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Silicification of `Cell Walls' of Certain Protistan Flagellates [and Discussion]

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Silicification of 'cell walls' of certain protistan flagellates

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[Plates 1 and 2]

Silica deposition is described for two protistan flagellates, *Synura petersenii* (Chrysophyceae, algae) and *Stephanoeca diplocostata* (Choanoflagellida, Protozoa). Both taxa produce silica units intracellularly and subsequently assemble them outside the protoplast to form a 'cell wall'. In *Synura* the cell wall consists of a scale case to which scales are added throughout the cell cycle. In *Stephanoeca* individual siliceous, costal strips are accumulated outside the protoplast and assembled into a lorica once sufficient strips have been produced. In both taxa silica is laid down within silica deposition vesicles (s.d.vs) of uncertain origin. Microtubules are involved in the orientation and support of s.d.vs during early stages of silica unit biogenesis. Detailed comparisons of silica deposition are made between *Synura* and *Stephanoeca* and between these and other silica-depositing protistans.

Introduction

Members of several distinct protistan groups use silica for the production of skeletons or 'cell walls'. Within the algae the diatoms, silicoflagellates and some members of the Chrysophyceae are major users of silica although certain taxa in other algal classes may in minor ways incorporate silica into their cell walls. Within the Protozoa the major silica users include the Radiolaria, Heliozoa, testate amoebae and the loricate choanoflagellates. These groups have been much studied by microscopists since the mid-nineteenth century on account of the aesthetic and biological interest of their silicified components.

However, of all the protistan groups mentioned above, the ultrastructure, physiology and biochemistry of silica deposition have only been studied extensively in the diatoms (Werner 1977). The major reason for this is the relative ease with which many diatoms can be cultured, in some cases axenically and synchronously. These organisms are also important ecologically in marine and freshwater habitats and play an important role in aquatic silica cycles.

This imbalance in information is now being redressed by current interest in other groups of silica-depositing organisms such as the Chrysophyceae (algae) and Choanoflagellida (Protozoa), which are to be discussed here (see Leadbeater (1981); McGrory & Leadbeater (1981); for references). For present purposes, one representative has been chosen from each group: *Synura petersenii* Korshikov from the Chrysophyceae and *Stephanoeca diplocostata* Ellis from the Choanoflagellida. These will be described separately and then comparisons will be made in the discussion.

Description of *Synura petersenii* (Chrysophyceae)

This species is a motile, colonial, pigmented chrysomonad common in freshwater. Each colony consists of up to 16 cells joined together by their posterior cytoplasmic stalks. Each cell

bears two unequal flagella coated with annular scales. In addition, the longer flagellum bears a bilateral array of heterokont hairs. Klaveness & Guillard (1975) demonstrated a silicon requirement for growth of *S. petersenii*. They also showed that the amount of silicon present in the scale case was comparable, per unit area, to some diatoms.

The scale case

Each cell is covered by a single layer of overlapping siliceous scales. Individual scales are oval to elliptical in shape, those on the stalk being smaller than those on the remainder of the cell. Although minor variations in morphology and patterning do occur, especially in relation to the age and physiological condition of a culture, the general features of all scales can be seen in figure 1, plate 1. Each scale consists of a flat base plate with a central hollow chamber, prolonged into a point anteriorly, present on the outward facing (distal) surface. An upturned, inflexed rim is more or less limited to the posterior end of the plate. Additional features include a series of transverse ridges crossing the plate and many tiny perforations in the plate itself with fewer and larger equivalents in the wall of the chamber. The rim itself is without perforations but is sufficiently transparent for those in the underlying plate to show through. It is unnecessary in the present context to deal in detail with the complex morphology of the chamber except to note its asymmetrical position, the prolongation into a spine at one end (known to be anterior) and the large hole in the subtending plate visible beneath the spine.

Entire scale cases can be cleanly isolated by treatment of cells with 1% (by volume) Triton X-100 detergent. Scales forming a case are arranged in diagonal rows with a precise and consistent imbrication. So when a scale case is viewed from the outer surface with the anterior (flagellar) pole forwards, the anterior edge of each row of scales can be seen to overlap the posterior edge of the row in front.

In most sections of scales, the silica of the base plate and chamber appears disjointed in a manner almost certainly expressing the perforations already noted (figures 2 and 4). At average

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Description of plate 1 (*Synura petersenii*)

Figure 1. Shadowcast whole mount of two scales. (Magn. × 10000.)

Figure 2. Vertical section of scale showing base plate, rim (r.) and median chamber (c.). (Magn. × 50000.)

Figure 3. Vertical section of HF treated scale. (Magn. × 50000.)

Figure 4. Attachment of two adjacent scales. Arrows denote layer of adhesive material holding scales together. (Magn. × 50000.)

Figure 5. Part of a s.d.v. not associated with a chloroplast. Note deposit of electron-opaque material on either side of cisterna and microtubules near the s.d.v. (Magn. × 50000)

Figure 6. Vertical section of a scale-shaped s.d.v. Densely staining filaments subtending s.d.v. except in mid-region. Microtubules overlie s.d.v. Periplastidial e.r. denoted p.e.r. (Magn. × 50000.)

Figure 7. Part of a s.d.v. before silica deposition. Note electron-opaque granules on inner surface of bounding membrane (arrows). (Magn. × 70000.)

Figure 8. Part of the s.d.v. from figure 6 showing microtubules superimposed over rim. Early stage of scale deposition inside s.d.v. (Magn. × 75000.)

Figure 9. Intermediate stage in scale deposition. (Magn. × 75000.)

Figure 10. Mature scale in s.d.v. Note trilaminate substructure of scale rim and layer of diffuse material on distal surface of rim. (Magn. × 75000.)

Figure 11. Shadowcast whole mount of HF treated scales. Scale base plate (b.) and rim (r.). (Magn. × 10000.)

Figure 12. Whole mount of misshapen scales from a cell treated with colchicine (compare with figure 1). Note deformities to rim and median chamber. (Magn. × 10000.)

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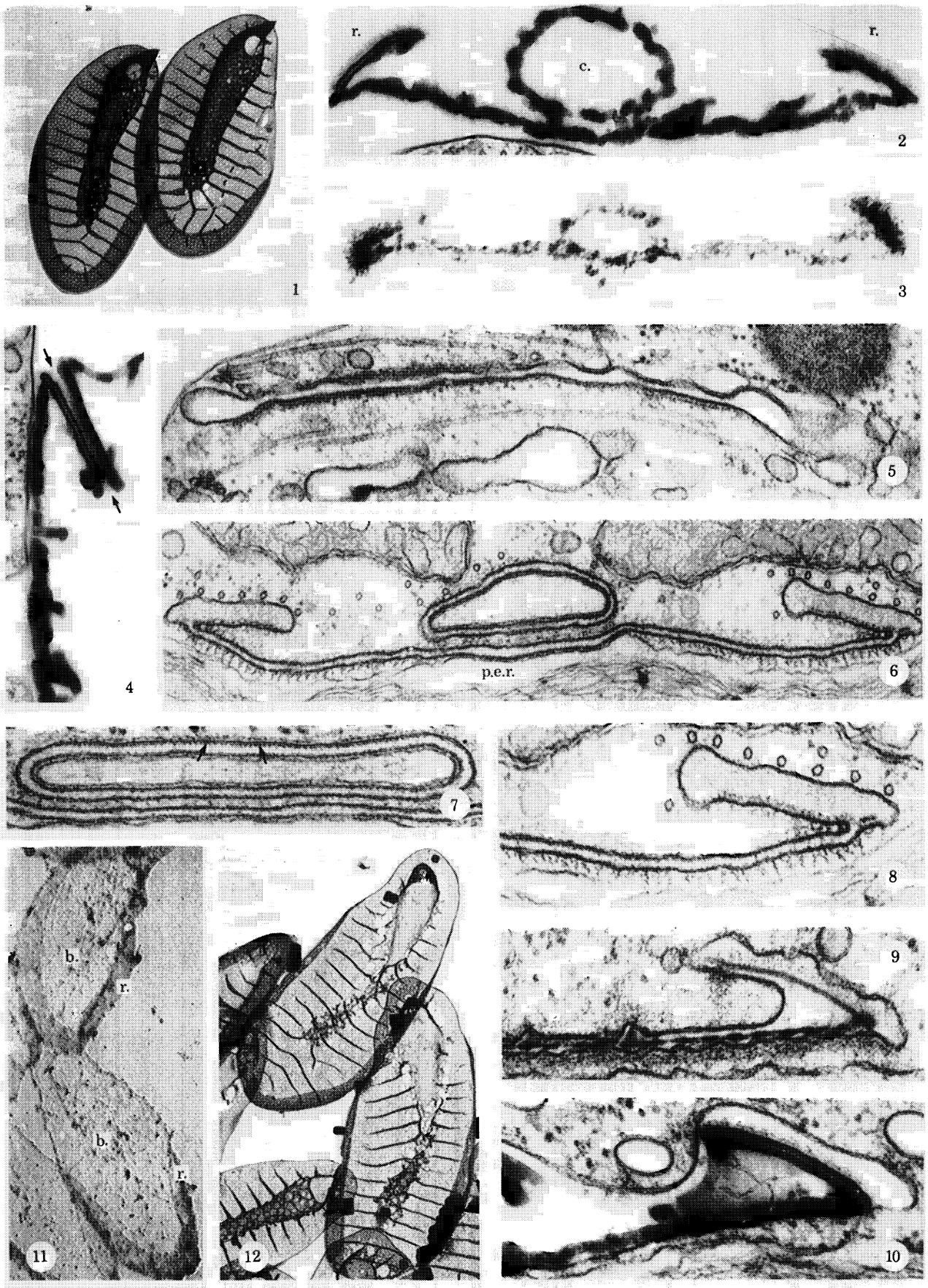

FIGURES 1–12. For description see opposite.

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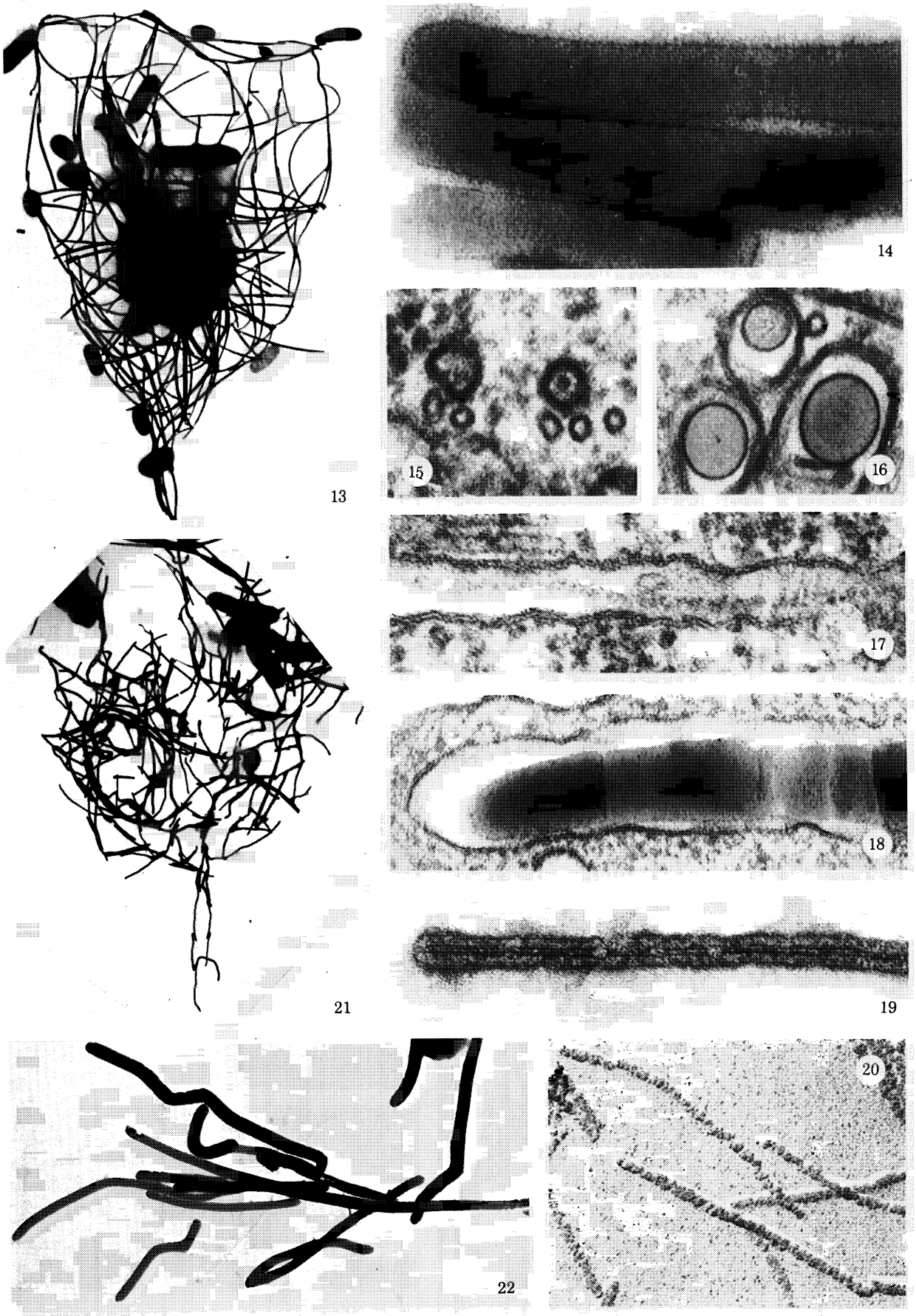

FIGURES 13–22. For description see opposite.

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magnifications, little internal differentiation of the silica can be observed in regions other than the rim. In contrast, the upturned rim has a trilaminate internal substructure with continuous silica separated by a layer of minute spherical chambers (figures 2 and 4). The outward facing surface of the rim is distinctive in possessing a superficial deposit of diffuse material arranged in one or two layers. In intact scale cases, this material is sandwiched between overlaps of adjacent scales (figure 4, arrows) and is probably the adhesive substance that keeps the case intact.

Treatment of scales with hydrofluoric acid

Silica is completely removed from scales after treatment with 2% (by volume) HF for 10 min. Scale shaped, thin, electron-transparent plates then remain but features such as the rim and median chamber can still be discerned though with difficulty (figure 11). The non-siliceous component responsible for this appearance can also be seen in sections of scales treated with HF before embedding. Under these conditions (figure 3) the substance of the base plate and chamber wall appears to be irregularly fibrillar, the rim being denser and more compact.

Biogenesis of scales

There are now several independent descriptions of scale production in *Synura* and related genera (see, for example, Schnepf & Deichgräber 1969; Wujek & Kristiansen 1978; McGrory & Leadbeater 1981; Mignot & Brugerolle 1982). Salient, agreed findings of these workers can be summarized as follows. Scales are produced individually within membrane-bounded, silica deposition vesicles (s.d.vs), which at early stages of development are closely appressed to the periplastidial endoplasmic reticulum (p.e.r.) on the outer surface of only one of the two chloroplasts. Before silica deposition, the s.d.v. is a flattened cisterna, but this shape is soon altered into that of a 'mould' of the mature scale. During this process, the periphery of the s.d.v. becomes upturned and inflexed while the centre takes the shape of the median chamber. The latter involves the active intervention of the p.e.r., which penetrates the region of the plate from beneath thereby producing a diverticulum in the shape of the future median chamber. At this stage in s.d.v. development, rows of densely staining filaments become conspicuous in the gap between the s.d.v. and p.e.r. except in the central region of the future scale. Concurrently the distal surface of the s.d.v. is covered by microfilaments and is overlain by a system of spaced microtubules. Mignot & Brugerolle (1982) have further shown that there

DESCRIPTION OF PLATE 2 (*Stephanoeca diplocostata*)

FIGURE 13. Whole mount of cell. (Magn. × 5000.)

FIGURE 14. Longitudinal section of newly formed costal strips showing homogeneous substructure and densely staining surface. (Magn. × 150000.)

FIGURE 15. Transverse section of early stages in strip deposition showing s.d.vs subtended by two microtubules. An extra microtubule on the right is associated with a s.d.v. not seen in this section. (Magn. × 150000.)

FIGURE 16. Transverse section of late stage in deposition. Note minute markings in centre of strips. (Magn. × 150000.)

FIGURE 17. Longitudinal section of juvenile strip in s.d.v. before rigidity is achieved. (Magn. × 150000.)

FIGURE 18. Nascent mature strip in s.d.v. (Magn. × 150000.)

FIGURE 19. Partially dissolved costal strip showing central longitudinal channel. (Magn. × 150000.)

FIGURE 20. Strips produced by cell grown in silica deficient medium. (Magn. × 10000.)

FIGURE 21. Misshapen lorica formed from misshapen costal strips produced by a colchicine treated cell. (Magn. × 5000.)

FIGURE 22. Misshapen costal strips produced as a result of colchicine treatment. (Magn. × 20000.)

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is a helical, antero-posterior movement of developing s.d.vs so that, in general, newly formed s.d.vs are to be found at the anterior end of the cell and almost mature scales towards the posterior end.

The results presented here (figures 5–10) confirm the general findings outlined above together with some new observations. These include more precise information relating to the s.d.v. and developing scale. The earliest stages in the formation of the s.d.v. that can be unequivocally recognized as such show the cisterna to be flattened and covered on both surfaces by a layer of diffuse, opaque material (figure 5). At this stage, part at least of the cisterna may not be in contact with chloroplast surface although e.r. and microtubules are usually present close by. The s.d.v. may be close to the dictyosome though not parallel to its cisternae. Occasionally, scale-shaped s.d.vs have been observed in an extreme anterior position though generally at this stage of development they are positioned laterally. As the shape of the s.d.v. changes from flat to that of the scale, many densely staining filaments become evident between the proximal surface of the s.d.v. and the p.e.r. (figure 6). Microtubules are also common at this stage. They are oriented parallel to the longitudinal axis of the scale and although they are not exclusively associated with the rim or median chamber they are particularly evident in these regions (figures 6 and 8). Arms on the microtubules, possibly associated proteins, appear to link the microtubules with the surface of the s.d.v. in the region of the rim (figure 8). Also present, but not always clearly observable, are microfilaments spread over the upper surface of the s.d.v.

Scale deposition in the shaped s.d.v. begins with the appearance of a thin layer of electron-opaque material in the rim (figure 8) and of a layer of electron-opaque granules on the inner surface of the s.d.v. in regions other than the rim (figure 7, arrows). Subsequent scale development proceeds more or less evenly throughout the s.d.v. After silica deposition has begun, alternating patches of electron opacity indicate onset of the system of perforations previously noted, especially in the base plate (figure 9). The initiation of transverse ridges is also detectable. At this stage the scale rim is less electron opaque than the denser parts of the base plate, presumably thereby denoting a difference in composition (figure 9). Throughout these early stages the s.d.v. fits closely around the base plate and chamber, although it is always loose around the rim (figure 9). Once silica deposition has begun, the thick filaments subtending the s.d.v. and the overlapping microtubules disappear. As silica deposition proceeds, the anatomy of the scale becomes increasingly similar to that of mature scales outside the plasmalemma. In particular, the transverse ridges increase in size and the rim acquires its trilaminate substructure (figure 10). The coating of diffuse material also appears on the distal surface of the rim so that, even in this respect, similarity with mature, extruded scales is achieved.

Mature scales apparently ready for extrusion are usually located around the mid-region of the cell where they can be found near to the plasmalemma but separated from the plastid surface. The exact manner in which they are extruded is unknown as is the subsequent fate of the bounding membrane of the s.d.v. Another outstanding problem is the exact manner in which a new scale is incorported into an already existing scale case. Some mechanism permitting an intercalary insertion of a new scale seems likely to be involved.

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Colchicine treatment

Treatment of cells with a sublethal dose of colchicine (1.5×10^{-3} M) had an effect on the morphology of subsequently produced scales. Minor effects were mostly confined to the rim and median chamber. These structures could be misshapen, incomplete or even absent (figure 12). Major effects resulted in uneven silica deposition and gross deformation of all scale parts.

Description of *Stephanoeca diplocostata* (Choanoflagellida)

Loricate choanoflagellates (Acanthoecidae), to which *S. diplocostata* belongs, characteristically possess a basket-like lorica (cell wall) composed of siliceous ribs or costae usually arranged in longitudinal, transverse or spiral arrays. Costae are further composed of rod-shaped units known as costal strips. Lorica characters are of major taxonomic importance, the colourless protoplasts of different taxa being remarkably uniform in appearance. Loricate taxa are exclusively marine or brackish water inhabitants.

The sequence of events involved in the formation of a lorica is similar for all taxa so far investigated (see Leadbeater (1981) for references). Costal strips are laid down intracellularly within membrane-bound s.d.vs, extruded to the outside of the protoplast and accumulated until sufficient strips have been produced to form a lorica. Assembly of the lorica then takes place rapidly, the process only taking a few minutes. However, the interpolation of cell division into this sequence varies. In 'nudiform' taxa the protoplast divides to produce a naked swarmer, which later deposits costal strips and ultimately assembles them into a new lorica. On the other hand in 'tectiform' species costal strips are produced by a parent cell already possessing a lorica. Strips are accumulated at the top of the collar. After division, one of the daughter protoplasts is pushed out of the parental lorica carrying all the accumulated strips with it and when free, it assembles them into a new lorica (Leadbeater 1981; Manton & Bremer 1981). *S. diplocostata* undergoes tectiform replication.

Under normal conditions of growth, *S. diplocostata* takes up soluble (reactive) silicate from the medium to produce costal strips that are subsequently used to build a lorica (Leadbeater 1984). The absence of silica from the medium has no effect on growth rate but newly divided cells lack loricae. Thus the deposition of silica and the possession of a lorica are not obligate requirements for this species.

The lorica

Descriptions of lorica of *S. diplocostata* have been given elsewhere (see, for example, Leadbeater 1979). Figure 13, plate 2, illustrates the organization of costae and the morphology of the two chambers separated from each other by a waist. A lorica contains between 150–200 costal strips, each more or less identical in size and shape. Strips are curved and tapered towards the ends, which are rounded. In newly-produced mature strips, the highly amorphous silica is evenly distributed throughout (figure 14). In these, sections generally reveal a homogeneous substructure, especially when seen in longitudinal section (figure 14). In transverse section (figure 16), one or more central markings are apparent but profiles in both planes of section show intense surface staining (figures 14 and 16). Whether or not these markings denote the presence of non-siliceous components is not known. However, hydrofluoric acid treatment of whole mounts and of cells before embedding totally removes costal strips leaving no traces of a non-siliceous component.

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Once in contact with sea water costal strips begin to dissolve in a characteristic fashion. The surface and centres of strips are simultaneously affected. The surface becomes progressively roughened and pitted while a central longitudinal channel becomes apparent (figure 19). With progressive dissolution the central channel enlarges and extends from one end of a strip to the other. Eventually strips lose their rigidity and fall apart. Complete strip corrosion may only take 5–10 d according to conditions (Leadbeater & Davies 1984).

The mechanism by which strips are attached to each other is not fully understood. Strips can be separated by treatment with concentrated nitric acid, which suggests the presence of an organic cement. Mann & Williams (1982) have suggested that fine silica threads may also join adjacent strips.

Biogenesis of costal strips

Costal strips are deposited intracellularly and singly, each within a s.d.v. Exactly where the s.d.vs originate is not known but early stages are often close to cisternae of both the e.r. and the Golgi apparatus. Before and during early stages of strip production, two microtubules accompany each s.d.v. (figure 15), running along its full length on the side furthest from the plasmalemma. These microtubules may perhaps orientate and support the s.d.v. while the developing strips lack rigidity (figure 17). Once the strips have become rigid the microtubules disappear (figure 16). Fully mature strips are extruded and subsequently moved to the top of the collar. The ultimate fate of the s.d.v. is unknown.

Effect of silica limitation on biogenesis of costal strips

S. diplocostata is a very efficient user of silica and even minute quantities, unmeasurable by conventional colorimetric means, can be used. Furthermore, silica is continually recycled as a result of lorica dissolution. However, eventually, in the complete absence of reactive silicate, cells produce thinner strips, which have a diagonal or transverse cross banding (figure 20). In this state there may be some reduction in strip length though they are uniformly thin. Very thin strips lack rigidity. Absence of reactive silicate in itself has no affect on growth rate but strip production eventually ceases and cells without loricae are produced. Under conditions of complete silica starvation s.d.vs cease to be formed and the whole silica depositing apparatus is shut down.

Effect of microtubule poisons on biogenesis of costal strips

The effect of microtubule poisons, such as colchicine and podophyllotoxin, on the form of costal strips once applied to cultures is rapid. Initially affected strips are of normal length but misshapen (figure 22). Later, strips are usually shorter, thicker and highly misshapen (figure 22), presumably as a result of lack of support by microtubules during development. Microtubule poisons do not interfere with the extrusion of strips or with their subsequent movement by the protoplast and, provided mitosis is not blocked, the juvenile protoplast resulting from division will build a misshapen lorica from misshapen strips (figure 21).

Discussion

Although the pigmented Chrysophyceae and colourless Choanoflagellida are phylogenetically very remote there are, nevertheless, many similarities in the ultrastructural events related to silica deposition in these two groups. In both *Synura* and *Stephanoeca*, silica destined for the 'cell wall' is laid down in units and assembled later outside the plasmalemma. Deposition of each

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unit occurs with a s.d.v. of uncertain origin. At present there is no unequivocal evidence that a s.d.v. is solely derived from either the Golgi apparatus or the e.r. Other possibilities exist, they may be derived from a separate membrane system or by coalescence of vesicles from other organelles in the cell. In both groups silica deposition occurs evenly throughout the wall unit.

Microtubules and microfilaments are important at various stages during silica deposition. Microtubules may possibly serve the dual functions of supporting and orienting the s.d.vs before silica deposition. Once the deposited silica has sufficient rigidity the microtubules disappear, their function having been performed in the most economical manner. The effects of microtubule poisons on the biogenesis of scales or costal strips, as determined by changes in morphology, provide evidence in favour of the above interpretation of microtubular function. In *Synura* the greatest effects are to scale morphology and in particular to the shaping of the rim and chamber, exactly the places where microtubules are most prominent during their development. In *Stephanoeca*, the misshapen strips probably result from lack of support before rigidity was achieved and the short, thick strips from a lack of shaping of s.d.vs at an early stage of development. Before these experiments can be fully interpreted appropriate controls are required to ensure that the results observed are a consequence of microtubule disturbance alone.

There are significant differences associated with silica deposition between *Synura* and *Stephanoeca*. In *Synura*, e.r. in the form of p.e.r. is intimately involved in shaping s.d.vs while in *Stephanoeca* there is no such e.r. involvement. In *Synura*, scales are used to produce a scale case, which increases in size and number of scales throughout the cell cycle, whereas in *Stephanoeca*, lorica assembly is a single event, no further strips being added once the lorica has been formed. In *Synura* the cement holding the scales together is obvious and substantial whereas in *Stephanoeca* no such cement has been observed. In *Synura* there is an obvious non-siliceous component in scales whereas in *Stephanoeca* there is no such equivalent fraction in costal strips. The presence of an organic component in scales may have some bearing on the apparent fact that scales of *Synura* do not dissolve readily whereas costal strips in choanoflagellates can completely dissolve within 10 d at 20°C (Leadbeater & Davies 1984).

Other groups of silica-depositing protistans also share characters in common with those outlined above for *Synura* and *Stephanoeca*. So in diatoms and testate amoebae silica is deposited in s.d.vs and microtubules are involved in their shaping and orientation (Pickett-Heaps *et al.* 1979; Hedley & Ogden 1973). Treatment of certain diatoms with colchicine also resulted in changes of frustule morphology, primarily in the region of the raphe (Schmid 1980). As in *Synura*, the scales of testate amoebae are held together by an organic cement (Hedley & Ogden 1973).

There are certain significant differences between diatoms on the one hand and *Synura* and *Stephanoeca* on the other. The bounding membrane (silicalemma) of the s.d.v. has a more elaborate function in diatoms than the equivalent membrane in other groups. So, while during early stages of frustule deposition in diatoms the s.d.v. encloses the developing frustule but is separate from it, at later stages the silicalemma itself becomes part of the organic coating of the frustule (Volcani 1981), a function not apparent in either *Synura* or *Stephanoeca*. There is no equivalent in *Synura* and *Stephanoeca* to the organic layer coating the frustule. In *Synura* where there is a non-siliceous component, this material appears to be present within the scale rather than as a surface layer. During deposition of silica in diatoms, thin feathery fibrils, possibly of carbohydrate, extend between the deposited silica and silicalemma. These fibrils may act

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as a framework created by the silicalemma for the deposition of silica (Pickett-Heaps *et al.* 1979). No exact equivalent has been seen in *Synura* or *Stephanoeca* although it is possible that granules on the inner surface of the s.d.v. of *Synura* (figure 7) are part of a deposition framework.

Protistan flagellates have much to contribute to our understanding of the use of silica in biological systems. They have the advantage of being unicellular organisms; they are relatively easy to grow and they respond rapidly to experimental treatments. Experimental and ultrastructural work is now in progress on both *Synura* and *Stephanoeca* to investigate some of the outstanding problems associated with silica use by these species.

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Discussion

W. C. Jones (*School of Animal Biology, University College of North Wales, U.K.*). Does the curvature of the costal rods of *Stephanoeca* always lie in a single plane and, if so, how is the plane of curvature related to the arrangement of the pair of microtubules alongside the vesicle in which the costal rib is formed? I am interested because megascleres of siliceous sponges exhibit uniplanar curvature, as do the rays of calcareous sponge spicules, and it is possible that microtubules are involved in the determination and maintenance of the plane of curvature.

B. S. C. Leadbeater. The curvature of the costal rods is indeed uniplanar, but the relation, if any, between the plane and the pair of microtubules has not as yet been examined.

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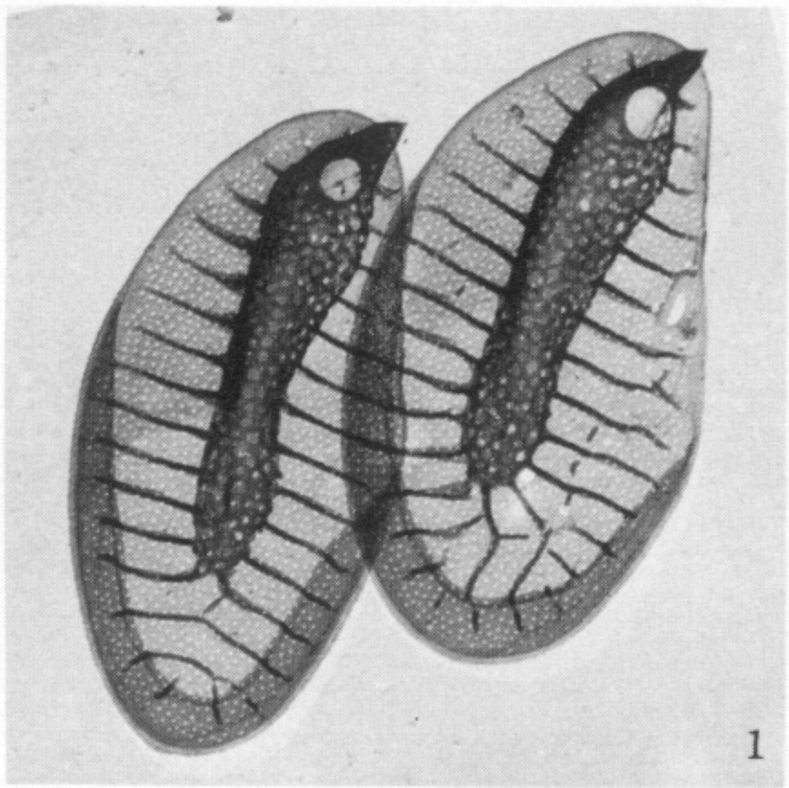
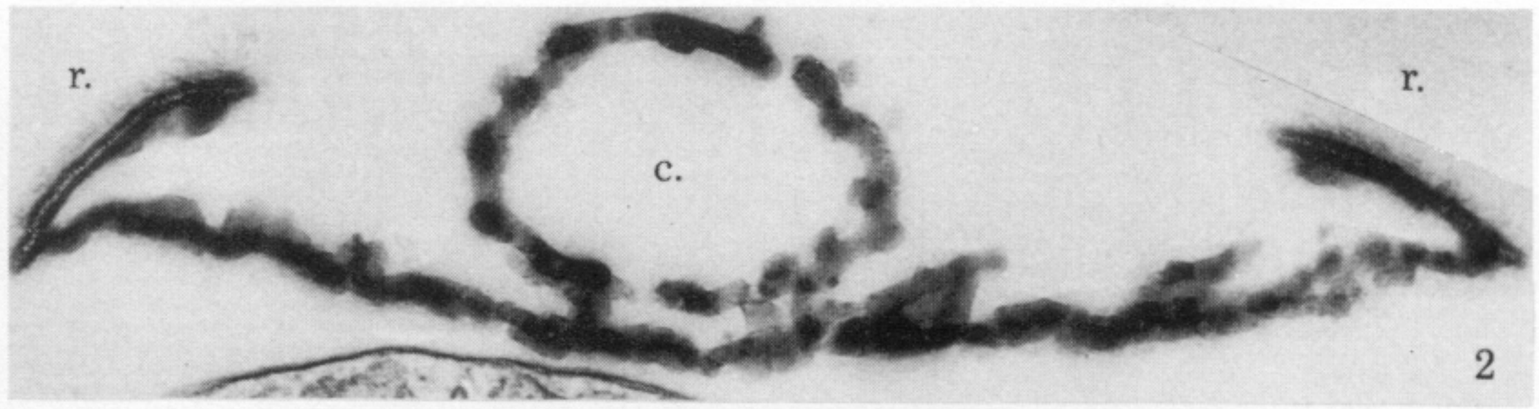
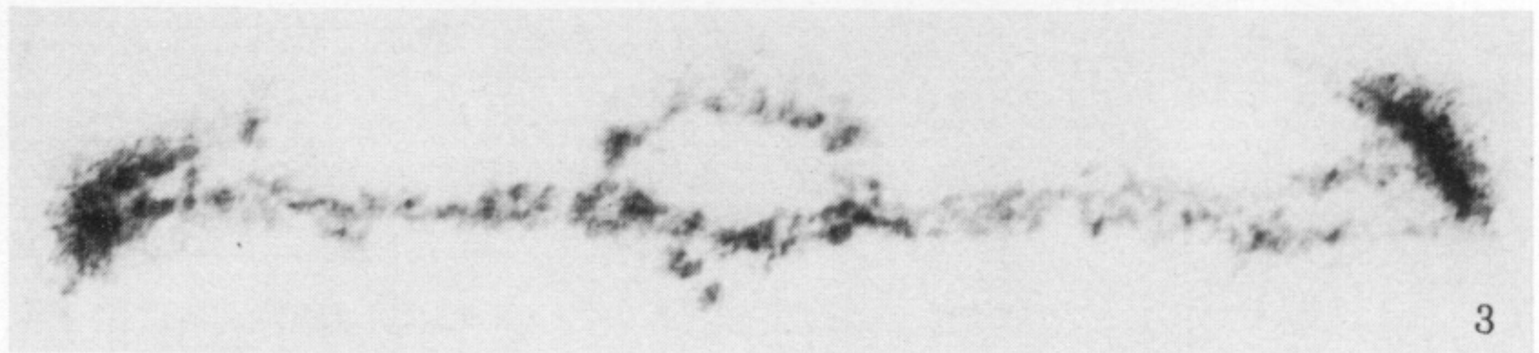
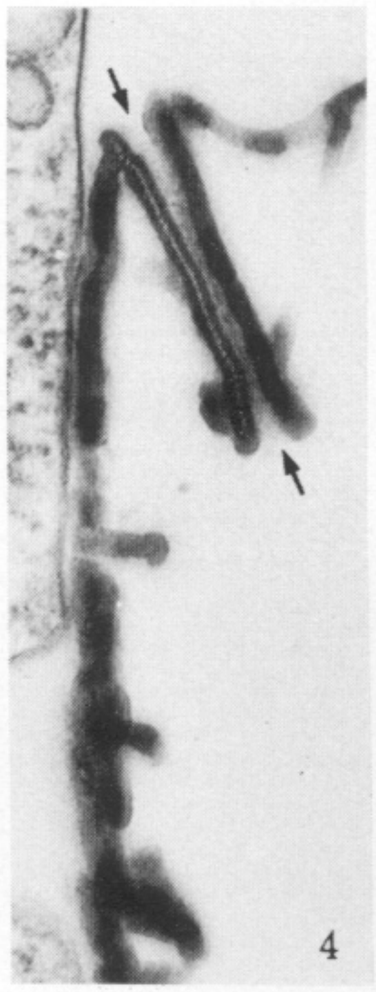
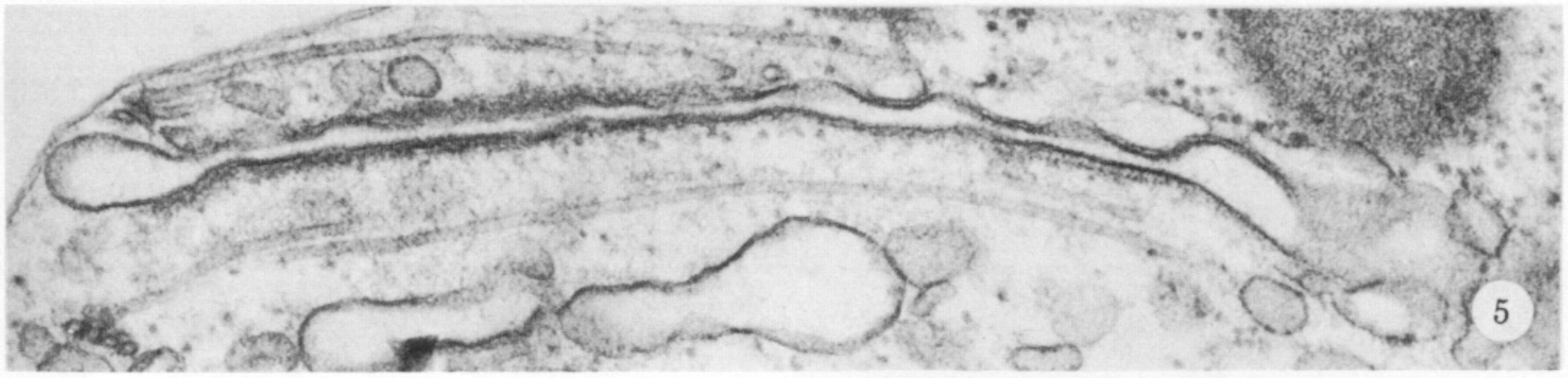
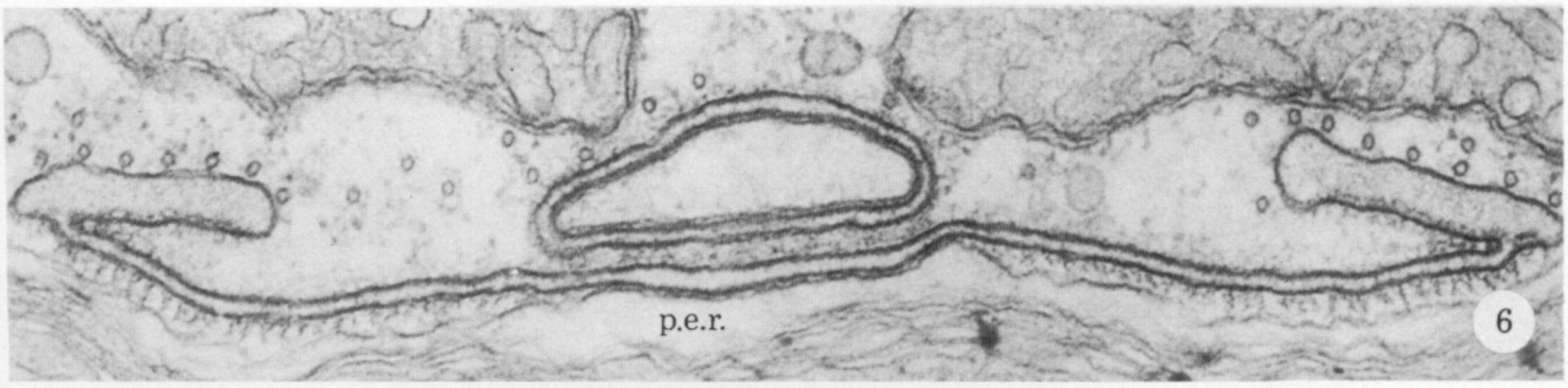


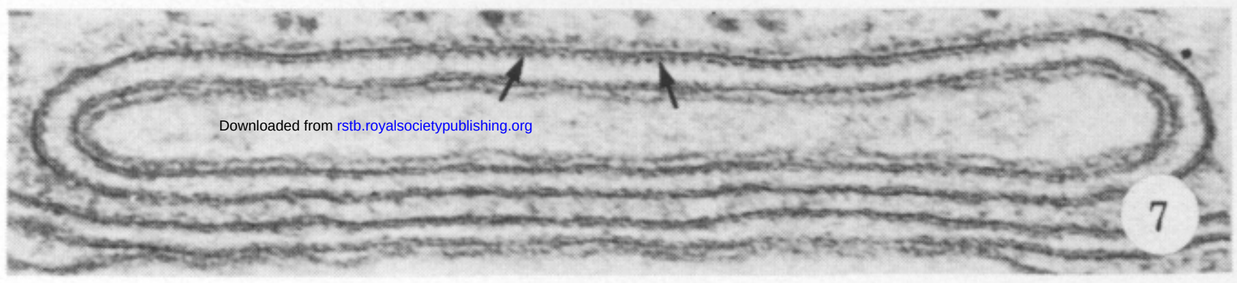


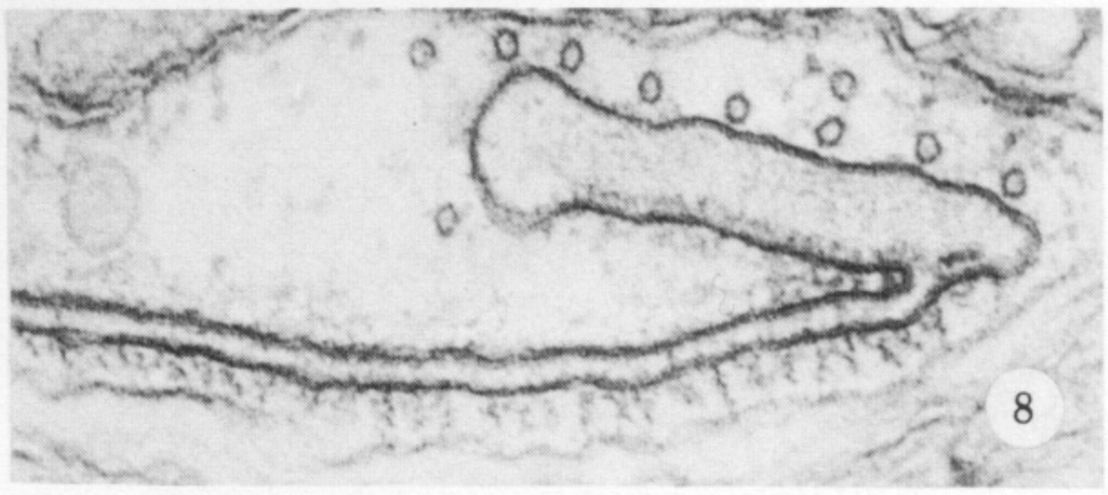


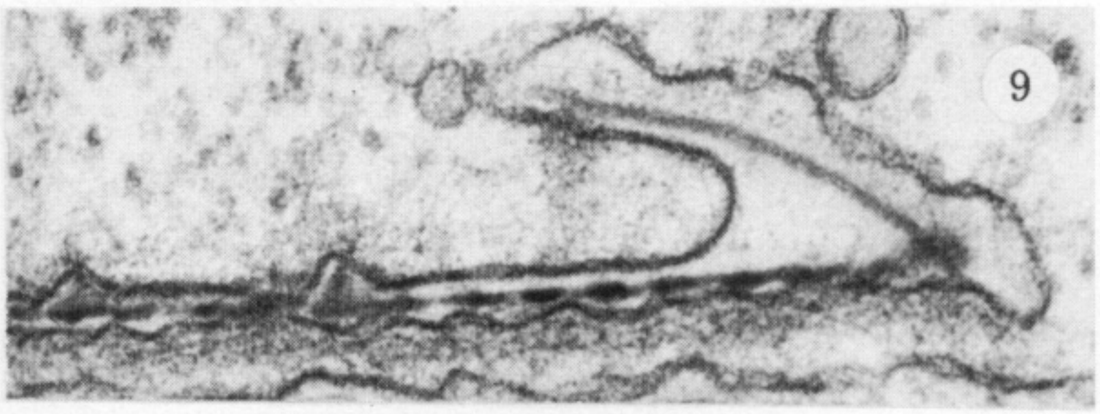


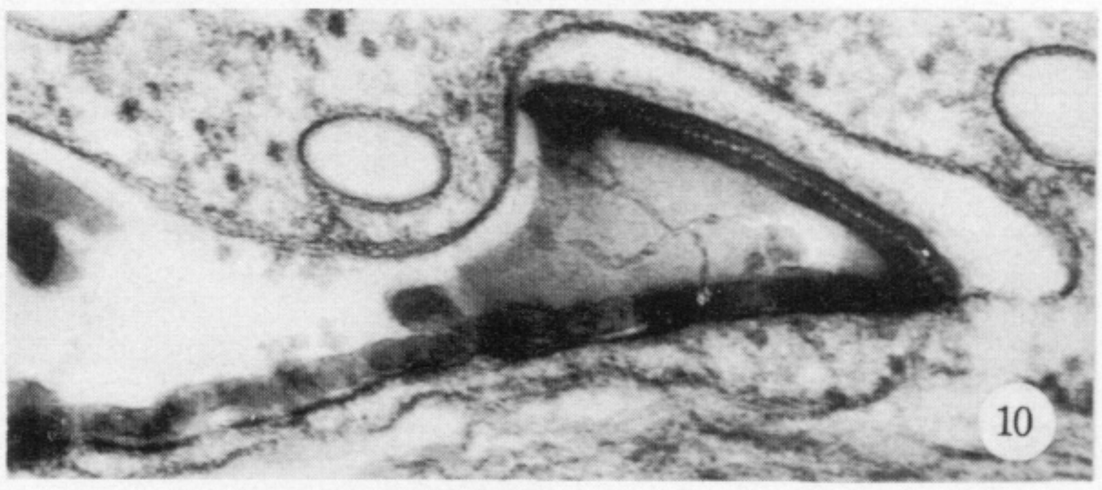


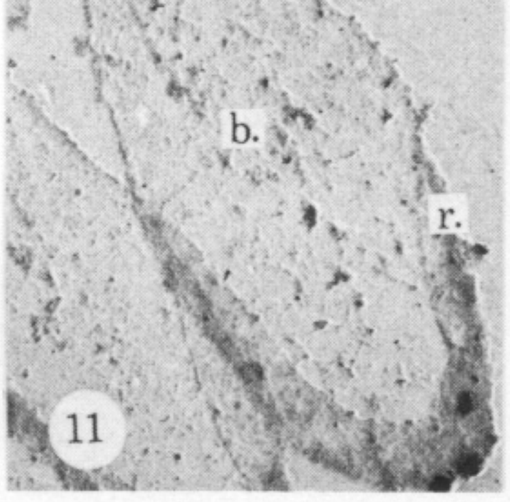


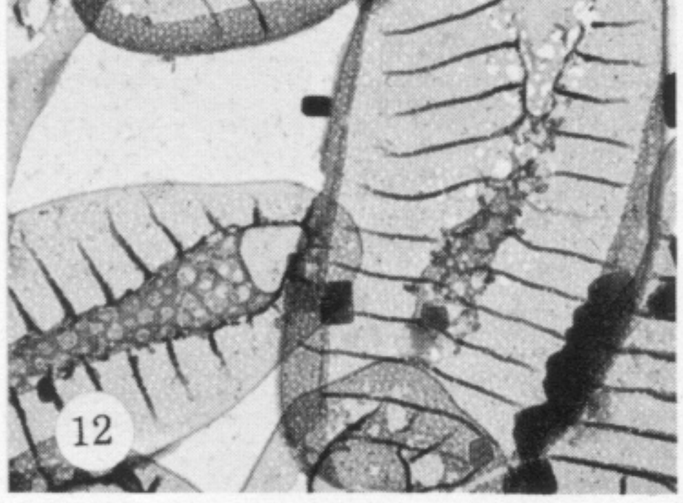




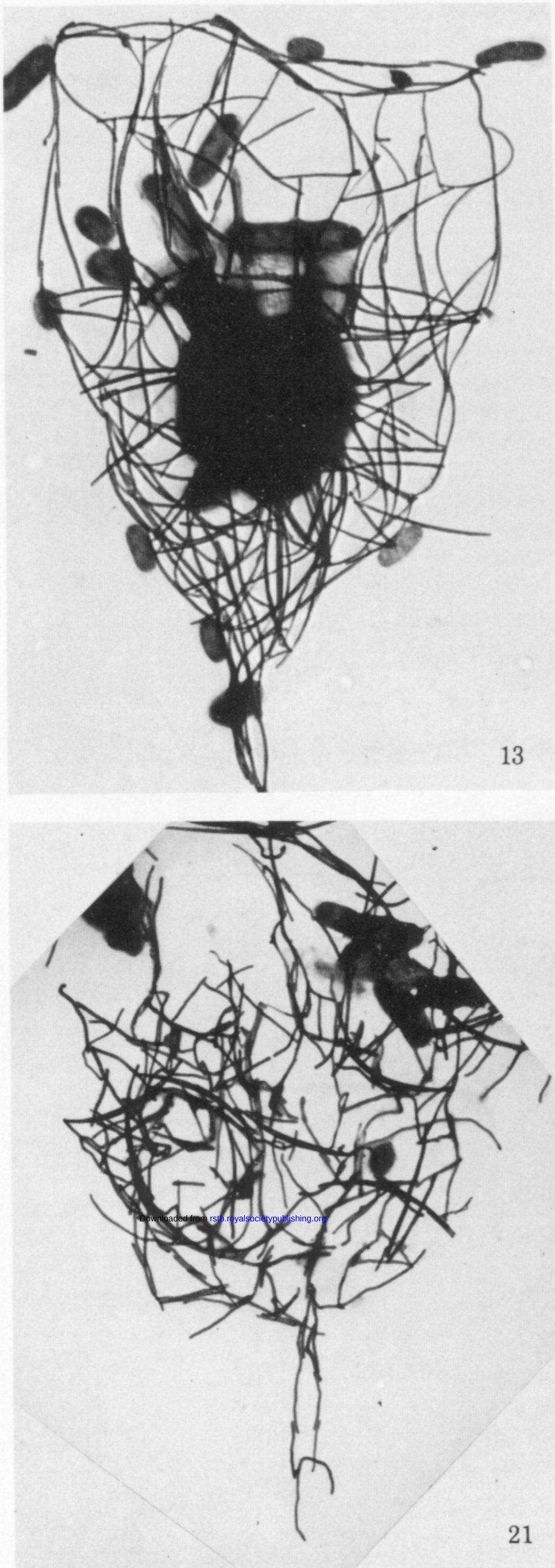








Figures 1–12. For description see opposite.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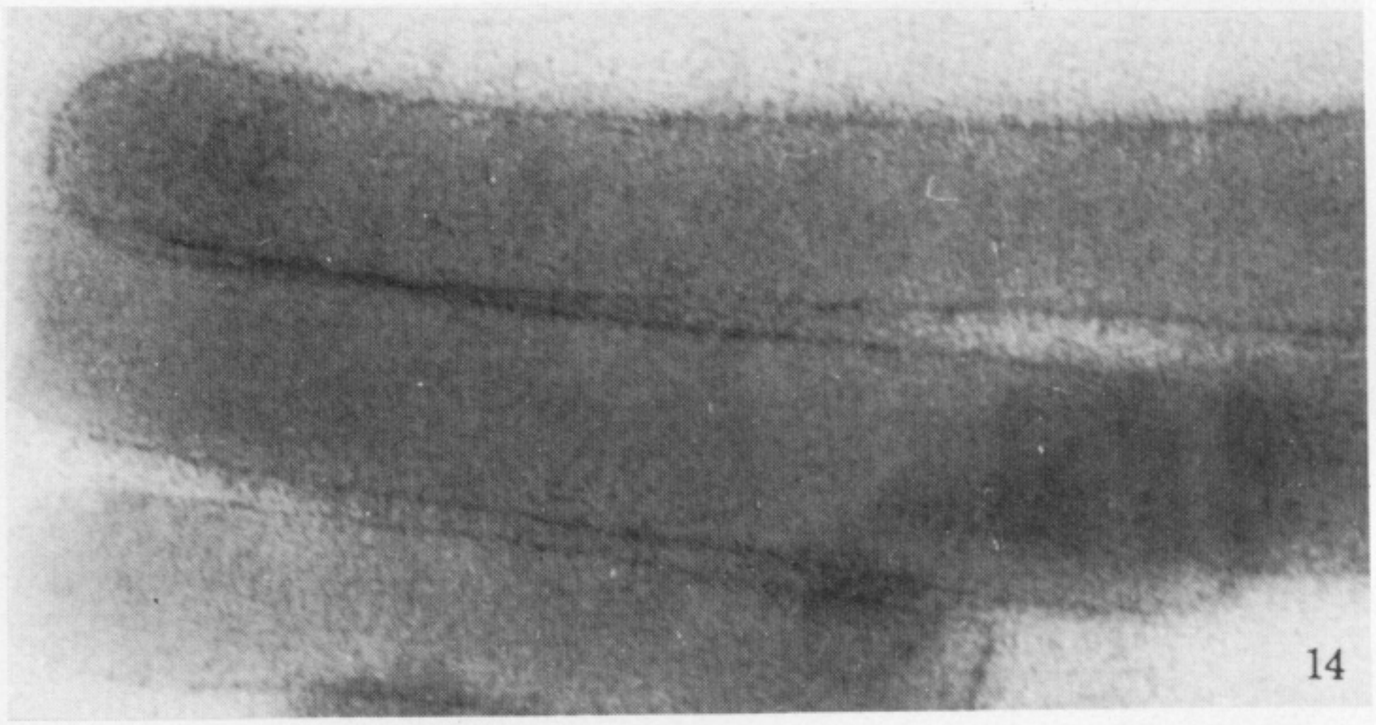
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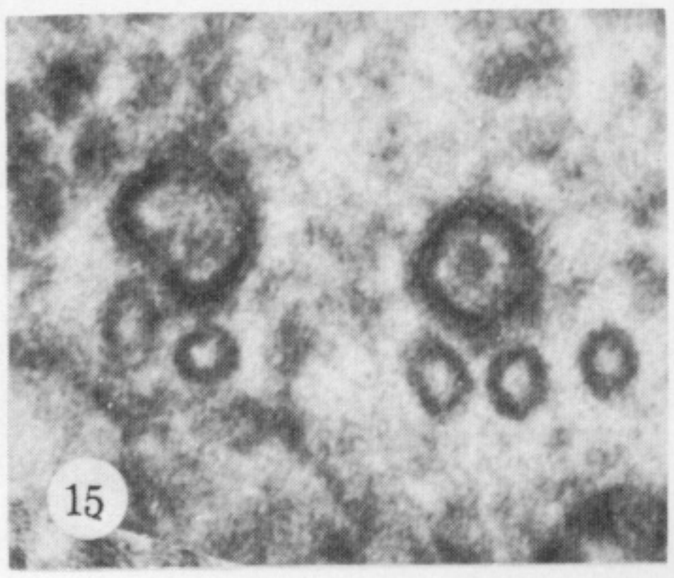
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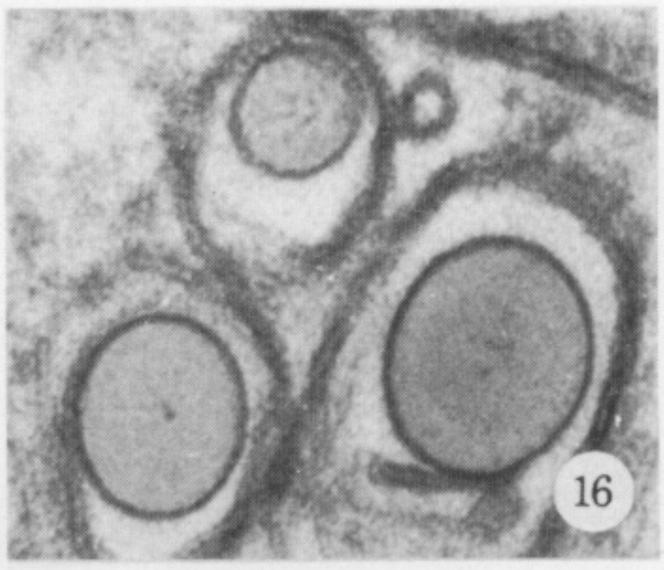
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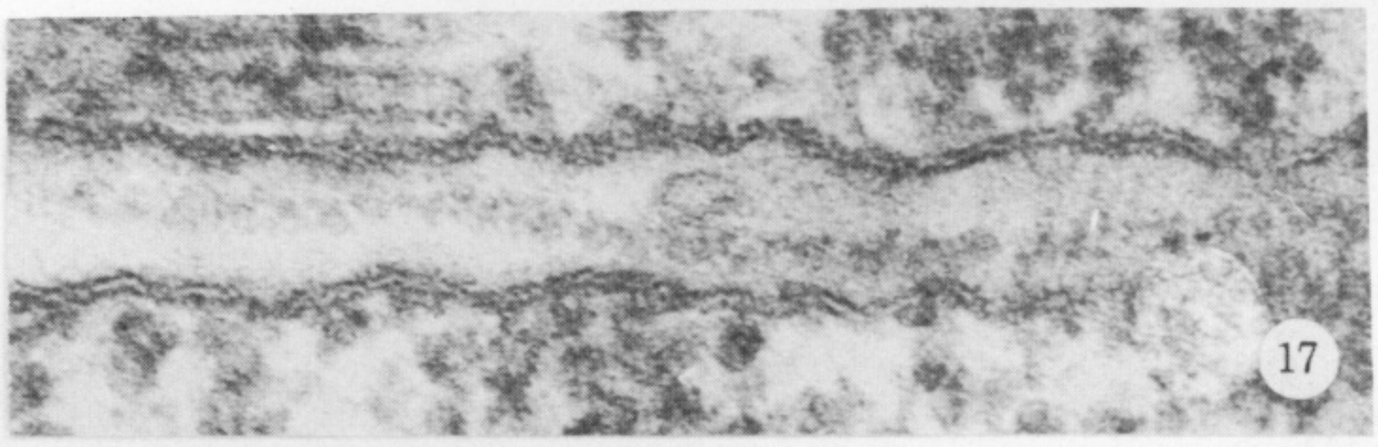
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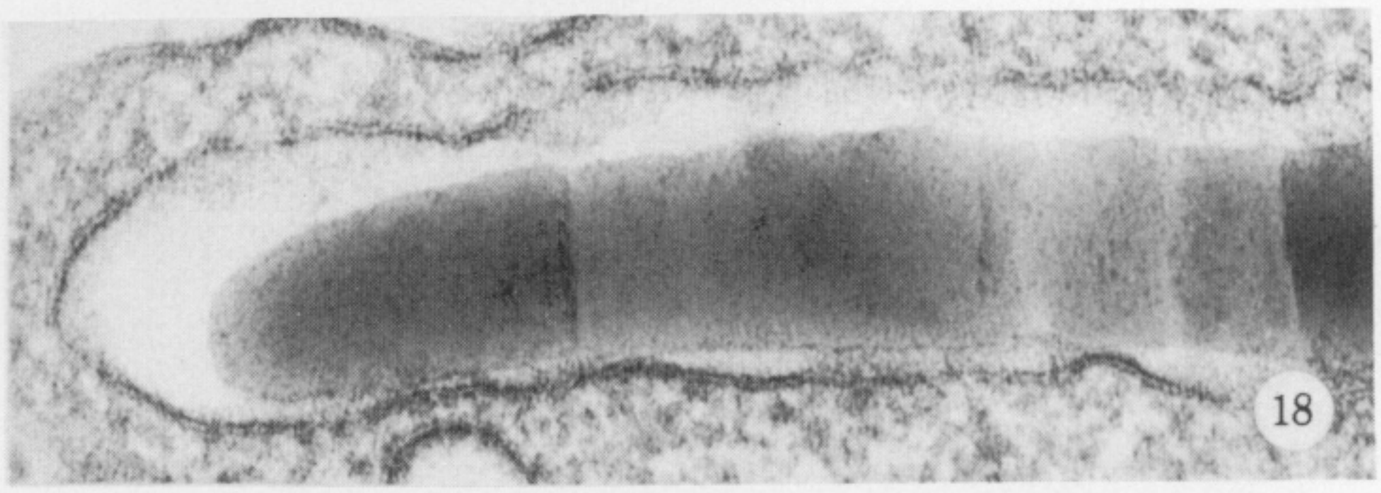
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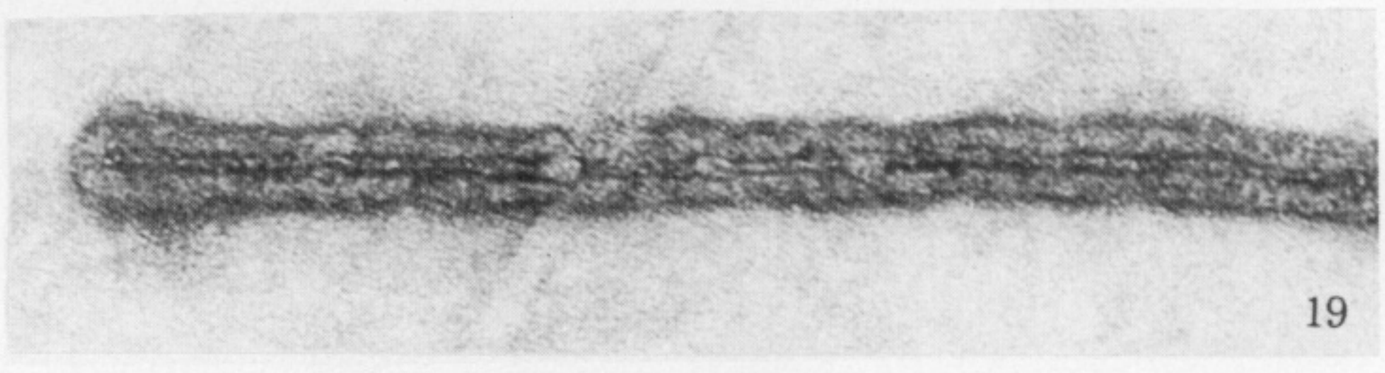
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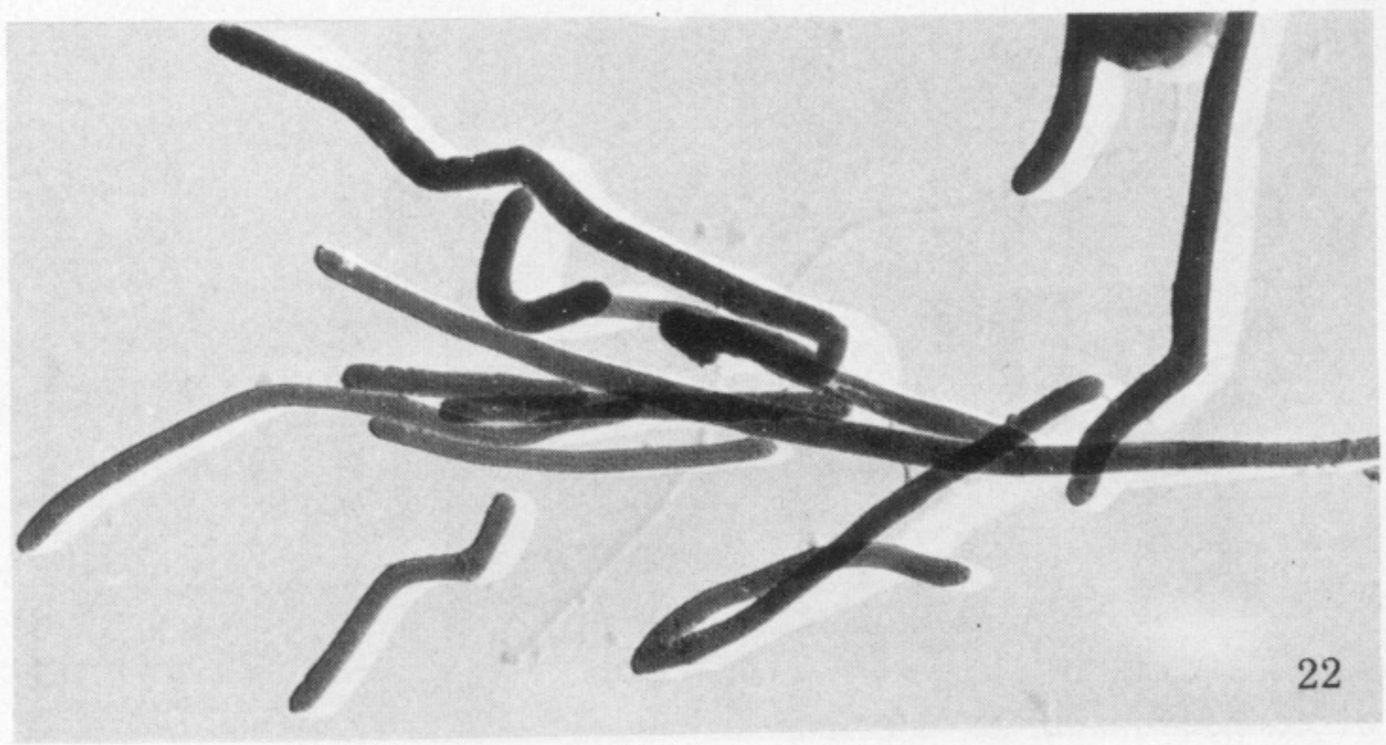
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FIGURES 13–22. For description see opposite.