

CELL DIVISION IN *OEDOGONIUM*

III.* GOLGI BODIES, WALL STRUCTURE, AND WALL FORMATION IN *O. CARDIACUM*

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Summary

The structure of the ring in *O. cardiacum* is described; it is laid down adjacent to a circumferential weakening in the inner wall layer (present in interphase cells as well) which predicts precisely the rupture site. In basal daughter cells, this weakening is derived originally from a discontinuity in the basal lip of the ring itself. Two types of caps are formed; the classical series of single caps in sequence in apical daughter cells is matched by the invariable formation in basal daughter cells of a single large cap, which has added to it one tier per division, the next ring being formed each time adjacent to this "tiered" cap. The golgi bodies become hypertrophied early in mitosis (after the ring has been initiated); they remain thus during cell extension that follows wall rupture. However, once the septum reaches its final position forming a new cross wall, the golgi bodies in the basal daughter cell revert to the interphase condition, while the golgi bodies in the apical daughter cell remain hypertrophied during further extension. The cytochemical staining properties of the cell wall with silver-hexamine are described; peroxidation before staining induces a reaction in two types of golgi vesicles, and also in a diffuse fibrillar material in the vacuole, as well as in the cell wall structures. The septum vesicles are essentially unreactive, but the new cross wall reacts very strongly. The possible relationship of the golgi apparatus to both cell wall deposition and turgor pressure control is discussed.

I. INTRODUCTION

This paper and a previous one concerning nuclear division in *O. cardiacum* (Pickett-Heaps and Fowke 1970) represent an extension of our earlier study (Pickett-Heaps and Fowke 1969a) of an unidentified local species of *Oedogonium*, called for convenience species A. We will deal here with some aspects of the structure and synthesis of the wall in *O. cardiacum*, and particularly with observations on the golgi apparatus.

"Basal" and "Apical" Cells.—Filaments of *O. cardiacum* exhibit polarity; in particular, the ring is invariably laid down at the apical end (i.e. away from the direction of the basal holdfast). Following rupture of the wall, the "basal" cell of the two daughter cells is enclosed by the older wall, while the "apical" cell is obviously enclosed by the stretching material of the ring (e.g. see Figs. 15, 16, 17). These cells hereafter will be called the "basal daughter cell" and the "apical daughter cell" respectively.

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II. METHODS AND MATERIALS

The material used, culture methods, and processing techniques have all been described in detail elsewhere (Pickett-Heaps and Fowke 1969, 1970). In addition, we also utilized the following techniques to obtain micrographs:

- (1) Thick ("blue-green") sections of Araldite-embedded tissues (fixed in glutaraldehyde-osmium as normal) were mounted on clean glass slides; some were peroxidized with either 1% periodic acid (0.5–1 hr) or 0.4% potassium periodate (1 hr) at room temperature, and some of these were subsequently "blocked" by exposure to chlorous acid (0.2M sodium chlorite in 1N acetic acid) for 1 hr at 60°C (this oxidizes aldehyde groups to carboxylic acids—Rappay and van Duijn 1965; O'Brien, personal communication 1969). These periodized, peroxidized-blocked, and some of the original (unoxidized) sections were then all concurrently stained with Schiff's reagent (1 hr, room temperature), washed briefly in metabisulphite solution and longer in water before being dried, mounted, and photographed with a Leitz Wetzlar light microscope.
- (2) Thin sections were stained with the silver-hexamine reagent for the ultrastructural detection of polysaccharides (for methods, see Pickett-Heaps 1967b, 1968). In particular, sections were stained without oxidation, or following peroxidation (1% periodic acid for 0.5–1 hr, or 0.4% potassium periodate for 1 hr at room temperature). Other peroxidized sections were blocked as in (1) above before staining. After oxidations, staining with silver-hexamine, clearing with thiosulphate and washing (Pickett-Heaps 1968), the sections were mounted on coated grids and examined without further (i.e. lead or uranium) staining at 50 kV in an Hitachi HU-11E electron microscope.
- (3) Filaments of live material were mounted on a glass slide under a cover slip, and photographed with phase optics.

III. OBSERVATIONS AND DISCUSSION

(a) Wall Rupture in Live Cells

In *Oedogonium* species A, rupture of the cell wall *in vivo* was quite violent with subsequent wall extension being quite rapid for some while (Pickett-Heaps and Fowke 1969a). As before, this phenomenon was very difficult to record; however, two sequential light micrographs of *O. cardiacum* (Figs. 1.1 and 1.2) show the extension of the ring material that occurred in 2 min after splitting of the wall at the ring (cf. Pickett-Heaps and Fowke 1969, Figs. 18.1–18.5).

(b) Wall Microtubules

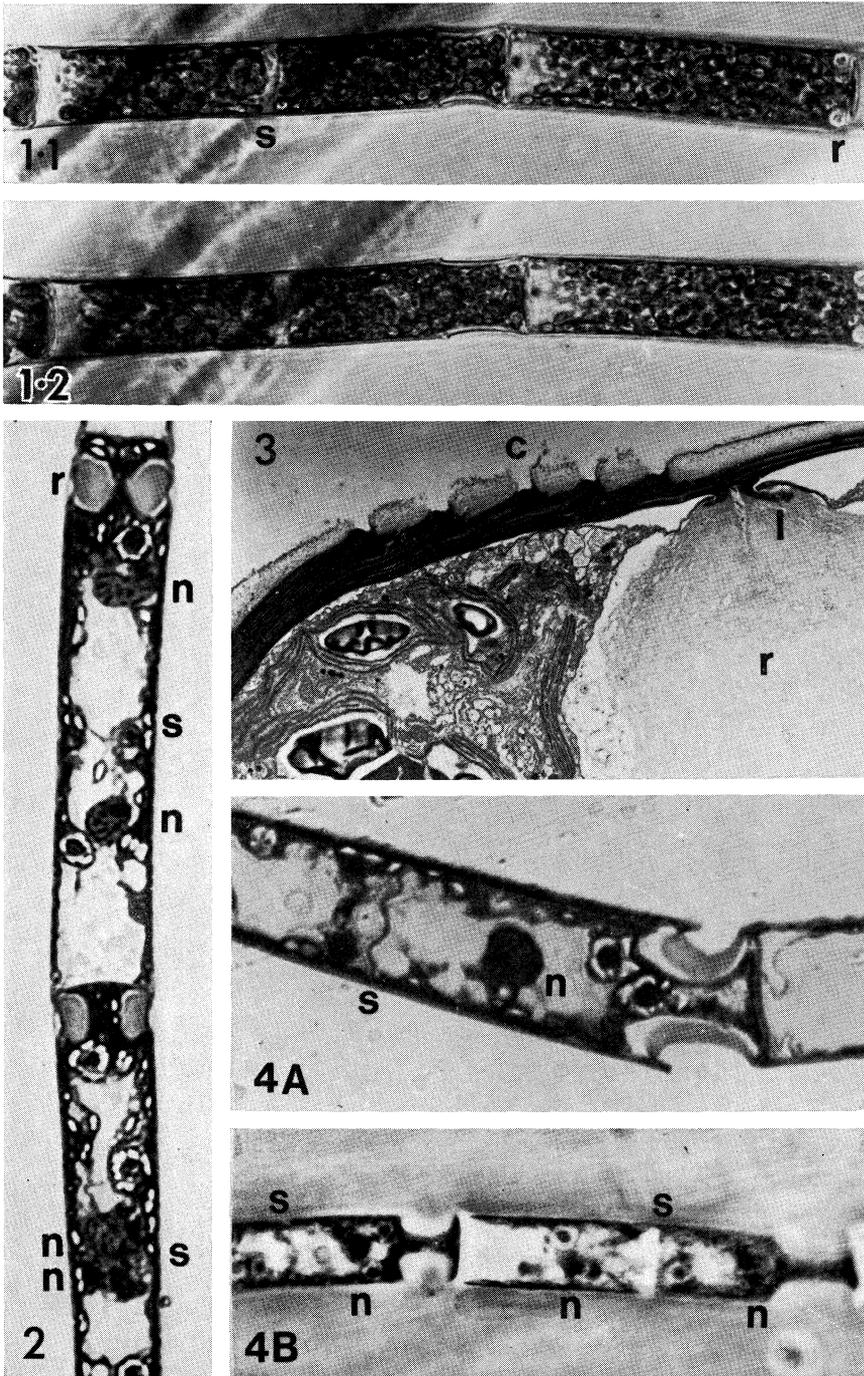
Wall microtubules in interphase cells of *O. cardiacum*, as in species A, were oriented longitudinally in contrast to their normal transverse orientation in other

Fig. 2.—Light micrograph, post-telophase cells. The nuclei (*n*) which are close together during septum (*s*) formation—lower cell—separate before wall-splitting at the ring (*r*)—upper cell. $\times 730$.

Fig. 3.—Ring (*r*), almost full size, typically situated a certain specific distance below the lowest of five caps (*c*) formed from five previous cell divisions—compare with Figure 8. Note lips (*l*) in ring, and the two wall layers. $\times 5,900$.

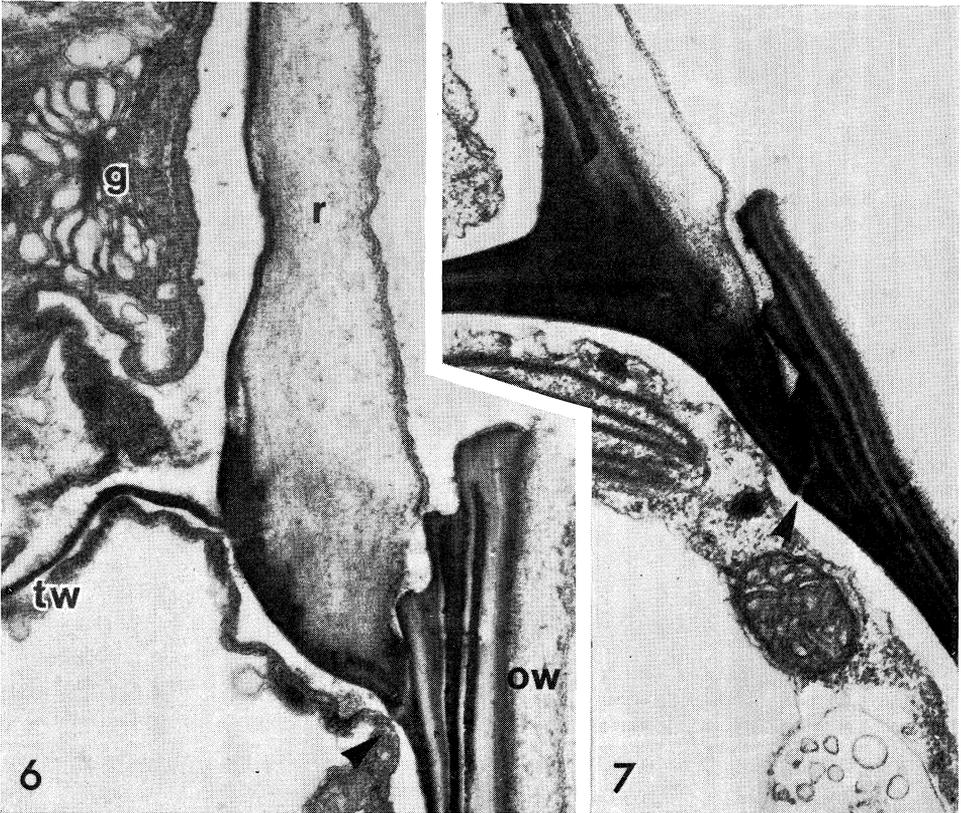
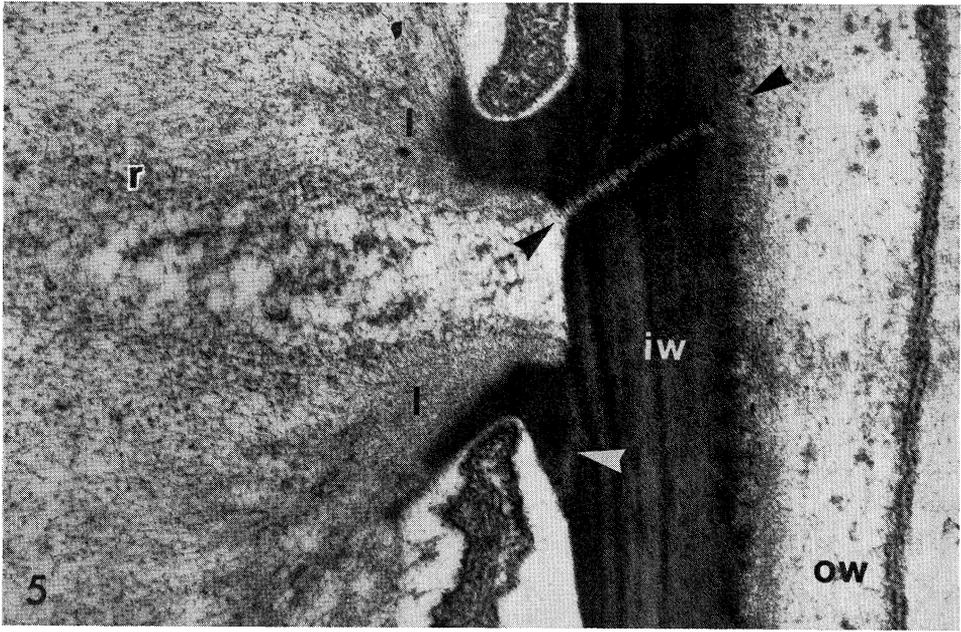
Fig. 4A.—Light micrograph; inner wall has just split, filament bending easily at the ring material. Plasmolysis damage is typical, being very difficult to avoid. $\times 750$.

Fig. 4B.—Whole fixed cells, photographed in Araldite block before sectioning; compare with Figure 4A. Inner wall has just split in left cell; elongation in right cell has increased cytoplasmic density on apical side of septum (*s*)—see Figure 14. $\times 350$.



In all figures except Figures 12 and 13, sections were cut longitudinally. Abbreviations used on these figures are as follows: *c*, caps; *cp*, cell plate; *g*, golgi bodies; *iw*, inner layer of cell wall; *k*, kinetochore; *l*, lips (in ring structure); *n*, nucleus; *ne*, nuclear envelope; *ow*, outer layer of cell wall; *p*, pyrenoid; *r*, wall ring; *s*, septum; *tw*, transverse wall; *v*, vesicles.

Figs. 1.1 and 1.2.—Light micrographs taken 2 min apart of live cell undergoing expansion after wall-splitting at ring; note elongation. Septum (*s*), and ring (*r*) in adjacent cell are visible. $\times 550$.



filamentous algae (e.g. *Chara*: Pickett-Heaps 1967a; *Spirogyra*: Fowke and Pickett-Heaps 1969) and higher plant cells (see however, Newcomb and Bonnett 1965). In *Oedogonium*, the cell's wall extension occurs mainly (or possibly entirely) during one distinct, quite short period of time (i.e. after breakage of the wall at the ring), an extraordinary method of cell growth; wall structure and microfibril orientation may therefore not necessarily be similar to those in higher plants or other algae. Unfortunately, we have no information concerning: (1) orientation of wall microfibrils and general structure of this cell wall, and (2) whether the interphase cell wall can undergo extension. Thus while microtubules may have their usual relationship with wall microfibrils (Ledbetter and Porter 1963, and others) their longitudinal orientation could be a reflection of an unusual type of wall structure.

(c) *Movement of Nuclei Prior to Wall Rupture*

As in *Oedogonium* species A, the two daughter nuclei were closely apposed to one another during septum formation (Pickett-Heaps and Fowke 1969, 1970); they then moved far apart before wall rupture at the ring, leaving the septum between them as a thin cytoplasmic strand (compare top and bottom cells in Fig. 2).

(d) *Wall Structure*

As in *Oedogonium* species A, the wall was composed primarily of two distinct layers (Figs. 3, 5-8, 10, etc.); we consistently observed, however, another very thin, membrane-like coat on the outer layer (e.g. Figs. 5-7, 10, etc.). The thick outer layer of the wall, derived from the material of the ring as in species A (Figs. 4A, 4B, 6, 17), is probably a layer of mucilage-like material, being particularly extensible and bendable (Figs. 4A, 4B) after wall-splitting at the ring. The inner layer usually (but not always) stained quite heavily (Figs. 5-8); if the algal filaments were bent during processing, this wall buckled and so it probably represented the tough cell wall proper; it was not laid down until cell elongation was (as far as we could tell) nearing completion (Figs. 6, 22, 24). A build-up of turgor pressure might be responsible for wall rupture and subsequent extension (Figs. 1.1 and 1.2; see also Pickett-Heaps and Fowke 1969); if so then the inner wall would need to be fairly rigid and inextensible.

Fig. 5.—Reinforced lips (*l*) of wall ring (*r*) are shown in their usual position, the apical one adjacent to the weakening (black arrows) in the inner wall layer (*iw*); the angle of this weakening matches precisely the angle of previous breakages (i.e. shown in the caps—Fig. 3). Outer wall layer (*ow*) enclosed by membrane-like structure (see Figs. 6, 7, 10, and others). Note incipient weakening in basal lip (white arrow); this will predict the site of the cap in the basal daughter cell (Figs. 6, 7, 8, and 18). Fibrous material of ring attached to the lips. $\times 51,000$.

Fig. 6.—Cell elongation almost complete; this division has added one tier to the single cap in the basal daughter cell. Transverse wall (*tw*) formed, inner wall layer forming inside ring material (*r*)—note typical hypertrophied golgi (*g*) in apical daughter cell. Site of next wall splitting in basal cell determined already (arrow) from incipient weakening of the basal lip—see in Figure 5. $\times 18,700$.

Fig. 7.—Interphase; note weakening (arrow) in inner wall predicting precisely the site of the next wall rupture (cf. Fig. 6) which would then have added another tier to the single cap. $\times 23,250$.

The existence of a discontinuity in the inner wall layer at the ring was suspected but not clearly demonstrable in species A. It has been found in all cells of *O. cardiacum* (Figs. 5-7); it predetermined precisely the position of the ring itself (Fig. 5), and the angle of the weakening matched the angle of previous wall splitting shown in the caps, where present (as in Figs. 3 and 8). It also widened appreciably just before wall rupture (Fig. 10). Since this weakening was present in interphase cells (Fig. 7) and in zoospores (Pickett-Heaps and Fowke, unpublished observations), it obviously can be utilized for zoospore release, when the cell wall also splits neatly (no ring is laid down in these circumstances). The formation of this discontinuity is described below.

(e) Structure of the Ring

The structure of the ring in *O. cardiacum* seems equivalent to that in species A (Pickett-Heaps and Fowke 1969), but it was often oval in profile (Fig. 8). The fibrous material of the ring was attached to the two reinforced lips of wall material (Fig. 5) which appeared vital both in controlling the extension of this material and in locating the septum in its final position (Figs. 16, 16A, 17; see also Pickett-Heaps and Fowke 1970). These two lips merged into the new inner wall layer deposited after cell extension was complete (see Figs. 6, 7).

Since the discontinuity in the inner wall layer predicted the site of the ring (see above), we tried to find how far back in the cell's history we could trace its existence. In the case of cells forming *tiered* caps (see below), the structure of the *basal lip* of reinforced wall material included this built-in weakness; for example, if one examines Figures 5 and 10 and visualizes a new inner wall layer being formed above the basal lip, then the faint electron-transparent region (Figs. 5 and 10, white arrow) is located in the right position to give the next weakened wall region in the daughter cell. Figure 6 shows a basal daughter cell soon after the transverse wall had been formed; note how this electron-transparent layer on the basal lip (Fig. 6, arrow)—now forming part of the new wall—is in precisely the right position for the next wall rupture (compare with Fig. 7, arrow). However, the equivalent situation in apical cells with a series of upper caps (Fig. 3) is not so easily explicable. The weakened region apparently must have been built into the new inner wall layer, its position being fixed a certain precise distance below the previously formed cap (Fig. 8). We do not know how this was achieved; however, a few other similar discontinuities elsewhere in the wall (apparent in Fig. 18—these were not artefacts) were always found, these having no obvious significance.

Hill and Machlis (1968) describe a "core" in the centre of the ring of *O. borisianum*, speculating that this functions in wall rupture. Our results show clearly that the

Fig. 9.—Tangential section, very early ring formation (well before prophase). Note small size of golgi bodies (*g*). $\times 18,000$.

Fig. 10.—Inner wall layer just about to rupture completely—note increasing size of the weakening (black arrow, cf. Fig. 5), into which some of the ring (*r*) material seems to have been forced. As before, incipient weakening (white arrow) for future basal daughter cell visible. $\times 24,200$.

Fig. 11.—Section tangential to *inner* surface of wall ring (*r*), prophase; the cytoplasm visible was very close to the ring, i.e. plane "x-x" in Figure 8. A large number of vesicles are normally found in this region—possibly derived from the golgi apparatus (Hill and Machlis 1968). $\times 12,200$.

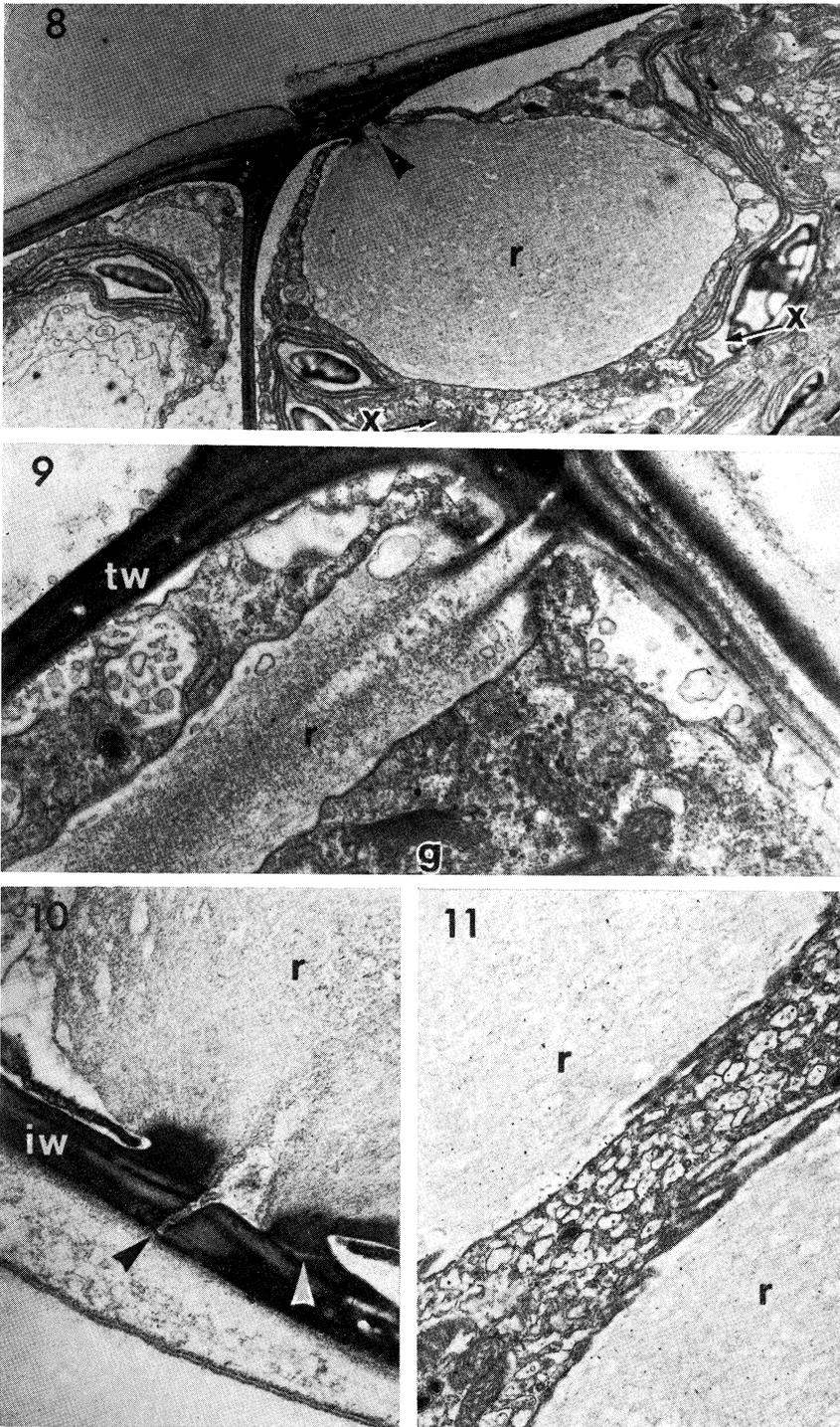


Fig. 8.—Wall ring (*r*) formed *adjacent* to the single, two-tiered cap (cf. Fig. 3). As usual, inner wall weakening (at arrow) predicts the rupture site. (Fig. 11 taken in the plane of “x-x”). $\times 8,900$.

wall is preweakened, which also explains zoospore release without ring formation (see above); a similar core in our micrographs (Figs. 5, 10) seems to be merely due to a region containing less densely organized ring material. Otherwise we are essentially in agreement with their results concerning ring structure.

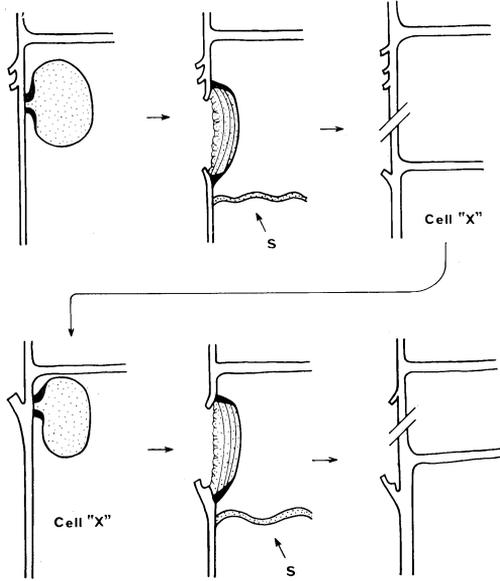


Fig. 12.—Diagram explaining tiered cap formation. Upper sequence shows how another cap is added to the two already present (the conversion of ring material into the outer wall layer is not represented here—see Pickett-Heaps and Fowke 1969, Fig. 54); in subsequent division in the basal daughter cell (cell “X”), the ring is laid down adjacent to the “upwards-facing” cap (see Fig. 8); wall-splitting, etc. leads to the addition of a tier to this single cap (lower sequence).

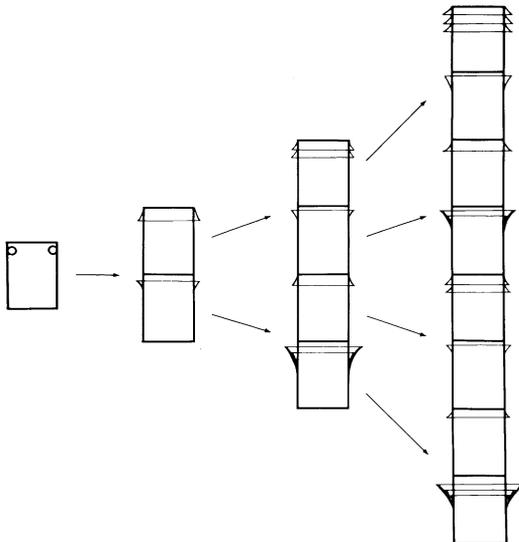


Fig. 13.—Diagram to show pattern of caps formed over three generations of cell division in a filament of *O. cardium*, all cells dividing. In addition to the classic series of upper caps, the formation of some single “tiered” caps is also invariable when basal daughter cells subsequently divide; see bottom sequence, Figure 12.

(f) Formation and Structure of the Caps

We have now established that (our two species of) *Oedogonium* forms two quite distinct systems of caps. In apical daughter cells, the next ring will always be situated a certain predictable distance below previous caps (Fig. 3); wall-splitting, etc. leads

to the formation of an apical daughter cell whose number of caps increases by one per division (top sequence in Fig. 12), the classical case described in all the literature. However, we also always encountered a small number of "tiered" caps (e.g. Figs. 6, 7, 8), these being ignored at first (as they were in species A). However, careful reflection soon revealed that these must invariably be formed in basal daughter cells undergoing further division, where the next ring is always laid down *adjacent* to the "upward facing" cap (Fig. 8); wall-splitting, etc. now leads to the formation of a single, slightly bigger cap whose *number of tiers* increases by one per division (bottom sequence in Fig. 12). Figure 13 shows how the pattern of these tiered caps in a filament of *Oedogonium* is related to the history of cell division (and of course to the pattern of typical caps) for three generations of cell division. Just why *Oedogonium* has evolved two different cap systems is not obvious. Possibly there is a subtle reason associated with the biophysical properties of the cell wall; in apical daughter cells, the system of caps probably strengthens the wall and if there is not enough space to form the next ring adjacent to the first-formed cap, then perhaps rings must be laid down below it to ensure reliable wall rupture.

(g) *Formation of the New Cross Wall*

As in species A, formation of a new cross wall in the septum occurred presumably following fusion of the vesicles within it, *after* the septum had moved to its final position (compare Figs. 16 and 17). Even when very thin, the newly formed cross wall (Fig. 17D) was easily detectable with the silver-hexamine staining procedure (see below—Fig. 17c). Whether the vesicles in the septum had been derived from the golgi bodies is not clear from our work.

(h) *Golgi Bodies*

The golgi bodies were invariably closely associated with endoplasmic reticulum which was devoid of ribosomes where adjacent to the golgi body, this being particularly noticeable in the thin peripheral cytoplasm of interphase cells, where these two organelles were isolated together. Small "coated" vesicles were apparently blebbing off the endoplasmic reticulum collecting at the adjacent face of the golgi body (Figs. 14A, 14B, 15B, 16C, and others); this may indicate how synchronized control over the golgi system is exercised (Pickett-Heaps and Fowke 1969).

In interphase cells, the golgi bodies and their heavily stained vesicles (as in Fig. 15A) were small. They remained thus before mitosis when early deposition of the ring was occurring (Fig. 9). However, by prophase, they became markedly hypertrophied, their vesicles increasing very considerably in size (as in Figs. 14A, 14B, and others). These vesicles sometimes contained a fibrous diffuse material or, less frequently, a small quite dense inclusion (shown in Figs. 21 and 22). As with species A, many of these large vesicles appeared to be discharged into the vacuole (e.g. Fig. 14A and others). However, many other vesicles containing the fibrous material were also concentrated around the wall ring (Fig. 11).

The golgi population in each of two daughter cells, separated by the septum, remained hypertrophied (Figs. 14, 14A, 14B) as the septum moved up the cells during cell elongation. The septum eventually came to rest at its final position above

the basal lip of reinforced wall material (Figs. 16, 16*B*, 17) derived originally from the ring (see Pickett-Heaps and Fowke 1969). During the septum's movement up the cell, the overall cytoplasmic density of the apical daughter cell always increased (compare Figs. 4*B*, 14, 15). When this movement of the septum was completed, the golgi bodies in the basal daughter cell reverted immediately back to the interphase state (Figs. 15 and 15*A*; 17 and 17*B*). However, in apical daughter cells the golgi bodies remained hypertrophied (Figs. 15 and 15*B*; 17 and 17*A*; also Fig. 6) for a considerable period whilst this cell was undergoing further extension. This difference in the two golgi populations was even detectable as the septum itself reached the lower lip; the cell shown in Figures 16 and 16*A* had no trace of vesicle fusion in its septum (Figs. 16*B*, 16*D*), yet the golgi bodies in the basal daughter cell were apparently just reverting back to the interphase condition (compare the golgi bodies labelled in Fig. 16*B* on each side of the septum, and those in Figs. 16*C*, 16*D*). This difference was always most pronounced by the time that the cross wall had been completed (as in Figs. 15, 15*B*). The significance of these observations is discussed below.

On a few occasions only, a phase of golgi replication by fission seemed to occur after cross wall formation had been completed. In the cell shown in Figure 15 almost all the golgi bodies in each cell were splitting (perhaps a synchronized event) although their secretory properties had changed in each daughter cell (Figs. 15*A*, 15*B*) as normal.

(i) *Histochemistry with the Light Microscope*

Unperoxidized sections did not stain with Schiff's reagent. Peroxidation of sections resulted subsequently in a very strong staining of the starch in the chloroplasts and pyrenoids (Fig. 24); the (inner) layer of the cell wall and the reinforced lips of wall material in the ring also stained quite strongly (Fig. 24), but the ring or material derived from it during cell wall extension was not detectably stained except where the new inner wall layer was forming (Fig. 24). Staining of the peroxidized wall structures and starch with Schiff's reagent did not occur if sections had been further oxidized with chlorous acid. However, this control may be invalid (see below).

(j) *Cytochemical Problems*

Cytochemical techniques, using basic silver-hexamine solutions for the detection of cell wall polysaccharides (Pickett-Heaps 1967*b*, 1968), have been used on sections of *O. cardiacum* to try to clarify functions of the golgi bodies.

Two technical problems need be considered: firstly, Dr. T. P. O'Brien (personal communication 1969) has followed the staining of the cuticle of some higher plants

Fig. 15.—Whole fixed cells in Araldite as before; transverse wall (*tw*) completed and quite thick. $\times 430$.

Fig. 15*A*.—Golgi body from that basal daughter cell shown in Figure 15 by arrow. It has reverted to the interphase condition. $\times 22,600$.

Fig. 15*B*.—As in Figure 15*A*, but the typical hypertrophied golgi body in the apical daughter cell in Figure 15. (Note the endoplasmic reticulum associated with the golgi body.) Most of the golgi bodies in each daughter cell (whether hypertrophied or not) appeared to be splitting. $\times 29,000$.

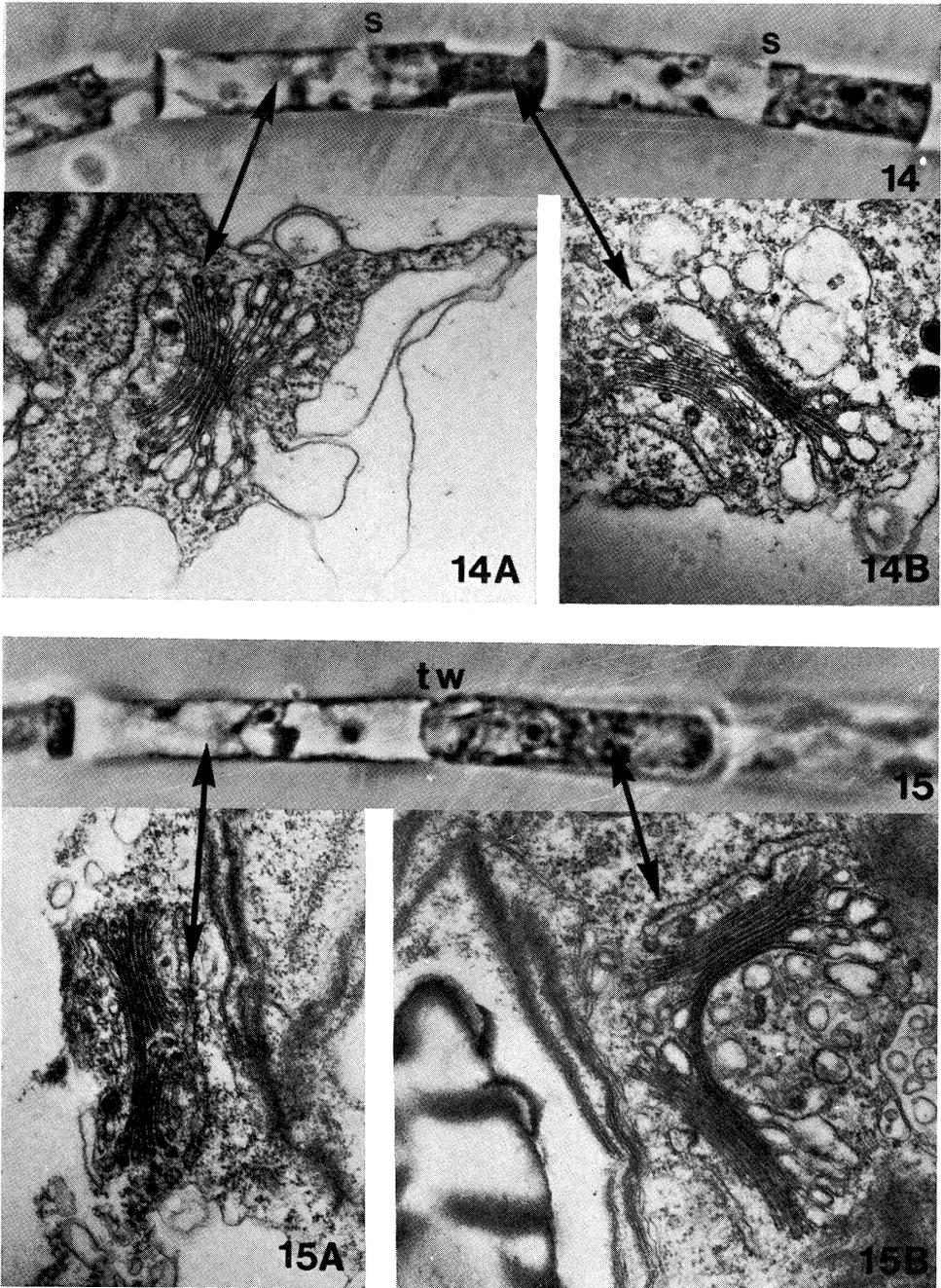


Fig.14.—Expanding cells, photographed whole in Araldite before sectioning; typical golgi shown below. $\times 375$.

Figs. 14A, 14B.—Golgi bodies from those basal and apical cells in Figure 14 indicated by arrows. Golgi bodies are all hypertrophied at this stage of cell elongation. $\times 29,000$.

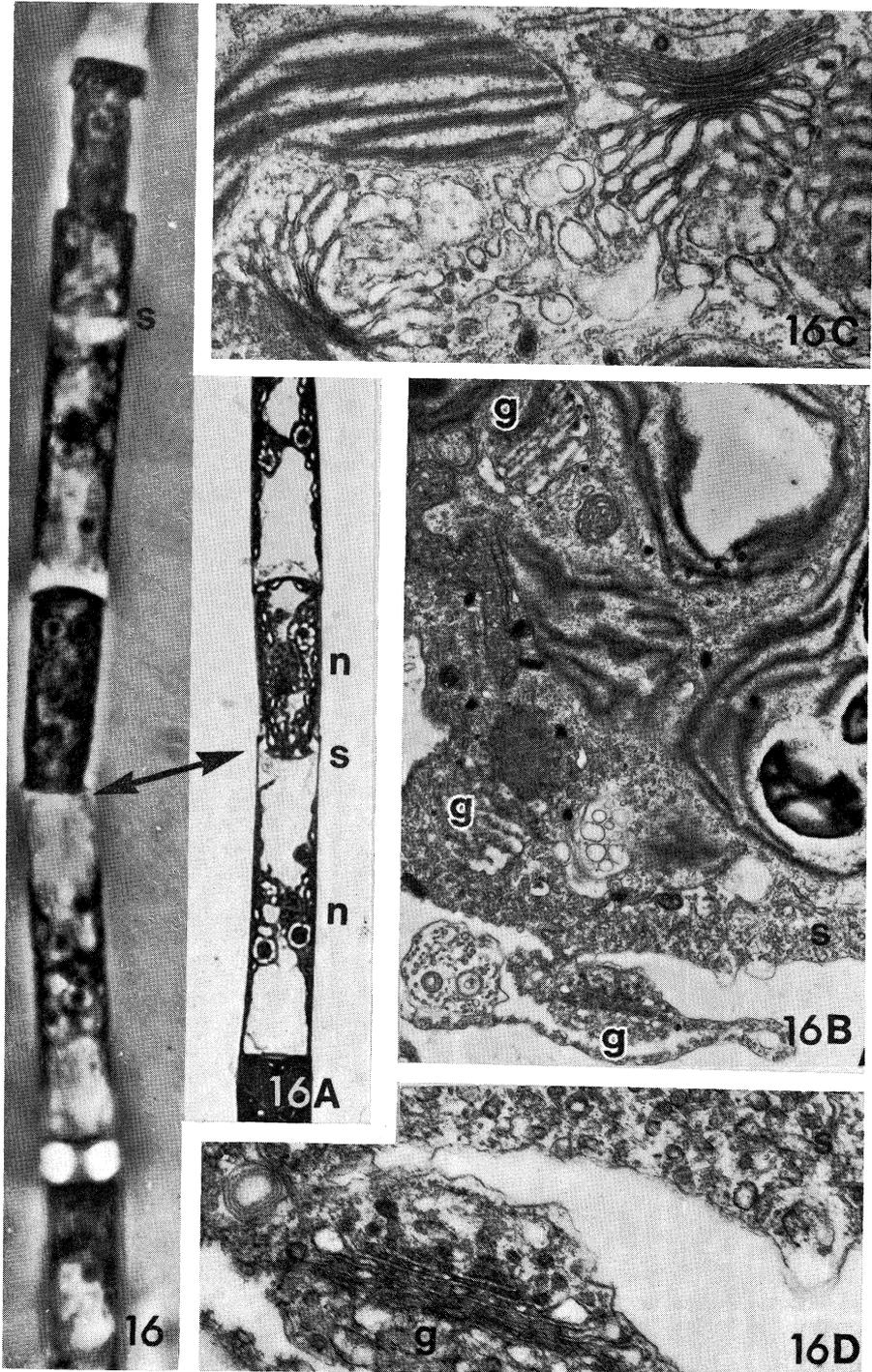


Fig. 16.—As before, whole cells in Araldite. $\times 450$.

Fig. 16A.—Light micrograph, section of cell indicated by arrow. Septum (*s*) had reached its final position, although plasmolysis damage has pulled it slightly away from the lower lip of the former ring. $\times 400$.

(wheat, barley, etc.) in Araldite sections concurrently with toluidine blue and silver-hexamine, obtaining convincing evidence that polyuronides, for example, are *easily extracted* by several reagents (e.g. the silver-hexamine buffer and peroxidative and blocking solutions); furthermore the thickness of the section was sometimes crucial in determining whether sufficient polysaccharide was retained to give certain staining reactions. In *O. cardiacum*, starch was always removed from electron microscope sections following peroxidation (being present in unoxidized, stained sections). For such reasons, what appear to be good negative controls (i.e. blocking after peroxidation) could be the result of efficient extractive procedures. Secondly, certain *highly specific* regions of cell walls may be very reactive towards silver-hexamine without prior peroxidation in glutaraldehyde-osmium fixed specimens (Figs. 18, 19—see below), a phenomenon confirmed in some higher plants by O'Brien.

It will, however, be fairly confidently presumed that polysaccharides are mainly responsible for the silver-hexamine staining described below since:

- (1) a very marked morphological specificity of staining was obtained even without peroxidation when only starch grains and certain vesicles and specific parts of the cell wall stained strongly.
- (2) peroxidation as expected, conferred reactivity on other wall regions, as well as other components.

(k) *Cytochemical Results*

(i) *Sections not Peroxidized*

The contents of certain small vesicles (a few being present in all cells) always stained very heavily, with (Figs. 21, 25, 26) or without (Fig. 18) peroxidation; these were otherwise difficult to distinguish from the various other vesicular inclusions. Certain very specific regions of the wall (and sometimes starch) stained strongly in such unoxidized sections. These were the end walls, reinforced lips of the ring, and the caps; the apical inner wall layer was also reactive to a varying extent always decreasing along the cell, (Figs. 18 and 19). The septum vesicles were unreactive, but two thin cross walls stained quite strongly (as in Fig. 17C). Surprisingly, the entire inner wall layer of an *interphase* cell, fortuitously present in one filament, reacted very strongly (Fig. 19). This staining pattern in dividing cells was consistent although somewhat variable in extent in six different batches of sections.

This remarkable reactivity of some unperoxidized wall regions (and starch) was initially very puzzling. An oxidation of polysaccharide by osmium tetroxide is inferred (see Criegee 1936). Thus, *Oedogonium* apparently has in certain very specific regions of its cell wall a polysaccharide susceptible to oxidation by osmium tetroxide, which may be easily extractable or degraded or both (see below). Whilst

Fig. 16B.—Septum dividing daughter cells shown in Figures 16, 16A; vesicles are unfused—no cross wall present. Note difference in golgi bodies (*g*) on each side of the septum. $\times 12,000$.

Fig. 16C.—Typical golgi bodies in apical daughter cell of this pair. $\times 24,000$.

Fig. 16D.—Detail of Figure 16B; vesicles and microtubules of septum (*s*) are visible. Golgi body just reverting back to interphase condition. $\times 32,000$.

appearing in the whole inner wall of an interphase cell (Fig. 19) it was confined to the apical regions of adjacent dividing cells, an intriguing observation whose significance may be considerable once the chemical intricacies of the staining reaction are elucidated. The silver-hexamine technique is capable of distinguishing between different components in higher plant cell walls (Pickett-Heaps 1968).

(ii) Sections Peroxidized

Peroxidation removed starch grains and often reduced the intense staining of those particular wall regions described above (probably a destructive or extractive effect of peroxidative solutions or both). Sodium periodate (pH *c.* 7) appeared less extractive than periodic acid (pH *c.* 1) in this regard.

Elsewhere, peroxidation induced staining of the outer as well as the whole inner layer of the wall, and also of the ring material (Figs. 17*C*, 21, 22, 26). The young cross wall in the septum was conspicuously reactive (Fig. 17*C*) although the septum vesicles were not (Fig. 25).

The vesicles at the edge of golgi cisternae now showed two quite distinct stained contents, a diffuse fibrillar or a quite dense compact component (Figs. 21, 22, 26); they could both be found on the one golgi body (Fig. 21), and both were recognizable in ordinary sections stained conventionally with lead and uranium. Vesicles free within the cytoplasm reacted similarly; these vesicles, some quite large, were often concentrated close to the wall ring (Fig. 26).

The vacuole in dividing cells of *O. cardiacum* contained a rather diffuse, fibrillar material in many regions (e.g. Fig. 17*B*). Peroxidation caused these fine fibrils to react, demonstrating the localization this staining method can achieve (Figs. 20, 23). Some micrographs suggested that vesicles from the golgi bodies might have fused together, forming prevacuolar bodies (Fig. 22) which then collected in the vacuole (Fig. 20), perhaps rupturing and releasing their contents into it (Fig. 23).

(iii) Sections Blocked after Peroxidation

Treatment of sections with chlorous acid (see Section II) after peroxidation, prevented almost all staining with silver-hexamine. This control may be invalid (see above).

(l) The Function of Golgi Bodies in Oedogonium

We have obtained no evidence concerning the origin of the septum vesicles. Since they do not react with silver-hexamine (Fig. 25), they may contain fairly

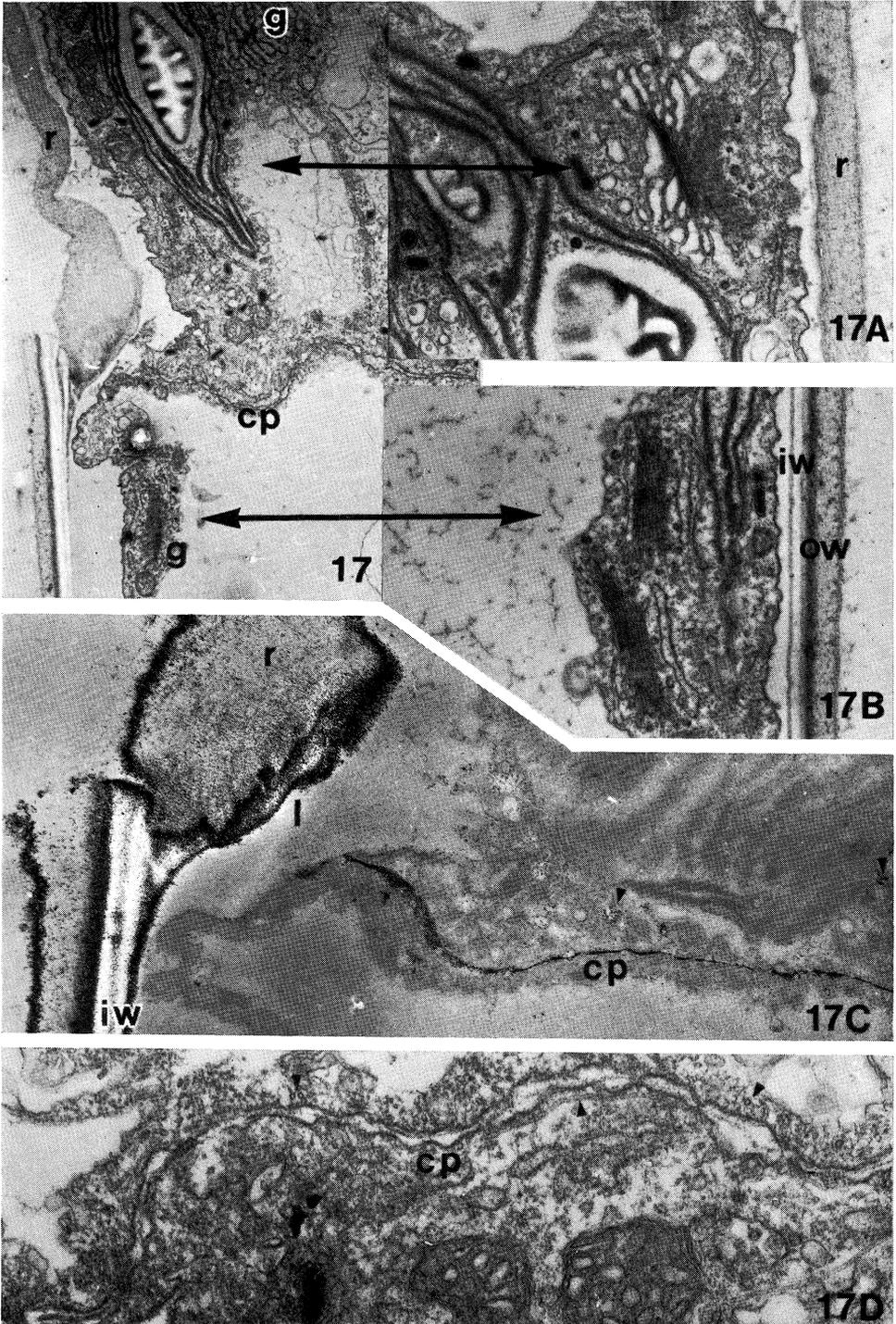
Fig. 17.—Cross wall formation—cell plate (*cp*) visible, ring material (*r*) still stretching considerably. Note difference in golgi bodies (*g*) on each side of cell plate. $\times 9,300$.

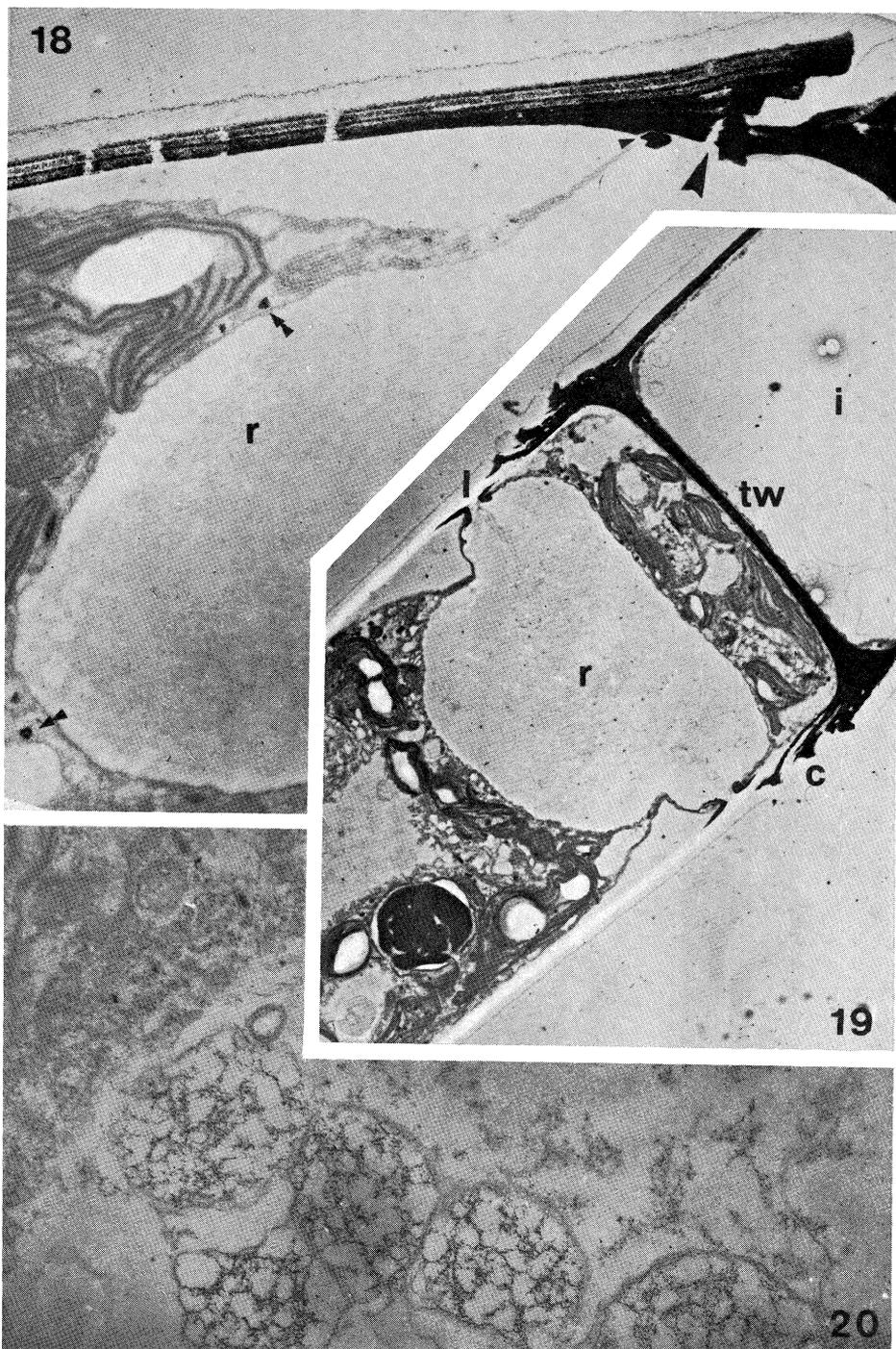
Fig. 17*A*.—Golgi in apical daughter cell of Figure 17; note the adjacent ring material (*r*) has no trace yet of the inner wall layer. $\times 16,900$.

Fig. 17*B*.—Basal daughter cell in Figure 17. Interphase-type golgi, near typical cell wall. $\times 16,900$.

Fig. 17*C*.—Equivalent cell to that in Figure 17. Section peroxidized (periodic acid, 30 min), then stained with silver-hexamine. Thin layer of wall in the cell plate is highly reactive; some vesicles nearby show a weak staining reaction (arrow). $\times 16,900$.

Fig. 17*D*.—Same cell as in Figures 17–17*B*. Thin cell plate (*cp*) is still surrounded by the transversely oriented microtubules (arrows) of the septum. $\times 35,600$.





soluble wall precursors; when they fuse, the young transverse wall is very reactive (Fig. 17C) indicating that the wall materials are now relatively insoluble. This staining behaviour seems exactly analogous to that of the vesicles and new wall in the cell plate of wheat (Pickett-Heaps 1968). Similar vesicles could also be involved in deposition of the inner wall layer; we cannot yet directly implicate the golgi bodies in this process as Hill and Machlis (1968) have done.

Hill and Machlis also provided clear morphological evidence (which we have been unable to obtain due to slight plasmolysis in our cells) for the incorporation of vesicles, probably from the golgi, into the growing ring of *O. borisianum*. Since the staining with silver-hexamine of fibrous material in vesicles matches that of the fibrous material of the ring (Fig. 26), our observations confirm their results. The ring material is soft and extensible, and is probably mucilaginous (Pickett-Heaps and Fowke 1969); Hill and Machlis found it stained with ruthenium red. Thus the golgi bodies are likely to be involved in secretion of mucilage as in some higher plants (Mollenhauer, Whaley, and Leech 1961; Northcote and Pickett-Heaps 1966).

However, as in species A, we also gained a very strong impression that golgi vesicles were being discharged directly into the vacuole (Figs. 14A, 14B). This is very significant since the violent wall rupture and cell expansion (Figs. 1.1, 1.2) appear to result from a build-up of turgor pressure within the cell (Pickett-Heaps and Fowke 1969; see also Hill and Machlis 1968, p. 270).

The cytochemical results described above support this impression as fibrillar staining material similar to that in golgi vesicles is found in the vacuole (Figs. 20 and 23). Some micrographs suggest a preliminary concentration of this material in prevacuolar bodies (Fig. 22), these prevacuolar bodies collecting in the vacuole too (Fig. 20) before contributing their material to it. Furthermore the hypertrophied golgi bodies in basal daughter cells revert back to the interphase condition immediately the septum stops moving up the cell, whilst in the still expanding apical daughter cell, the golgi remain conspicuously hypertrophied (see above). To our mind, these observations indicate a role of the golgi in turgor control.

Alternatively, it could be argued firstly that the appearance of golgi vesicles being discharged into (and the detection of fibrillar material within) the vacuole is artefactual, resulting from some (so far unavoidable) disruption of the tonoplast during fixation which allows golgi vesicles to leak into it (this would not explain how the large vesicle in Fig. 22 was formed). This line of argument could secondly explain the different secretory products of the golgi in daughter cells (as shown in Figs.

Fig. 18.—Unoxidized section stained with silver-hexamine. Typical very intense staining of certain cytoplasmic vesicles (double arrows), transverse wall, lips of the ring, and to a lesser and variable extent, the inner wall layer at the apical end. Ring (*r*) and outer layer of wall unstained. The inner wall layer was just rupturing (large arrow) and the incipient weakening in the basal lip of the ring shows up well (small arrow). $\times 13,200$.

Fig. 19.—As for Figure 18. The whole wall of the upper interphase cell (*i*) reacted strongly as did the lips (*l*), caps (*c*), and transverse wall of a dividing cell (*tw*). $\times 3,750$.

Fig. 20.—Section peroxidized (periodic acid, 30 min) and stained with silver-hexamine. The vacuole of these cells often contains a diffuse fibrillar material often enclosed by membranes in many regions. This material reacts with silver-hexamine after peroxidation. $\times c. 28,000$.

15-15*B*; 16-16*D*; 17-17*B*) in terms of the differing need for wall material in each cell once elongation had been completed (i.e. the apical daughter cell needing continued deposition of wall materials). However, it does not explain why the golgi bodies are initially hypertrophied in the basal daughter cell (Figs. 14, 14*A*) where there is little need or evidence for wall deposition. The golgi bodies may be forming vesicles with at least two different contents (see Fig. 21); perhaps, therefore, they are more versatile than normally suspected, performing two functions at once, contributing material to the wall (ring) and also being involved in turgor pressure control. A role in vacuolation in other algal cells has already been suggested [see Pickett-Heaps and Fowke (1969) for references], and such a dual function is quite logical; for example, secretion of acidic (pectic) polysaccharides could be utilized for the concurrent elimination of ions from the cell; other polysaccharides could contribute to the turgor pressure of the vacuole. Further speculation is unwarranted; however, *Oedogonium*, because of its unique and fascinating method of cell division, may prove ideal for further study into vacuolation and turgor pressure control.

IV. ACKNOWLEDGMENT

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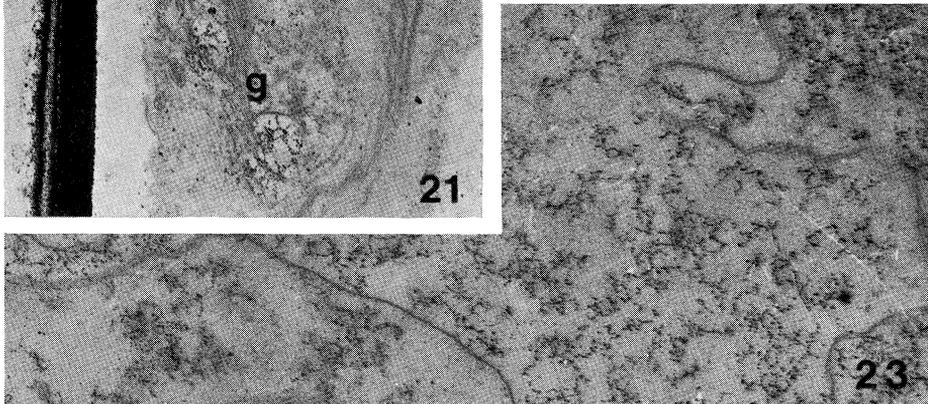
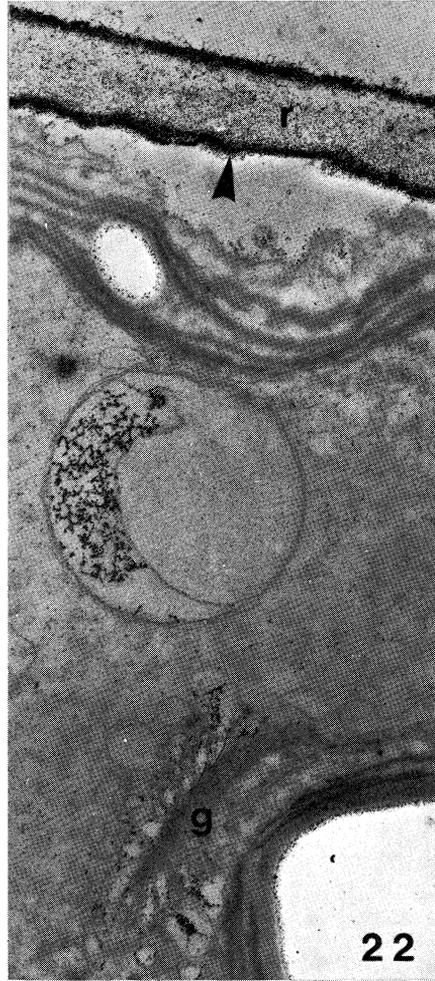
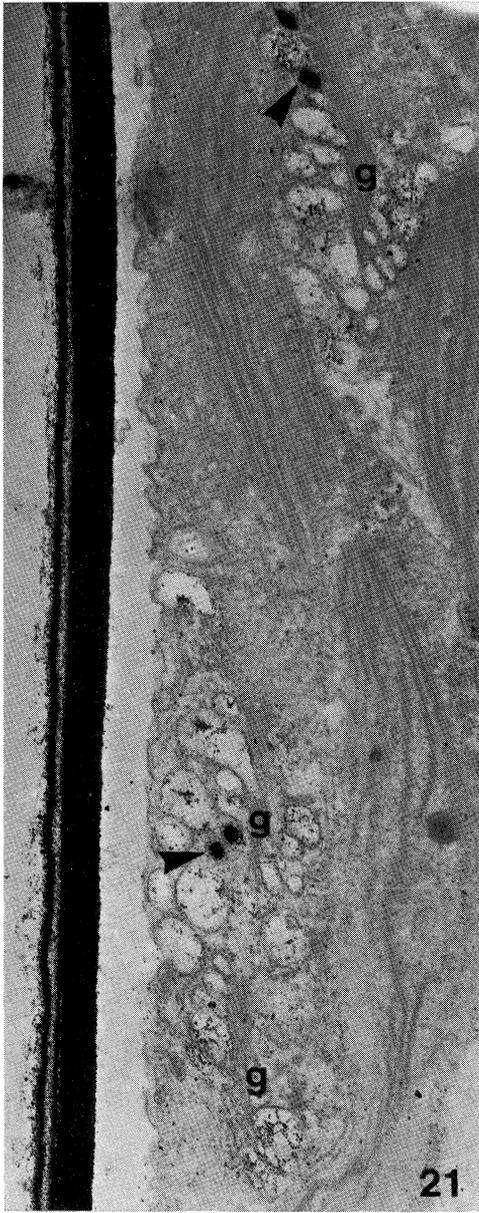
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Fig. 21.—Basal daughter cell, soon after wall rupture. Section peroxidized as before and stained with silver-hexamine. The vesicles near the golgi bodies (*g*) contain either a small densely reacting component (arrows) or diffuse fibrils which stain. $\times 18,800$.

Fig. 22.—Apical daughter cell, very thin inner wall layer (arrow) being laid down inside stretched ring material (*r*); section peroxidized and stained as before. The contents of the golgi vesicles have reacted, as has the fibrillar material within some (probably damaged) membrane-bounded inclusions. $\times 22,600$.

Fig. 23.—Section treated as before; fine fibrils in the vacuole of this anaphase cell have reacted with silver-hexamine. $\times c. 47,200$.



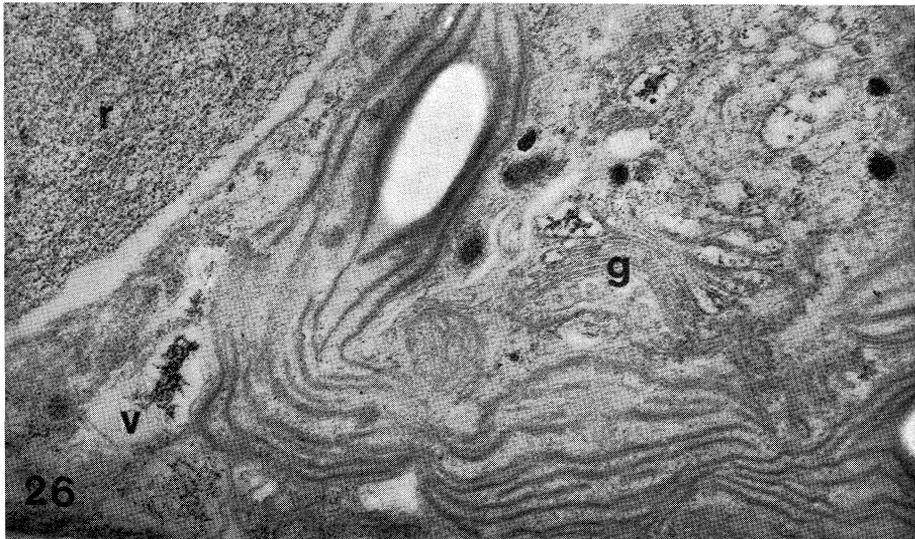
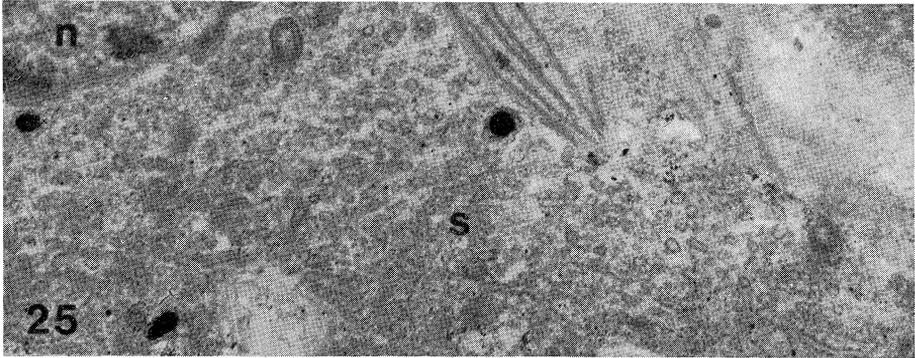
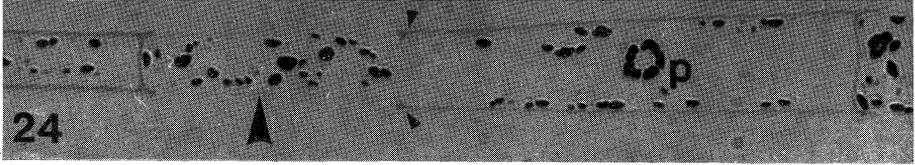


Fig. 24.—Light micrograph; thick Araldite section peroxidized (periodic acid, 30 min) and stained with Schiff's reagent. Starch reacts very strongly; inner wall layer (and lip—small arrows—derived from the ring) stain in these elongating cells; the material of the ring (here considerably stretched) does not stain detectably, but a very thin layer of new inner wall is just visible (large arrow). $\times 600$.

Fig. 25.—Section peroxidized, stained with silver-hexamine. Post-telophase nuclei (*n*) close together on each side of septum (*s*)—as in Figure 2, lower cell. Vesicles of septum show little if any reactivity (compare with the thin wall in Fig. 17C). Three of the ubiquitous, densely stained vesicles are visible. $\times 27,000$.

Fig. 26.—Premitotic cell; section peroxidized and stained as before. Ring (*r*) reacts as do the contents of vesicles at the golgi bodies (*g*), and nearer the ring (*v*). However, no direct evidence for vesicle absorption into the ring could be obtained. $\times c. 18,000$.

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