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Aspects of Silicification in Wall Morphogenesis of Diatoms

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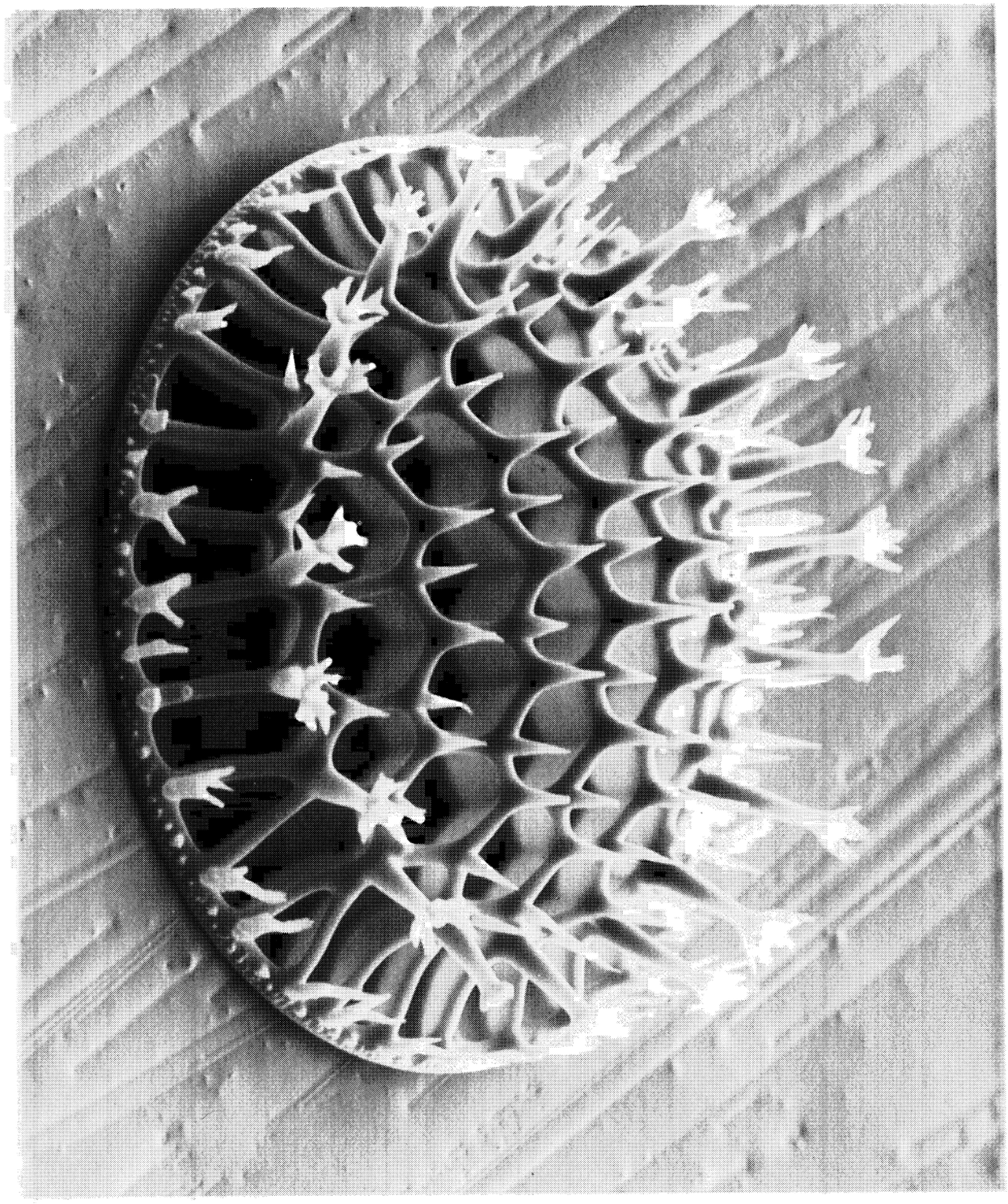

SILICA

Stephanopyxis sp. Eocene marine deposits made of hydrous silica from the South Atlantic. Single valve of an unidentified species found in a deep sea core made by the Glomar Challenger.

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Aspects of silicification in wall morphogenesis of diatoms

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[Plates 1 and 2]

The siliceous cell wall of diatoms is formed in a silica deposition vesicle that is delimited by a membrane, the silicalemma. Once the siliceous wall matures, it is expelled and a new plasmalemma is formed underneath. Underlying wall formation is a multitude of events and processes, some of which are now known. A comparative study on wall morphogenesis in seven centric diatoms leads to the following conclusions: (1) the silica deposition vesicle is formed by the coalescence of small vesicles; (2) the silicalemma becomes part of the organic casing of the mature siliceous wall; (3) at least four morphological forms of deposited silica can be seen during the development of wall components; (4) microtubules, serving as cytoskeletons, are associated with the formation of certain wall components initiated from a cytoplasmic protrusion. These events are discussed in detail.

Introduction

Among a number of organisms that produce siliceous structures (for example, chrysophytes, amoebae, silicoflagellates, radiolarians, sponges, and some vascular plants), diatoms are perhaps the most interesting. They are known to have an absolute requirement for silicon, and have delighted generations of microscopists by the endless diversity and baroquely beautiful architecture of their species-specific siliceous cell walls. Until about 20 years ago, it was thought that the complex pattern of the diatom cell wall was the result of physicochemical processes. Now we know, however, that the siliceous structures are produced by a hitherto unsuspected type of mineralization *in vivo*, which involves polymerization and deposition of silicic acid, $Si(OH)_4$.

The diatom wall consists of a pair of box-and-cover-like structures, the valves, plus sets of girdle bands (which may vary in number) that constitute the side walls. The valves are often elaborately patterned with varied arrangements of ribs, pores, and a variety of processes such as tubes. The wall itself consists of two components: a siliceous portion, and a tightly adhering organic casing. Diatoms are divided into two major groups according to the pattern of perforations on the valves: the 'pennate' diatoms, characterized by bilateral symmetry, and the 'centric' diatoms by tri- or omni-radiate symmetry. The degree of silicification in both groups varies widely, ranging from the heavily silicified species to the almost non-silicified, in which the valves are entirely organic and only the girdle bands or the raphe, or both, contain silica.

Although the morphogenesis and fine structure of the diatom wall have been explored in considerable detail, the data, as so often occurs in science, have produced as many controversies as answers, and have opened up new areas for exploration. In this paper we shall deal with three major aspects of wall formation.

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The first concerns the formation of the silicalemma, the membrane that bounds the silica deposition vesicle, and its relation with the plasmalemma during the period when the mature wall is expelled to the cell exterior. A second aspect involves the morphological forms in which the deposited silica occurs. The mineralized component of the diatom wall is usually said to be composed of 'amorphous' silica, a description that often has little value. However, various forms of silica have been observed during early stages of morphogenesis, though they cannot be found in the mature wall, and until recently these forms *per se* have not been investigated. A third aspect concerns the participation of the microtubules; we have some evidence that they play a crucial role in several fundamental steps of wall formation.

With the recent surge of interest in silicon both as an essential trace element and a pathogenic agent in mammals, diatoms have become the model system for studying silicon metabolism, and answers to these questions can shed some light on the new fields of silicon biochemistry and silicon mineralization *in vivo*.

The formation of the silica deposition vesicle

Morphogenesis of the diatom's siliceous cell wall begins during or after cytokinesis, when a silica deposition vesicle (s.d.v.) appears beneath the newly exposed plasmalemma. The vesicle is delimited by a membrane, the silicalemma (figure 2(*a*), plate 1); the s.d.v. progressively extends as silicic acid is polymerized and deposited within it. The silicalemma is an enigmatic membrane, and although it occurs in all organisms that form siliceous structures (except higher plants) (Simpson & Volcani 1981), very little is known of its origin, its ultimate function, its fate, or even of its ultrafine structure. The only study of its structure reports that freeze–fracture of *Thalassiosira eccentrica* shows numerous particles on the surface of the silicalemma arranged in a pattern similar to that of the cell wall perforations (Schulz 1978). The origin and formation of this membrane, on the other hand, has been the subject of much investigation and continuing controversy. Dawson (1973) reported that in *Gomphonema parvulum*,

Description of plate 1

Figure 1. Central part of the valve of *Ditylum brightwellii*, showing the centrally located labiate process (l.p.) and the marginal ridge (m.r.), which delimits the valve face (v.f.) and valve mantle (v.m.). The marginal ridge consists of an elevated ring and pairs of spines. Scanning electron micrograph (magn. ×4400).

Figure 2. Schematic drawing of early stages of valve formation in *D. brightwellii*. (*a*) A siliceous ring and a cytoplasmic layered structure (l.s.) are formed. The small vesicles (v.) will fuse with the s.d.v.; pl., plasmalemma; sl., silicalemma; Si, silica. (*b*) The siliceous ring expands by the radiating ribs (r.).

Figure 3. The end valve of *Chaetoceros rostratum*. Cells of *C. rostratum* form a filamentous colony by siliceous intercellular linkage (i.l.; see figures 4, 15) between intercalary valves, but the end valves of a colony lack i.l. The end valve shows the eccentrically located labiate process (l.p.) and the seta (s.), which are about 300 μm long. Scanning electron micrograph (magn. ×2300).

Figure 4. Schematic drawing of early stages of valve formation in *C. rostratum*. (*a*) A siliceous ring is formed within the s.d.v. The small vesicles (v.) will fuse with the s.d.v. (*b*). The siliceous ring expands in a diagonal pattern by branching ribs (r.), and the intercellular linkage (i.l.) starts to form; sl., silicalemma.

Figure 5. The valve of *Odontella sinensis*, showing the polar location of the labiate processes (l.p.) and elevations (e.). Scanning electron micrograph (magn. ×500).

Figure 6. Schematic drawing of the early stage of valve formation in *O. sinensis*. (*a*) The slender s.d.v., which extends pole to pole; the detail of its polar region is shown in *b* and the detail of its central region is shown in *c*. (*b*) Short radiating ribs and two long parallel ribs with regularly spaced unilateral branches grow from a polar elliptic siliceous ring. The small vesicles (v.) will fuse with the s.d.v.; sl., silicalemma. (*c*) In the central region of the s.d.v., ribs from opposite poles grow toward each other and will eventually fuse.

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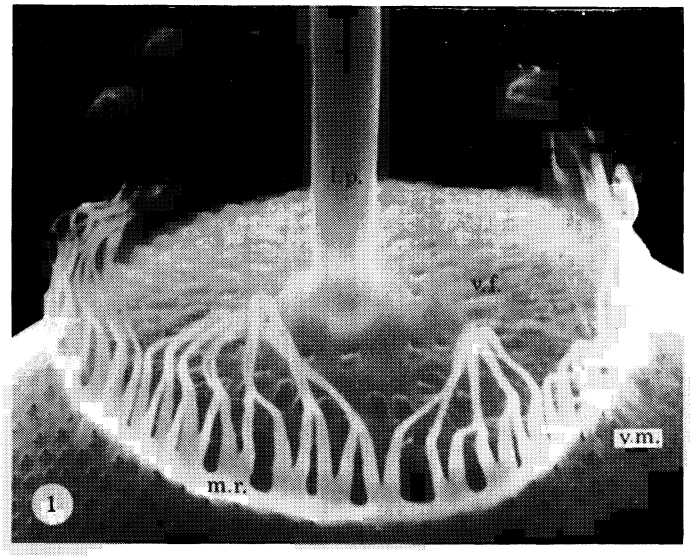

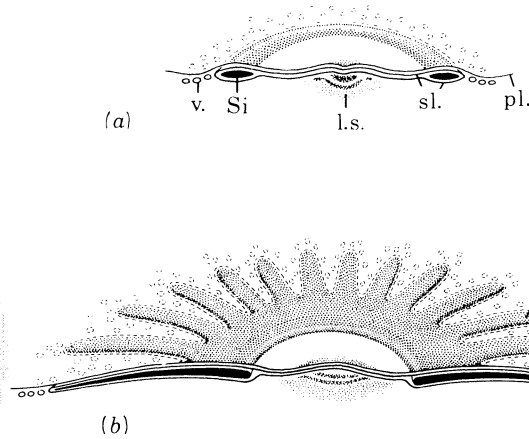

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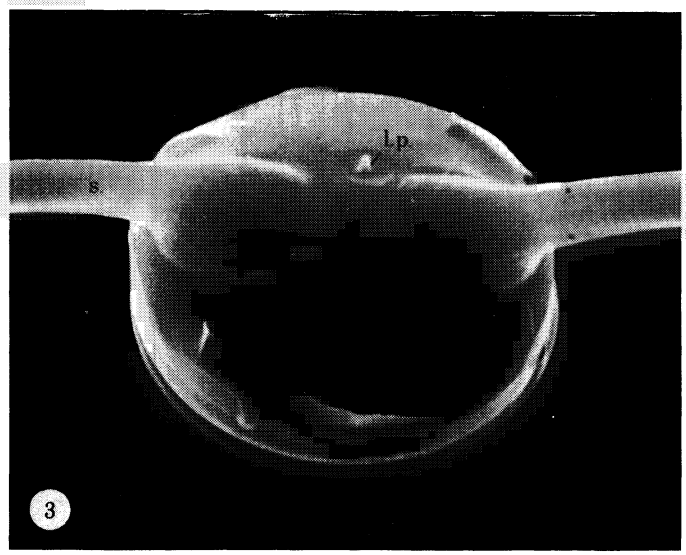

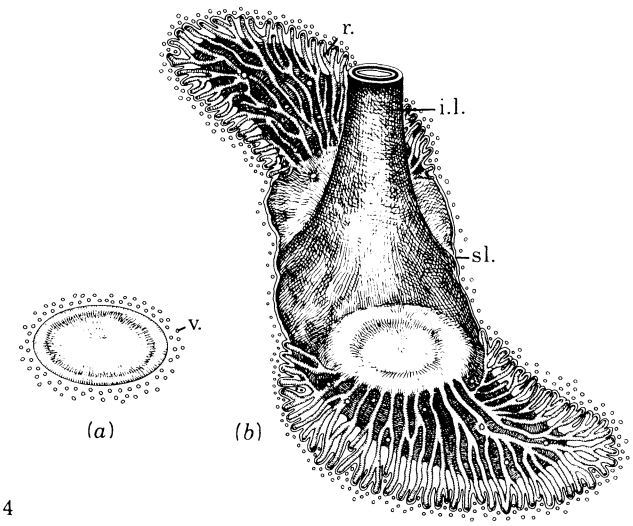

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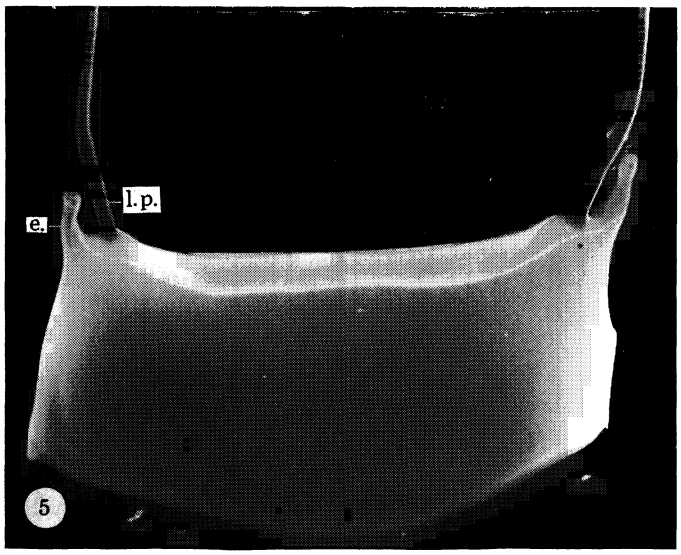

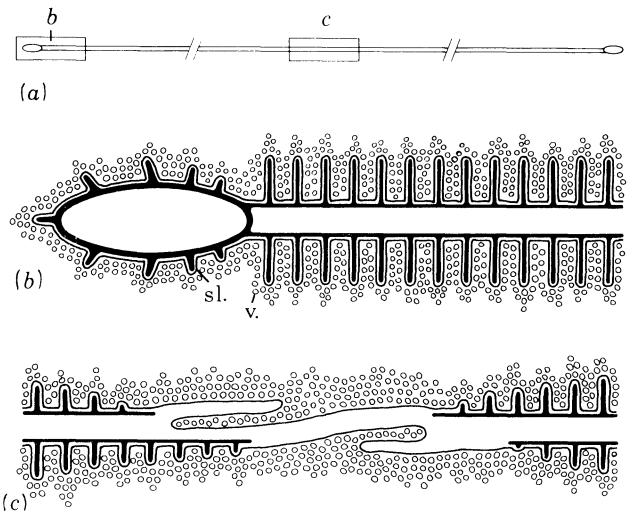

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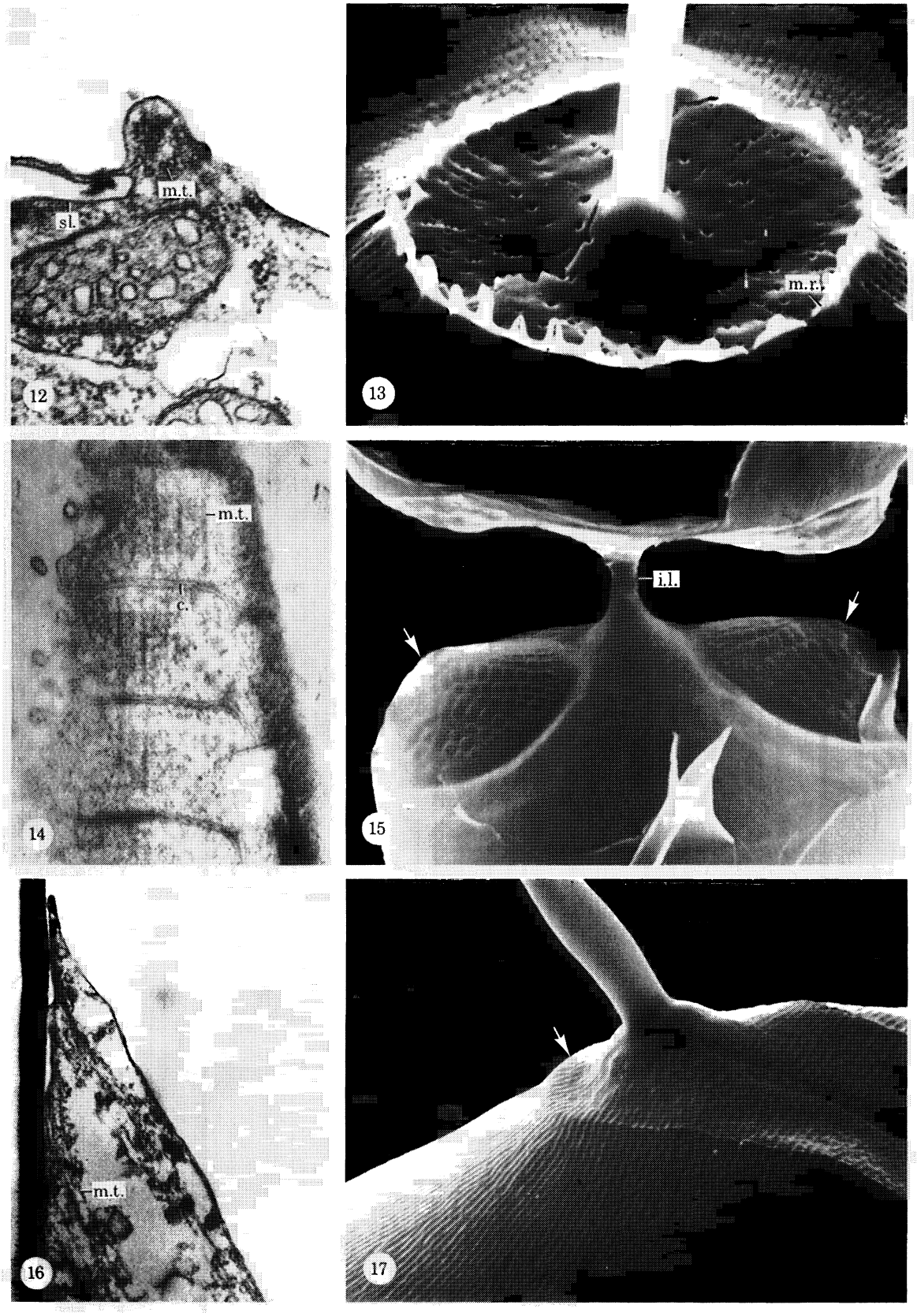

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electron-lucent vesicles collect and coalesce to form the silicalemma; however, fusing vesicles have not been seen in *Melosira nummuloides* (Crawford 1974), *Diatoma vulgare* (Pickett-Heaps *et al.* 1975), *Navicula pelliculosa* (Chiappino & Volcani 1977), *Pinnularia maior* and *P. viridis* (Pickett-Heaps & Tippit 1979), or *Hantzschia amphioxys* (Pickett-Heaps & Kowalski 1981). Pickett-Heaps and his co-workers (1975) think that Dawson's (1973) micrographs are equivocal and that these vesicles were artifacts produced by disruption and vesiculation of the endoplasmic reticulum during permanganate fixation. They later suggest that the silicalemma is equivalent to a giant, highly evolved 'Golgi' vesicle that can expand autonomously (Pickett-Heaps *et al.* 1979). However, Schmid & Schulz (1979) report that in *T. eccentrica* small electron-dense vesicles (30–40 nm in diameter) called 'silicon transport vesicles' coalesce to form the silicalemma and release their siliceous content into the s.d.v. to form individual spheres. Schnepf *et al.* (1980) also report the fusion of small vesicles (30–80 nm in diameter) to form the s.d.v. in *Attheya decora*, but found no indications that the fusing vesicles contain 'silicon'.

By studying the developing cell wall at very early stages and by using serial sections, we have been able to demonstrate the occurrence of fusing vesicles in six centric diatoms: *Ditylum brightwellii*, *Odontella sinensis*, *Odontella* sp., *Melosira nummuloides*, *Stephanopyxis turris*, and *Chaetoceros rostratum*. These fusing vesicles are not artifacts of fixation, since their appearance unequivocally coincides with the developing s.d.v. in each species. The fact that five of these six diatoms belong to different families suggests that in all diatoms the s.d.v. is formed in this way, and the suggestion by Pickett-Heaps *et al.* (1979) that the s.d.v. is in fact a giant Golgi vesicle is not confirmed. Judging from the number of these fusing vesicles, we have concluded that their major function is to provide the membrane for the expanding s.d.v. rather than to supply siliceous material; they cannot rightly be called 'silicon transport vesicles'.

The spatial relation between the s.d.v. and the fusing vesicles at early stages of valve formation in three diatoms is shown in figures 1–6. In *D. brightwellii*, a diatom with one central labiate process† on each valve (figure 1), the earliest form of the s.d.v. (figure 2 (*a*)) is a centrally

† The labiate processes are found in almost all of the centric, and in some pennate, diatoms. Each consists of an opening in the valve wall or an outer tube that projects through it, and an inner structure with a slit between two lips. Although the number, morphology, and position of the labiate processes vary greatly in different diatoms, we have found that these structures are phylogenetically related by a common cytoplasmic layered structure (figure 2) associated with their formation. Our observations indicate that the silicification in centric diatoms starts from the area where the labiate processes are located.

Description of plate 2

Figure 12. In the region where the marginal ridge in *D. brightwellii* is formed, a cytoplasmic protrusion is seen first; cross sections of microtubules (m.t.) can be seen in the protrusion. The s.d.v. has not reached the protrusion yet; sl., silicalemma. Transmission electron micrograph (magn. ×56000).

Figure 13. Colchicine-treated developing cells of *D. brightwellii* forms a new valve with aberrant marginal ridge (m.r.); for comparison see figure 1. Scanning electron micrograph (magn. ×4400).

Figure 14. Superficial longitudinal section close to the top of a developing seta in *C. rostratum*, showing the arrangement of microtubules (m.t.) between the forming costae (c.). Transmission electron micrograph (magn. ×56000).

Figure 15. Colchicine-treated developing cells of *C. rostratum* form new valves with no seta (arrows); for comparison see figure 3. Scanning electron micrograph (magn. ×4000).

Figure 16. In the region where the elevations in *O. sinensis* is formed, a cytoplasmic protrusion is seen first, numerous microtubules (m.t.) can be seen in the protrusion. The s.d.v. has not reached the protrusion yet. Transmission electron micrograph (magn. ×19000).

Figure 17. Colchicine-treated developing cell of *O. sinensis* forms a new valve with no elevation (arrow); for comparison see figure 5. Scanning electron micrograph (magn. ×1850).

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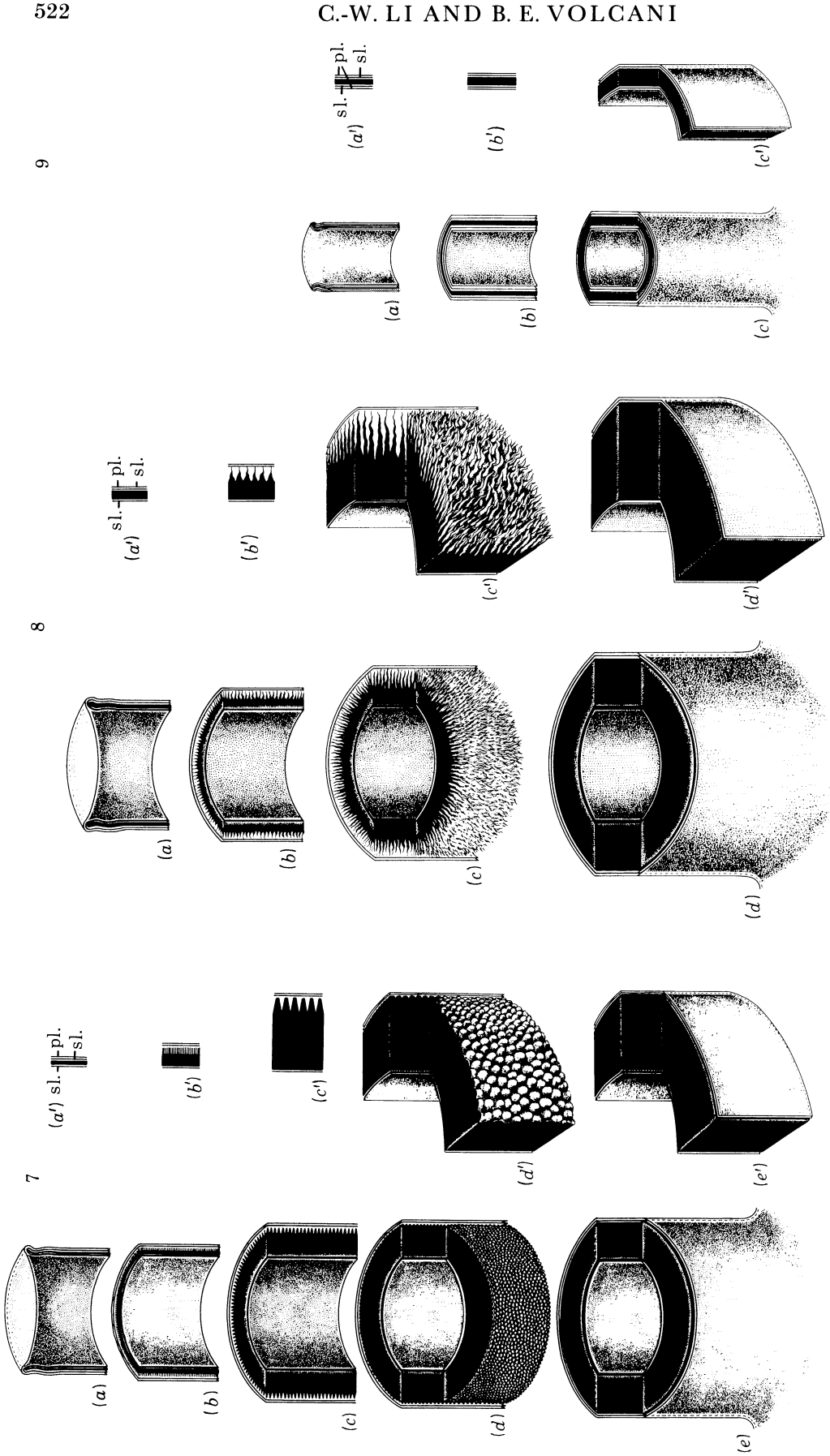

FIGURES 7–9. Schematic drawings of the different morphological forms of deposited silica observed during the development of the outer tube of the labiate process in three centric diatoms. For full description, see opposite.

located flat round bag with a siliceous ring inside it, surrounded by a few small vesicles (35 nm in diameter). The s.d.v. then begins to expand by fusion with the small vesicles, and the siliceous ring grows centrifugally as ribs radiate from it to form the central area of the valve; small vesicles occur not only along the outer boundary of the s.d.v., but also between the ribs (figure 2(*b*)). In *C. rostratum*, a diatom with one eccentric labiate process on each valve (figure 3), the earliest form of the s.d.v. (figure 4(*a*)) is an eccentrically located flat round bag with a siliceous ring inside it, surrounded by a few small vesicles (16 nm in diameter). The s.d.v. is expanded by fusion with the small vesicles; the siliceous ring is expanded in a diagonal pattern as the radiating ribs extend outward; the s.d.v. is still surrounded by small vesicles (figure 4(*b*)). In *O. sinensis*, a diatom with two polar labiate processes on each valve (figure 5), the earliest form of s.d.v. (figure 6(*a*)) is a very slender flat bag that extends pole-to-pole, surrounded by small vesicles (35 nm in diameter). Inside the s.d.v. at each polar area, there is an elliptical siliceous ring with several short radiating ribs and two long parallel ribs with regularly spaced unilateral ribs branching from them (figure 6(*b*)); at the central part of the s.d.v., the long parallel ribs, which grow from opposite polar rings, have not fused (figure 6(*c*)).

The origin of the fusing vesicles is not known. Since the activity of the Golgi bodies in diatoms has no profound effect on changes in the cell cycle (Pickett-Heaps *et al.* 1975; C.-W. Li and B. E. Volcani, unpublished data), by judging from their vesiculation, the suggestion that the fusing vesicles are derived from the Golgi bodies (Dawson 1973; Schnepf *et al.* 1980) is not confirmed. Because no contents are visible in electron micrographs of the fusing vesicles, it is so far impossible to determine their origin or trace their early development.

The fate of the silicalemma after wall formation has been completed is controversial. Chiappino & Volcani (1977) and Volcani (1981) consider that the plasmalemma and silicalemma are probably integrated with the organic casing of the newly expelled cell wall, and that a new plasmalemma is formed underneath the new wall. In contrast, Stoermer *et al.* (1965) and Crawford (1981) suggest that both the plasmalemma and the outer silicalemma are lost before the new wall matures, that further silicification occurs on the expelled wall, and that the inner silicalemma remains as the functional plasmalemma of the new cell. This would require silica deposition to occur outside the s.d.v., and would involve a radical change in function for the inner silicalemma. However, neither of these phenomena are confirmed by recent studies. Changes in the morphological forms of deposited silica (see next section) are good indicators of wall development up to the stage of wall maturation, but using this criterion, we conclude that no silica deposition occurs outside the s.d.v. (C.-W. Li and B. E. Volcani,

Description of figures 7–9

Figure 7. *Ditylum brightwellii*. (*a*), (*a*′) Deposited silica within the s.d.v. forming a thin continuous base layer; pl., plasmalemma; sl., silicalemma. (*b*), (*b*′) Siliceous microfibrils grow outward from the base layer. (*c*), (*c*′) Disappearance of microfibrils; the wall is thickened by exterior hexagonal columns. (*d*), (*d*′) Height of hexagonal columns decreases when silica deposition is about to be completed. (*e*), (*e*′) Completed silica deposition, showing undifferentiated structure.

Figure 8. *Odontella sinensis*, (*a*), (*a*′) Deposited silica within the s.d.v. forming a thin continuous base layer; pl., plasmalemma; sl., silicalemma. (*b*), (*b*′) Siliceous microfibrils grow outward from the base layer. (*c*), (*c*′) The wall is thickened by exterior microfibrils. (*d*), (*d*′) Completed silica deposition, showing undifferentiated structure.

Figure 9. *Stephanopyxis turris*. (*a*), (*a*′) Deposited silica within the s.d.v. forming a thin continuous base layer. (*b*), (*b*′) The cell is thickened by an undiscerned structural form of deposited silica. (*c*), (*c*′) Completed silica deposition, showing undifferentiated structure.

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unpublished data). Moreover, a freeze–fracture study shows that both inner and outer sides of the mature cell wall of *T. eccentrica* are still covered by the silicalemma (D. Schulz, personal communication 1982); we therefore assume, without any evidence to the contrary, that part of the plasmalemma is also retained, and that both membranes become part of the organic casing of the new wall.

Morphological forms of deposited silica

The many electron microscope investigations of diatom morphogenesis are beginning to make it clear that silica is deposited in several forms in the siliceous cell wall. This has been obscured by the fact that the mature siliceous wall is composed of amorphous silica and its surface is always smooth. However, during wall formation different forms of silica can be seen at different stages of development, and the forms differ from species to species. In most cases, for example, *G. parvulum* (Drum & Pankratz 1964), *Amphipleura pellucida* (Stoermer *et al.* 1965), *Pinnularia maior* and *P. viridis* (Pickett-Heaps *et al.* 1979), and *Hantzschia amphioxys* (Pickett-Heaps & Kowalski 1981), siliceous microfibrils can be seen on both the inner and outer surfaces of the developing wall. Pickett-Heaps *et al.* (1979) suggest that the microfibrils are the product of silica deposition on the fine strands of template (presumably polysaccharide) created by the silicalemma.

However, in other examples, siliceous spheres are found in certain wall components during their formation, for example, the girdle bands in *Phaeodactylum tricornutum* (Borowitzka & Volcani 1978) and on the outer surface of the valve in *T. eccentrica* (Schmid & Schulz 1979). Schmid (1979*a*) states that the valve of *Anomoeoneis sphaerophore*, *Surirella peisonis*, *Amphiprora paludosa*, and *Navicula radiosa* is composed of siliceous spheres 30–50 nm in diameter. However, the micrographs do not confirm this. Our recent study of silica deposition in *D. brightwellii* demonstrates that the previously described spheres (1–50 nm in diameter) in this diatom at least (Volcani 1981; figures 7–13 (*c*)) are cross section fragments of artificially fractured siliceous hexagonal columns. According to Schmid & Schulz (1979), in *T. eccentrica* each sphere is formed by condensation of the siliceous material carried by an individual 'silicon transport vesicle' when the latter fuses with the s.d.v. The spheres progressively aggregate and another form of silica is deposited, filling the spaces between the spheres. This hypothesis seems untenable in view of the fact that the vesicles described, 30–40 nm in diameter, cannot carry enough aqueous siliceous material to form a siliceous sphere 12–20 nm in diameter (C.-W. Li and B. E. Volcani, unpublished data). Another structural form of deposited silica, the 'mounds' or 'knolls', are found in the central nodule of the raphe in the developing *N. pelliculosa* (Chiappino & Volcani 1977). The mounds (30–40 nm in diameter) on the inner surface of the central nodule are more distinct than those on the outer surface. No similar forms have been reported from the developing central nodule of other diatoms.

In recent studies on cell wall formation of several diatoms, we have uncovered still another form in which silica is deposited, i.e. the hexagonal column. In our studies on *D. brightwellii*, we have found that deposited silica occurs in three different forms: as a thin base layer of homogeneous silica; as hexagonal columns; and as microfibrils. The base layer, the first morphological feature to be laid down in every part of the siliceous wall (figures 7 (*a*), (*a'*), 10 (*a*), 11 (*a*), 11 (*b*)), is a continuous thin structure (sheet). Although it can also be seen in the micrographs of other workers, for example, in the forming fibulae of *H. amphioxys* (Pickett-Heaps

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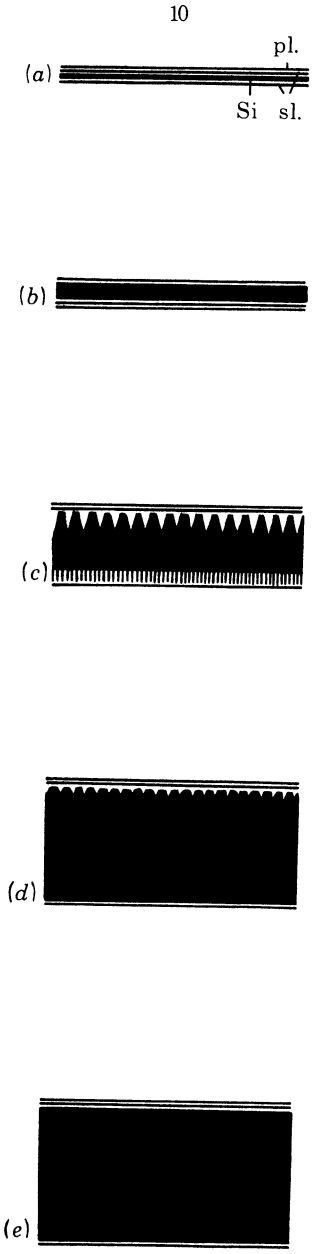

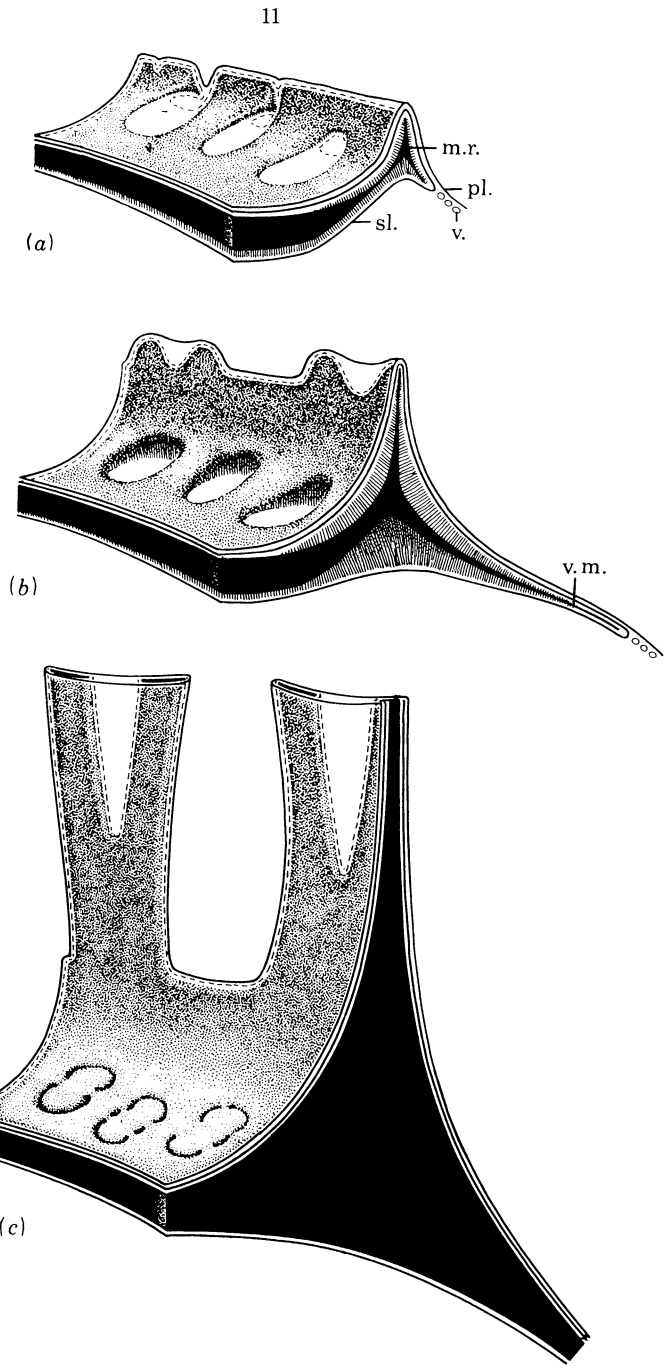

FIGURE 10. Schematic drawing of the different morphological forms of deposited silica observed during development of the valve face in *Ditylum brightwellii*. (*a*) Deposited silica within the s.d.v. forming a thin continuous base layer; pl., plasmalemma; sl., silicalemma; Si, silica. (*b*) The base layer is thickened by an undiscerned morphological form of deposited silica. (*c*) The wall is thickened on the exterior by the hexagonal columns and on the interior by microfibrils. (*d*) Microfibrils disappear and the height of the hexagonal columns decreases when silica deposition is about to be completed. (*e*) Completed silica deposition, showing undifferentiated structure.

FIGURE 11. Schematic drawing of the different morphological forms of the deposited silica on the marginal ridge and its sequential formation in *Ditylum brightwellii*. (*a*) Initiation of the marginal ridge (m.r.). The marginal ridge enlarges by the deposition of siliceous microfibrils. The small vesicles (v.) will fuse with the s.d.v.; pl., plasmalemma; sl., silicalemma. (*b*) Formation of paired spines and the valve mantle (v.m.). (*c*) Silica deposition has been completed at the basal part of the marginal ridge, showing undifferentiated structure. Tips of the spines of the marginal ridge continue to grow.

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& Kowalski 1981), the authors do not attempt to differentiate it from the microfibrils extending from it. The initial thickness of these base layers is about 14 nm on the outer tube of the labiate process, and about 7 nm on the valve face, marginal ridge, valve mantle, and girdle bands. It increases to about 90 nm on the valve face as additional silica is deposited; the morphological form of this silica cannot be discerned (figure 10(*b*)).

The hexagonal column, which displays a broad base (35 nm in diameter), and an attenuated and truncated tip, is a unique form of silica deposition, seen only in this diatom. Columns occur on the outer surface of the valve face (figures 10(*c*), 10(*d*)) and on the outer surface of the outer tube of the labiate process (figures 7(*c*), (*c*′), 7(*d*), (*d*′)). The hexagonal columns are arranged at regular intervals, and each column is usually surrounded by six others (figure 7(*d*′)). Their height increases with the deposition of additional silica, which also gradually fills the spaces between them; again, the physical form of this silica cannot be determined. The height of the hexagonal columns varies with the developing stages of the forming valve. The length of the outer tube is increased by apical growth, so that progressive thickening of the tube wall can be observed along the tube from the tip to the base part (figures 7(*a*)–(*e*)). The microfibrils (about 10 nm in diameter) occur on the outer surface of the outer tube of the labiate process (figures 7(*b*), (*b*′)) before the initiation of hexagonal columns, the inner surface of the valve face (figure 10(*c*)) and the valve mantle (figure 11(*b*)), and the whole surface of the marginal ridge (figures 11(*a*), (*b*)). No hexagonal column or microfibril can be seen when valve formation is complete (figures 7(*e*), (*e*′), 10(*e*), 11(*c*)).

In *O. sinensis*, *O. regia*, and *Odontella* sp., we have found two morphological forms of deposited silica: the base layer and the microfibrils. Since these three diatoms use similar morphological forms of deposited silica to build their cell wall and since the microfibrils only occur on the heavily silicified part of the wall, for example, the labiate process, only the sequential silica deposition of the labiate process in *O. sinensis* is illustrated (figure 8). Similar to that in *D. brightwellii*, the base layer in *O. sinensis* is the form first deposited in every part of the siliceous wall and increases in thickness as more silica is deposited. Initially, the base layer is about 35 nm on the outer tube of the labiate process (figure 8(*a*), (*a*′)) and about 10 nm on the rest of the valve. The microfibrils have a slightly wavy shape, with very fine tip and broad base (25 nm in diameter); they extend outward from the outer surface of the base layer on the outer tube of the labiate process and are irregularly arranged (figures 8(*b*), (*b*′), 8(*c*), (*c*′)). No microfibrils can be seen when tube formation has been completed (figures 8(*d*), (*d*′)).

In *S. turris*, a diatom with 6–20 labiate processes arranged in a concentric ring, silica appears to be deposited in only one form, the base layer. This layer constitutes every part of the siliceous wall; it thickens, as in other diatoms with further amorphous silica deposition. The progressive thickening of the outer tube of the labiate process is shown in figure 9.

From these studies, it is evident that diatoms use at least four forms of deposited silica in varying combinations to build their siliceous walls, even for wall component, for example, the labiate process. Since the morphological forms of silica are quite similar in the three *Odontella* species, but are very different from those in *Ditylum*, the form is apparently under genetic control, and study on this subject may provide an additional method for taxonomical classification of diatoms.

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Involvement of microtubules in cell wall morphogenesis

There is some evidence that microtubules are involved in valve formation in pennate diatoms (Pickett-Heaps *et al.* 1979; Pickett-Heaps & Kowalski 1981) and in formation of various siliceous structures in a number of other organisms (for example, choanoflagellates, radiolarians, sponges). However, there is no conclusive evidence as to their function, though it has been suggested that they play a role in nuclear migration or in moving the nucleus closer to the primary silicification site, or both; that they participate in valve formation by affecting cytoplasmic transport or by associating with the raphe fibre, a dense cytoplasmic structure within the forming raphe fissure (Pickett-Heaps *et al.* 1979), or that they are responsible for the patterning of the diatom valve (for a review, see Schmid *et al.* (1981)).

During mitosis in pennate diatoms, an organelle, the microtubule center (m.c.) can be seen at the polar region of the spindle with microtubules arising from it. In late cytokinesis, the m.c. migrates close to the primary silicification site; the microtubules now extend over the forming valve. As the valve develops, the microtubules are closely associated with formation of the raphe (Pickett-Heaps *et al.* 1979; Pickett-Heaps & Kowalski 1981). Exposure of dividing cells to microtubule inhibitors such as colchicine produces a wide variety of aberrations in cytokinesis, cell division (for example, binucleated cells) or wall formation, or both, particularly formation of the raphe (Coombs *et al.* 1968; Blank & Sullivan 1980; Schmid 1980). However, Blank & Sullivan (1983) conclude that these development aberrations are not due to interference with silicon supply, since disruption of microtubules has no effect on silicic acid transport or on the levels of incorporated silicon.

Microtubule inhibitors strongly affect valve formation in centric diatoms also. In post-telophase cells of *T. eccentrica*, exposure to inhibitors produces fragmented or defective valves, or both, (Schmid 1979*b*; Schulz & Wedemeyer 1981); however, the arrangement of microtubules associated with normal valve formation in this species is not known. Schnepf *et al.* (1981) failed to detect any microtubules in the developing horns of *A. decora*, although the horn formation is disturbed by colchicine; hence there may be another mechanism, as well, that is affected by this treatment.

When cells of the five centric diatoms of this study were treated with 1 μg/ml colchicine at a very early stage of development, we found the following consequences: in *Ditylum*, microtubules are found in a cytoplasmic protrusion (figure 12, plate 2) where the marginal ridge (figure 1) will be formed (C.-W. Li and B. E. Volcani, unpublished data), but treatment results in an aberrated marginal ridge in the daughter cells (figure 13). In *Chaetoceros*, a row of regularly spaced microtubules lies close to the s.d.v. near the tip of each developing seta where the space between costae has not silicified (figure 14) (C.-W. Li and B. E. Volcani, unpublished data), but treatment results in the absence of the setae in the daughter cells (figure 15). In *Odontella*, microtubules occur in the two polar cytoplasmic protrusions (figure 16) where the elevations (figure 3) will be formed (C.-W. Li and B. E. Volcani, unpublished data), but in the treated cells, the elevation is lacking on the new valve (figure 17). In these species, the microtubules associated with wall formation do not arise from the m.c.; they disappear when their associated wall components mature. Schulz & Wedemeyer (1981) propose that microtubules may affect the patterning in the diatom wall by participating in the translocation of fusing vesicles. Since our observations show that microtubules are associated only with the wall components that are initiated from cytoplasmic protrusions, it is apparent that they serve

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as cytoskeletons during wall formation and may have little to do with the translocation of fusing vesicles.

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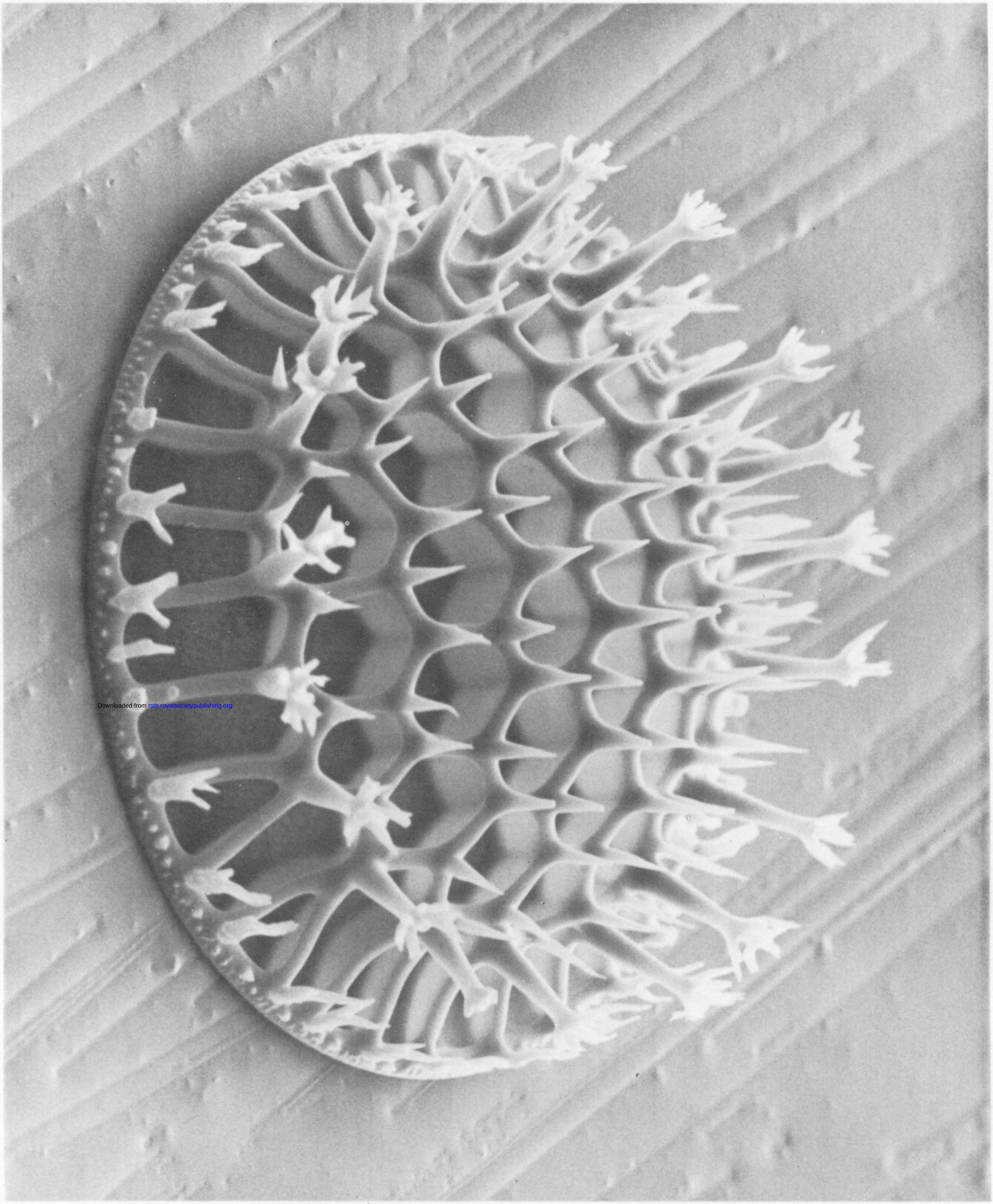

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SILICA

Stephanopyxis sp. Eocene marine deposits made of hydrous silica from the South Atlantic. Single valve of an unidentified species found in a deep sea core made by the Glomar Challenger.

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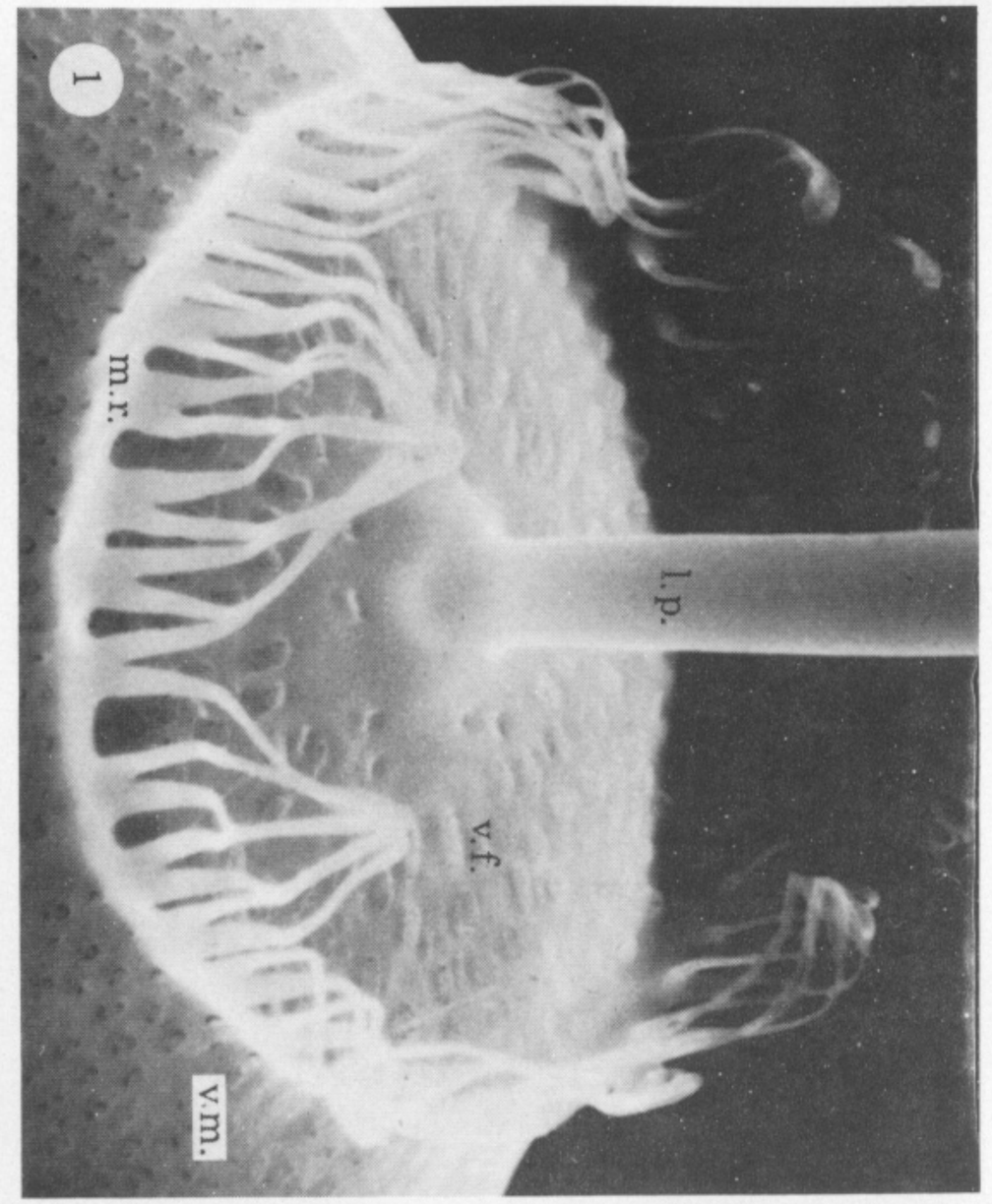

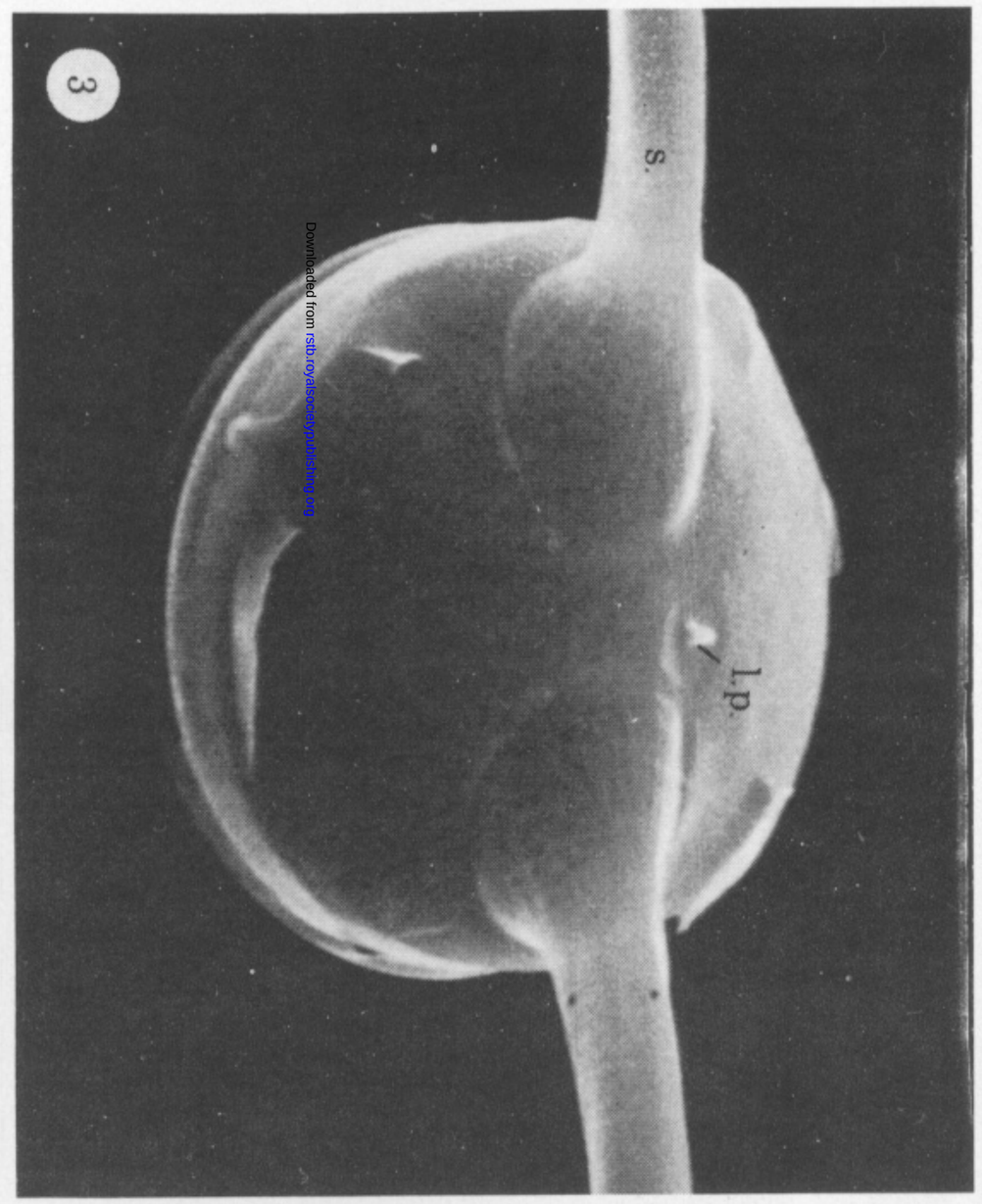

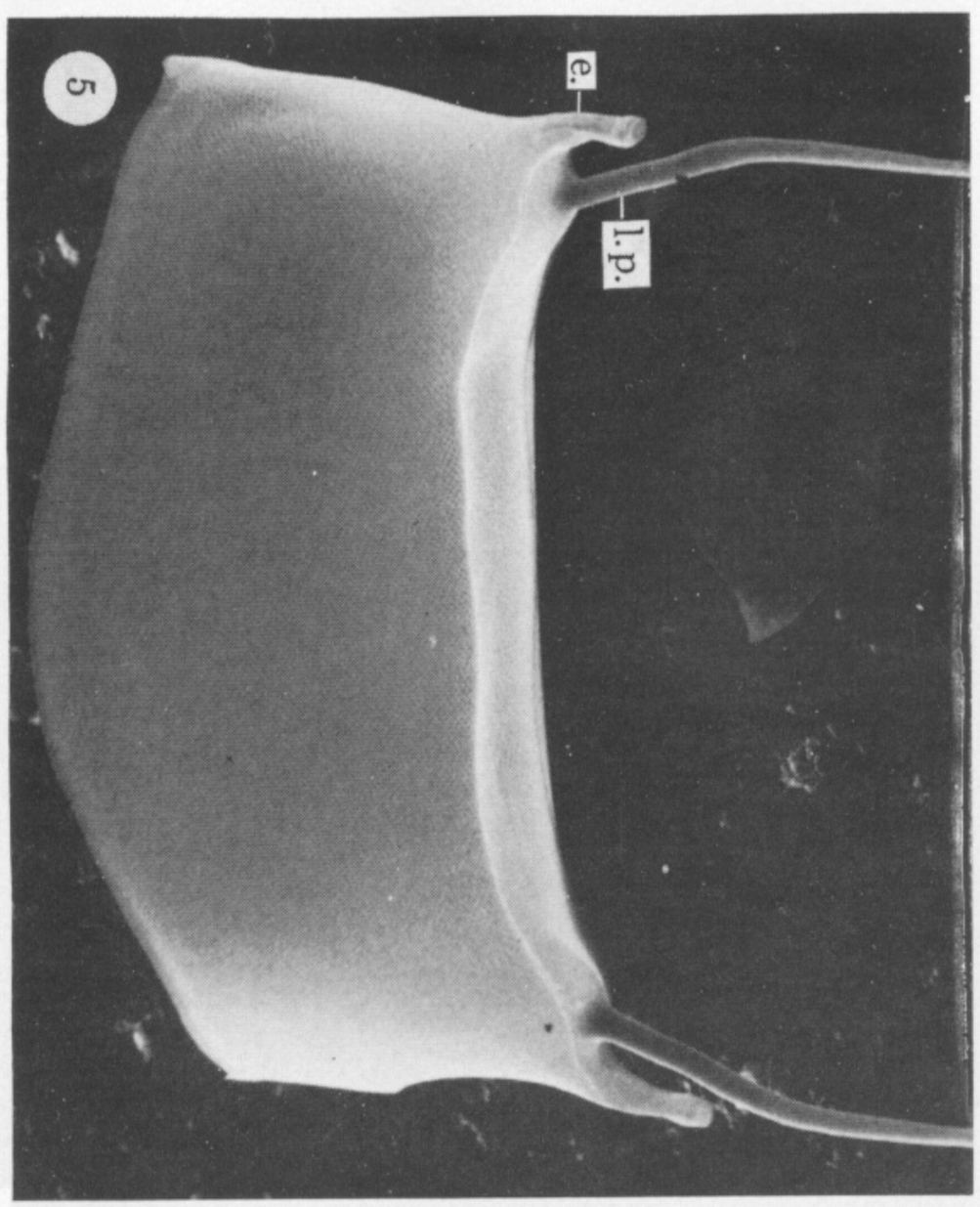

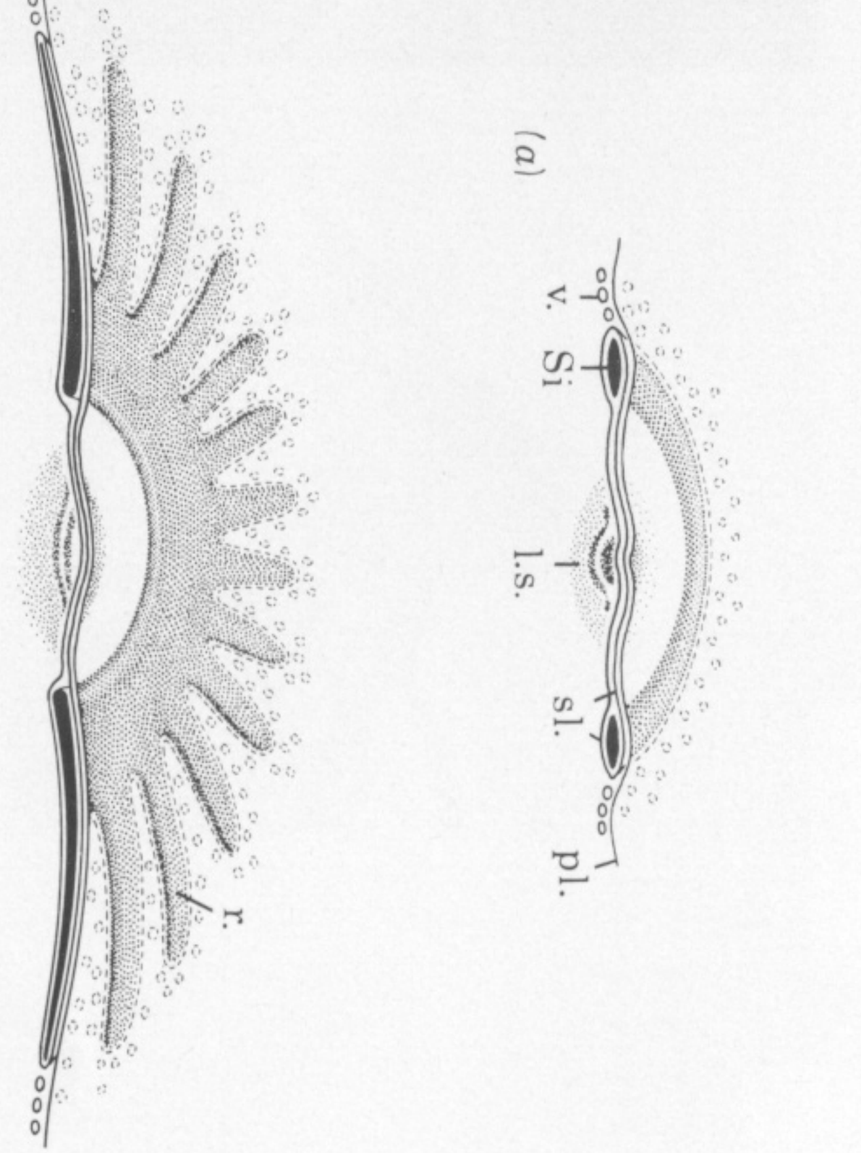

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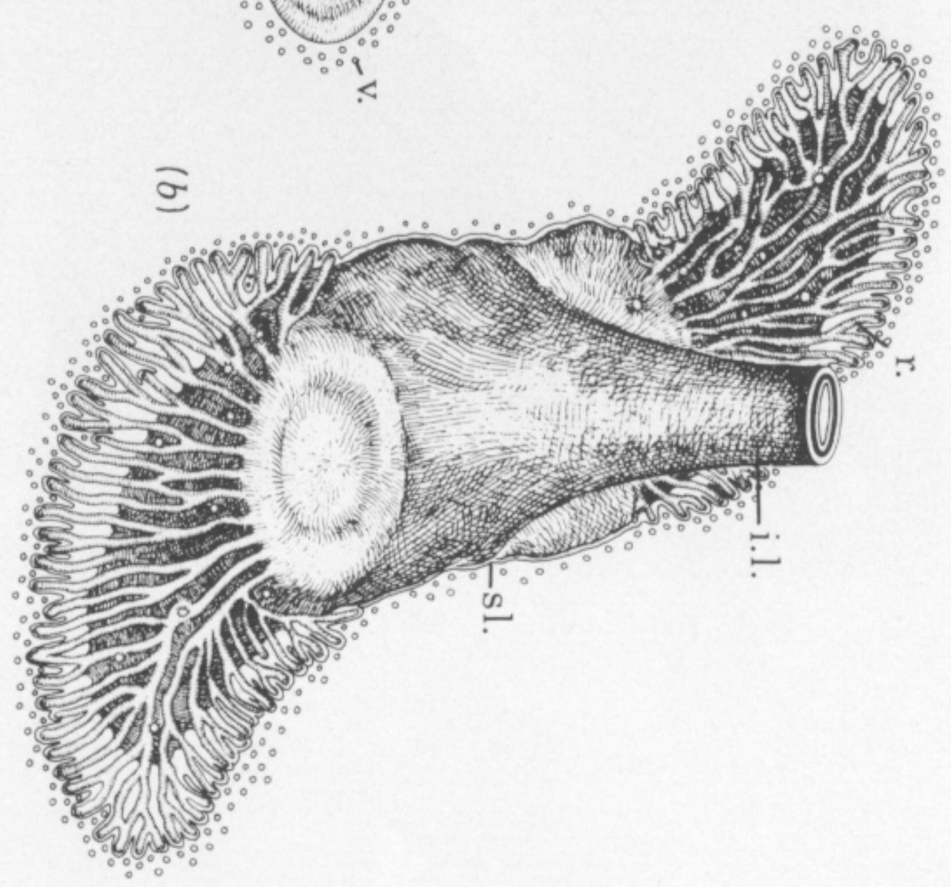

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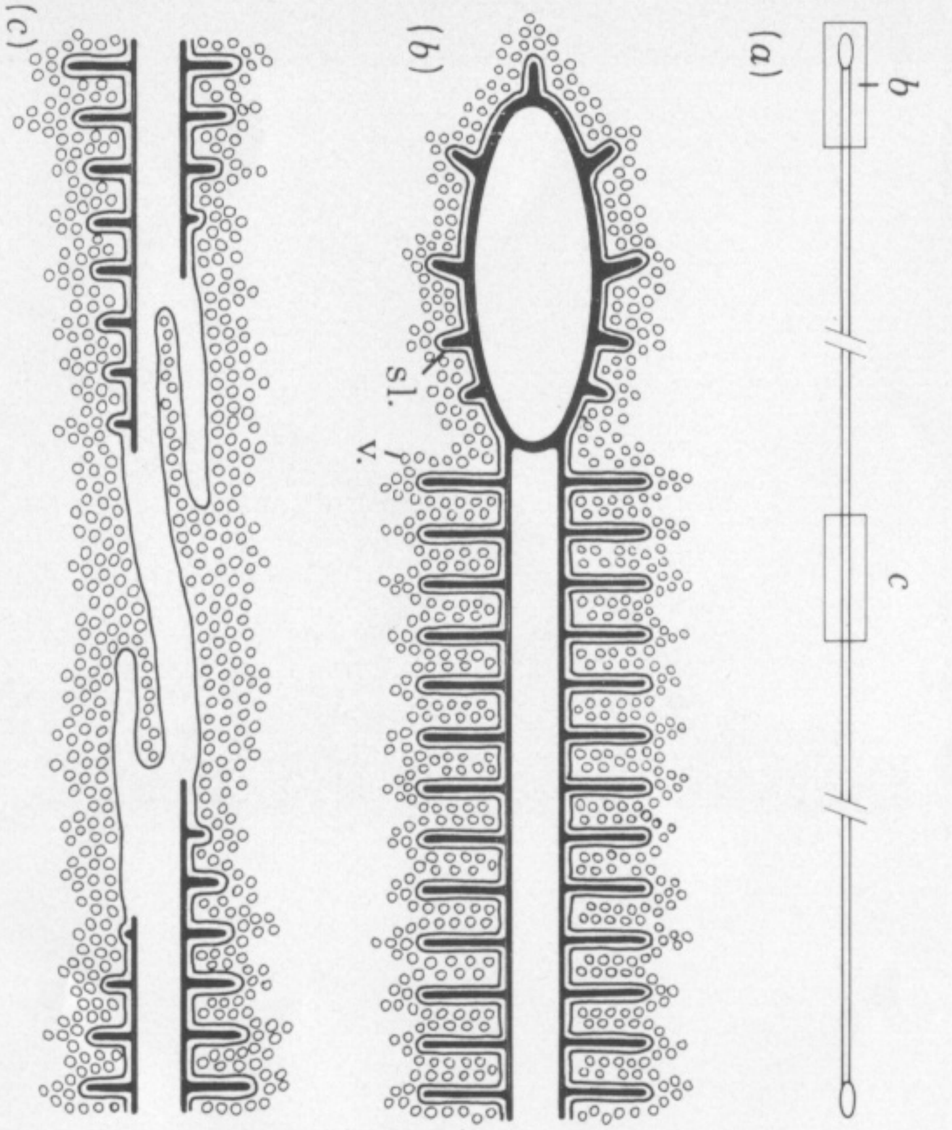

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