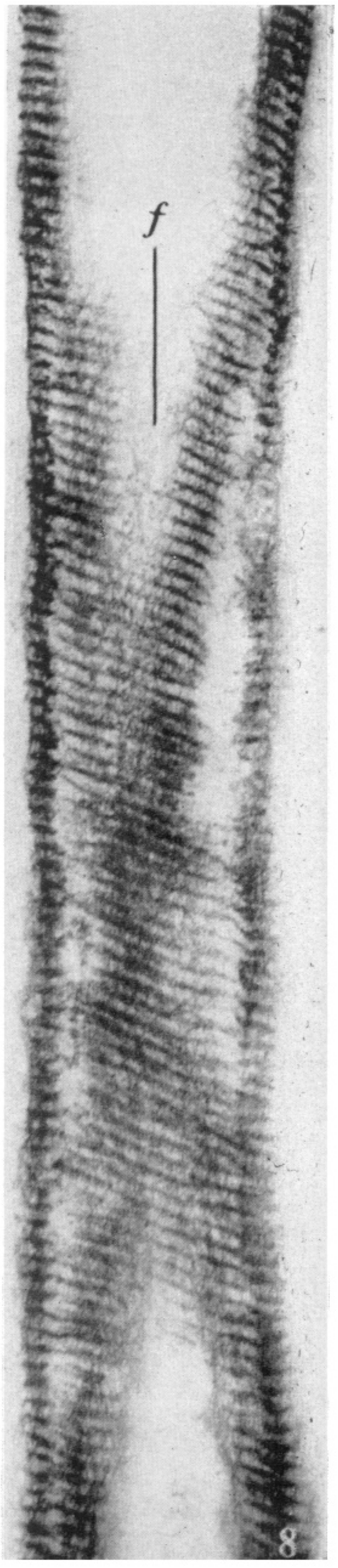
FIGURE 3. Photomicrograph of longitudinal section of bifurcated stalk as shown in figure 1. This illustrates the general continuity of the longitudinal structures through the junction: phase contrast (× 2750).

FIGURE 4. Photomicrograph of transverse section of single stalk. This confirms the tubular nature of the whole stalk inferred from figures 2 and 3: phase contrast (× 2750).

FIGURE 5. Low magnification electron micrograph of the junction between stalk *S* and zooid *Z*. Note the complex scopula organelles *SO* and their means of attachment to the stalk tubules. Comparison with figure 4 shows that this section was cut close to the surface, e.g. along *XX*, (× 21 520).







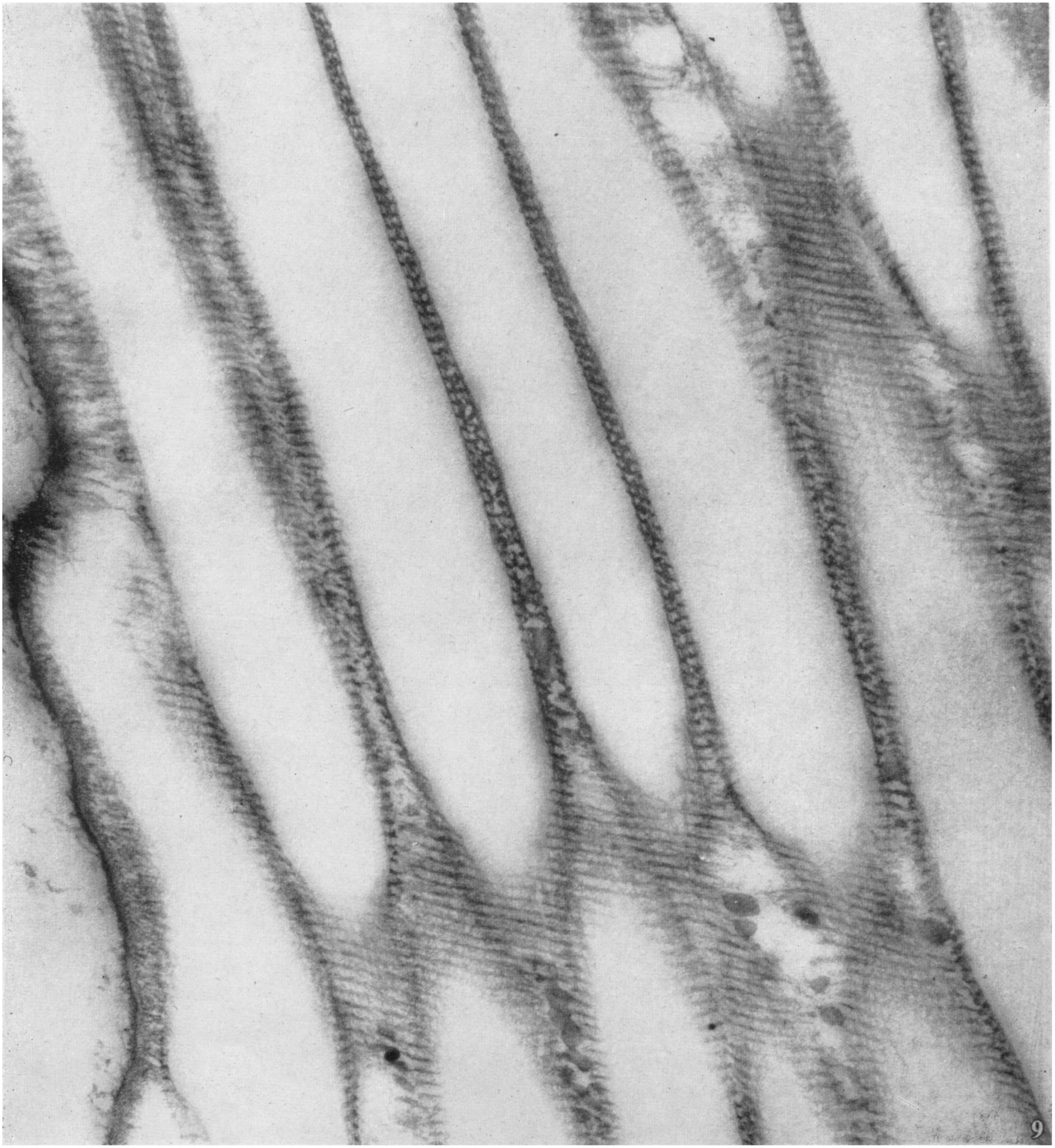
Epistylis plicatilis

FIGURE 6*a*. The stalk wall *W* and its attachment to the zooid *Z* (× 61 200).

FIGURE 6*b*. Enlargement of portion of wall *W* marked in figure 6*a*. The four layers *a*, *b*, *c*, *d* can be more clearly distinguished (× 122 400).

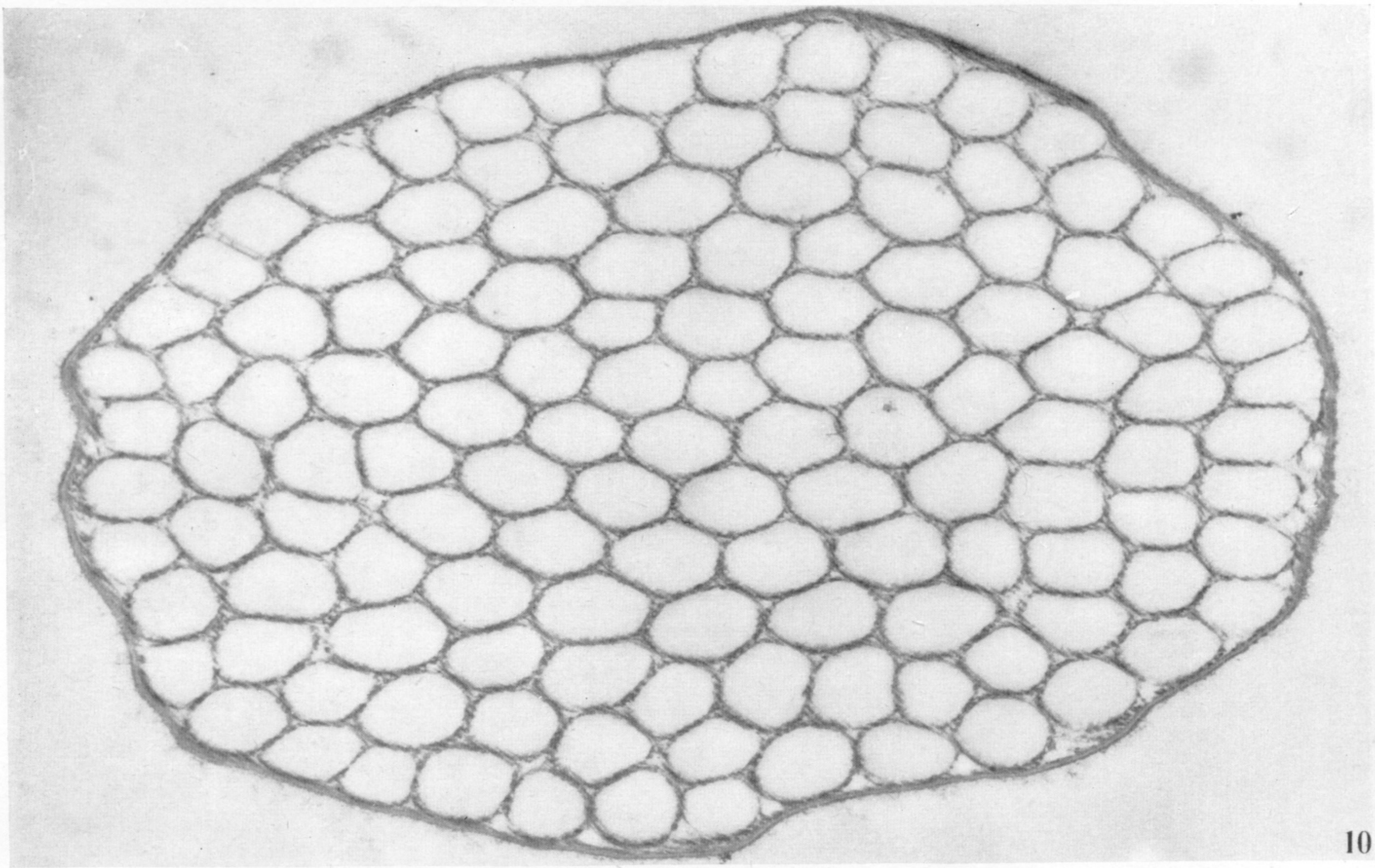
FIGURE 7. Low magnification electron micrograph of longitudinal section of stalk. Note the striations of neighbouring tubules which are in register with each other (as along the junction *tt*) and join them together (× 39 600).

FIGURE 8. Higher magnification electron micrograph of a single stalk-tubule showing a large area of transverse striation and many much finer longitudinal fibrils as at *f* (× 61 200).



Epistylis plicatilis

FIGURE 9. This oblique near-longitudinal section of stalk tubules shows the complexity of structure at the junctions of neighbouring tubules (× 61 200).



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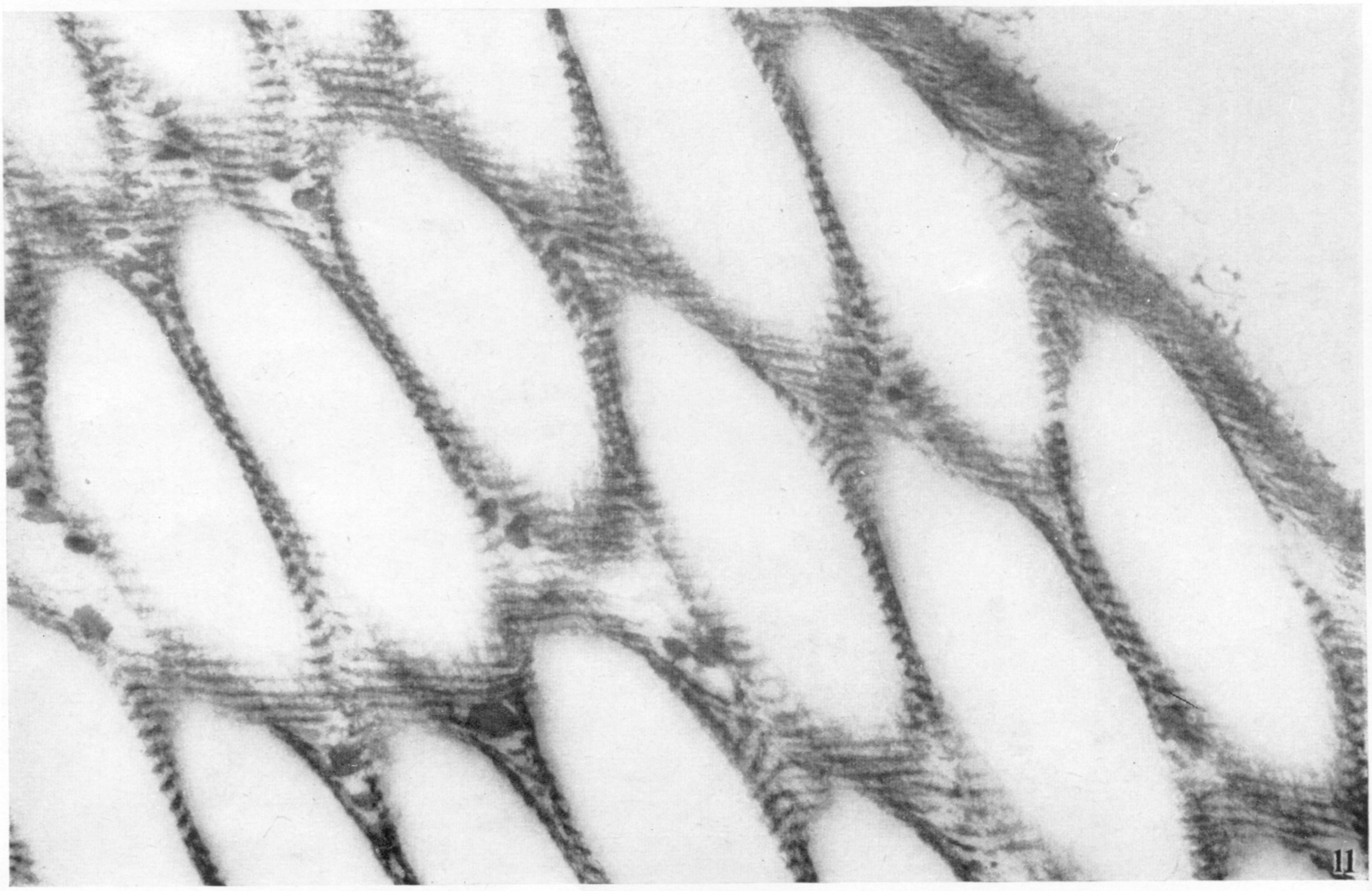

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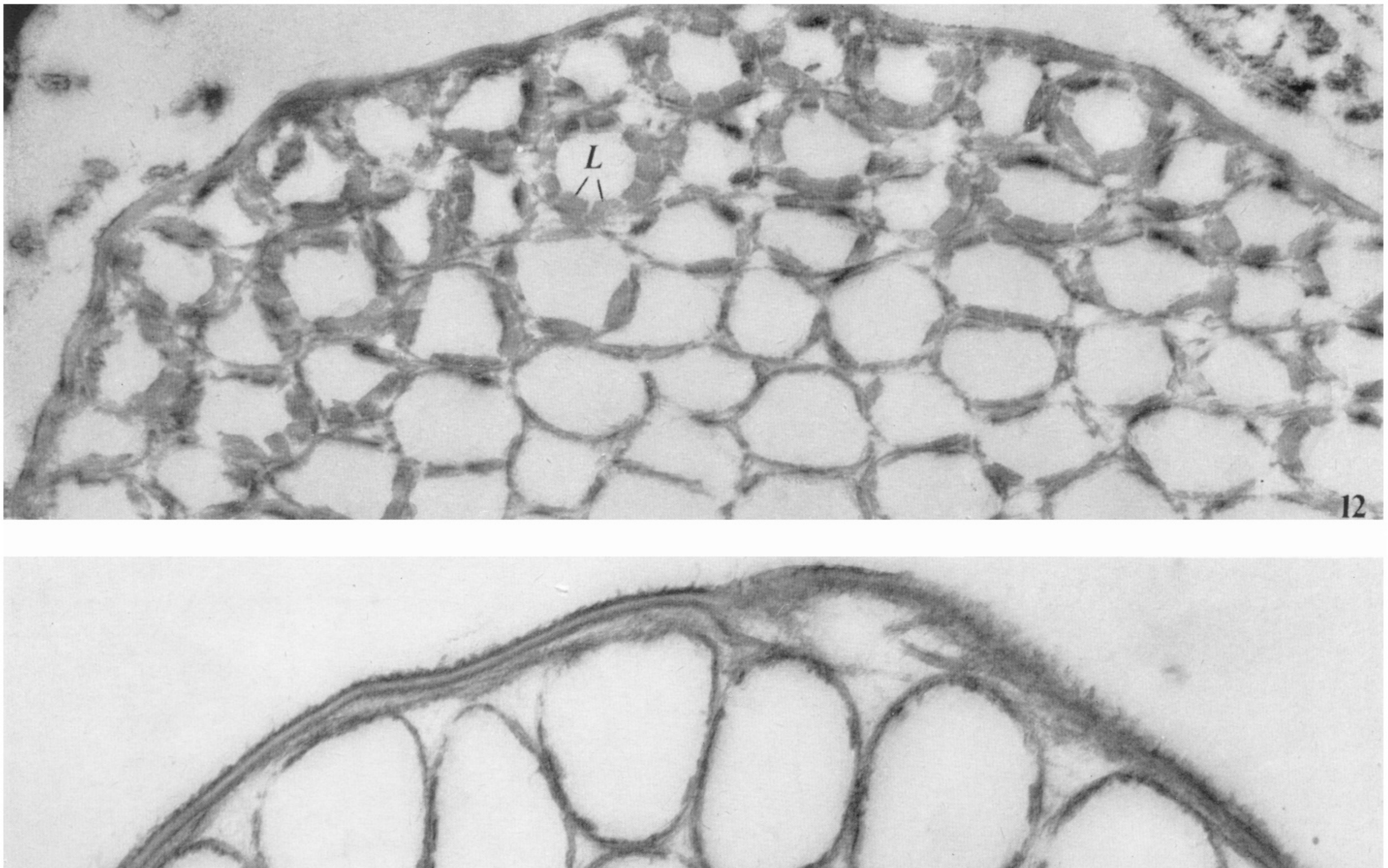
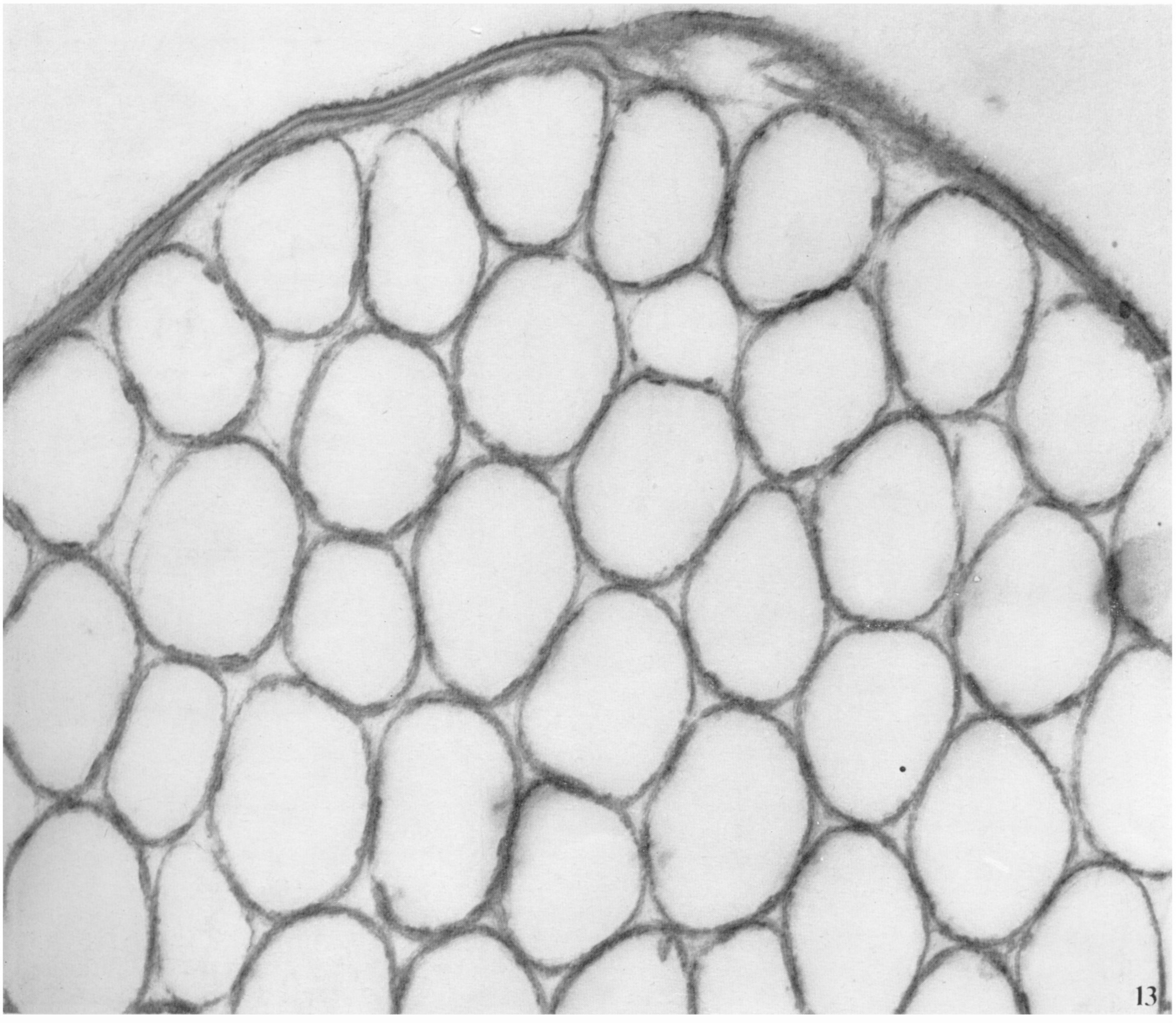
Epistylis plicatilis

FIGURE 10. Transverse section of stalk mid-way along its length. The tubules are close-packed and enclosed within a substantial wall (× 21520).

FIGURE 11. Near-transverse section of stalk at higher magnification (× 61200).

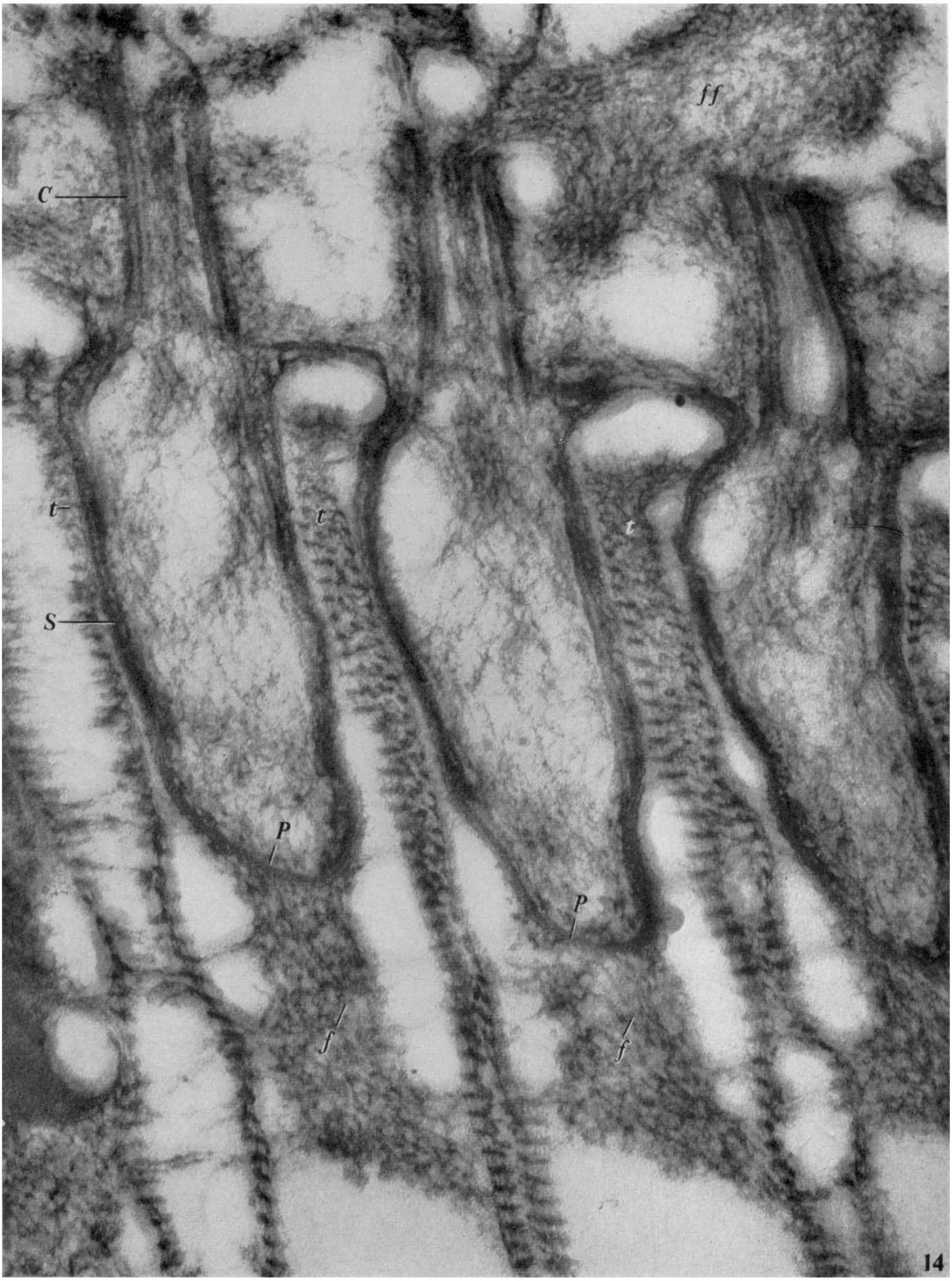




Epistylis plicatilis

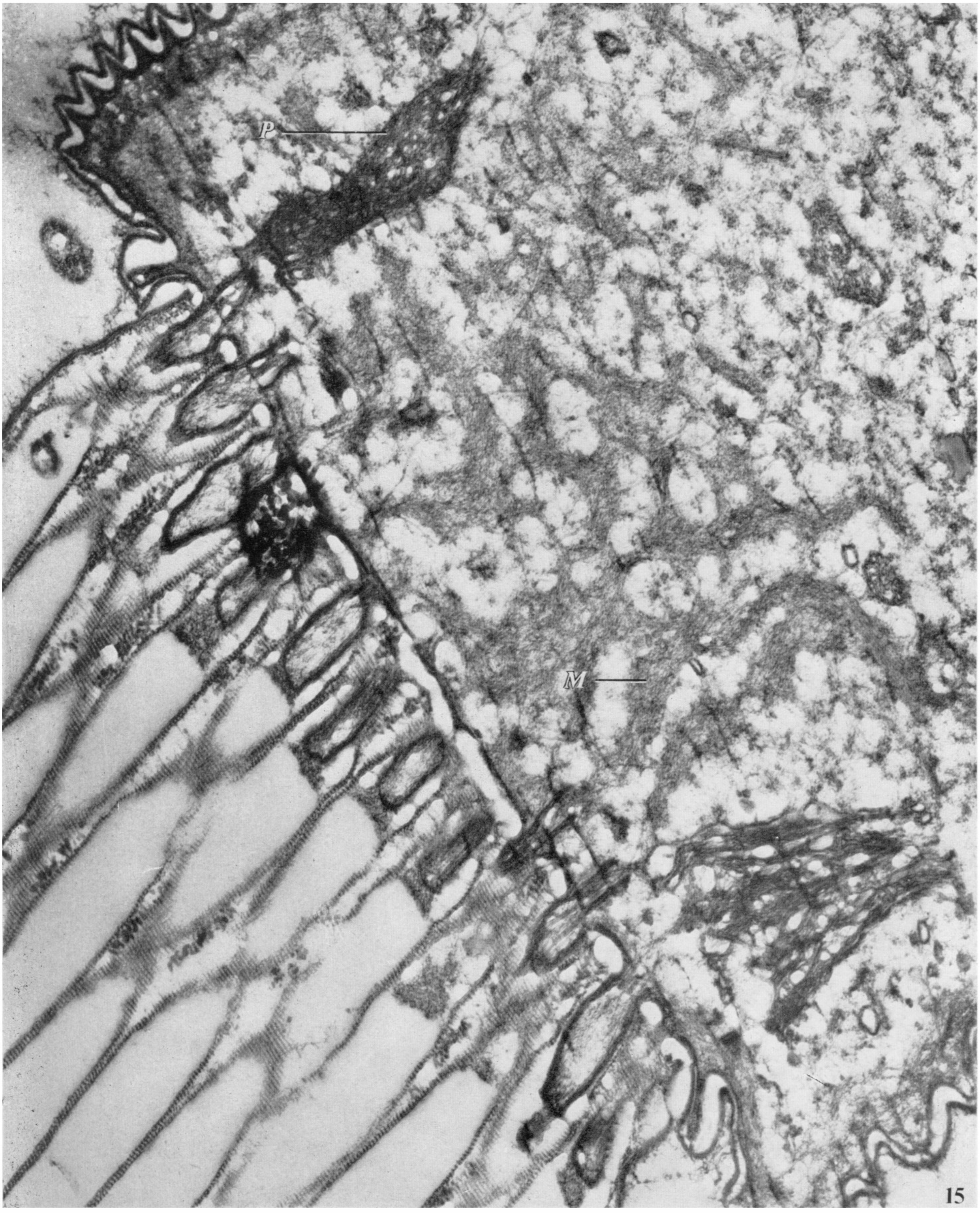
FIGURE 12. View of section of part of a stalk cut from the distal region. Each tubule in the outermost layers contains a number of elements often lozenge-shaped in section, as at *L* (× 36 000).

FIGURE 13. Remnants of the structures seen in figure 12 are present in this section from another region of the stalk. The complex wall of the stalk is also clearly shown (× 61 200).



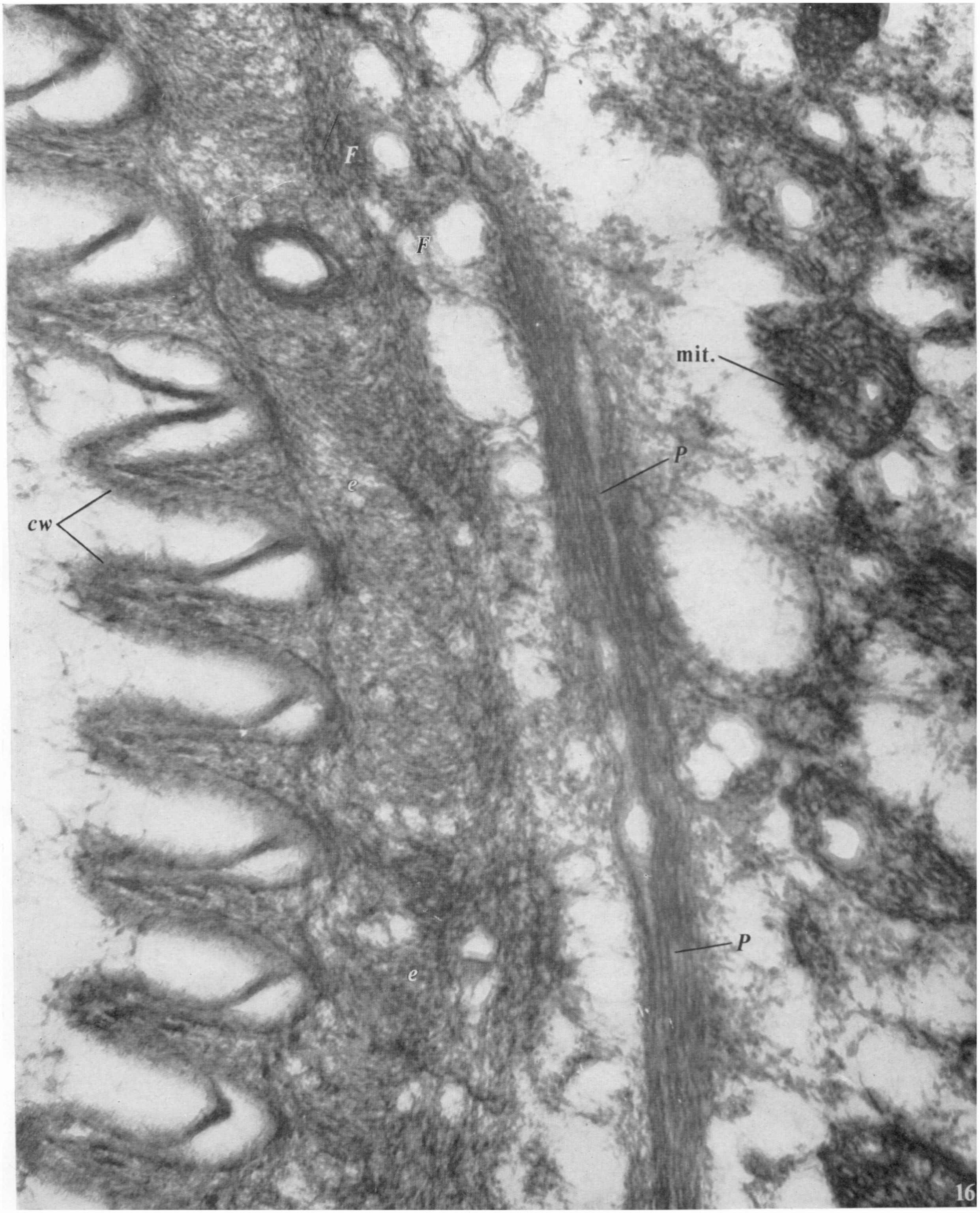
Epistylis plicatilis

FIGURE 14. Longitudinal section of scopula organelles, probably peripheral. They consist of an upper portion *C* in the form of a fibrous cylinder and a lower part or sac *S* depending into the stalk. The fibrils *ff* originating in the scopula organelles are eventually attached to the zooid wall and are probably concerned with zooid contraction. The stalk tubules (as at points *t*) fit closely over the sacs *S*. Fibrils *f* extend from these sacs into the stalk tubules possibly through the pore-like structures visible at *P*, *P* (× 114000).



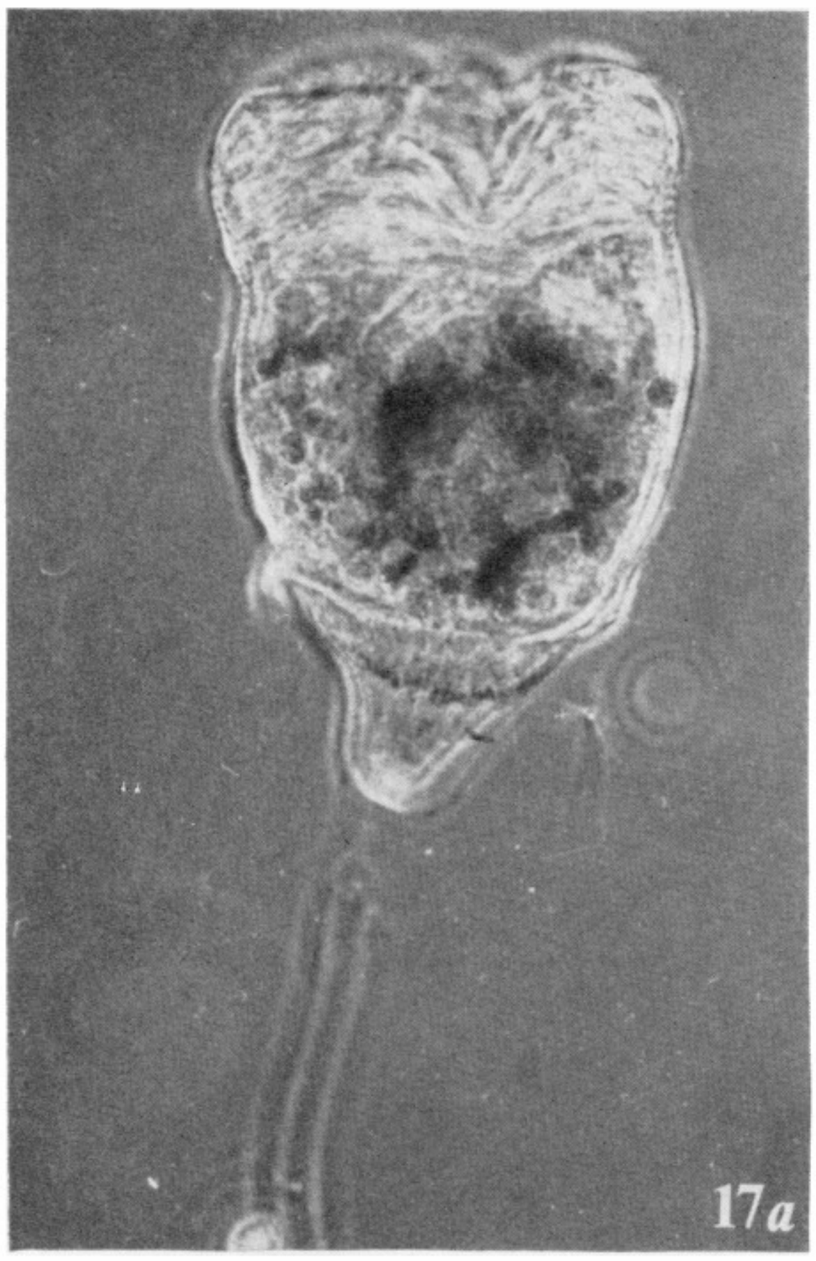
Epistylis plicatilis

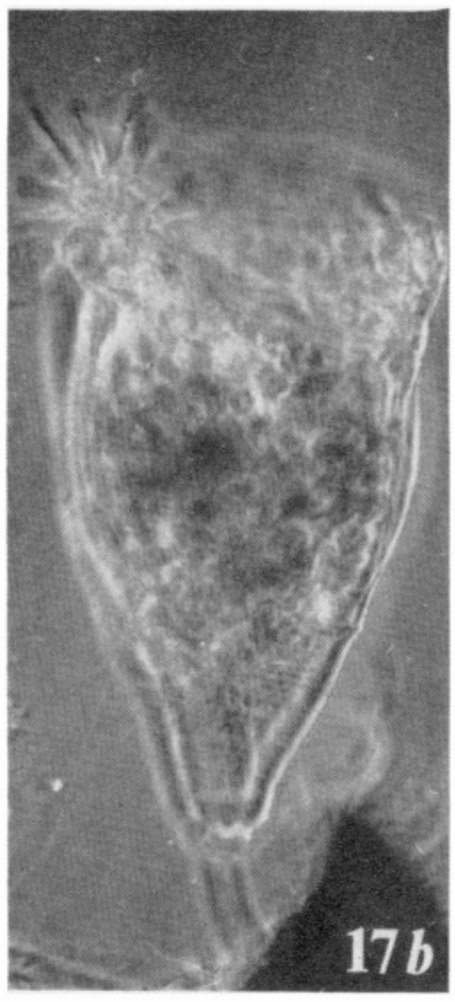
FIGURE 15. Longitudinal section through a stalk-zooid junction, probably cut through the middle of the stalk. The peripheral scopula organelles are seen to be different from the central ones (× 27 000). From the upper parts of the peripheral organelles long bundles of fibrils, *P*, extend into the zooid and termmate ultimately on or near its wall. Shorter bundles, *M*, are attached to the more central scopula organelles.

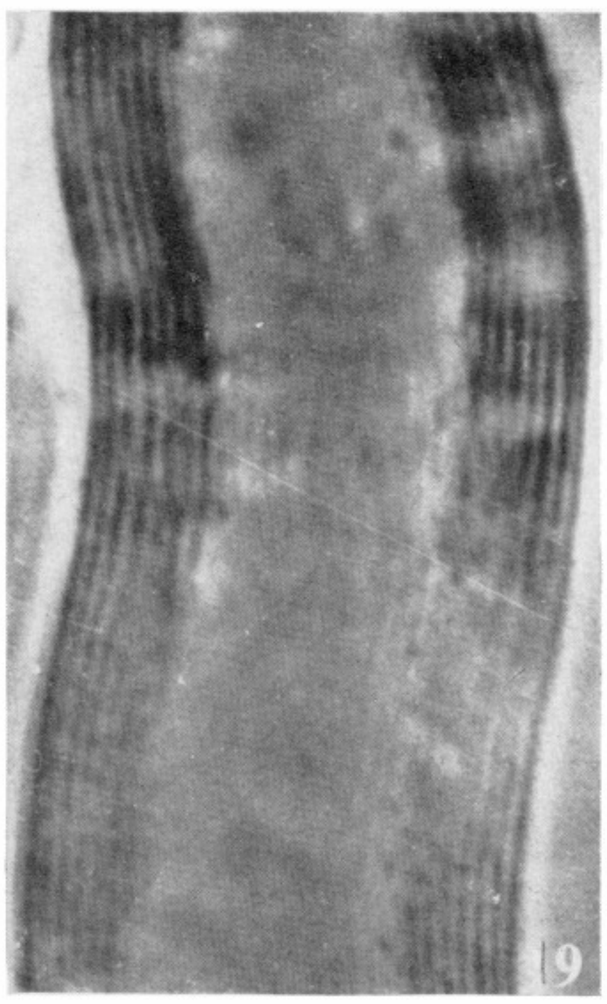


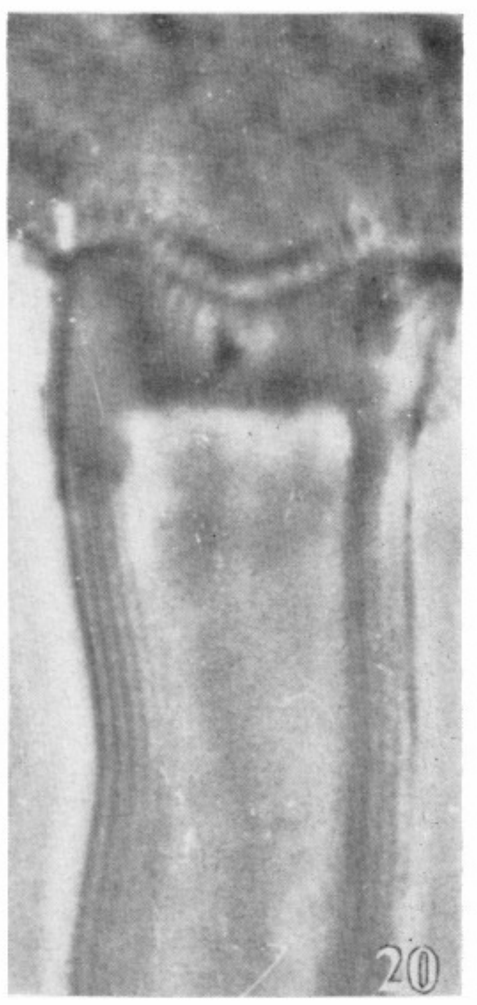
Epistylis plicatilis

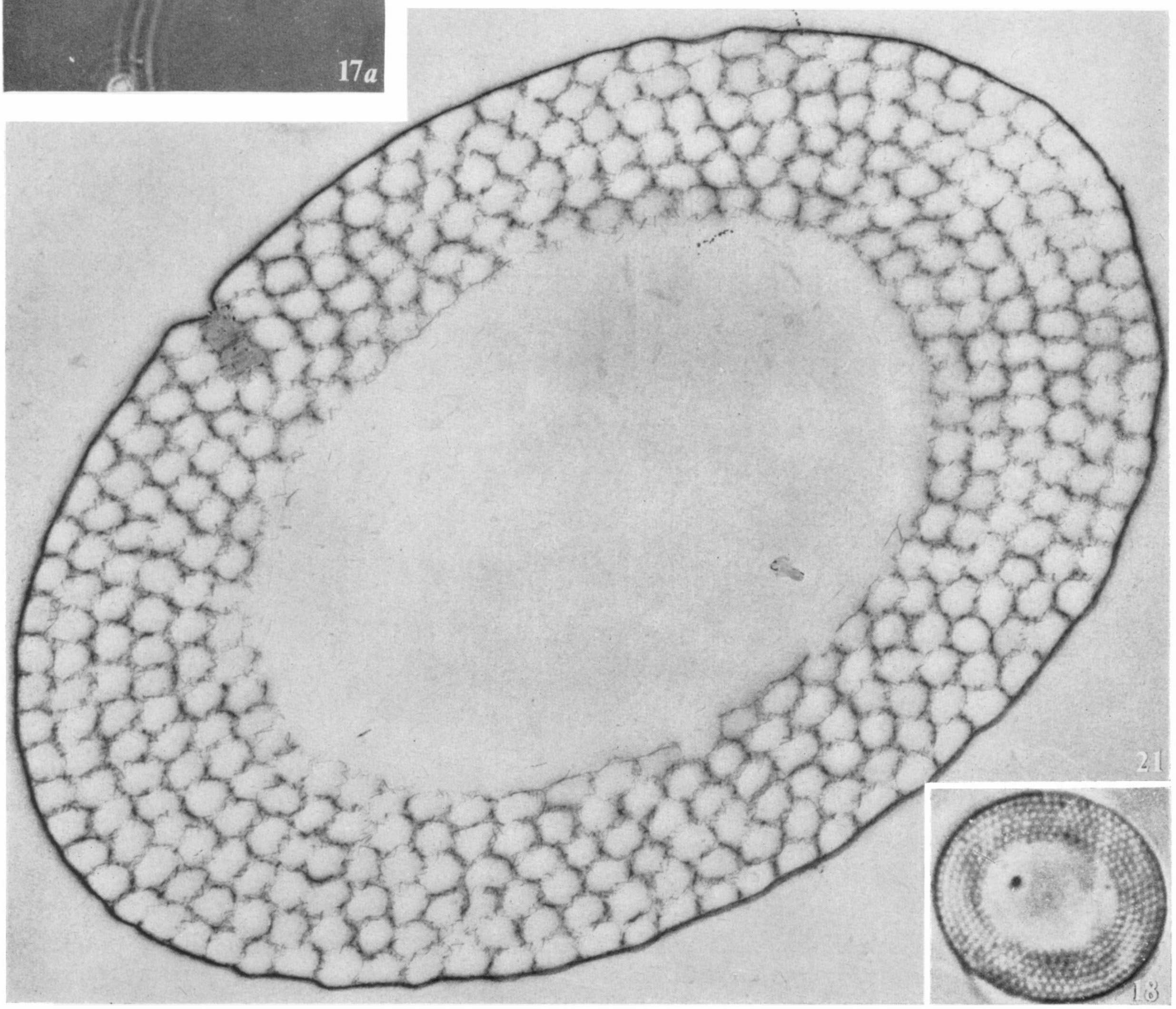
FIGURE 16. View of the crenellated wall *cw* of the fixed zooid with its ectoplasmic layer *ee*. The fibrillar bundle *PP* originating in the peripheral scopula organelles appears to fuse with this layer in the region *FF* (× 76500). Mitochondria such as mit. are commonly observed in the cytoplasm.











Campanella umbellaria

FIGURE 17*a*. Developmental stage with young stalk. The ciliary girdle of the free-swimming telotroch is still apparent (×320).

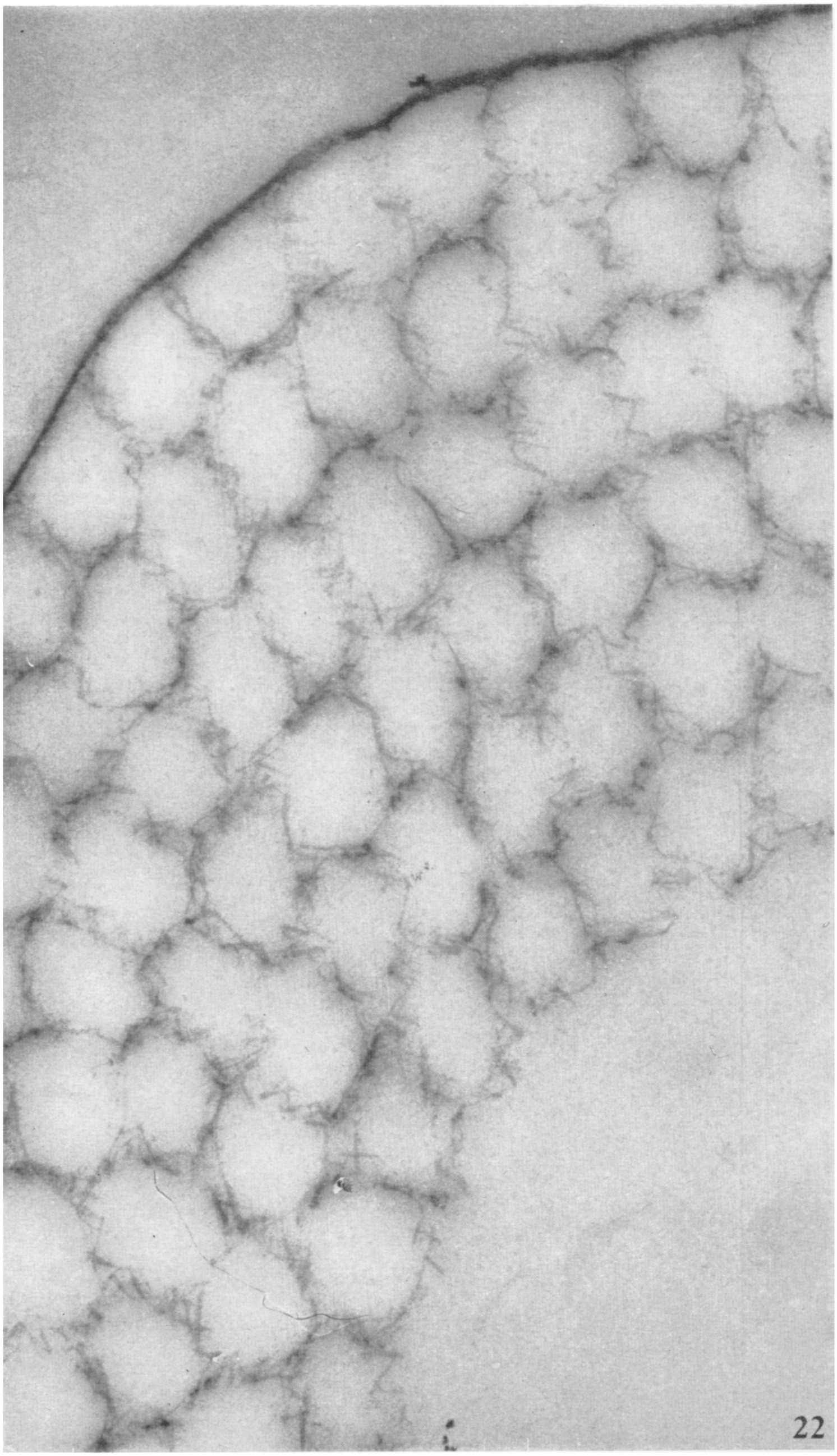
FIGURE 17*b*. Adult specimen to be compared with figure 17*a* (×300).

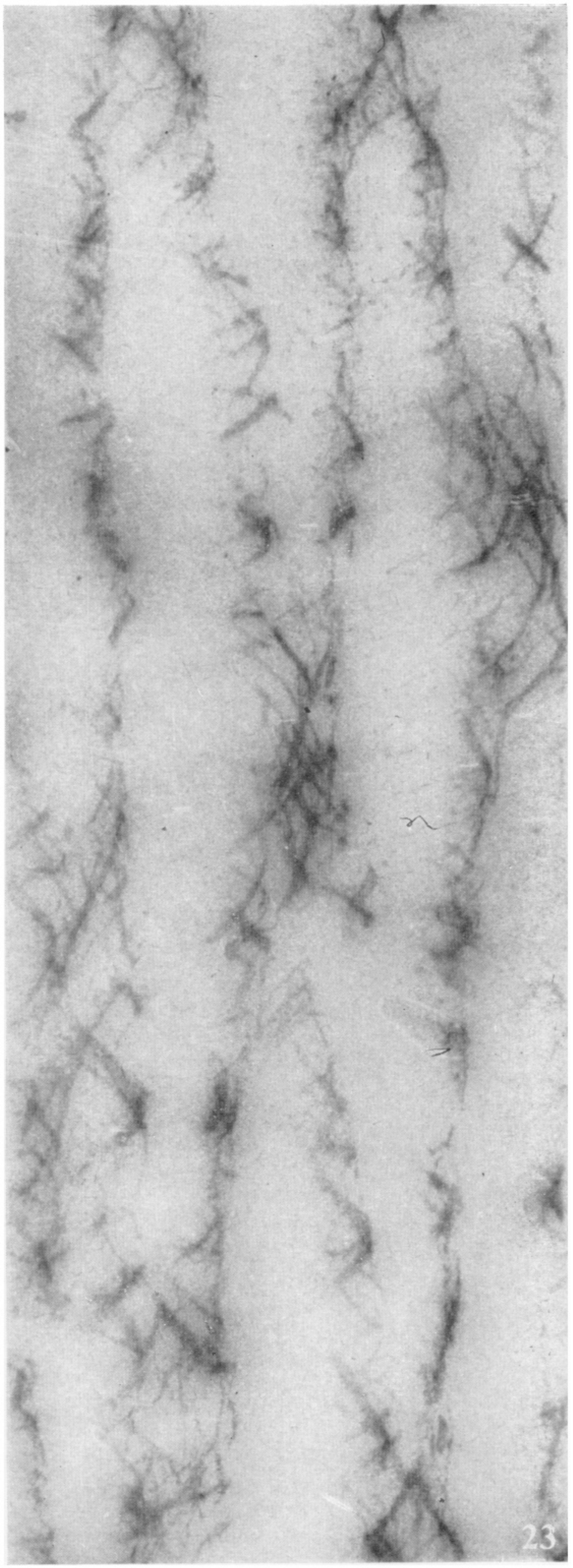
FIGURE 18. Photomicrograph of *Campanella* stalk that shows characteristic tubular structure with central structureless canal (×2750).

FIGURE 19. Longitudinal section of stalk seen in light microscope and consistent with figure 18 (×2750).

FIGURE 20. Longitudinal section of stalk and its junction with the zooid. At the junction the stalk tubules appear to extend over the whole section. At a distance of about 2 μ from the junction the inner tubules cease abruptly. Note the scopula organelles (×2750).

FIGURE 21. Low magnification electron micrograph of transverse section of stalk (×13600).

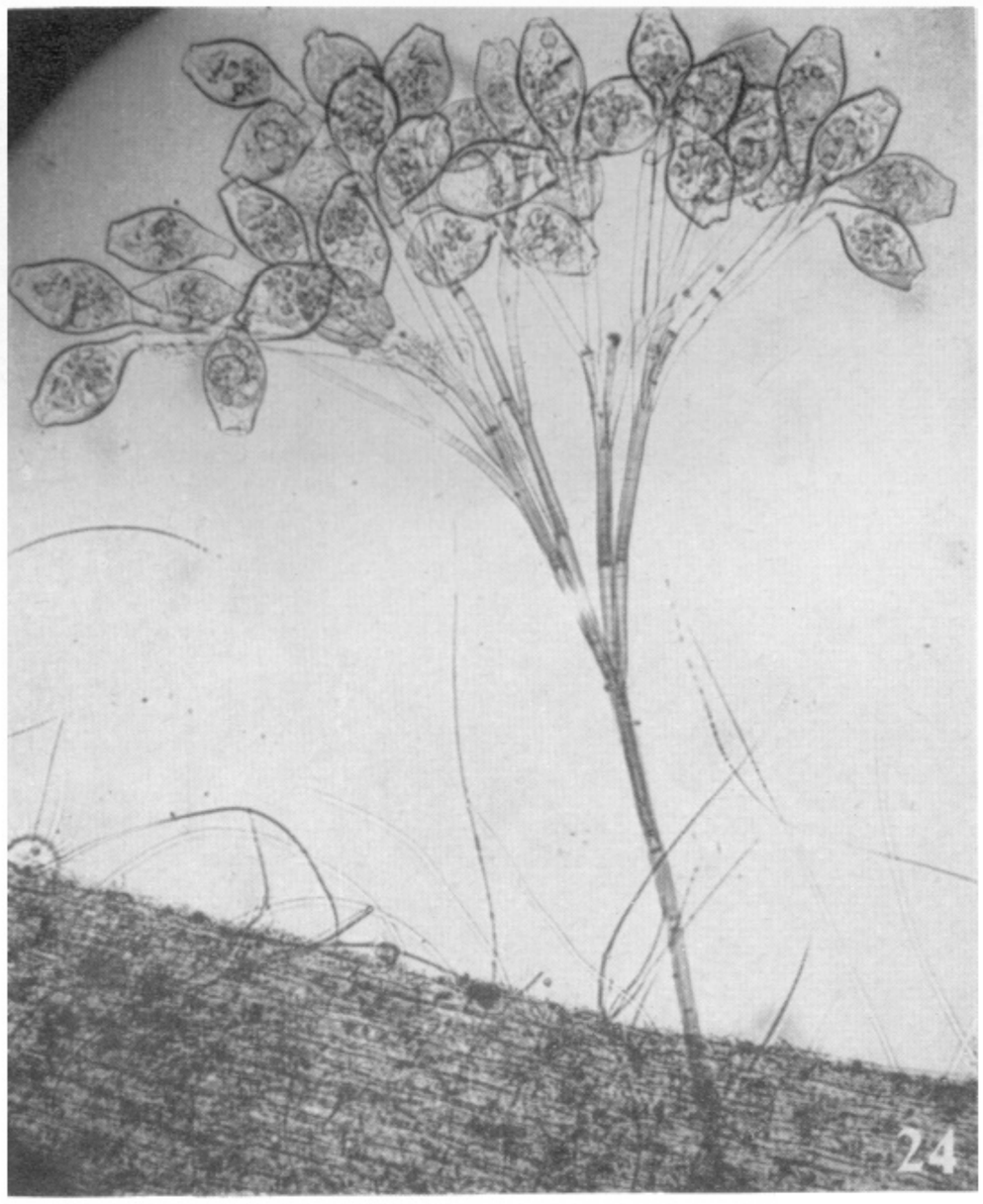


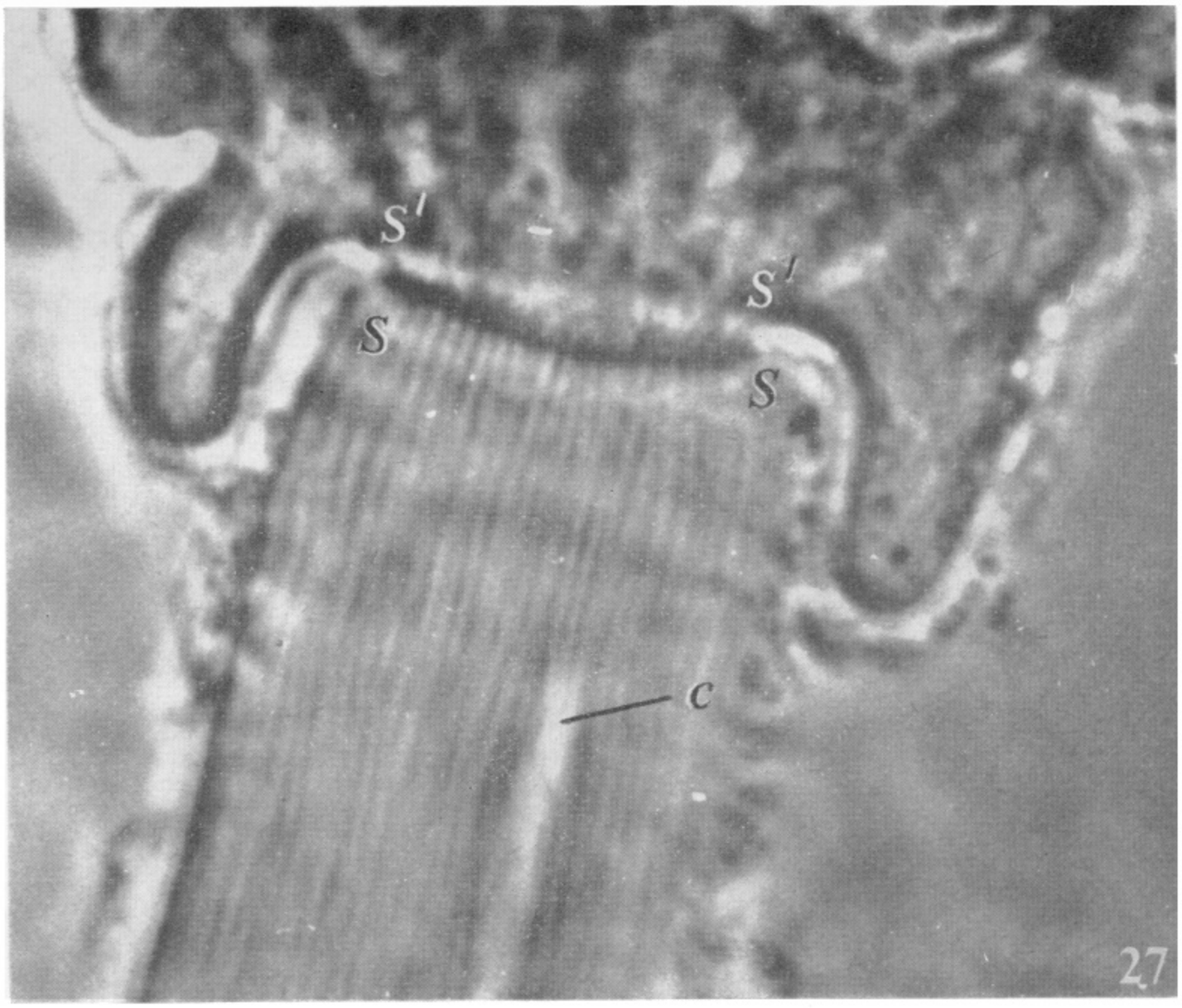


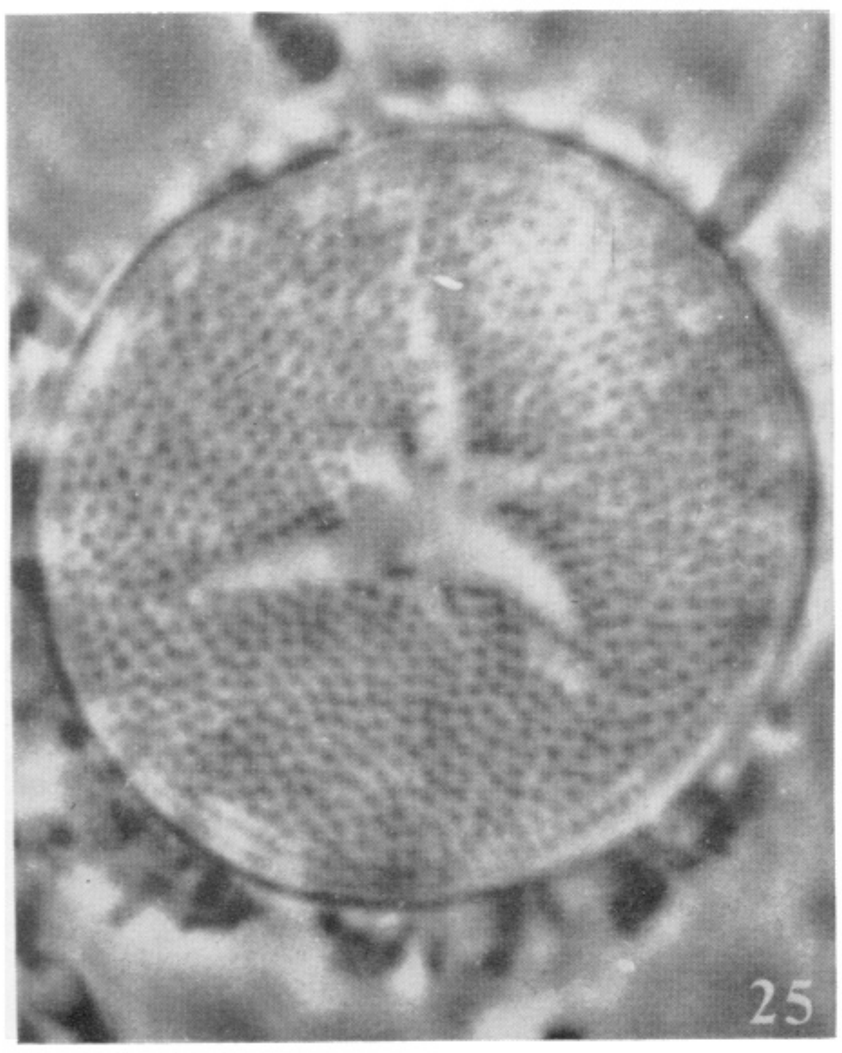
Campanella umbellaria

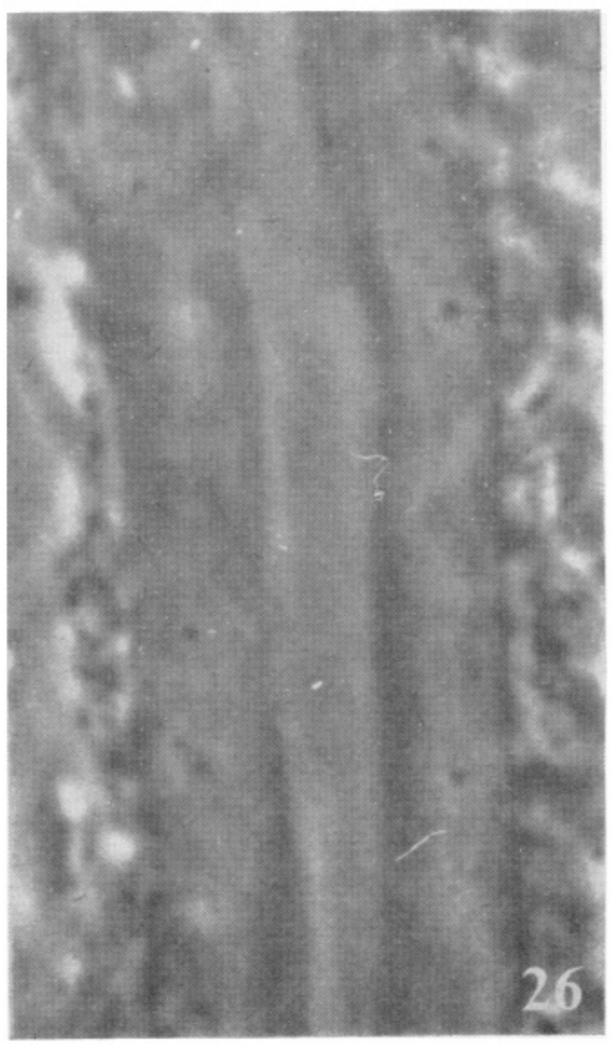
FIGURE 22. This transverse section of a sector of the stalk shows six peripheral rings of fibrous tubules and a canal in the centre of the stalk. The tubules are closely packed, devoid of structure rather polygonal in outline and of linear dimensions 0·3 to 0·5 μ (× 42 000).

FIGURE 23. Longitudinal section of fibrous tubules indicating as in figure 22 how one tubule is interlocked with its neighbours (× 60 000).









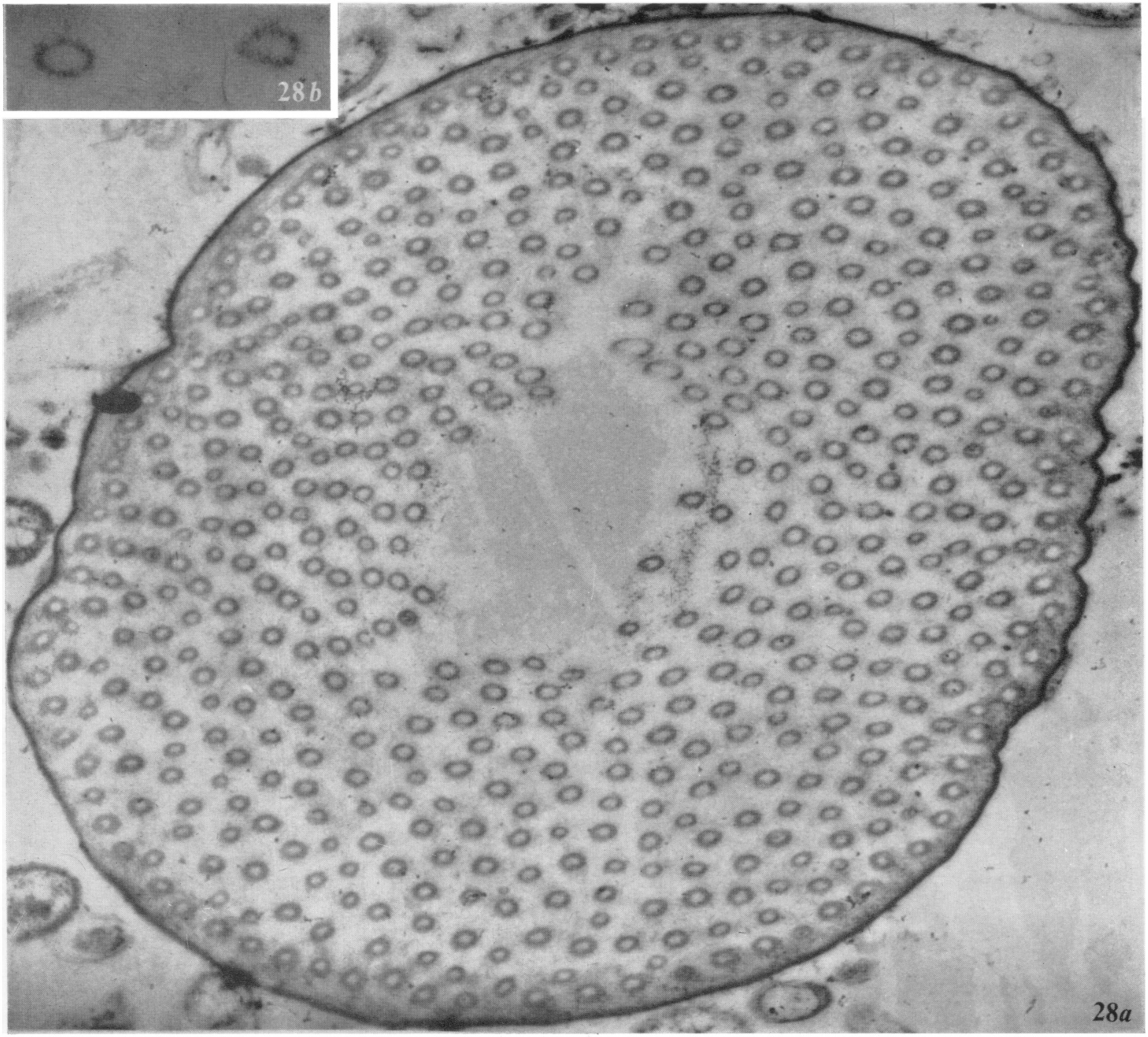
Opercularia plicatilis

FIGURE 24. Small living colony showing its tree-like character and the form of the individual zooid (× 112).

FIGURE 25. Photomicrograph of transverse section of stalk which shows the individual tubules. The more or less central canal is very irregular in this illustration and this may in part be artifact. See also figures 26 and 27 (× 2750).

FIGURE 26. Photomicrograph of longitudinal section of stalk. The central canal is prominent (× 2750).

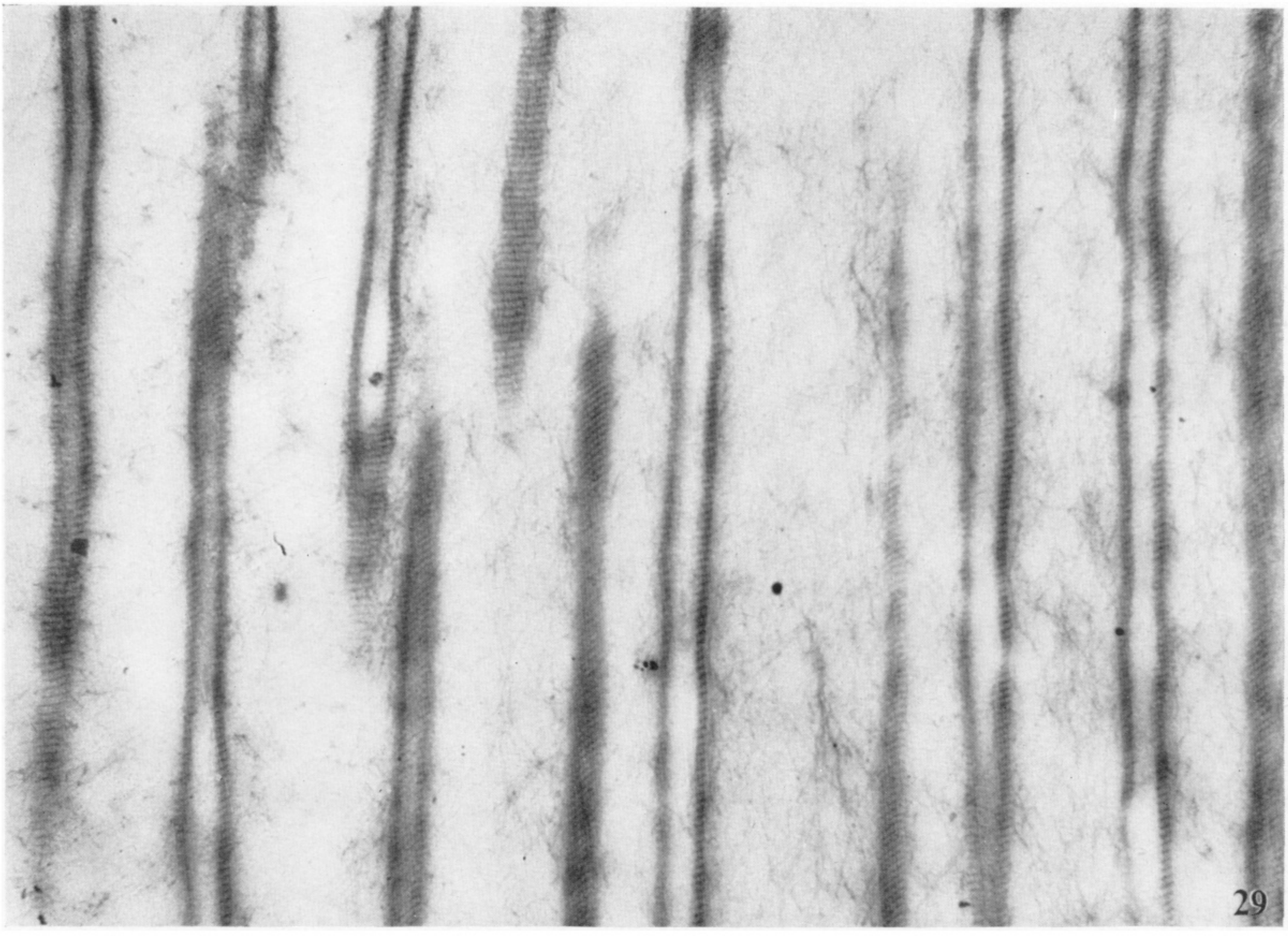
FIGURE 27. Photomicrograph of longitudinal section of junction between stalk and zooid. The central canal *c* does not appear to start at the junction (× 2750).

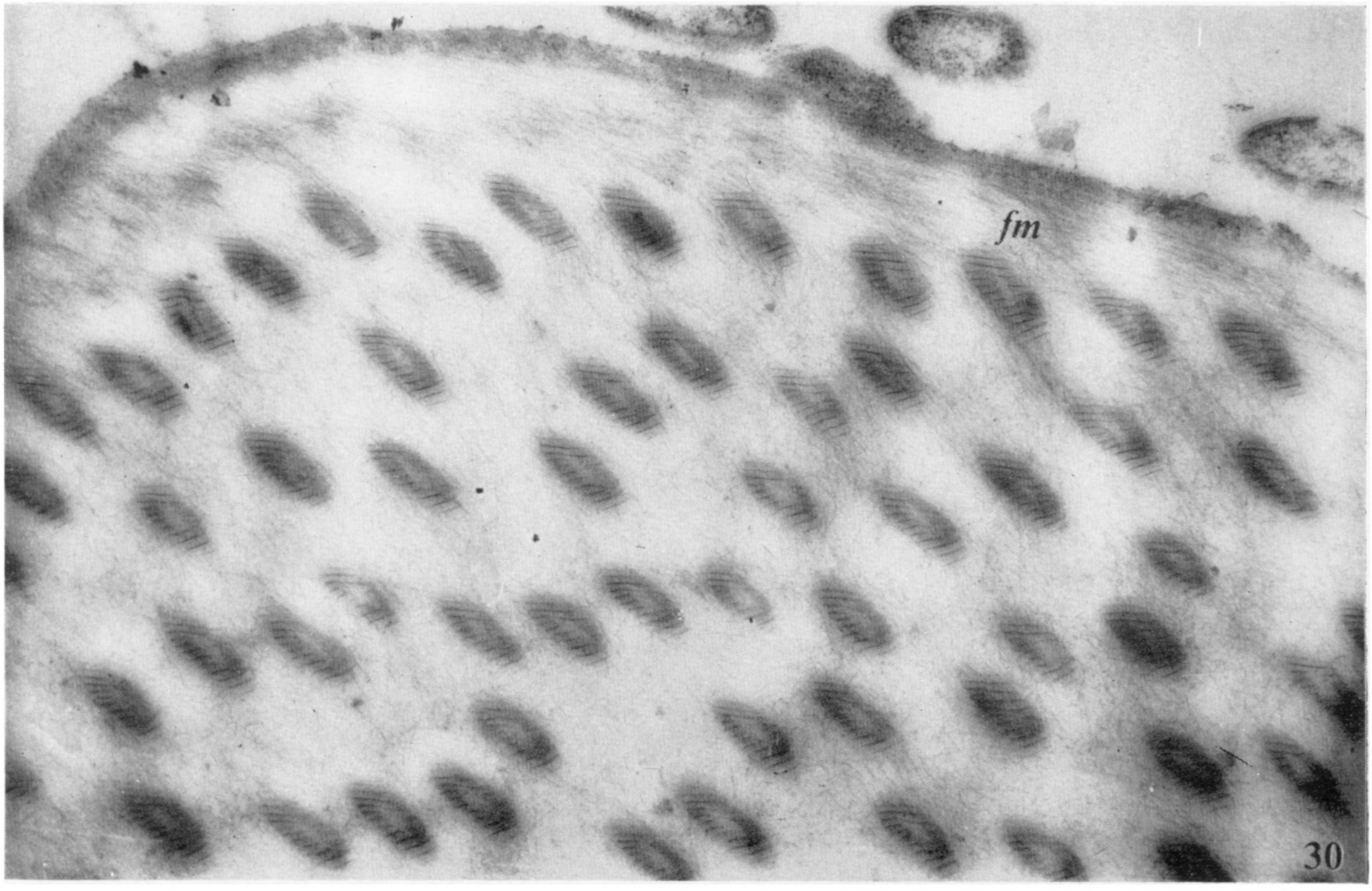


Opercularia plicatilis

FIGURE 28*a*. Low magnification electron micrograph of transverse section of stalk showing the many tubules and central canal (cf. figure 27). No membrane separates the canal from the annulus (× 13 600).

FIGURE 28*b*. Transverse section of two tubules at higher magnification indicating probable fibrous nature of the wall (× 27 200).

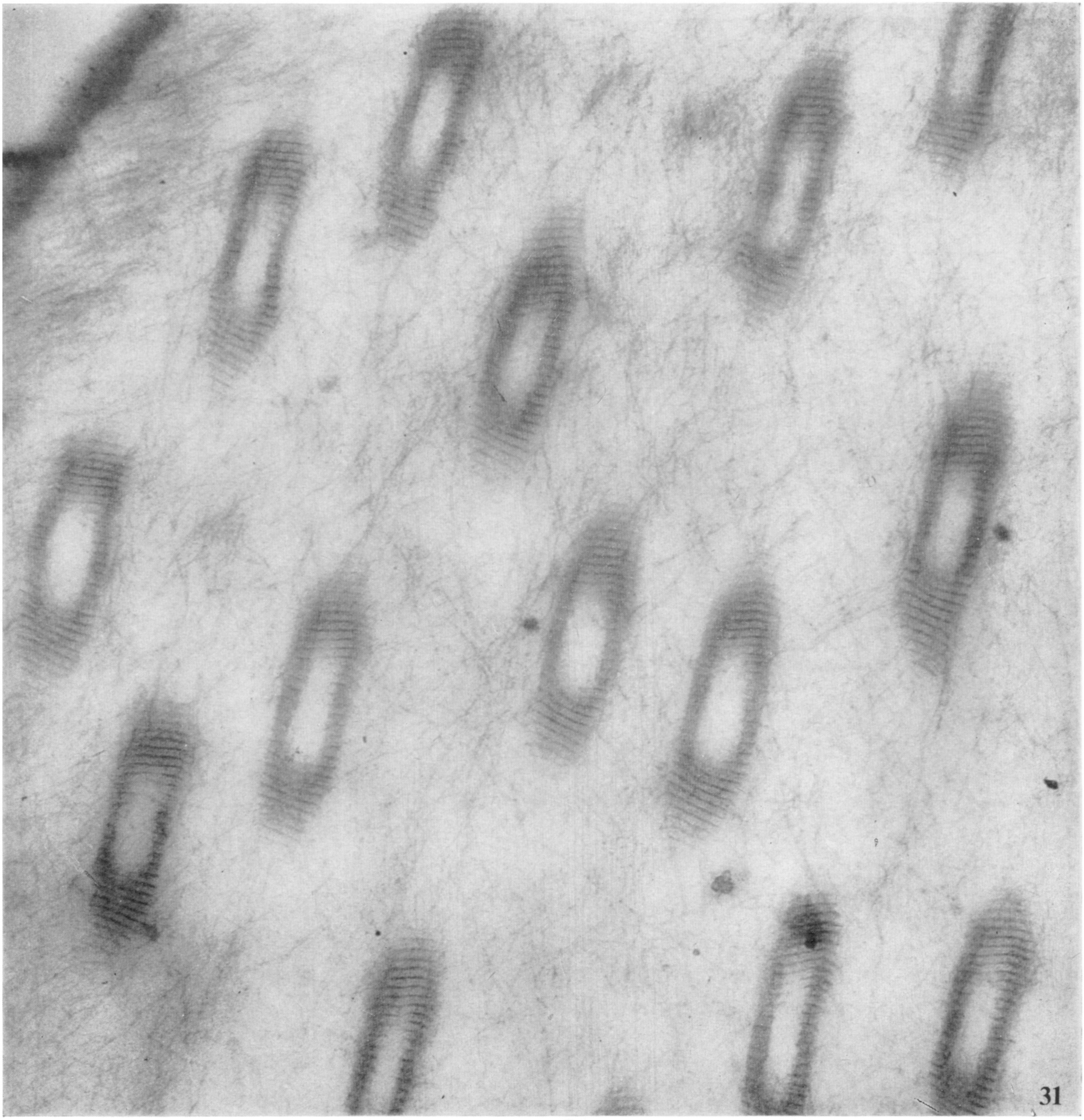




Opercularia plicatilis

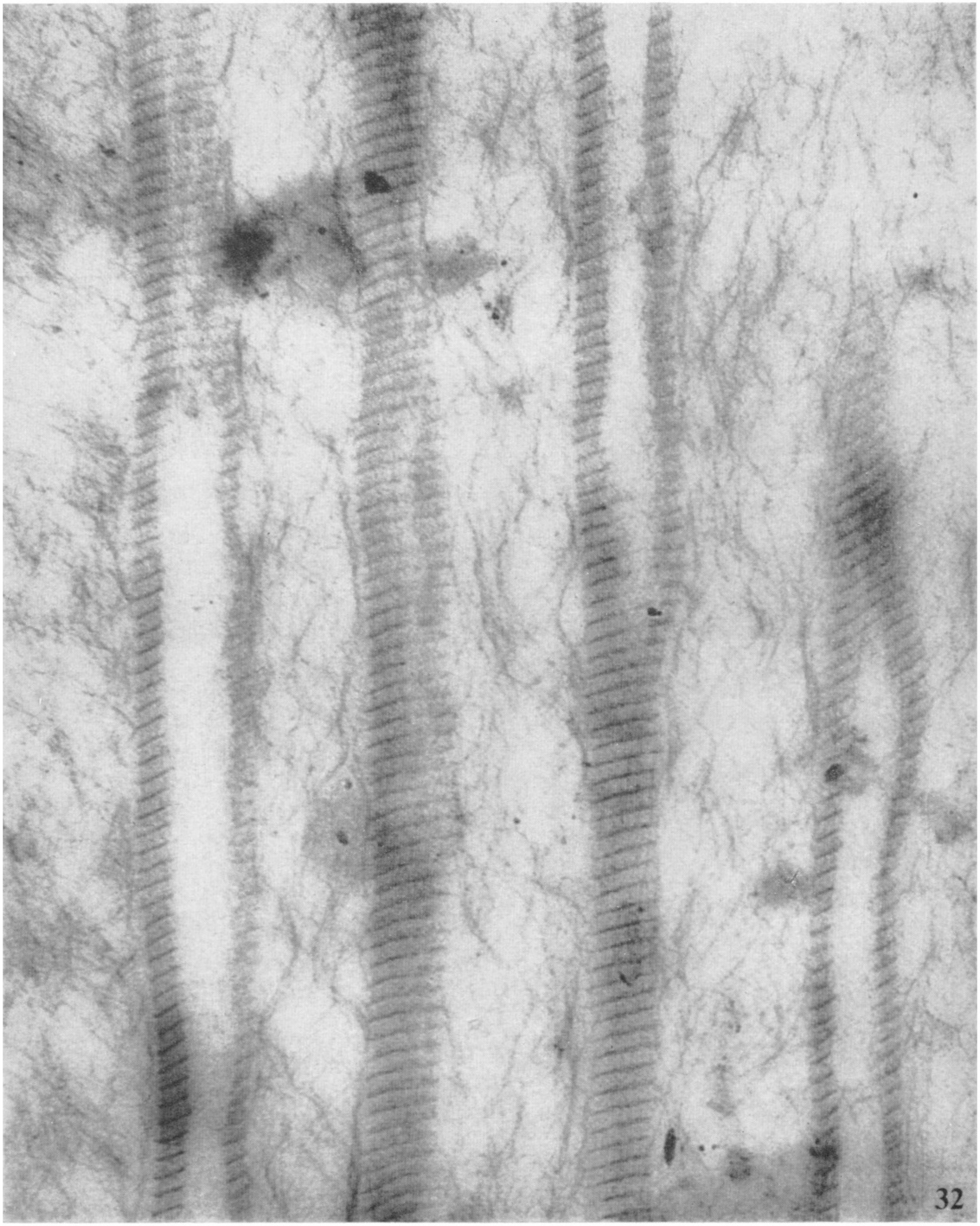
FIGURE 29. The stalk tubules in longitudinal section showing transverse striations (period 400 Å) and fibrillar matrix (× 27 200).

FIGURE 30. Oblique section of the stalk in which the striations of figure 29 are also visible as well as the attachment of the fibrillar matrix *fm* to the wall (× 27 200).



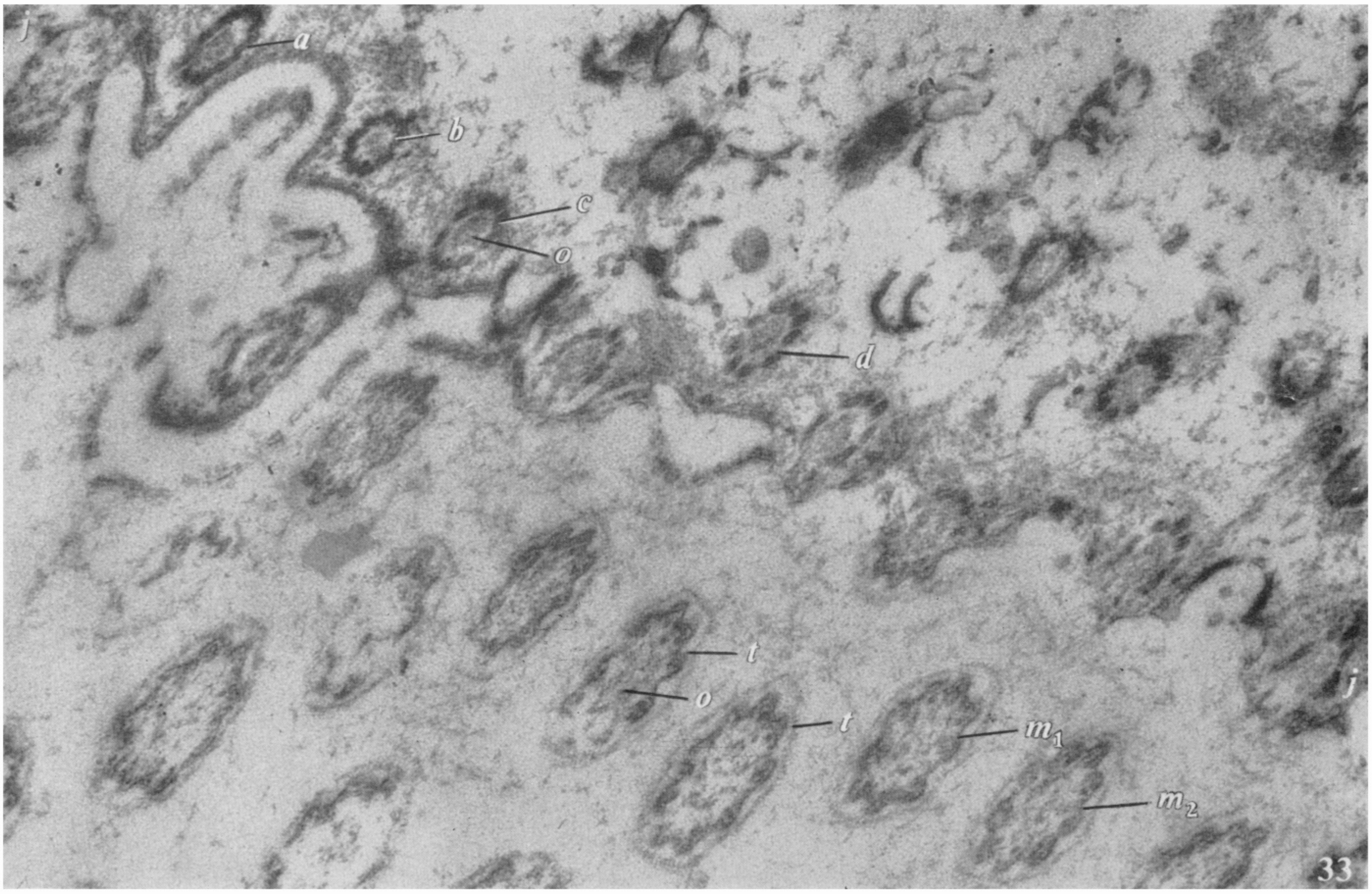
Opercularia plicatilis

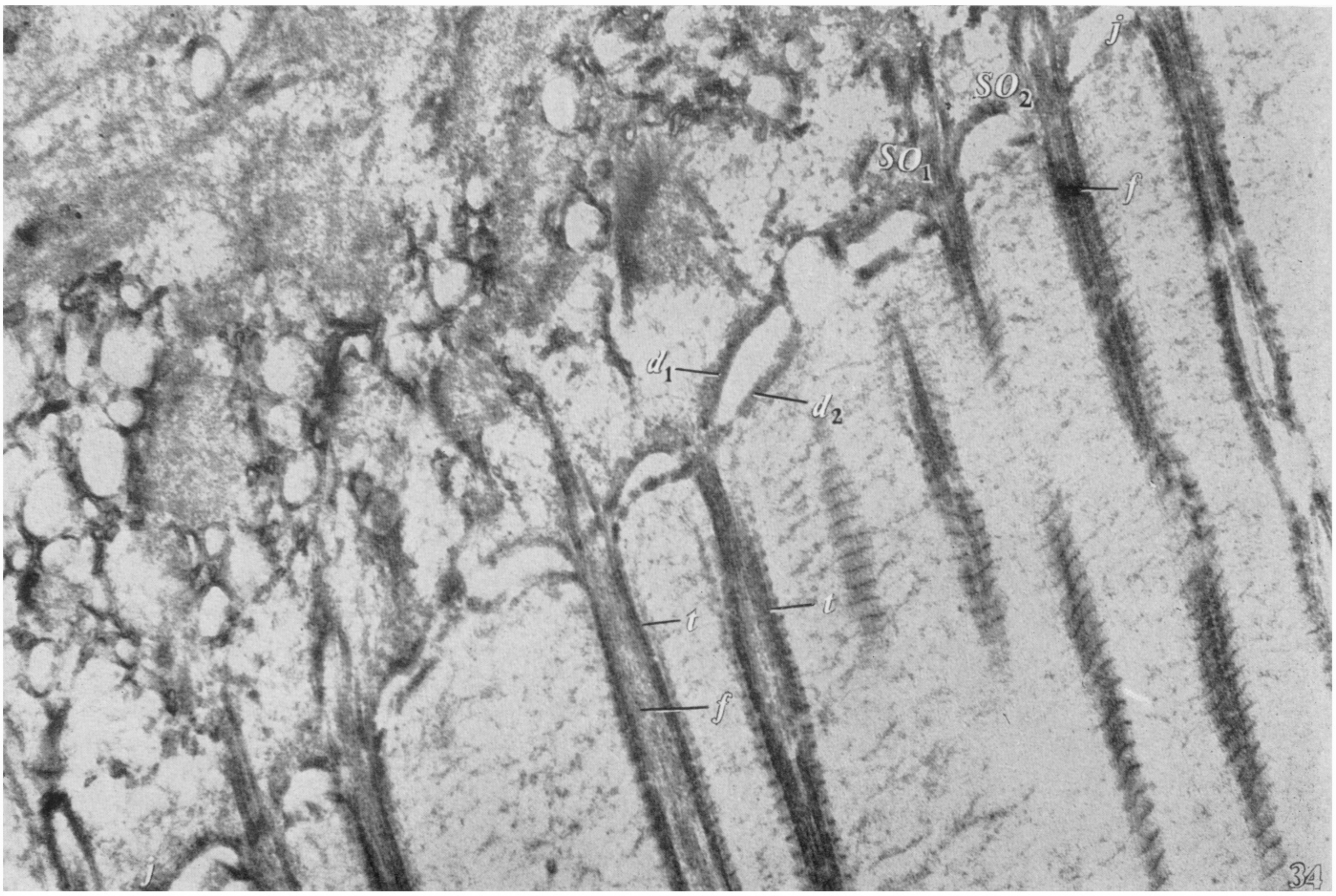
FIGURE 31. This section shows (*a*) striations of tubules each about 60 Å wide and 400 Å apart, (*b*) matrix fibrils which are not striated, and (*c*) component longitudinal fibrils of the tubules (× 55 500).



Opercularia plicatilis

FIGURE 32. Demonstrates the regularity of the transverse striations over considerable lengths of tubule and the unstriated nature of the matrix (× 66 600).

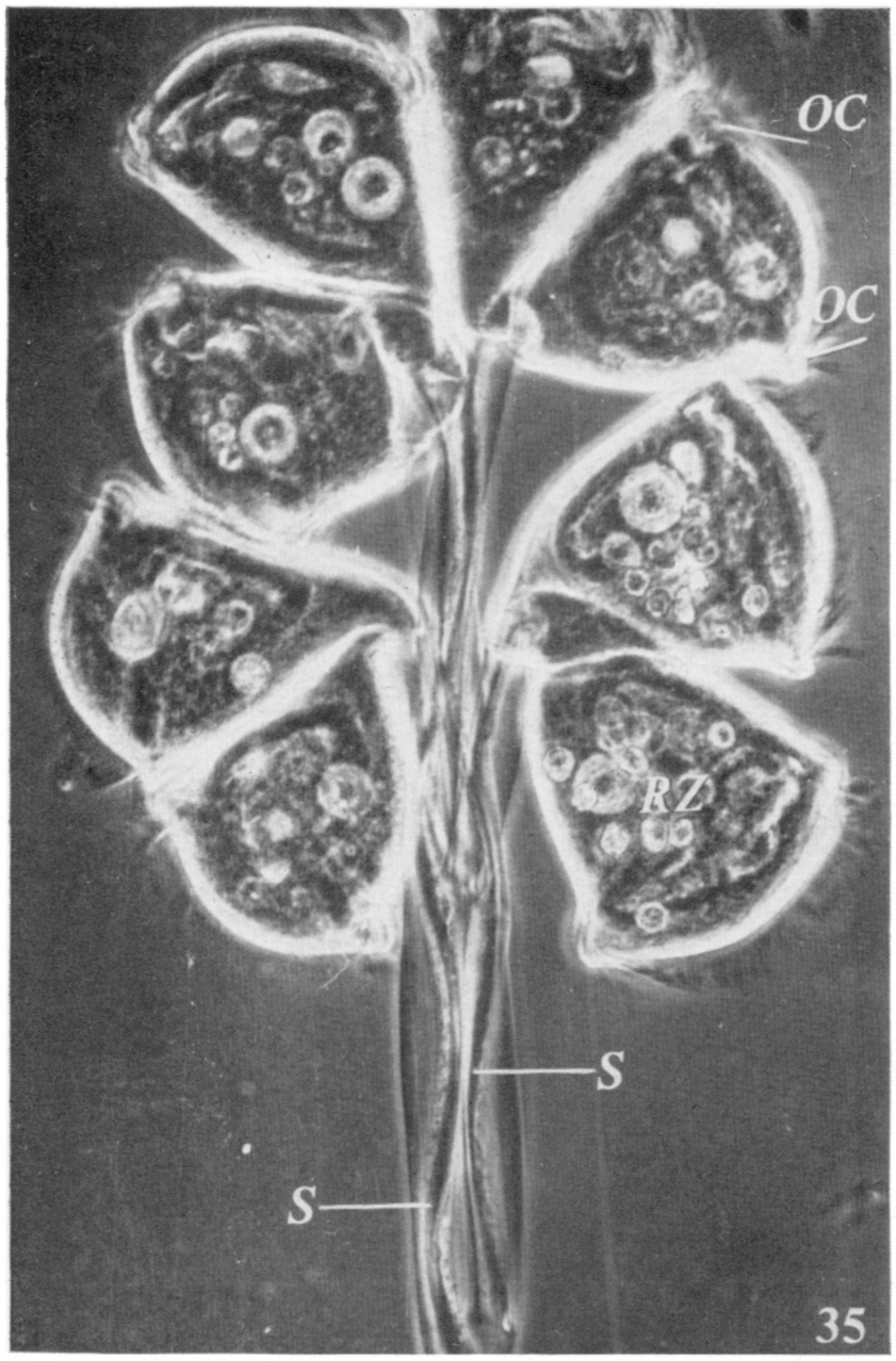


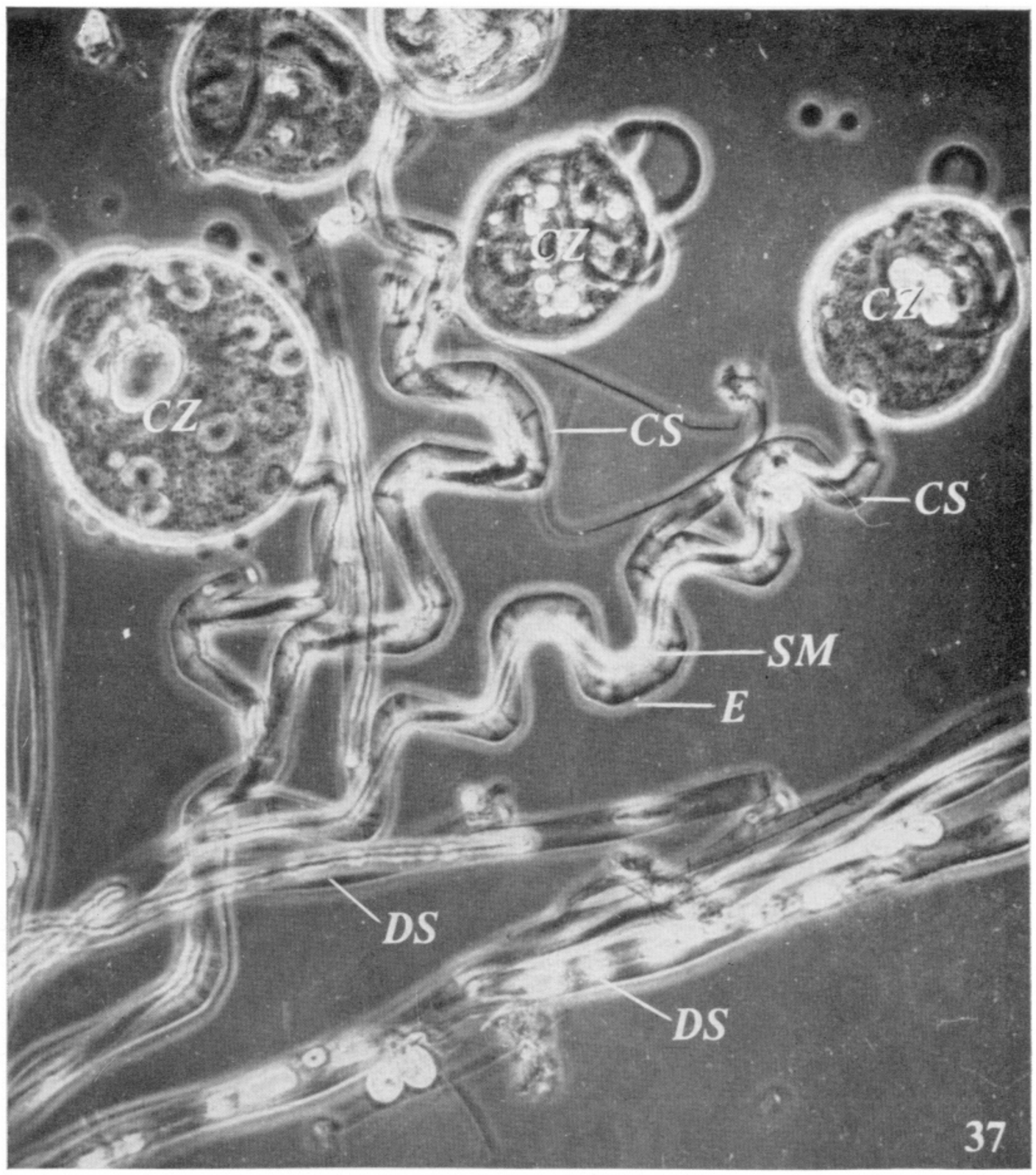


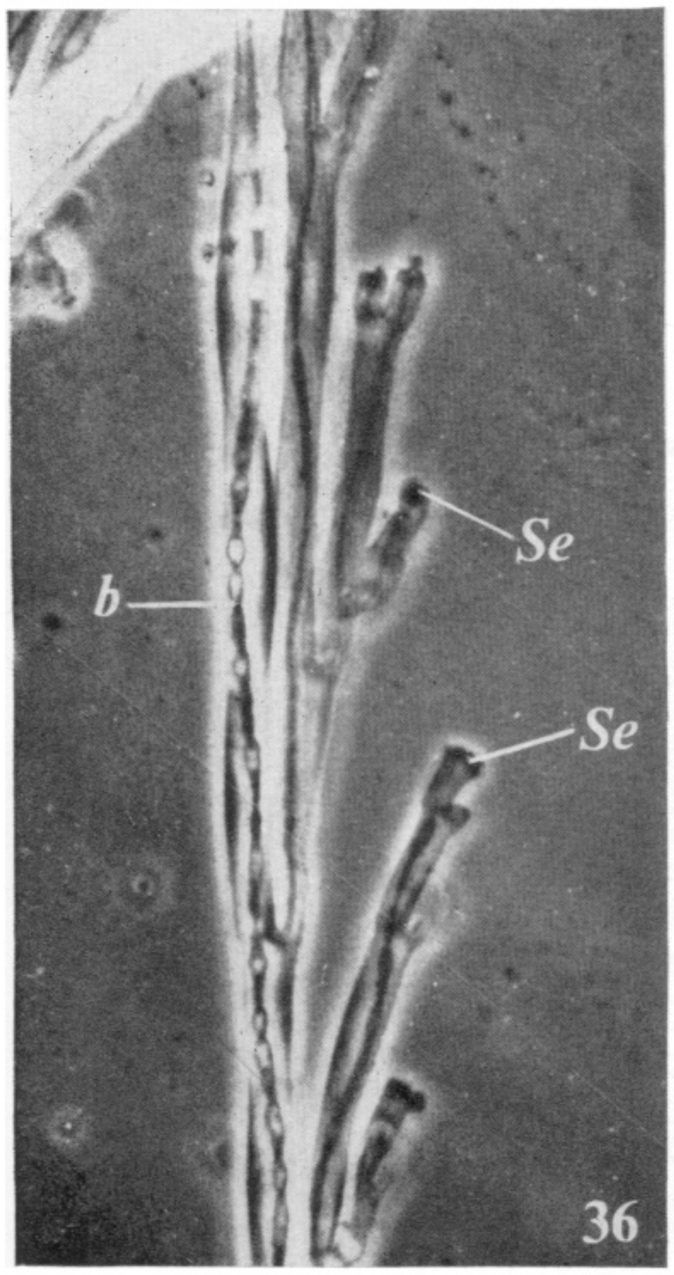
Opercularia plicatilis

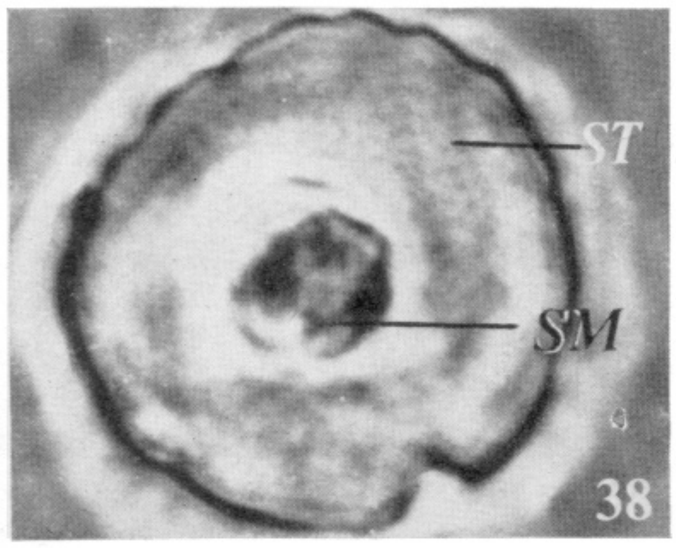
FIGURE 33. Oblique section of the junction *jj* between stalk and zooid. Parts of scopula organelles lying within the zooid are visible at *a*, *b*, *c* and *d*, and are structurally analogous to basal bodies. The component fibres are not enveloped by a membrane above the junction but lower parts of the *SO* as at *t*, *t* are. Material, possibly fibrous, is also visible within the fibrous peripheries of the *SO*, as at *O*, both above and below the junction plane *jj*. The main fibrils of the *SO* below the junction appear to be surrounded by a scalloped membrane (m_1, m_2) and are probably attached to it (×44400).

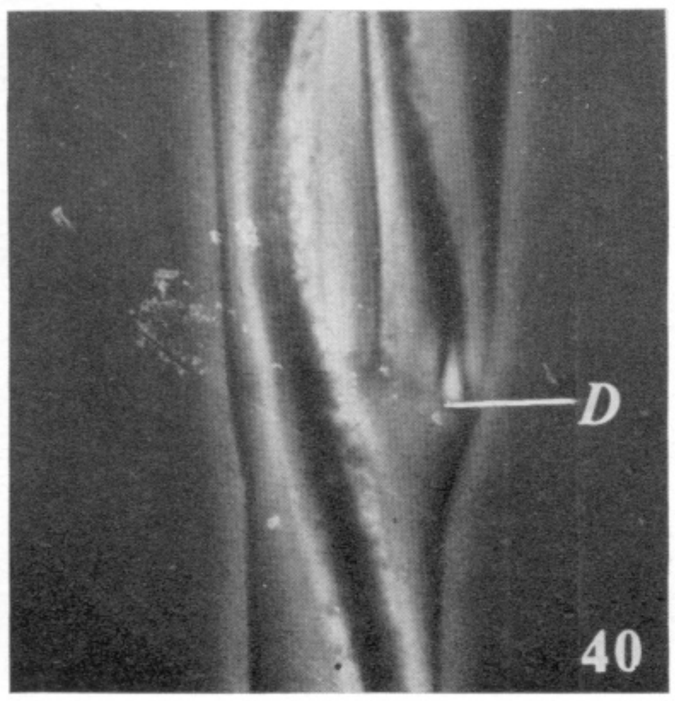
FIGURE 34. This longitudinal section through the junction *jj* shows the duplex nature of the junction membrane (d_1, d_2) and the fibrous interior of the *SO* as at *f*, *f*. The stalk tubules are recognizable at *t*, *t* by their striations and fit closely over the elongated bases of the scopula organelles. This manner of attachment is similar to that adopted in *Epistylis* (cf. figure 14, plate 19 (×44400).

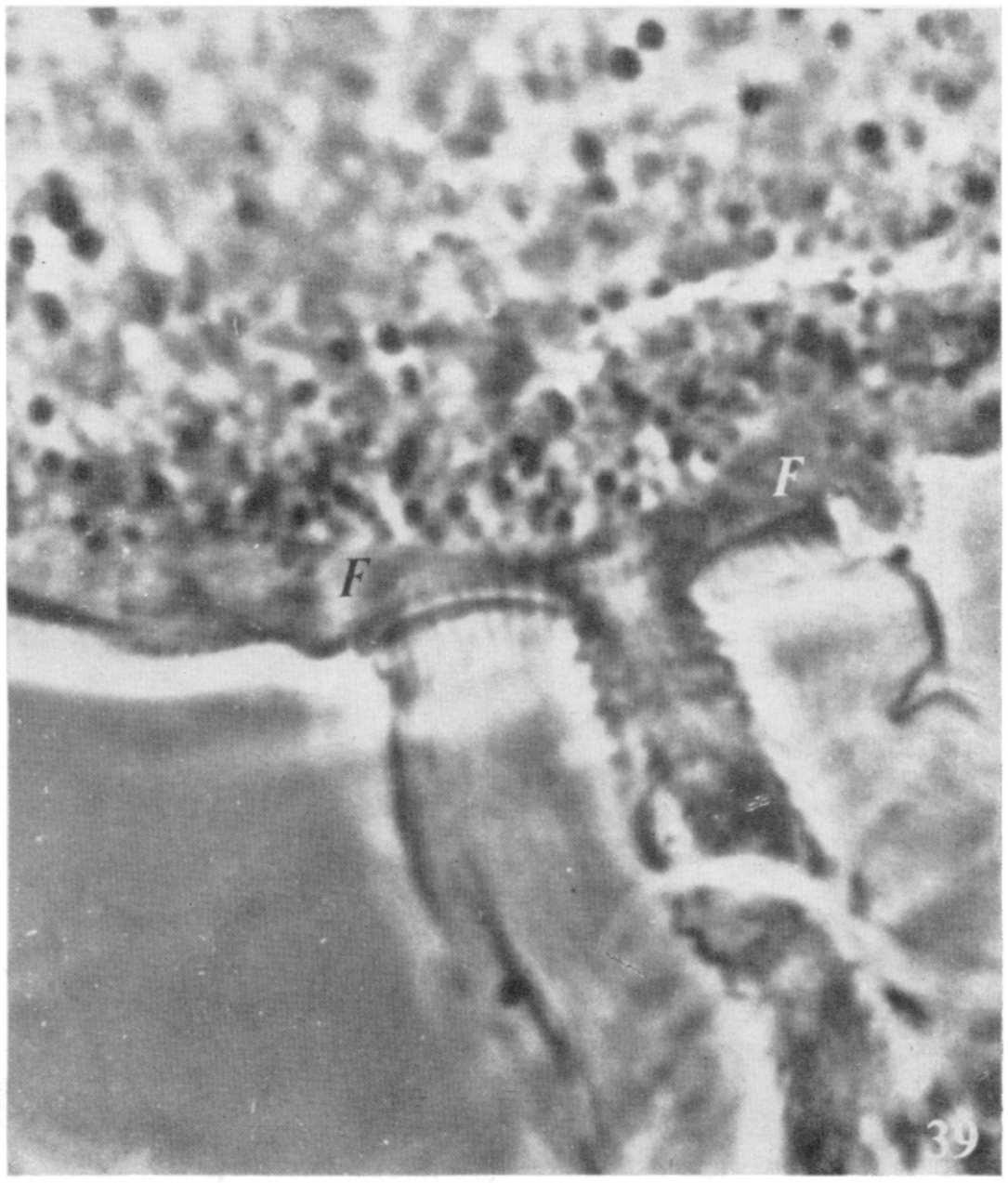












Carchesium polypinum

FIGURE 35. View of portion of living colony with the stalks extended or relaxed. The spasmonemes of individual stalks can be seen at points *S*, *S*, and the oral cilia at *OC* (×380).

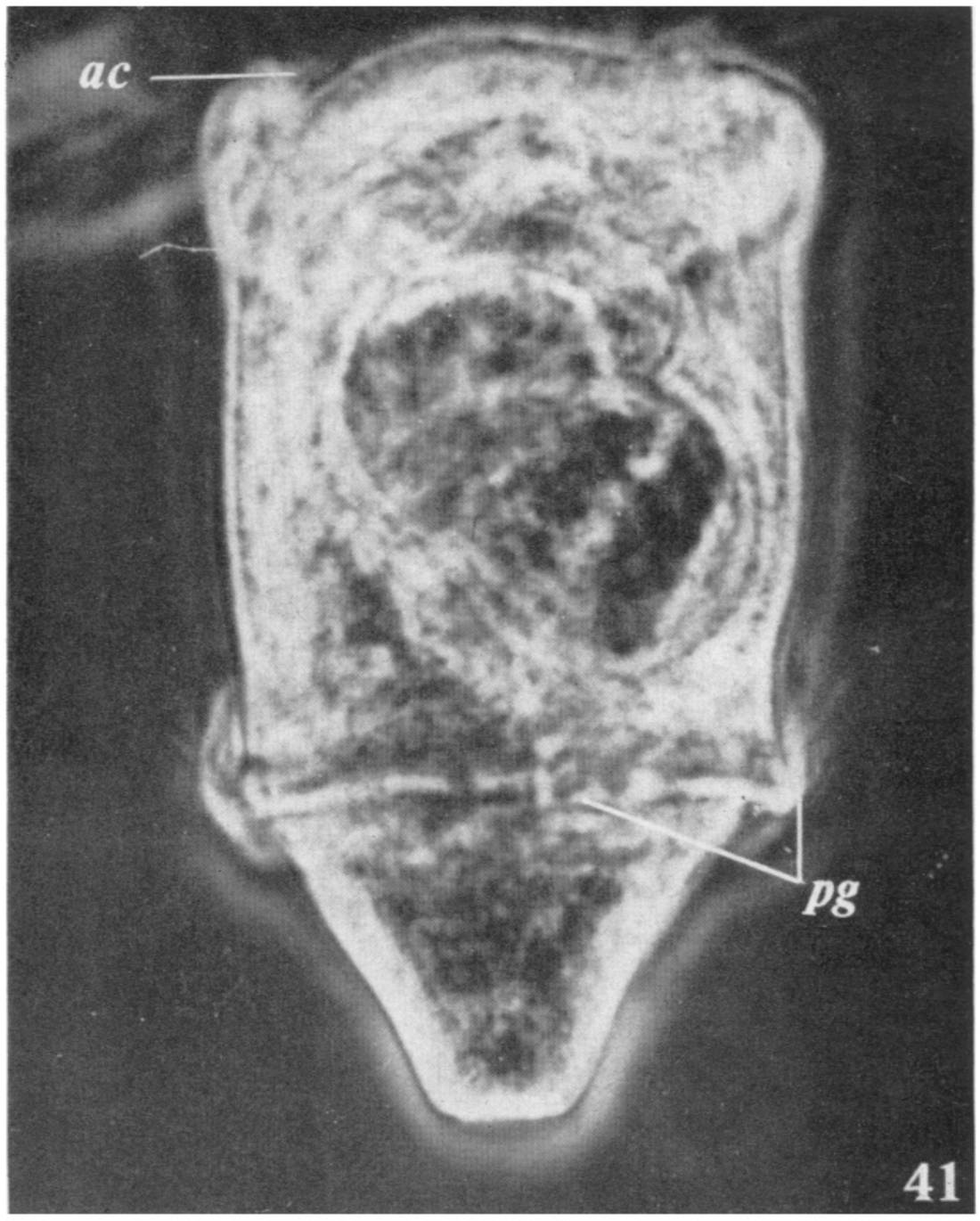
FIGURE 36. Portion of living colony that has lost its zooids. The exposed ends of the stalks are visible at *Se*. The stalks so deprived are always extended and the spasmoneme is often beaded as at *b*.

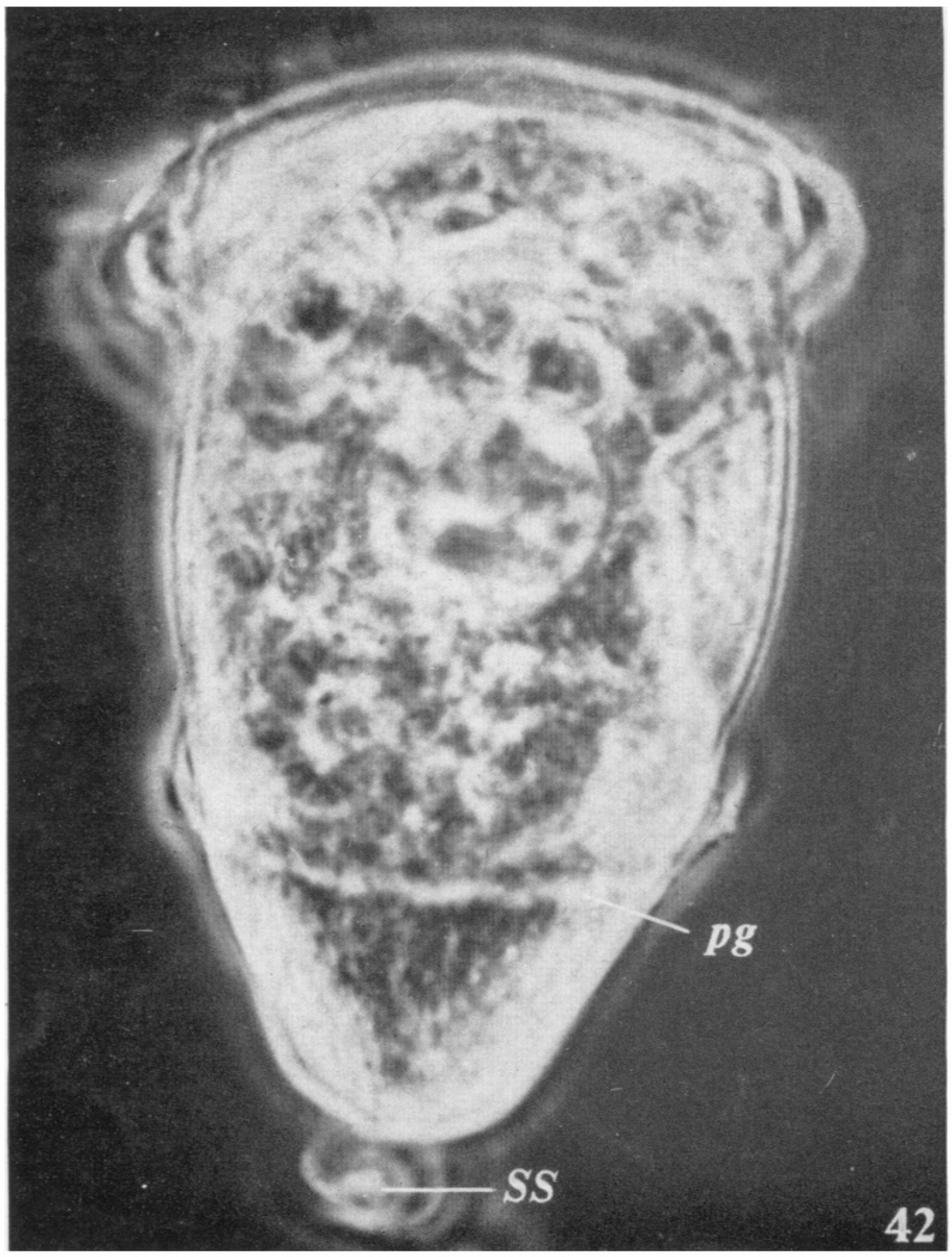
FIGURE 37. Photograph of living colony in contraction. Note the convoluted contracted stalks *CS* and that the spasmoneme *SM* takes the shortest path within the envelope *E* of the stalk. The zooids *CZ* have all changed shape and are quite different from the 'relaxed' zooids *RZ* of figure 35. The oral cilia are also withdrawn (×208).

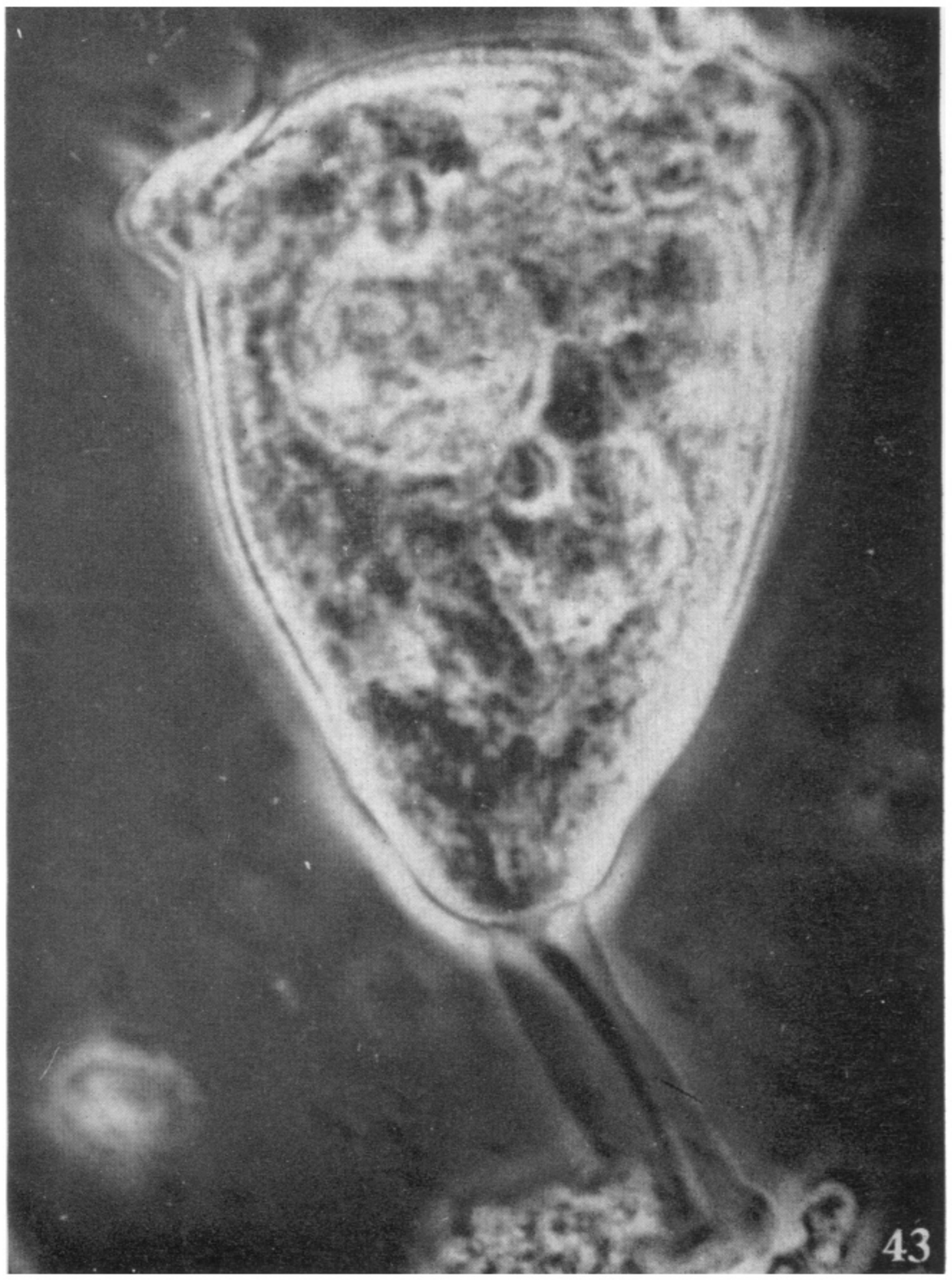
FIGURE 38. Photomicrograph of transverse section in which both the internal spasmoneme *SM* and the annular stalk tubules *ST* can be seen (×2750).

FIGURE 39. Longitudinal section of stalk and scopula showing spasmoneme and individual scopula organelles. The spasmoneme is seen to fan out in to the zooid at *F* and *F*: photomicrograph (×2750).

FIGURE 40. Enlarged photograph of stalk with spasmoneme discontinuity *D* at bifurcation (×950).





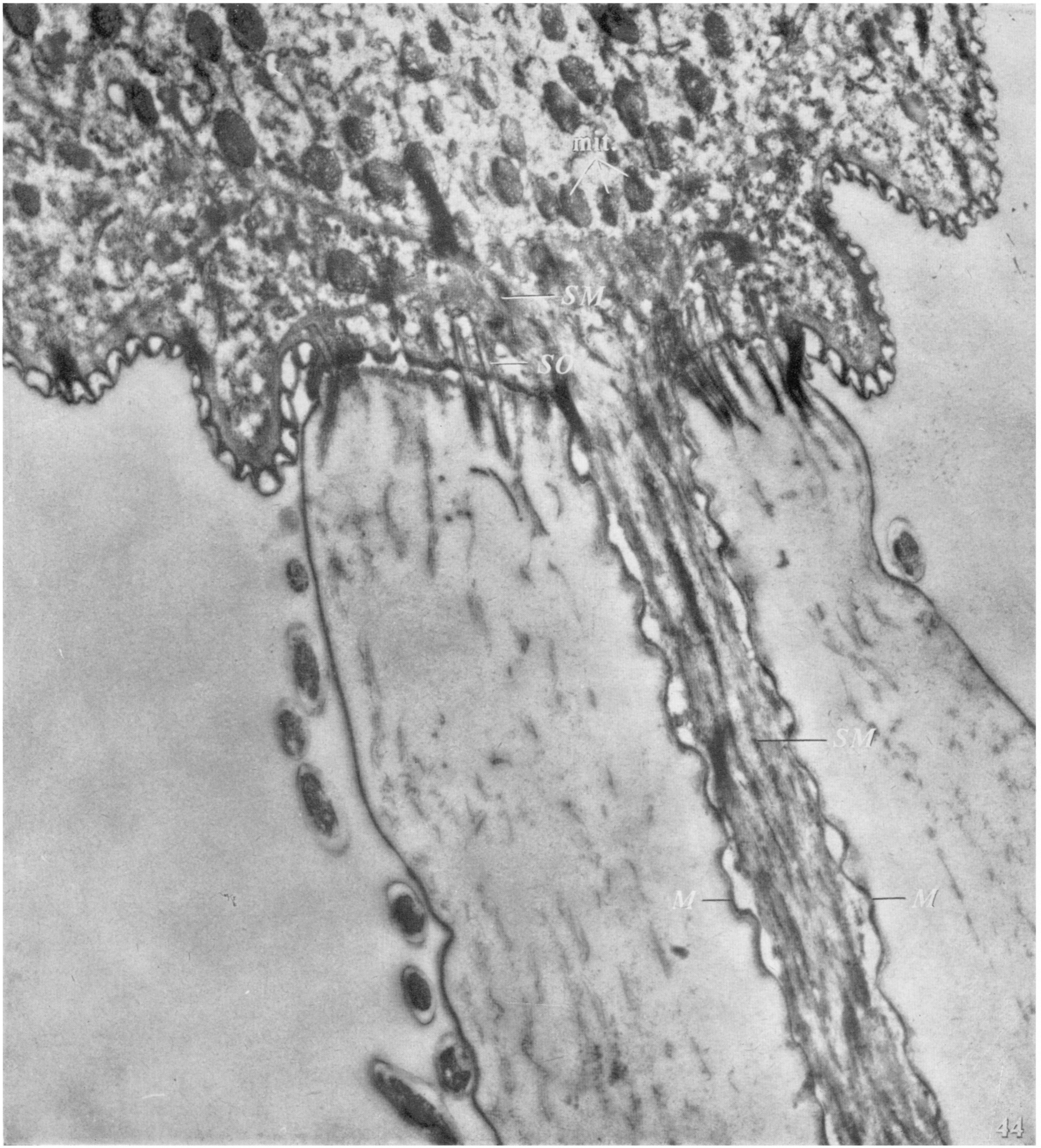


Carchesium polypinum in development

FIGURE 41. Free-swimming telotroch with apical cilia at *ac* and posterior girdle at *pg* (× 950).

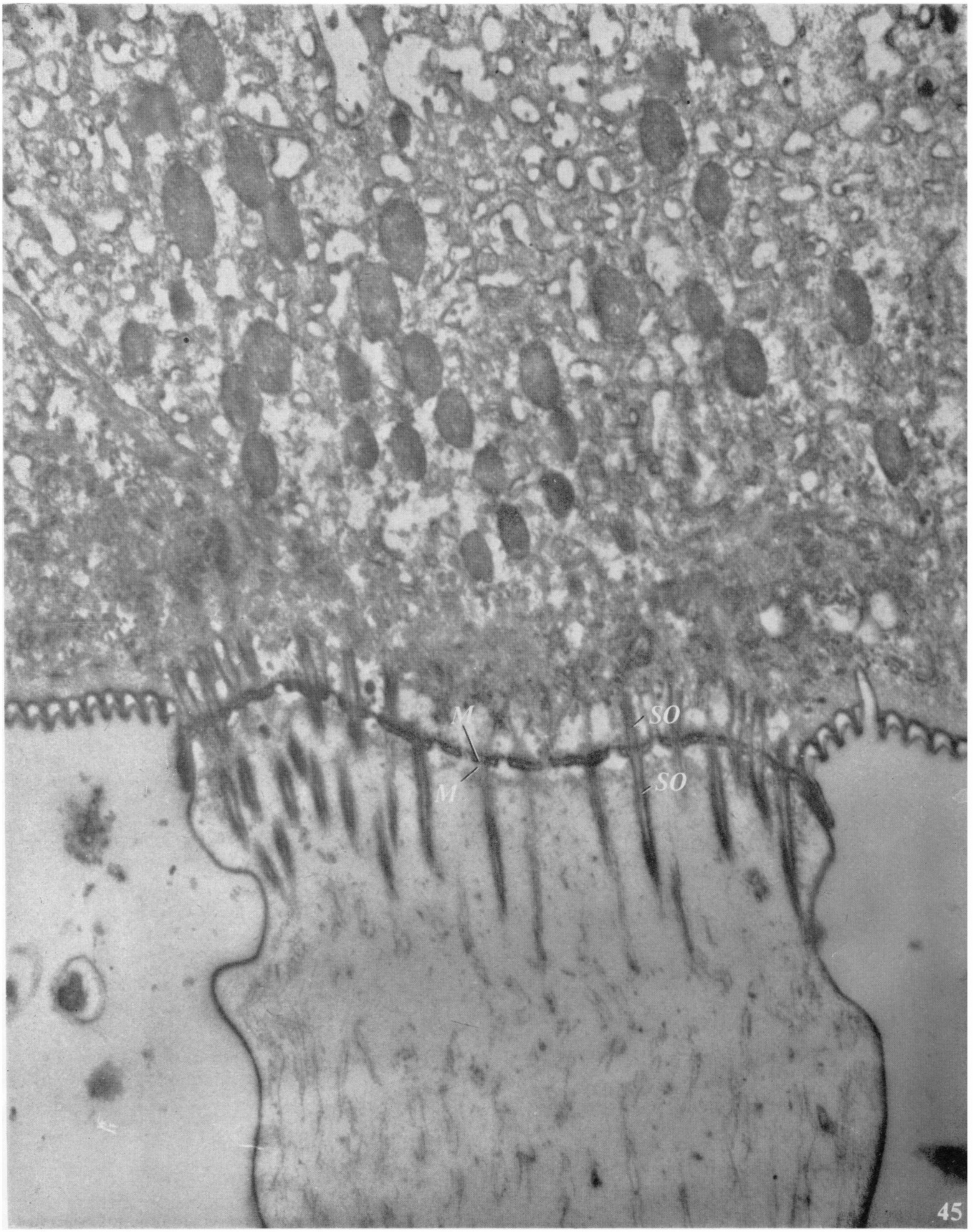
FIGURE 42. Telotroch with very short stalk *SS* attached. The posterior girdle of cilia *pg* is still present and has not yet regressed obviously (× 950).

FIGURE 43. Young adult with longer stalk. The posterior girdle has disappeared and the overall shape of the zooid has changed (× 950).



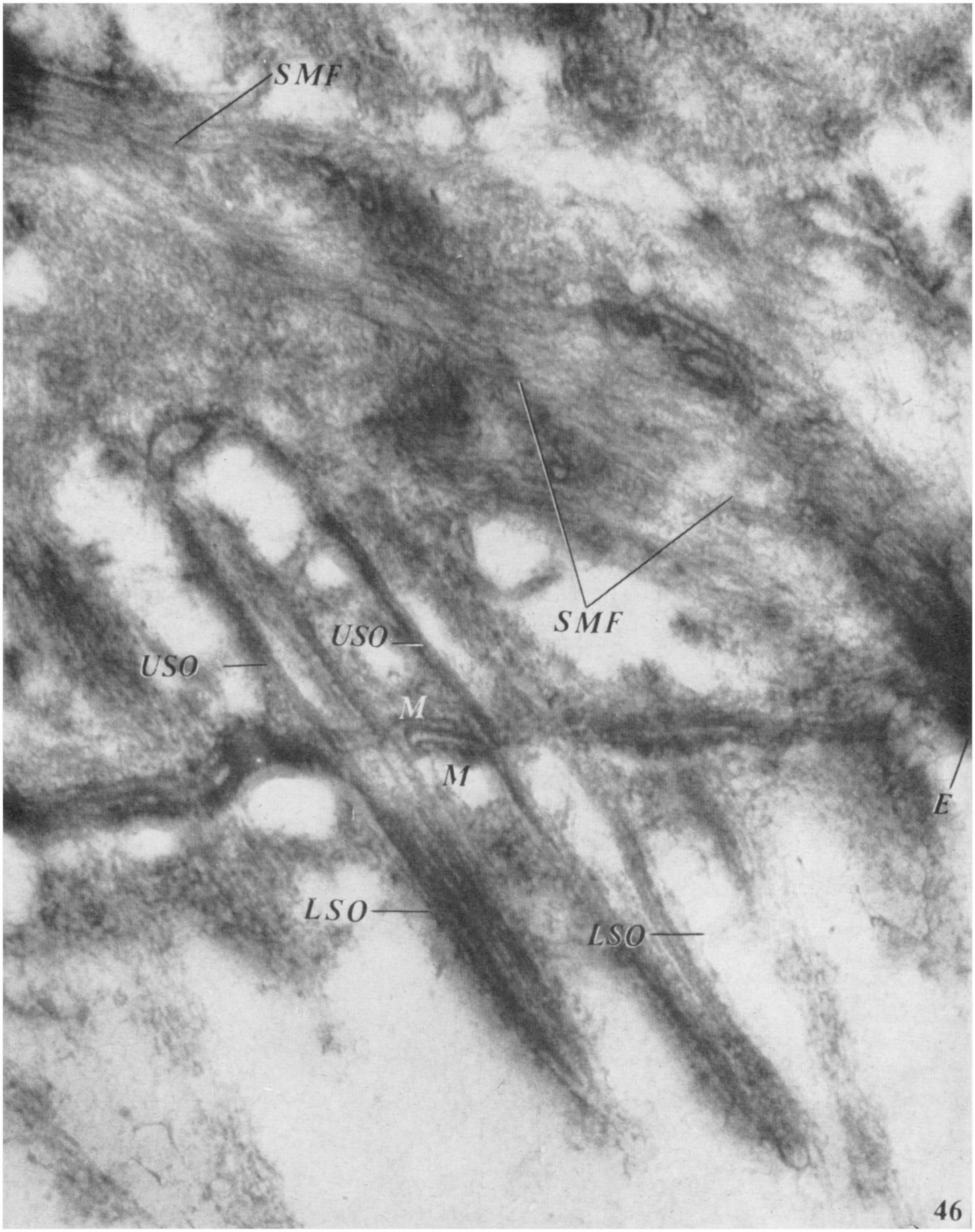
Carchesium polypinum

FIGURE 44. Longitudinal axial section of stalk, scopula and part of zooid. The spasmoneme *SM* is visible within its membrane *M* and can be seen to pass into the zooid. Scopula organelles *SO* and mitochondria (mit.) are also visible (× 13 600).



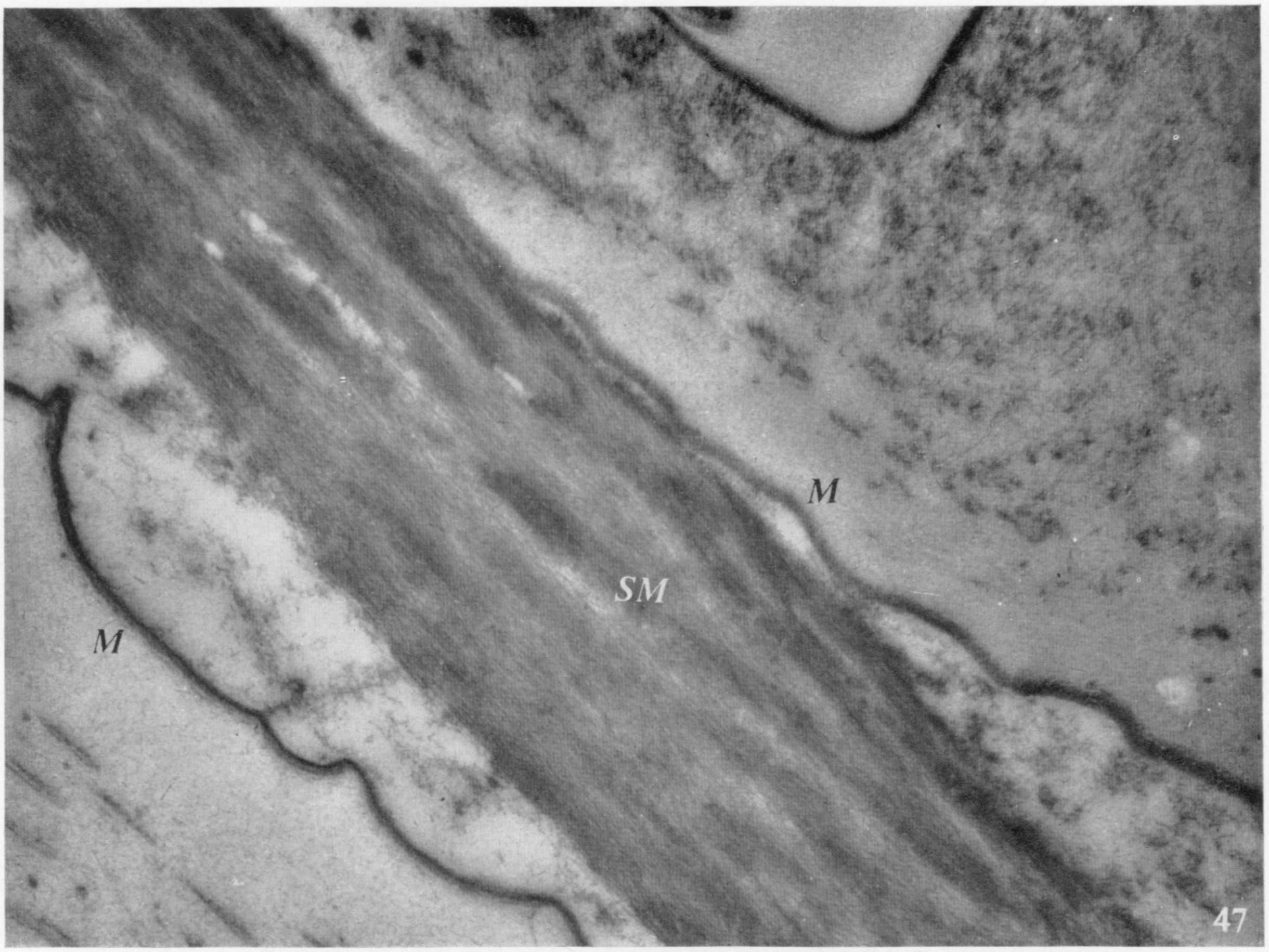
Carchesium polypinum

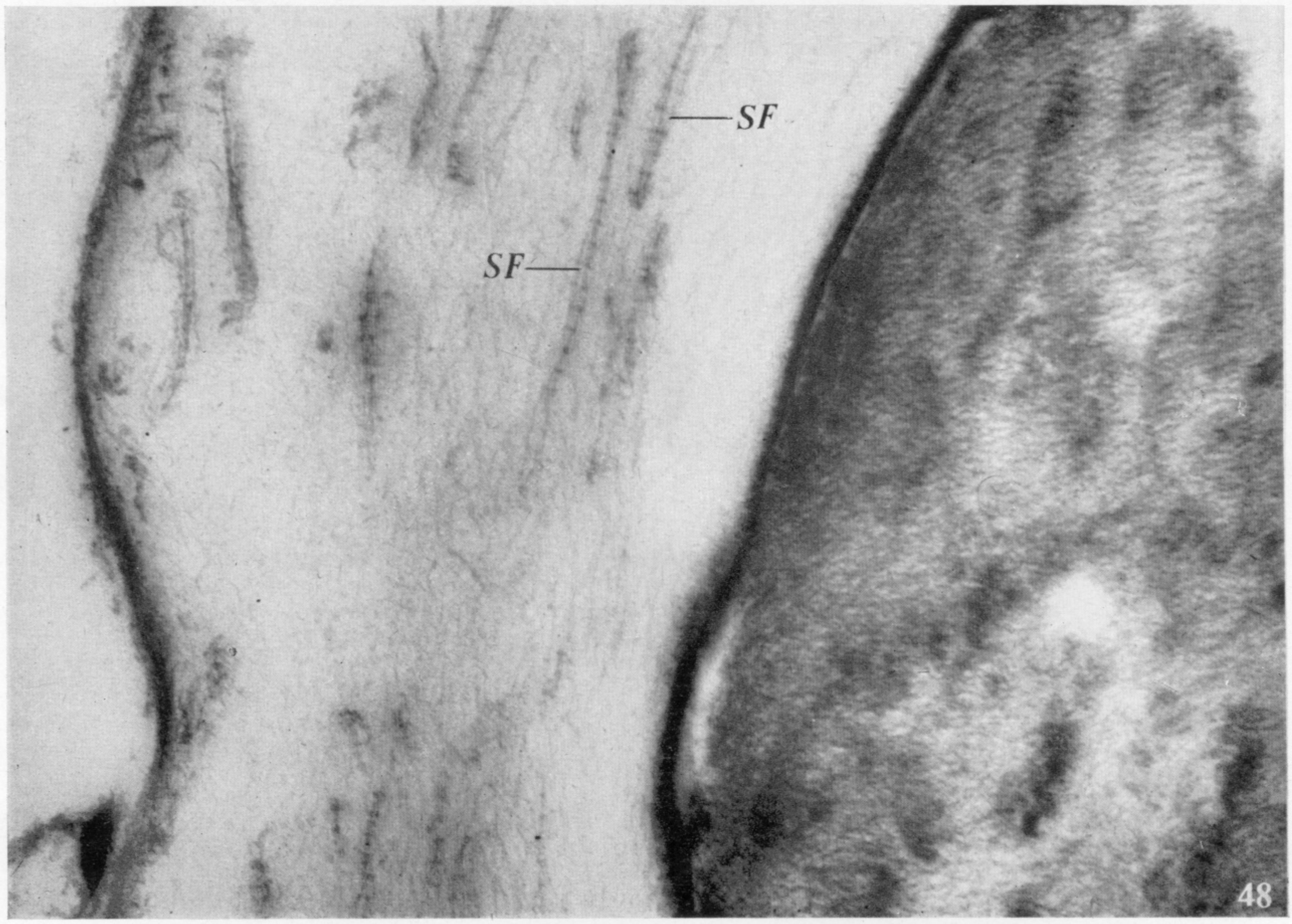
FIGURE 45. Off-axis longitudinal section in which the spasmoneme is not visible because the section is cut closer to the surface of the stalk. This micrograph is particularly useful in demonstrating the specialized form of scopula membrane *MM* and scopula organelles *SO* (× 28000).



Carchesium polypinum

FIGURE 46. Higher magnification electron micrograph of part of scopula and zooid. The upper (*USO*) and lower (*LSO*) parts of two scopula organelles are shown and the complex scopula membrane can be seen at *MM*. This micrograph shows part of the spasmoneme emerging from the stalk into the zooid at *E*. The spasmoneme fibrils *SMF* spread out fan-wise to the upper left hand corner of the plate (× 90 800).

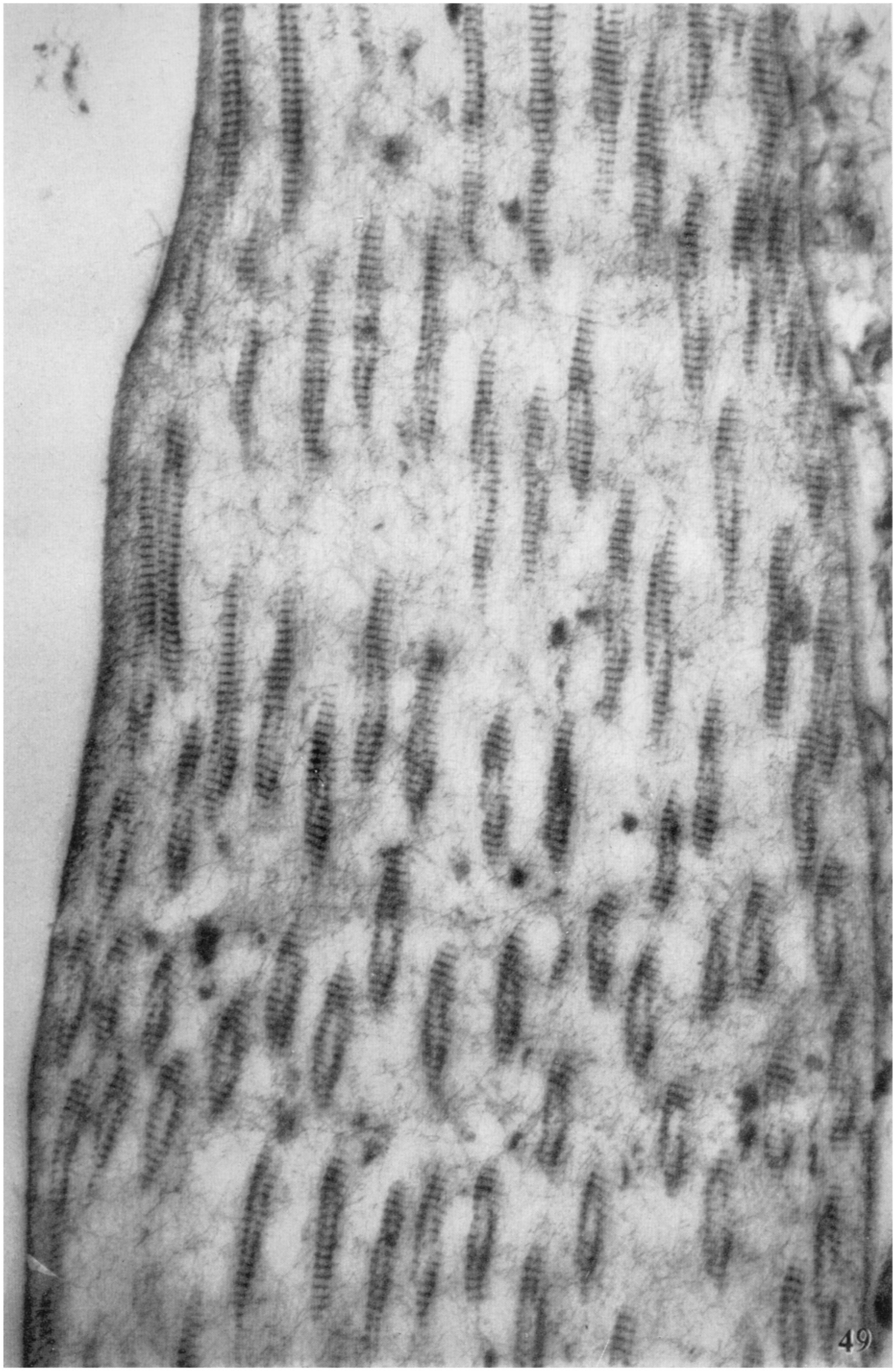




Carchesium polypinum

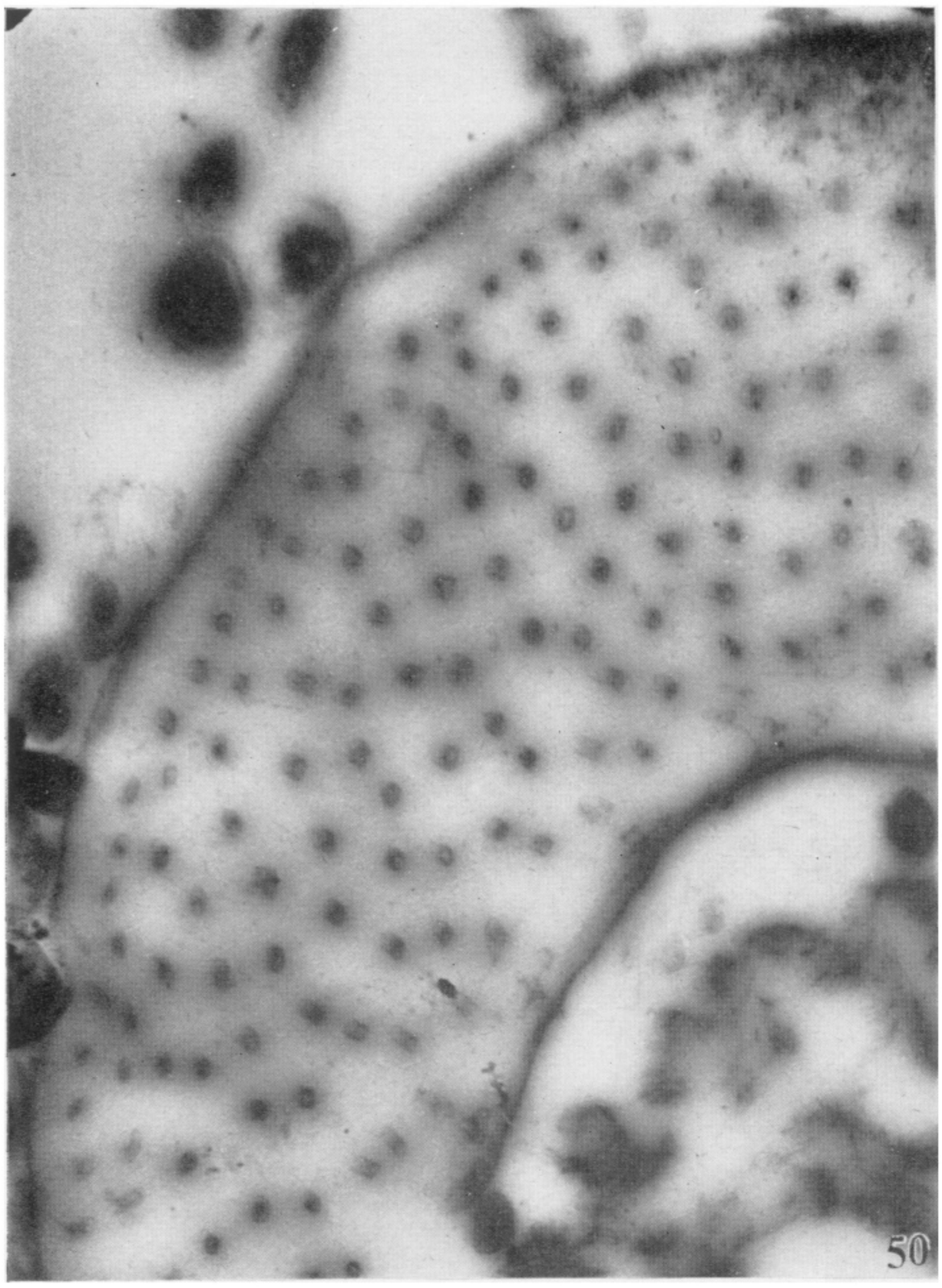
FIGURE 47. Longitudinal section of part of stalk: individual fibrils of the spasmoneme *SM* are visible (× 27 200). The irregular membrane of the spasmoneme canal is seen at *M*, *M*.

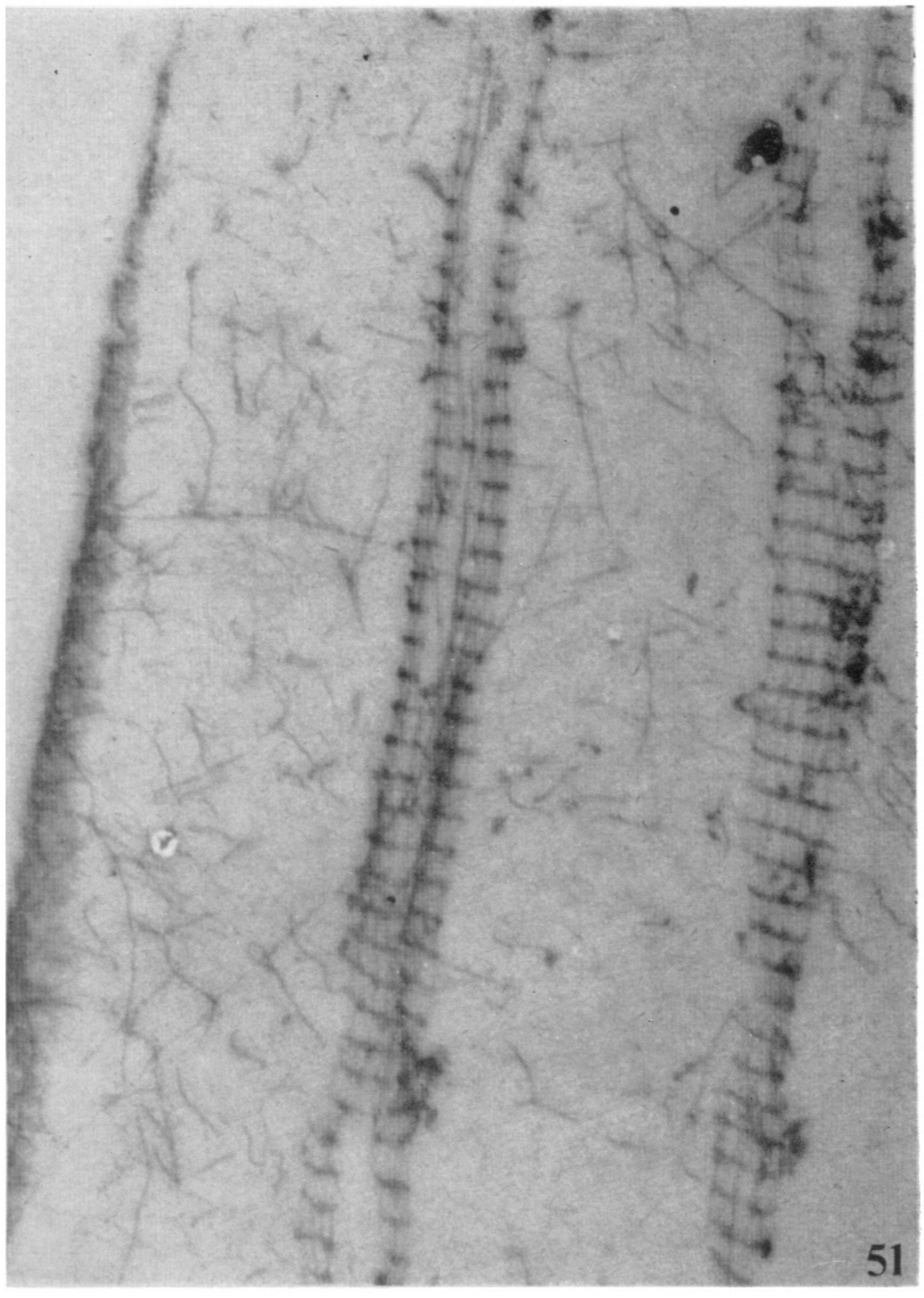
FIGURE 48. Oblique section of stalk. Note individual fibrils of spasmoneme and striated fibrils *SF* of the annular tubules (× 58 000).

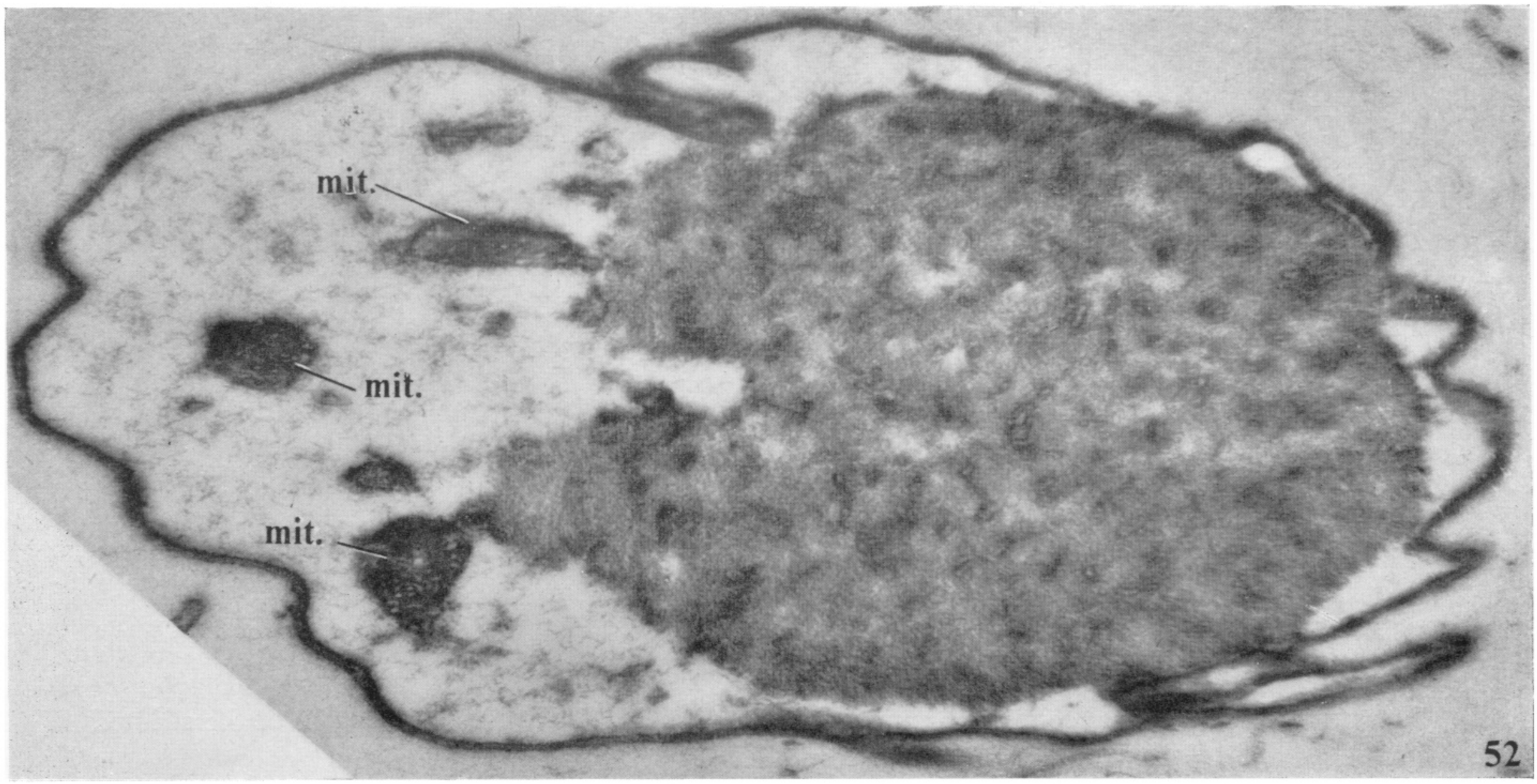


Carchesium polypinum

FIGURE 49. Longitudinal section of annular region of stalk filled with short lengths of the striated tubules. The matrix contains finer fibrils (×36000).





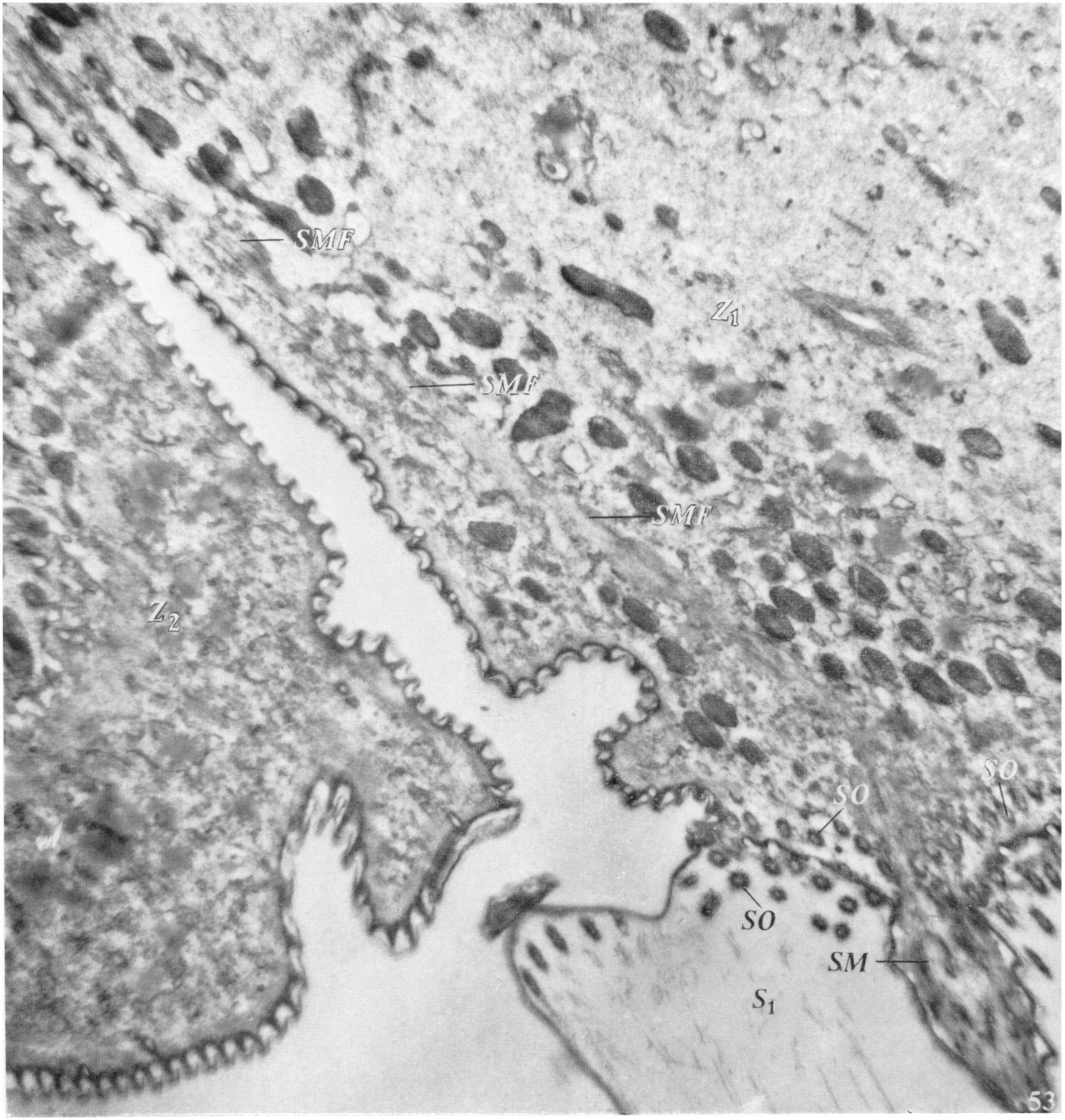


Carchesium polypinum

FIGURE 50. Transverse section of sector of stalk, showing chiefly the annular tubules (× 10 000).

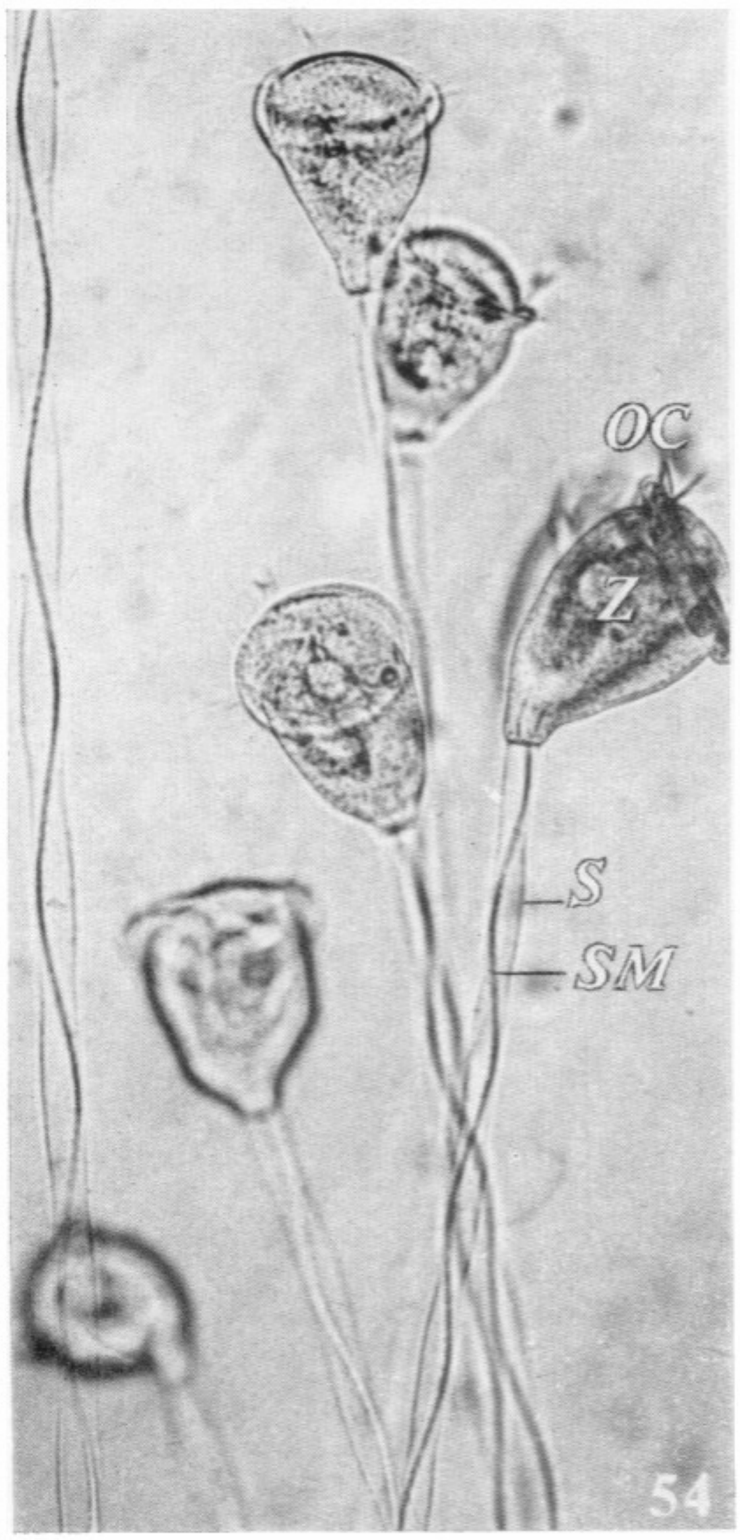
FIGURE 51. Longitudinal view at higher magnification of individual tubules. The striations and fine component fibrils are clearly visible (× 60 000).

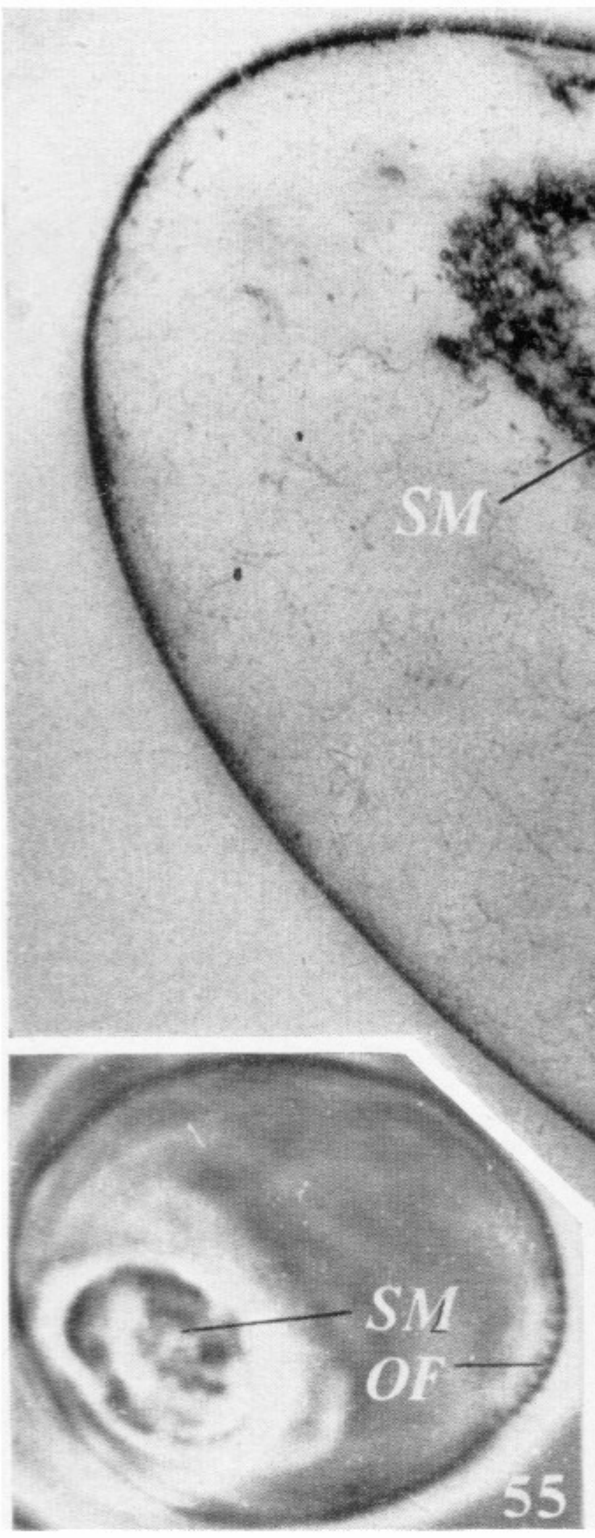
FIGURE 52. Transverse section of stalk core in which can be seen mitochondria (mit.) (× 27 200).

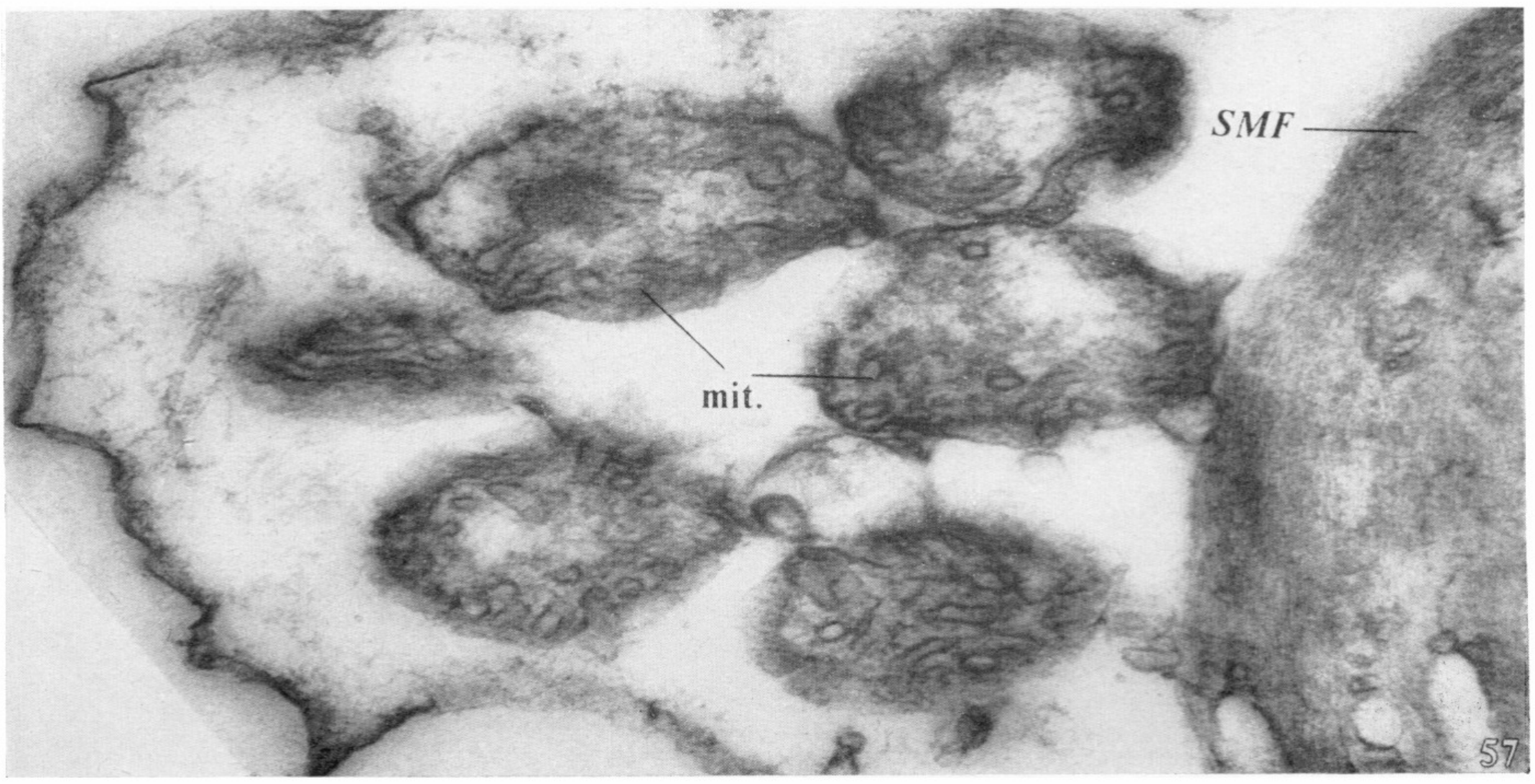


Carchesium polypinum

FIGURE 53. Oblique section through part of a zooid Z_1 and its stalk S_1. Another zooid Z_2 shows at the left of the plate. Almost transverse sections of scopula organelles at points *SO* shows them to contain component peripheral fibrils. The spasmoneme *SM* passes into the zooid and its fibrils may be seen to extend to points *SMF* (× 44400).







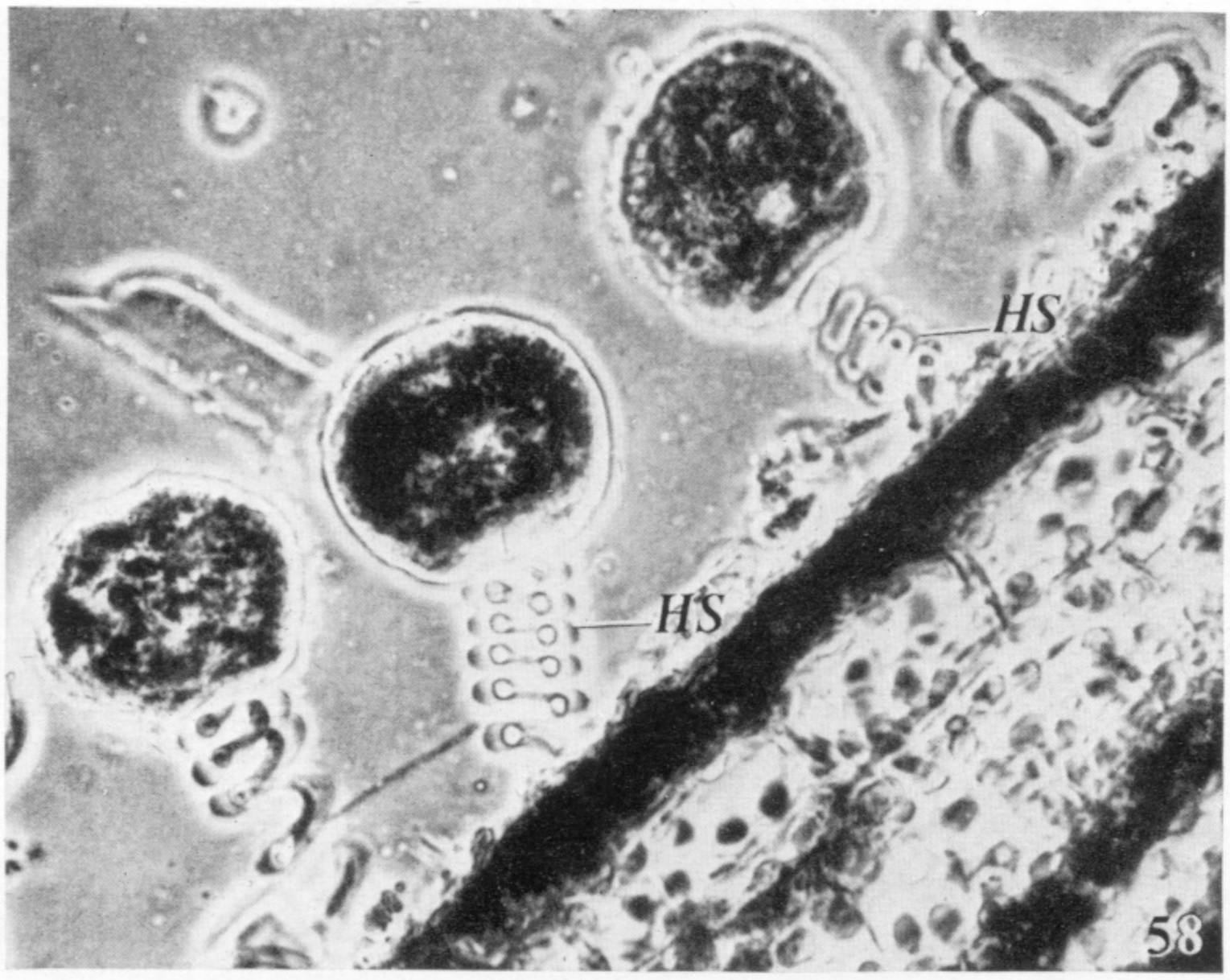
Vorticella campanula

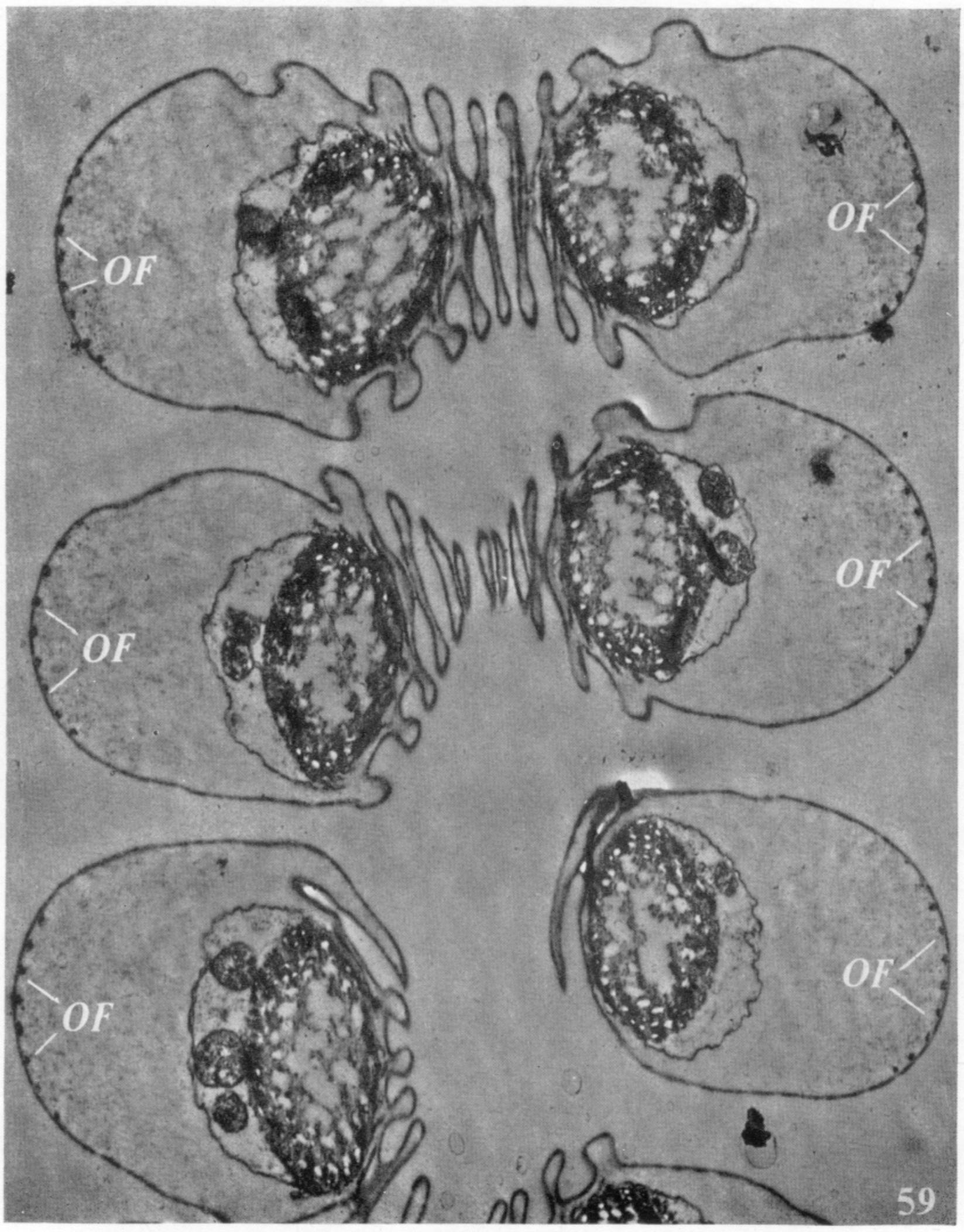
FIGURE 54. Living group of individuals. Note the zooid *Z* with the oral wreath of cilia *OC* and the curved (helical) path of the spasmoneme in the stalk *S* (× 400).

FIGURE 55. Photomicrograph: transverse section of stalk. Spasmoneme *SM* and outer fibres *OF*, cf. figure 56 (× 2750).

FIGURE 56. Electron micrograph low magnification. Transverse section of stalk with poorly fixed spasmoneme *SM*, outer fibres *OF* and matrix fibrils *mf* (× 30 000).

FIGURE 57. Oblique section of stalk core with individual fibrils *SMF* of spasmoneme resolved. The stalk canal contains mitochondria (mit.) (× 76 400).





Vorticella campanula

FIGURE 58. Fixed organisms in section to show the helical character of the contracted stalks, *HS* (× 350).

FIGURE 59. Low-power electron micrograph. Longitudinal section of stalk through several turns of contracted helix. The *OF* fibrils are always on the outer rims of the coils (× 6320).

Vorticella campanula

FIGURE 60. Slightly oblique section of outer part of stalk. The *OF* fibres are composite and the matrix fibrils beaded (× 46 200).

FIGURE 61. Oblique section. Composite *OF* fibres (× 36 000).

FIGURE 62. Almost longitudinal section of one *OF* fibre (× 58 000).

FIGURE 63. Almost transverse section of three *OF* fibres. This sections also shows the triple layer character of the stalk wall (× 70 200).

FIGURE 64. Somewhat oblique section of *OF* fibres and of stalk wall which is finely striated (× 108 000).