CELL DIVISION IN *OEDOGONIUM*

I. MITOSIS, CYTOKINESIS, AND CELL ELONGATION

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Summary

Development of new processing techniques has enabled a light- and electron-microscopic study of cell division in *Oedogonium* to be made; many unusual or unique features are described. A series of light micrographs confirms the sequence of events described by earlier workers. Splitting of the cell wall at the ring has been observed *in vivo*; the rupture is often quite violent, and subsequent elongation is rapid for a short while. At the ultrastructural level, the premitotic movement of the spherical nucleus towards the wall ring coincides with the appearance of some nearby microtubules. The preprophase nucleus enlarges and becomes flattened and spindle-shaped as a sheath of microtubules envelopes it. The nuclear membrane at each pole is very drawn out, appearing as two closely apposed membranes undulating into the cytoplasm; between the membranes is a dense amorphous material, and around them are longitudinally oriented microtubules. During prophase, chromatin condensation accompanies nucleolar dispersion. Microtubules start appearing within the nuclear membrane which remains essentially intact throughout subsequent division. At prometaphase, intranuclear microtubules increase and some are attached to diffuse scattered kinetochores. By metaphase, many bundles of microtubules are attached to paired kinetochores, which have a complex, layered structure. Separated kinetochores are visible at anaphase. At telophase, the spindle becomes very elongated, and many interzonal microtubules appear. A granular and heavily staining "midbody" is found between the nuclei, and this later disperses. The daughter nuclei then come very close together, and between them is found a complex of vesicles and microtubules. A septum is then formed across the cell, evidently by these microtubules (accompanied by the vesicles) pushing out the tonoplast across the vacuole till partitioning is accomplished. The nuclei then separate prior to wall splitting. The ring formed at one end of the cell has a highly characteristic structure with two lips of toughened material at its outer edge attached to the outer wall; splitting of the older wall occurs between these lips. A proliferation of large, electron-transparent golgi vesicles was apparent at this stage; these were apparently being discharged into the vacuole, and so may have been associated with a possible build-up of turgor pressure within the cells. The soft material of the ring is drawn out between the lips during cell elongation. The septum (still composed of vesicles and microtubules) moves up the cell like a diaphragm, eventually reaching the bottom lip of the extended ring structure. Coalescence of vesicles then occurs in the septum to form the new transverse wall attached to the lower lip from the ring. The extensible material of the ring becomes the outer layer of the cell (mucilage?); the cell wall proper is evidently formed as a thinner layer on the inside edge of this diffuse material. It is therefore suggested that the formation of wall rings, characteristic of cell division in *Oedogonium*, represents an adaptation of more usual processes of algal mucilage secretion.

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I. Introduction: Cell Division in Oedogonium

Cell division in the Oedogoniales, a classic subject for study in botany, is uniquely characterized by the formation of a ring of wall material laid down at one end of the cell. Following mitosis, the older wall splits circumferentially at the ring and cell elongation occurs as the material of the ring is stretched and thereby fashioned into the new wall. Such a curious sequence of events is of great interest, and was studied by many botanists in the past. Fritsch (1935) and Smith (1938) list many early papers on this subject.

These and other highly vacuolated, filamentous algae, however, present the biologist with a most refractory material for study using standard histological techniques. This is attested to by the lack of thin-section work in the literature. If normal techniques of fixation and embedding (either in wax or plastics) are followed, the cell contents invariably collapse. When this fixed material is compared to the delicate structure of the living cell, such gross damage renders observations on sectioned material at best highly suspect. However, recent advances in histological techniques, coupled with careful observation on the material throughout processing (to pinpoint sources of damage), have enabled the authors to achieve reasonable preservation of both *Spirogyra* (Fowke and Pickett-Heaps 1969) and *Oedogonium*. The light and electron micrographs presented here are considered an improvement on what has been achieved previously with such algae; careful examination of electron micrographs, however, clearly reveals room for further improvement (e.g. broken tonoplast membranes).

The procedures used for fixation and embedding are based on the methods of O'Brien (1967) and McCully (personal communication). The basic requirement seems to be great care and time spent over processing, with mechanical disturbance kept to a minimum and changes of solutions being made gradually.

II. Materials and Methods

(a) *Materials*

Several local species of *Oedogonium* were obtained from Lake Burley Griffin, Canberra, and one of these, hereafter called species A, proved particularly suitable for cytological investigation, since it had large, highly vacuolated, and rather short cells. This species has not been identified as yet; no sexual stages of reproduction have so far appeared. The best cytological results were obtained when this material was fixed directly upon removal from the lake (i.e. without subculturing). We have been unable to emulate the nutritional qualities of this natural environment (due in part to the proximity of drains). Our cultural methods, though reasonably effective, never provided material that gave as good cytological results as the native plants although it appeared quite healthy under the light microscope *in vivo*. This has turned out to be *vitally* important; preliminary work using cultured *Oedogonium cardiacum* and *O. foveolatum* yielded results far inferior to those described below, although just recently considerable success has been obtained with these species also. Some effects of nutrition on the cytology of these algae, and a more complete description of *O. cardiacum* and *O. foveolatum*, will soon follow in another paper.

Many variations of culture media were tried. All species we used were relatively easy to maintain on a soil–water medium (steamed twice), to which a small amount of calcium carbonate had been added (e.g. see Starr 1964). They were kept at 20°C, with an 18-hr day supplied by fluorescent lighting (four 40-W daylight bars) from underneath, and diffused above the cultures with white cardboard. Vitamin B_{12} was also added to some cultures [as recommended by Machlis (1962)]. However, such subcultured material was almost always unsuitable for microscopy, as

was material grown on many other artificial media. The only exception to this rule applied to very young cultures (i.e. a few days after subculture), when the growth of filaments (mostly derived from germlings, following zoosporogenesis) was very active. For electron microscopy, therefore, we strongly recommend use of either very young cultures, or better, material gathered in the natural state.

(*b*) *Methods*

All material was placed into 1·5% glutaraldehyde in 0·025M sodium phosphate buffer (pH 6·8) at room temperature for 1–2 hr. It was transferred to 5–6% glutaraldehyde in the same buffer at room temperature for 3–10 hr, and then into at least three changes of the buffer over about 24 hr; these buffer solutions were kept on ice. The specimens were post-fixed in 1% osmium tetroxide in the same buffer, on ice, for 12–18 hr. They were washed twice with cold buffer (about ½ hr), transferred to cold distilled water, and dehydrated gradually with cold methyl cellosolve; they remained in this iced solvent (2 changes) for 24–48 hr. They were transferred gradually through to cold absolute alcohol, and then remained 24–48 hr in two more changes of this solvent. Cold propylene oxide was added dropwise over 6 hr until an approximate 2 : 1 (v/v) ratio was reached. The specimens were transferred to cold propylene oxide, and left on ice overnight. They were brought to room temperature, and the propylene oxide was changed twice. Araldite resin mixture was then added dropwise over 6 hr to about 50% v/v with the propylene oxide, and the latter was evaporated off overnight. The material was transferred to fresh resin in polythene boats and left for a day; the resin was then polymerized at 60°C. A few strands of the algae at this stage were often mounted whole on microscope slides with cover slips for subsequent photography.

Sections were cut on glass knives, stained with uranyl acetate and lead tartrate, and examined in an Hitachi HU-11E electron microscope. For light microscopy, thicker ("blue-green") sections of the same material were placed on a drop of water on a clean glass slide. The water was evaporated away by gentle heating, and the sections were stained with a few drops of toluidine blue (1% w/v in 1% borax solution) by gentle heating again for a few seconds. The sections were then washed with distilled water, dried, mounted, and photographed as necessary. In some cases, whole specimens were photographed in the Araldite block (Figs. 55, 56, and 57), so that these micrographs could be compared with sections taken subsequently of the same cells (Figs. 55*a et al.*). Live material was mounted in water under a coverslip and photographed using phase-contrast optics.

III. Observations

(*a*) *Selection of Material for Microscopy*

We have chosen one of the local species of *Oedogonium* (sp. A) for especially close study. Its image under the microscope (when material had been fixed directly from the lake) was by far the best. There was usually a deterioration in the image of this same material when fixed after successful subculturing (see above). We have concluded, therefore, that for good results it is *very important* that the algae are as healthy as possible prior to fixation. The ease of handling of this species in Araldite has also enabled observations to be made on a considerable number of dividing cells. Mitosis in both *O. cardiacum* and *O. foveolatum* has been examined more recently; the results will soon be presented separately. These corresponded closely with the local species, and the three are probably representative of other species of *Oedogonium*. The main difference between the three studied lay in the size and shape of the cell and degree of vacuolation. Both *O. foveolatum* and *O. cardiacum* have long narrow cells; *Oedogonium* sp. A, direct from the lake, had shorter and broader, highly vacuolated cells; the nucleus and spindle was always situated on one side of the cell. This is

perhaps more typical (cf. the classical description of mitosis in the literature—Tuttle 1910; Ohashi 1930; and others).

Ten fairly distinct stages in the division cycle have been recognized. These stages are useful in that they clarify the continuous sequence of events of nuclear division; a complicated process of cytokinesis follows (step xi). The stages will be described, firstly as they appear under the light microscope, and then the ultrastructure of such cells will be detailed.

(*b*) *Light Microscopy* (*Sectioned Material*)

The morphological criteria adopted to distinguish the stages are shown; important coincidental electron microscopical observations are added briefly in parentheses. These light micrographs can be compared with more classical descriptions in the literature (e.g. Strasburger 1880; Tuttle 1910; Kretschmer 1930; Ohashi 1930); the similarity between our light micrographs and their drawings at every stage is very striking.

(i) *Interphase* (*Fig. 1*)

No wall thickening was present. The nucleus was small, spherical or slightly flattened against the wall, and always situated on one side of the cell, equidistant from the ends.

(ii) *Preparative Stage* (*Fig. 2*)

Wall thickening was synthesized; its growth continued into the earlier stages of division. The thickening was much more easily seen after fixation and embedding, as it looked much smaller in living cells (compare Figs. 18 and 55, for example). The spherical nucleus moved towards the wall thickening as preprophase set in. (Some microtubules appeared close to the nucleus.)

(iii) *Preprophase* (*Figs. 3 and 4*)

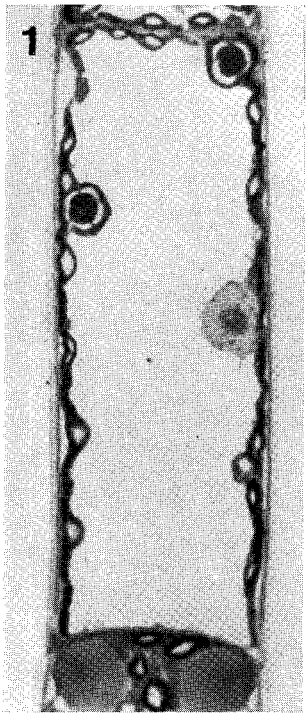
Nucleus enlarged considerably, while becoming elongated and spindle-shaped. The nucleus and spindle were flattened against the wall of the cell. Radial sections of the cell show a long, thin nuclear profile, but if the section approached the cell more tangentially, the nucleus, when included in this plane, appeared quite broadened (as in Figs. 5–7). (Sheath of microtubules appeared around nucleus, and nuclear membrane began differentiating.)

(iv) *Prophase* (*Fig. 5*)

Early condensation of chromatin into chromosomes was visible while the nucleolus was dispersing. (Nuclear membrane differentiated at poles; some microtubules appeared inside the nuclear envelope.)

All sections shown in Figs. 1–53 and 55–57 are longitudinal.

Figs. 1–7.—Light micrographs of stages in cell division in *Oedogonium*. *1*, Interphase (wall ring belongs to *lower* cell). *2*, Preparative stage: ring initiation, nuclear migration. *3* and *4*, Preprophase: nuclear enlargment; note drawn-out poles (arrows). *5*, Early prophase: nucleolus dispersing. *6*, Prometaphase. *7*, Metaphase: paired chromosomes just visible. All figures approx. ×920.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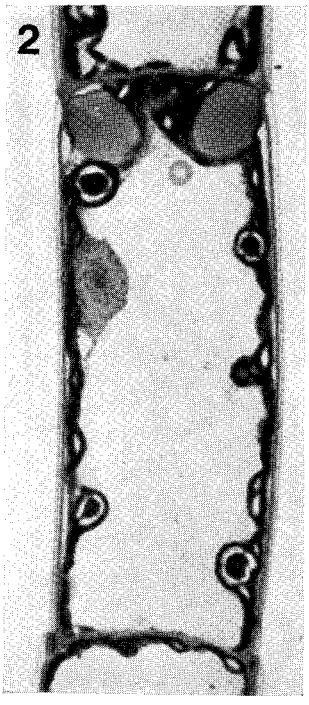
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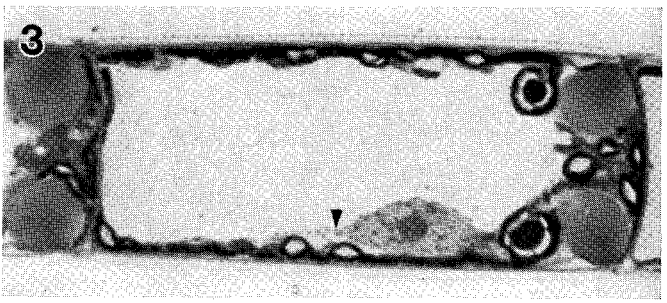
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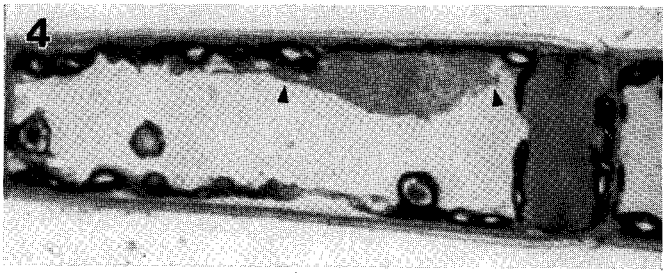
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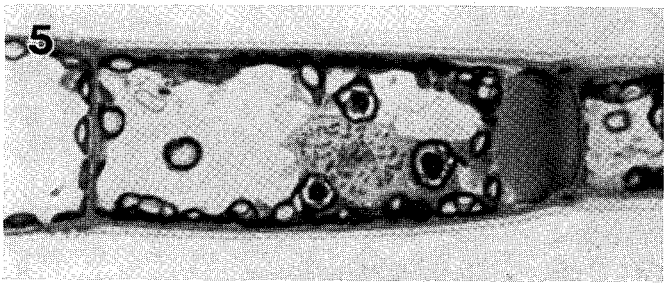
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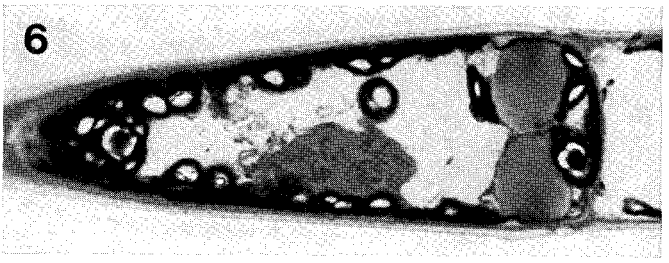
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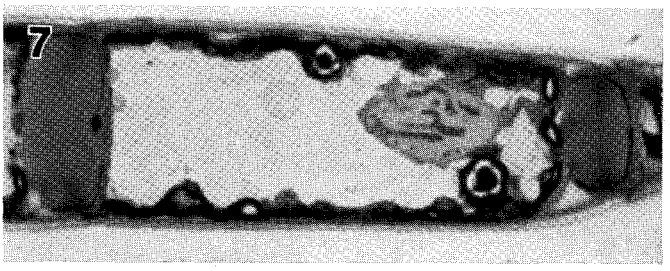
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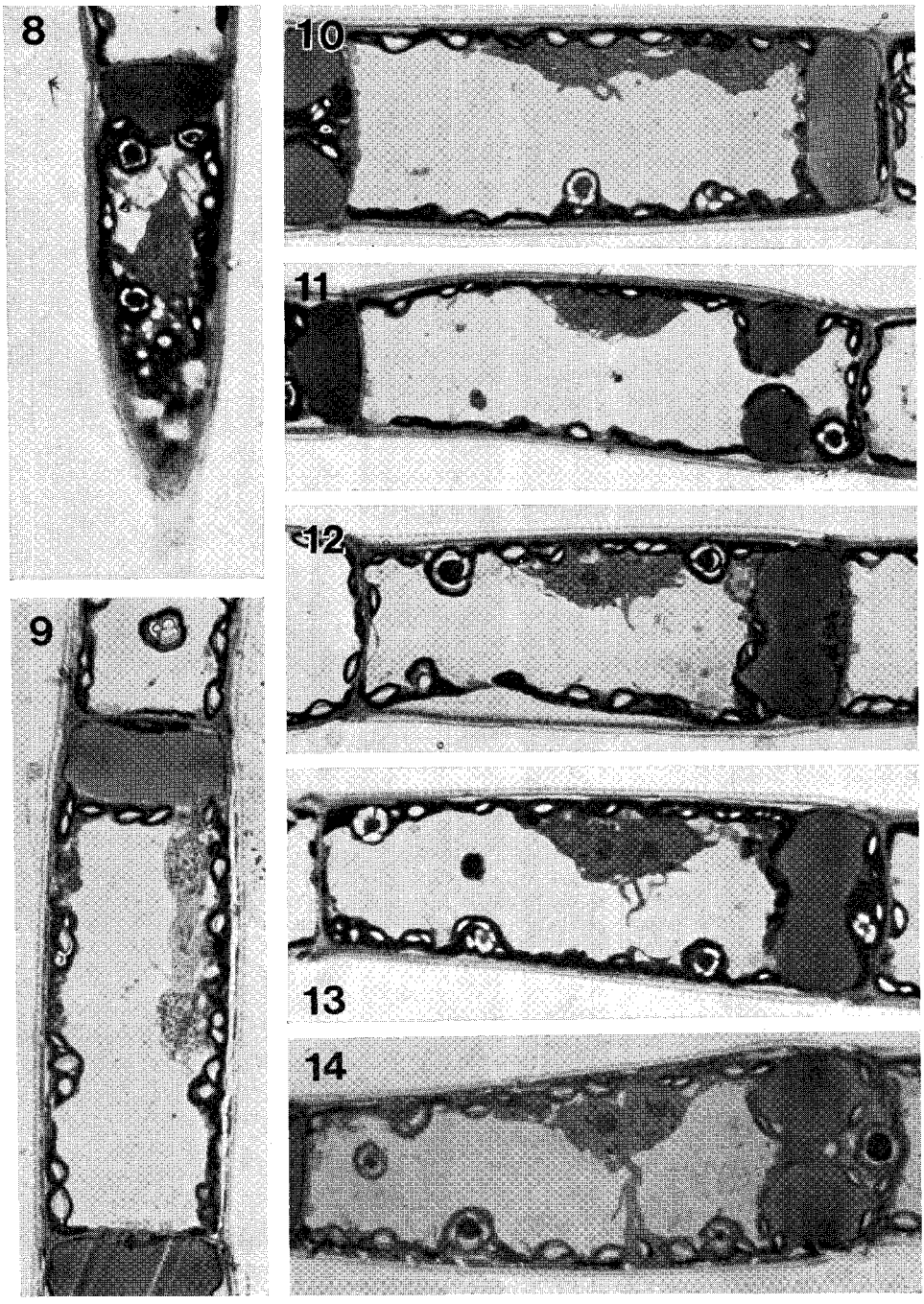

Figs. 8–14.—Light micrographs of stages in cell division in *Oedogonium*. *8*, Anaphase. *9*, Early telophase: midbody visible. *10*, Telophase: midbody dispersing. *11*, Late telophase. *12–14*, Septum formation. All figures approx. ×920.

(v) *Prometaphase* (*Fig. 6*)

Dispersal of the nucleolus completed. (Scattered, paired kinetochores present; nuclear envelope remained essentially intact throughout division.)

(vi) *Metaphase* (*Fig. 7*)

Paired chromosomes clearly evident. (Paired kinetochores, with microtubules attached, were very obvious; spindle contained many microtubules.)

(vii) *Anaphase* (*Fig. 8*)

Separation of (V-shaped) chromosomes accomplished. (Kinetochores now separate.)

(viii) *Early Telophase* (*Fig. 9*)

Spindle became very elongated (dumbell-shaped) with reforming nuclei at each end and a central body occasionally detectable which soon dispersed (Fig. 10). (Increasing number of interzonal microtubules, and a "midbody" component appeared.)

(ix) *Late Telophase* (*Fig. 11*)

Nuclei moved very close together; the septum was initiated during or after this movement. (Microtubules appeared between nuclei, in the plane of the future cell plate.)

(x) *Septum Formation* (*Figs. 12, 13, and 14*)

Strand of cytoplasm crossed cell, between nuclei, which then separated again (Figs. 15 and 16). (Microtubules and vesicles were present in large numbers in the septum; however, transverse cell wall was *not* formed.)

(xi) *Ring Breakage* (*Fig. 17*): *Elongation and Cytokinesis*

The outer cell wall at the ring broke circumferentially and cell elongation characteristically stretched the ring material into the form of the new cell. The septum moved up the cell (Fig. 17) to the edge of the broken wall, and then the new cross wall formed between daughter nuclei, in the septum. (Coalescence of vesicles in the septum occurred *after* this movement was complete.) The ring of wall material stained heavily with toluidine blue, and exhibited virtually no birefringence. However, during wall elongation it stained much more weakly, and later began to show very weak birefringence.

(c) *Light Microscopy* (*Live Material*)

Even in the vacuolated species A, we have been unable to examine the mitotic spindle *in vivo*, due to the number of chloroplasts and pyrenoids within the cell. However, we have been able to watch cell wall rupture and wall elongation in species A and in *O. foveolatum* and *O. cardiacum*. Careful and prolonged observation was necessary to catch this striking phenomenon, even in cultures which were largely

synchronized. The breakage was often quite explosive, jerking the filament; the ring sometimes tore unevenly, resulting in a quite violent kinking movement of the whole filament. As breakage was completed, the filament straightened and further elongation was detectable by eye for about half a minute. After this, much slower elongation, difficult to discern, continued for some while.

It has been very difficult to record this phenomenon photographically, since it is impossible to predict precisely which of the many late telophase cells on a slide was about to break. We did, however, manage to record one cell *in vivo* approximately 2 sec after wall splitting commenced (Fig. 18.1); the series of five micrographs (Figs. 18.1–18.5) were taken over a period of about 90 sec, each exposure lasting about 1·5 sec (elongation of the filament has blurred Fig. 18.2). The extension undergone in this period is quite clear, as is the appearance of the septum at the bottom edge of the final micrograph (Fig. 18.5).

During this wall elongation, the cytoplasm of the daughter cell enclosed by the older cell wall becomes stretched and very thin at each end. This is clearly seen both *in vivo* (Fig. 19) and in light and electron micrographs.

(*d*) *Electron Microscopy*

No centrioles have yet been found in these vegetative cells.

(i) *Interphase*

Detailed descriptions of interphase cells and their constituents are irrelevant to this paper. However, one interesting and unexpected finding was that cell wall microtubules were always found oriented longitudinally, never transversely as is usual in cylindrical plant cells. There were few if any microtubules near the spherical nucleus. Golgi bodies were quite small and looked rather inactive.

(ii) *Preparative Stage*

Little will be said concerning the synthesis of the wall thickening in this paper. The ring was always laid down a small distance below the lowest cap (of the series) that had been formed from previous divisions (Fig. 20). Deposition possibly occurred via incorporation of vesicular components into the wall, though this is not very clear from our own work. The golgi bodies definitely appeared more active at this stage (Fig. 22). There is no evidence that microtubules were intimately involved in the ring formation; it does not seem to be analogous to the formation of xylem wall thickenings (Pickett-Heaps 1967*a*). The ring of wall material itself always had two

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Fig. 15.—Daughter nuclei at various stages of separation after septum formation. ×420.

Fig. 16.—Nuclei separated, after septum formation. ×230.

Fig. 17.—Cell elongation after wall splitting—cytoplasm in lower cell becoming thin. Septum has moved up. ×230.

Figs. 18.1–18.5.—*Live* cells, phase contrast; sequence of micrographs taken over about 1·5 min. ×450. *18.1*, Splitting of wall at ring had just occurred (small arrows). Wall ring visible in adjacent cell (large arrows). *18.2–18.5*, Elongation of cell at stretching ring (small arrows). Figure 18.2 is blurred due to filament elongation during exposure. Camera orientation was not altered; filament in Figure 18.5 had twisted sideways due to another adjacent wall split. Septum (*s*) detectable in Figure 18.5.


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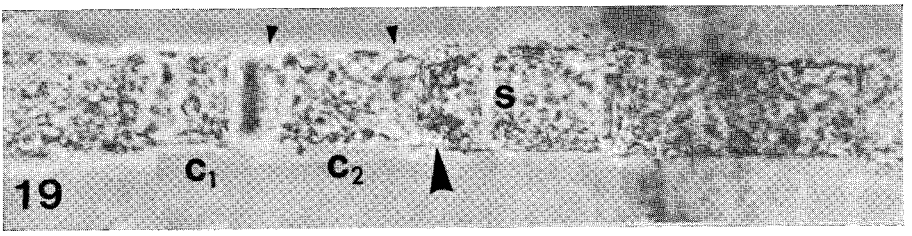
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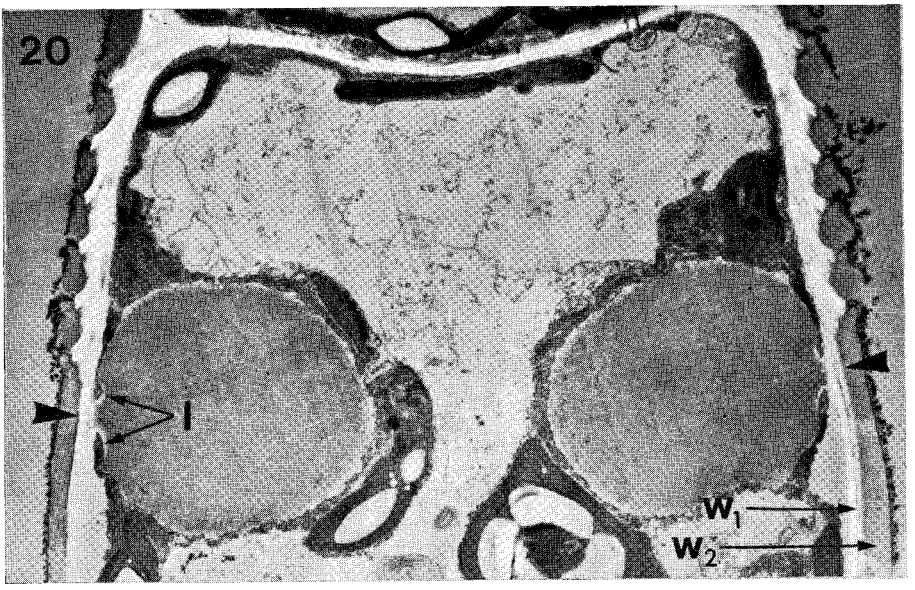
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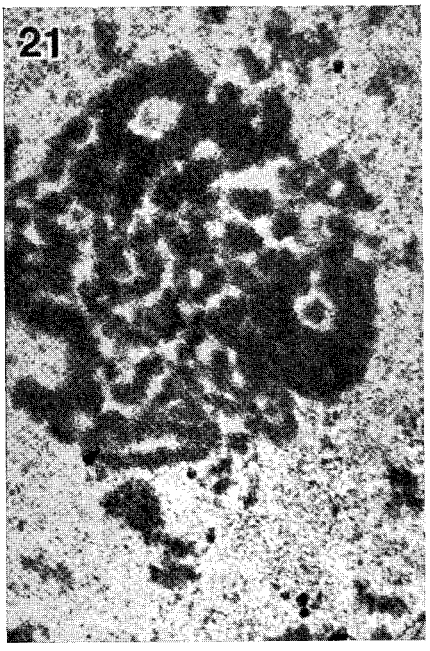
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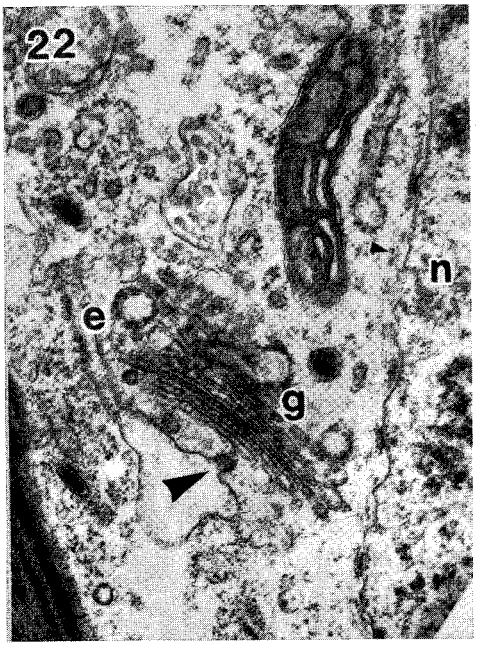
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characteristic "reinforced" lips of material at its inner edge which appeared identical in nature to the adjacent longitudinal wall (Fig. 20—see Figs. 51, 52, *et al.*). Later, the wall split between these two lips (Fig. 20—see below). The rest of the ring material appeared to be diffuse and faintly fibrous. As the nucleus (still spherical) moved towards the thickening, the first microtubules appeared around it and also in the nearby cytoplasm (Fig. 22).

(iii) *Preprophase*

A very characteristic ultrastructural feature of preprophase is the change of the nucleus from a spherical to a spindle shape, before the chromatin begins to condense (Figs. 2–4). As this elongation and flattening became more obvious, an increasing number of longitudinally oriented microtubules appeared very close to the nuclear envelope (as in Fig. 26), enveloping it as a sheath (Fig. 24 *et al.*). The microtubules were quite close together, but seldom more than two or three deep around the envelope. The source of these microtubules is not clear; nearby, somewhat scattered microtubules suggested that perhaps these organelles were being drawn from the other regions of cytoplasm (Figs. 23*a* and 23*b*). Polar differentiation (see below) of the nuclear membrane started at this stage (Figs. 23, 23*a*, and 23*b*), and sometimes was quite advanced. Disintegration of the nucleolus commenced (Figs. 21 and 23).

(iv) *Prophase*

The mitotic spindle, enclosed throughout division by a nuclear membrane, contained many microtubules. Consequently, it is of some interest to ascertain if possible how these organelles entered the spindle (assuming their subunits were not present all the time). While the nuclear envelope was retained throughout division, careful investigations revealed many small gaps in the membrane, although no intact microtubule was ever found running through such a gap. The first microtubules clearly visible within a dividing nucleus were generally seen at the two polar regions,

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The following abbreviations are used on Figures 19–57*b*: c_1 and c_2, daughter cell pair (following division); *ch*, chromosome; *g*, golgi body; *k*, kinetochore; *l*, lip of reinforced wall material in ring; *n*, nucleus; *nc*, nucleolus; *nm*, nuclear membrane; *m*, midbody material; *r*, material of the ring; *rn*, reforming nucleus (at telophase); *s*, septum; *sc*, components of the septum; *t*, microtubules; *v*, vacuole; w_1, inner layer of cell wall; w_2, outer (mucilagenous?) layer of cell wall; *w*, cell wall. The use of lower case letters after the figure number (e.g. Figs. 55, 55*a*, 55*b*) indicates that these are micrographs of the same cell.

Fig. 19.—Daughter cells (c_1, c_2) *in vivo* after ring extension; note stretched cytoplasm (small arrows) in c_2. Adjacent cell has septum and wall thickenings (large arrow). ×300.

Fig. 20.—Sectioned ring with its lip (*l*) of reinforced material is situated below lowest of the six caps, formed from six previous divisions. Two layers of the wall (w_1, w_2) are obvious; wall would have eventually split circumferentially at arrows. ×3,000.

Fig. 21.—Preprophase nucleolus, beginning to disintegrate (compare with midbody in Fig. 38). ×15,200.

Fig. 22.—Preparative stage: nucleus had moved towards thickening (as in Fig. 2); first extranuclear microtubules (small arrow) near it. Golgi body with small vesicles (cf. Figs. 49 and 50) characteristically associated with endoplasmic reticulum, which appears to be forming a coated vesicle (large arrow). ×26,000.

after the nuclear membrane had become very characteristically differentiated. This differentiation was apparently due to the membrane becoming drawn out into a long, narrow neck (Figs. 25, 25*a*, 27, 27*a*, and 28). Two profiles of the membrane were commonly seen running parallel, quite close together, undulating for some considerable distance into the cytoplasm (Figs. 27 and 27*a*). There sometimes appeared to be an opening at the end of the membranes which were always enveloped by microtubules (Figs. 23*a*, 23*b*, 25*a*, 27, and 27*a*). The region between the membranes was characteristically filled with amorphous material (Figs. 23*a*, 27, 27*a*, and 35). The paired membranes were easily identifiable even when not visibly continuous in a given section with the nuclear membrane (Figs. 23*b* and 27*a*). Occasionally two adjacent portions of this paired membrane arrangement were found in one section, suggesting that the paired membrane is somewhat folded (Fig. 35). However, we must emphasize that we have been unable to reconstruct the entire structure and organization of this very characteristic feature using serial sections, and it may be even more complex than it appeared. The microtubules lying around the nucleus at this stage became somewhat concentrated near the membranes, and their orientation was variable in this region (Fig. 27*a*). Chromosome condensation was evident during prophase (Figs. 25 and 28).

(v) *Prometaphase*

An increasing number of microtubules, often in bundles, appeared inside the nuclear envelope at prometaphase (Figs. 29 and 30). Some sections suggested that the kinetochores were being formed—rather ill-defined, paired structures were associated with microtubules and were rather widely scattered within the nucleus (Fig. 30). The nucleolus was becoming very diffuse.

(vi) *Metaphase*

By metaphase, large numbers of microtubules were seen in the nucleus. Most distinctive was the appearance of paired kinetochores, with groups of attached microtubules, lying roughly in the mid-plane of the nucleus (Fig. 31). The kinetochore structure was much more complicated than that normally found in plant cells, and contained several layers (Figs. 32 and 34). Many interzonal microtubules were also present; all the tubules converged towards the polar regions, generally in bundles (as in Fig. 29), but were not seen attached to any particular structure in this region. The differentiated nuclear membrane (Fig. 35) apparently disappeared at about this

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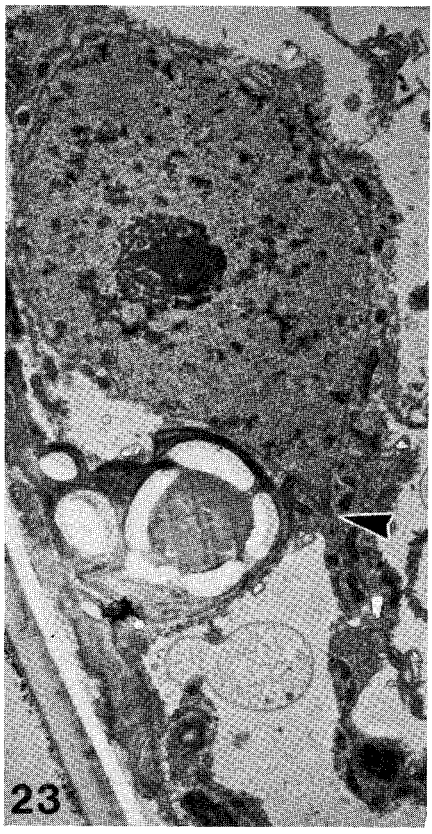
Fig. 23.—Early preprophase nucleus (note nucleolus), just becoming elongated. Polar differentiation of membrane is arrowed. ×4,200.

Fig. 23*a*.—Detail of polar membrane in Figure 23. Microtubules associated with membrane (arrowed) are visible away from nucleus. Other microtubules (*t*) collecting in nearby cytoplasm. ×20,000.

Fig. 23*b*.—Polar membranes, shown above in Figures 23 and 23*a*, sectioned at a different depth in the cell. Many microtubules are seen associated with this double membrane structure (arrow). ×21,000.

Fig. 24.—Early prophase nucleus (*n*) sectioned tangentially, showing large number of adjacent extranuclear microtubules. ×17,000.



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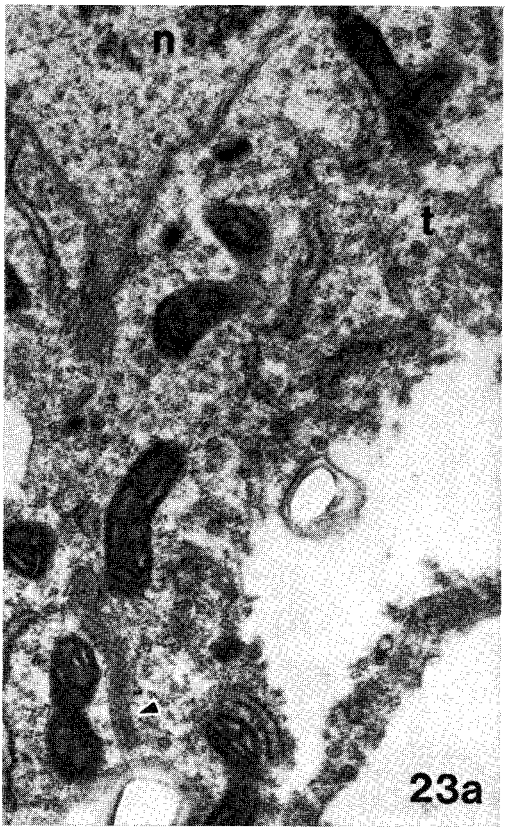

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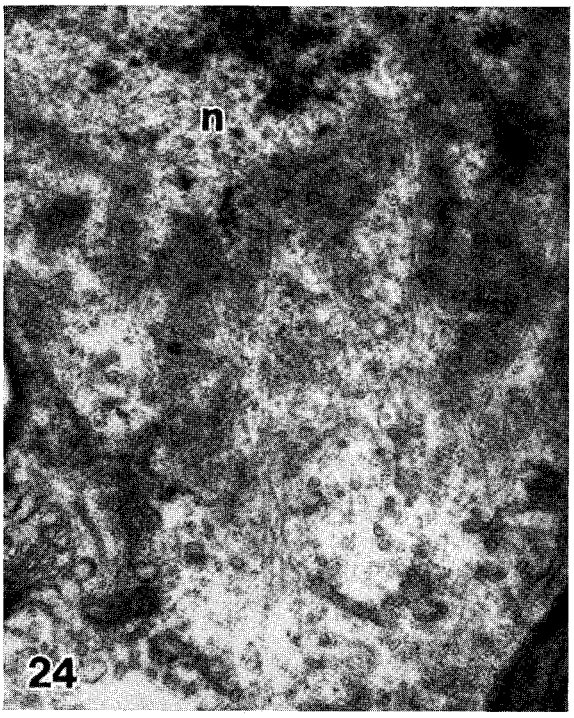

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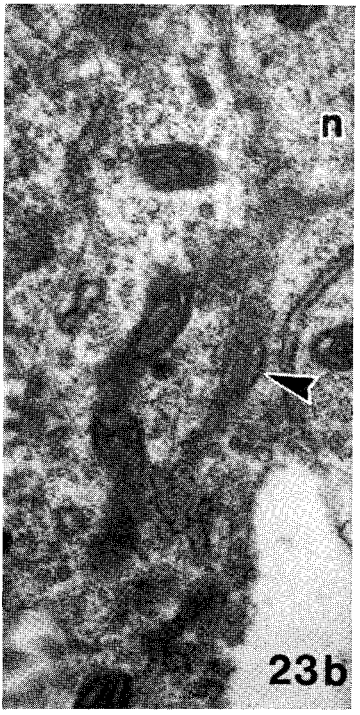

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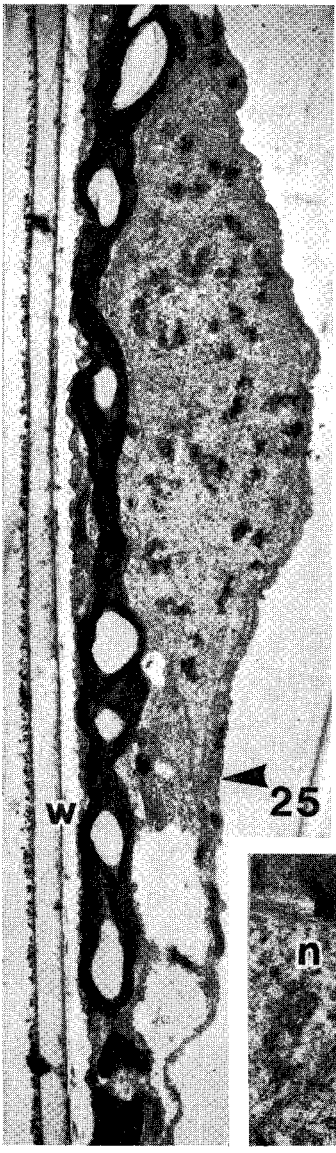
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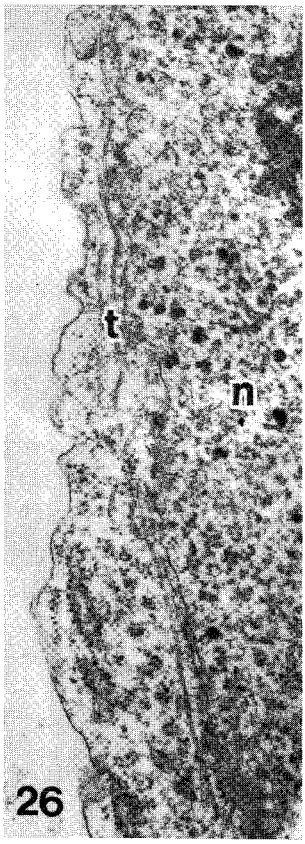
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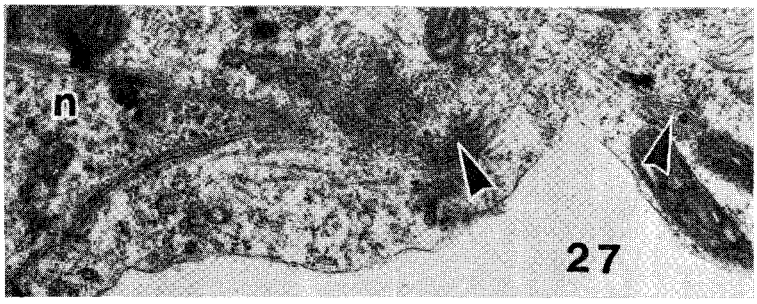
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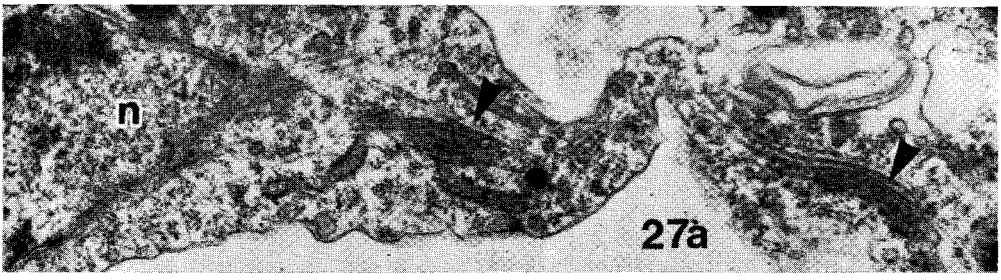
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time or later. Some microtubules remained outside the nuclear envelope, and throughout subsequent stages of mitosis (Fig. 33).

(vii) *Anaphase*

Chromosomal movement occurred within an intact nuclear membrane (Fig. 37). The structure and shape of the anaphase spindle with its kinetochores (Fig. 36) and intact nuclear envelope were more typical of animal cells than plant cells at this stage.

(viii) *Early Telophase*

Early telophase was characterized by a very considerable elongation of the spindle and the emergence of an amorphous, granular material midway between the nuclei (Figs. 38 and 39). The nuclear envelope appeared to contract around the chromosomes but some breaks were evident (Fig. 38). Interzonal microtubules proliferated between the nuclei (Figs. 38 and 38*a*), later to disperse (Figs. 39 and 40). The granular midbody material was often still partially enclosed by the nuclear membrane (endoplasmic reticulum?); the material later lost its staining characteristics to a large extent, and was apparently dispersing (compare Figs. 38, 39, 39*a*, 40, and 40*a*). Several micrographs suggested that septum formation was beginning in the region between the midbody and each nucleus (Fig. 39), where the orientation of microtubules was becoming irregular (they perhaps were tending towards the plane of the future septum) and an early concentration of vesicles was detectable (Figs. 39*a* and 40*a*).

(ix) *Late Telophase*

The separated nuclei, previously widely separated, moved very close together again at this stage [Fig. 41—Tuttle (1910) says this movement is "exceedingly rapid"]. However, we seem to have caught one pair of nuclei at this stage (Fig. 40); the material of the midbody, though still detectable, had almost disappeared (Fig. 40*a*). The septum of the cell was also being initiated very early (Figs. 40 and 40*b*). Between the nuclei, vesicles and various membranous profiles collected (Figs. 41, 41*a*, 42, 44, and 45). Microtubules were present, but these were becoming oriented *in the plane of the future septum* (Figs. 41 and 41*a*). ["Septum" is used here, because a cell plate is *not* formed at this stage—the term is used, for example, by Fritsch (1935)].

(x) *Septum Formation*

The strand of cytoplasm which extended across the cell between daughter nuclei (Figs. 12–14) was quite distinctive and cohesive in structure; it was rarely

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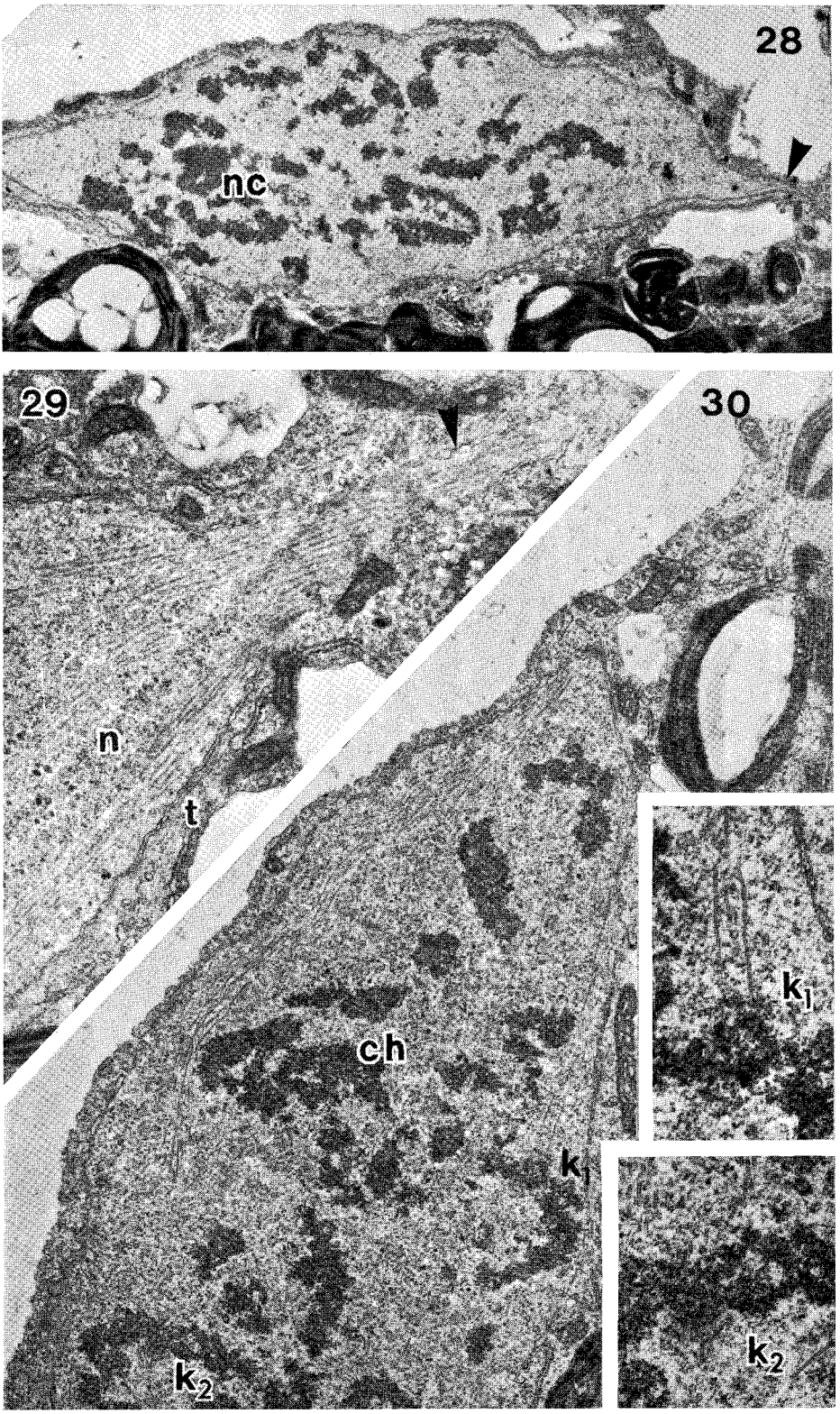
Fig. 25.—Very elongated, early prophase nucleus (radial section). Note typical elongated nuclear membrane profile (arrow). ×4,100.

Fig. 25*a*.—Detail of polar membranes of nucleus in Figure 25. ×26,000.

Fig. 26.—As for Figure 25, showing extranuclear microtubules (no intranuclear tubules present at this stage—cf. Fig. 33). ×29,000.

Figs. 27 and 27*a*.—Two serial (but not adjacent) sections of drawn-out polar nuclear membrane (arrows) at prophase, with associated microtubules. Both ×20,000.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disrupted during fixation and subsequent processing, even when very thin and when the membranes had been stripped from either side of it. Septum formation was initiated after the nuclei had come close together (Fig. 42), though occasionally a cytoplasmic protrusion was seen at earlier telophase (Figs. 40 and 40*b*). There was evidence of microtubule reorientation soon after the phase of spindle elongation (see above). The septum contained a few organelles such as mitochondria (Fig. 48). Most characteristic, however, was the presence of numerous vesicles (or tubular membranes) interspersed with a somewhat random array of transverse microtubules, the former being concentrated initially between the nuclei (Figs. 42*a*, 44, and 45). The microtubules and associated vesicles penetrated eventually through the cytoplasm right up to the older longitudinal walls (Fig. 47). The microtubules were always aligned in the plane of the septum (Figs. 42*a*, 43, and 46); nearer the older, longitudinal cell walls, they often diverged slightly into the cytoplasm (Fig. 41*a*). During early septum formation, sometimes several protrusions of cytoplasm were evident, all of these containing longitudinally oriented microtubules (Fig. 43). A few microtubules could generally be found in the cytoplasm directly opposite a forming septum. The source of the vesicles is not clear, but it is quite possible that they were derived from the nearby golgi bodies. The golgi bodies were almost invariably associated with a nearby element of endoplasmic reticulum (as in Fig. 22) which had no ribosomes on the membrane adjacent to the golgi body. A transfer of small vesicles between these two organelles might be deduced from the micrographs (as in Fig. 22).

(xi) *Ring Breakage, Cell Elongation, and Transverse Wall Formation*

After the septum was formed, the nuclei separated and moved into the middle region of their respective cells (Figs. 15 and 16). Then the older longitudinal wall split (Fig. 17) at the region between the two lips of reinforced wall material in the ring (Fig. 20); these lips appeared to be strong and quite firmly bound to the older wall (Figs. 51, 52, 55*b*, 56*c*, and 57*b*). As cell elongation proceeded and the wall ring stretched, the shearing force generated appeared to pull each lip perpendicular to the cell axis, forcing the rim of the older wall out into the characteristic cap of *Oedogonium* (Figs. 52, 54, 55*b*, 56*c*, and 57*b*). The algal filaments were frequently bent at the region of wall stretching, indicating that the wall material was softer at this region, as would be expected by its extensibility. The septum (Fig. 46) moved along the elongating pair of cells (Fig. 17); a region of thin ("stretched") cytoplasm was generally visible behind it. This stretched and thin cytoplasm is often very obvious in live cells (Fig. 19). The movement of the septum apparently ceased when it finally reached and then became caught behind the lip of reinforced wall material from the ring (Figs. 51, 53, 54, and 55). Thus, as the vesicles of the septum coalesced, the new cross wall coincided precisely with the position of the bottom cap (Fig. 54, cf. Figs. 55–57*b*).

Fig. 28.—Late prophase nucleus; chromosomes are apparent (cf. Fig. 25), as is typical polar membrane (arrow). Intranuclear microtubules also present. × 5,000.

Fig. 29.—Prometaphase pole. Nuclear membrane at pole is filled with tubules; extranuclear tubules also shown (*t*). Approx. × 14,000.

Fig. 30.—Prometaphase. Intranuclear bundles of microtubules; some scattered kinetochores (k_1, k_2) just detectable. Insets show k_1 and k_2 in more detail. × 10,000 approx.

Special attention was directed to organelles that might be concerned with vacuolation, since the mechanism of rapid cell elongation that occurred at this juncture is of obvious interest. The number of large electron-transparent vesicles associated with the golgi bodies appeared to increase considerably at this stage—often these vesicles were apparently being discharged into the vacuole (Figs. 49 and 50).

Microtubules appeared near the stretching wall, often in reasonable numbers; their orientation was variable, but generally longitudinal.

(*e*) *Cell Wall Structure: Relationship to the Material of the Ring*

Two major structural components of the cell wall were differentiated by their staining characteristics (e.g. Figs. 20, 41, 48, 51, 52, 55*b*, and 57*b*). The thicker outer layer was weakly staining with toluidine blue, and with the uranium–lead combination of stain used in electron microscopy. The inner layer stained heavily with toluidine blue to give a clear blue colour; it was generally electron-transparent after uranium-lead staining. However, prolonged exposure to fresh lead stain often caused this layer (and starch grains) to become much more heavily stained (Figs. 55*b*, 56*c*, and 57*b*). The reason for this is obscure, and the rather dirty, coarse overstaining was normally unwelcome except for this particular phenomenon. The lips of reinforced wall material of the ring always followed exactly the staining behaviour of the inner layer (Figs. 20, 51, 52, 55*b*, 56*c*, and 57*b*) which we believe represents the tough cell wall proper.

The light and electron micrographs indicate that the bulk of the ring material became the *outer* layer following ring breakage and cell elongation (Figs. 52, 55*b*, 57*a*, and 57*b*). When the elongation was almost complete, the formation of the layer was detectable (Fig. 56*c*) along the inner edge of the stretched ring material, this layer then merging into the lips of reinforced wall material from the ring (Fig. 57*b*, cf. Fig. 54). We have not yet been able to decide whether this new inner layer resulted from a new phase of cell wall deposition, or from an alteration of some of the pre-existing material derived from the ring. The new cross wall, formed in the septum after elongation, matched exactly the staining behaviour of the inner cell wall layer (Figs. 56*c* and 57*b*).

IV. Discussion

(*a*) *Material*

It is perhaps a pity that most observations described are on an (as yet) unidentified species of *Oedogonium*. However, this is relatively unimportant, since we believe it more useful to get good cytological results on an unknown species rather than poor results on a named species. Many classical descriptions of the behaviour of algae have concerned unidentified "local" species [Tuttle (1910), for example, used an unidentified species of *Oedogonium* obtained from "the basin of a public drinking

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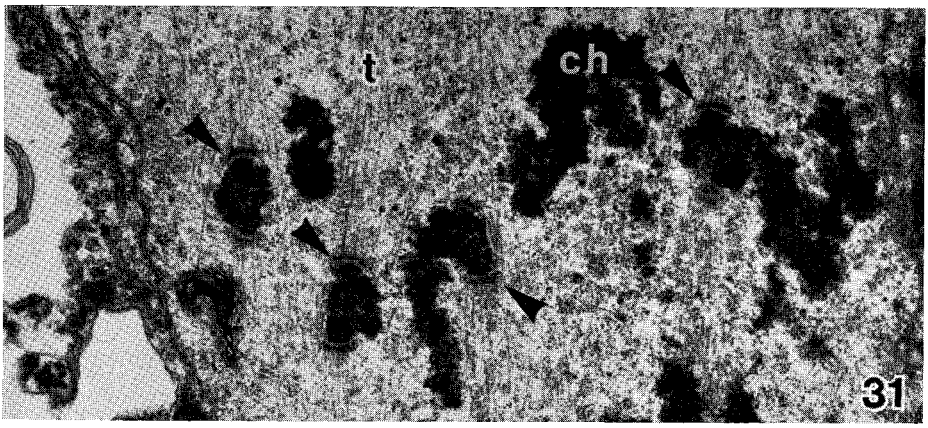
Fig. 31.—Metaphase; four paired kinetochores are shown (arrows). ×15,000.

Fig. 32.—Paired metaphase kinetochore in chromosome, with attached microtubules. ×32,000.

Fig. 33.—Intra- and extranuclear microtubules at metaphase. ×13,000.

Fig. 34.—Less dense print of another metaphase kinetochore to show substructure. ×34,000.

Fig. 35.—Two adjacent profiles of polar nuclear membrane at metaphase. ×26,000 approx.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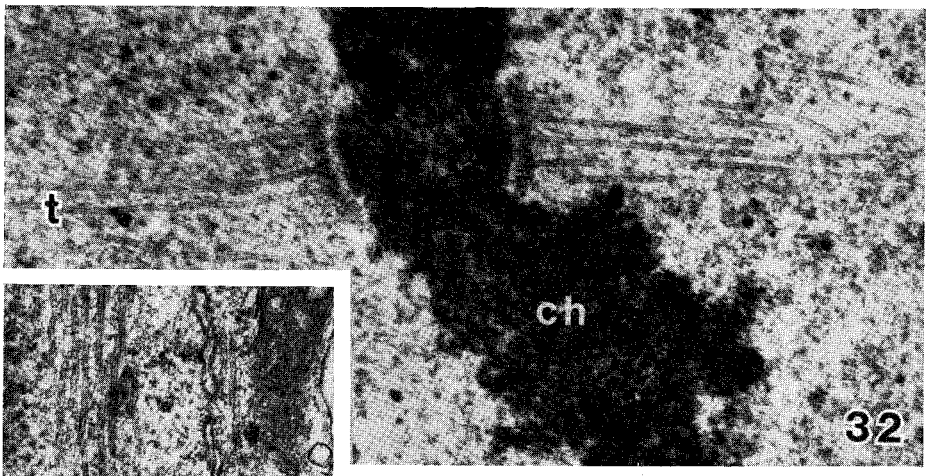
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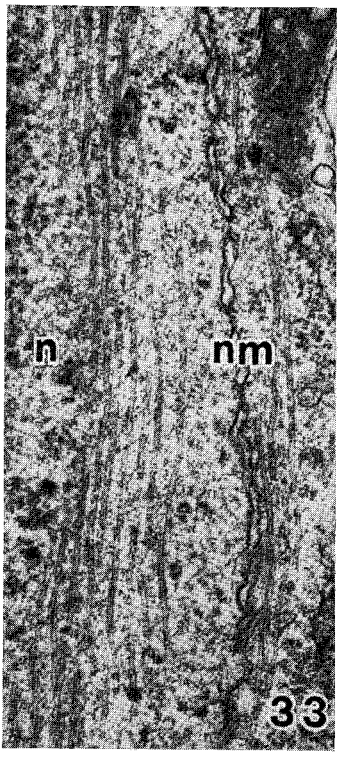
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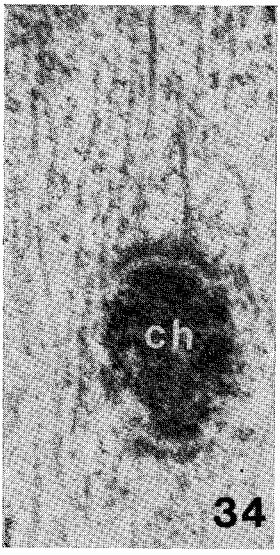
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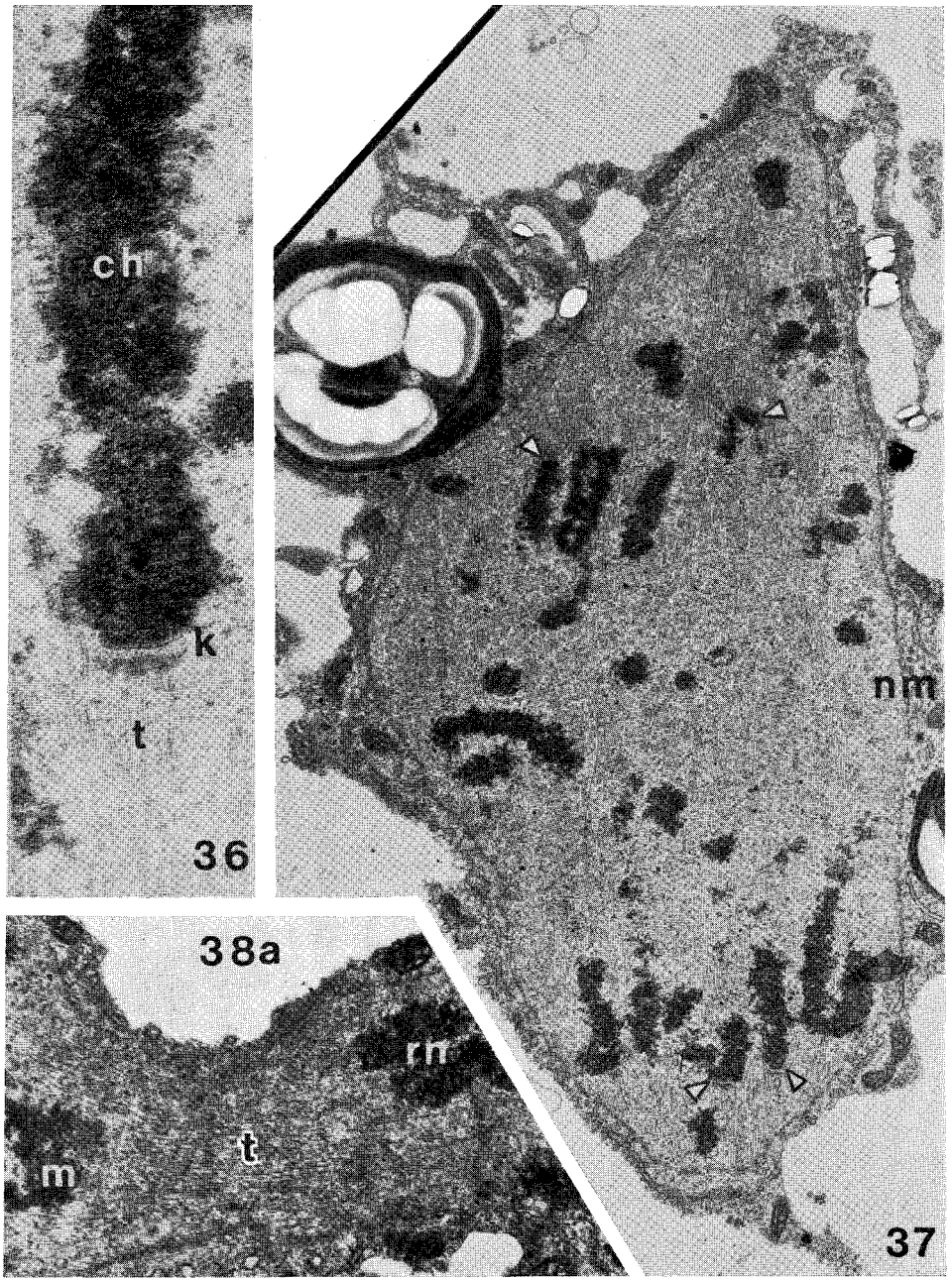


Fig. 36.—Single anaphase kinetochore and attached chromosome. ×44,000.

Fig. 37.—Anaphase spindle, with intact nuclear membrane. Some kinetochores apparent (arrows). ×8,000 approx.

Fig. 38*a*.—Detail of Figure 38 showing interzonal microtubules between midbody (*m*) and reforming nucleus (*rn*). ×11,000.

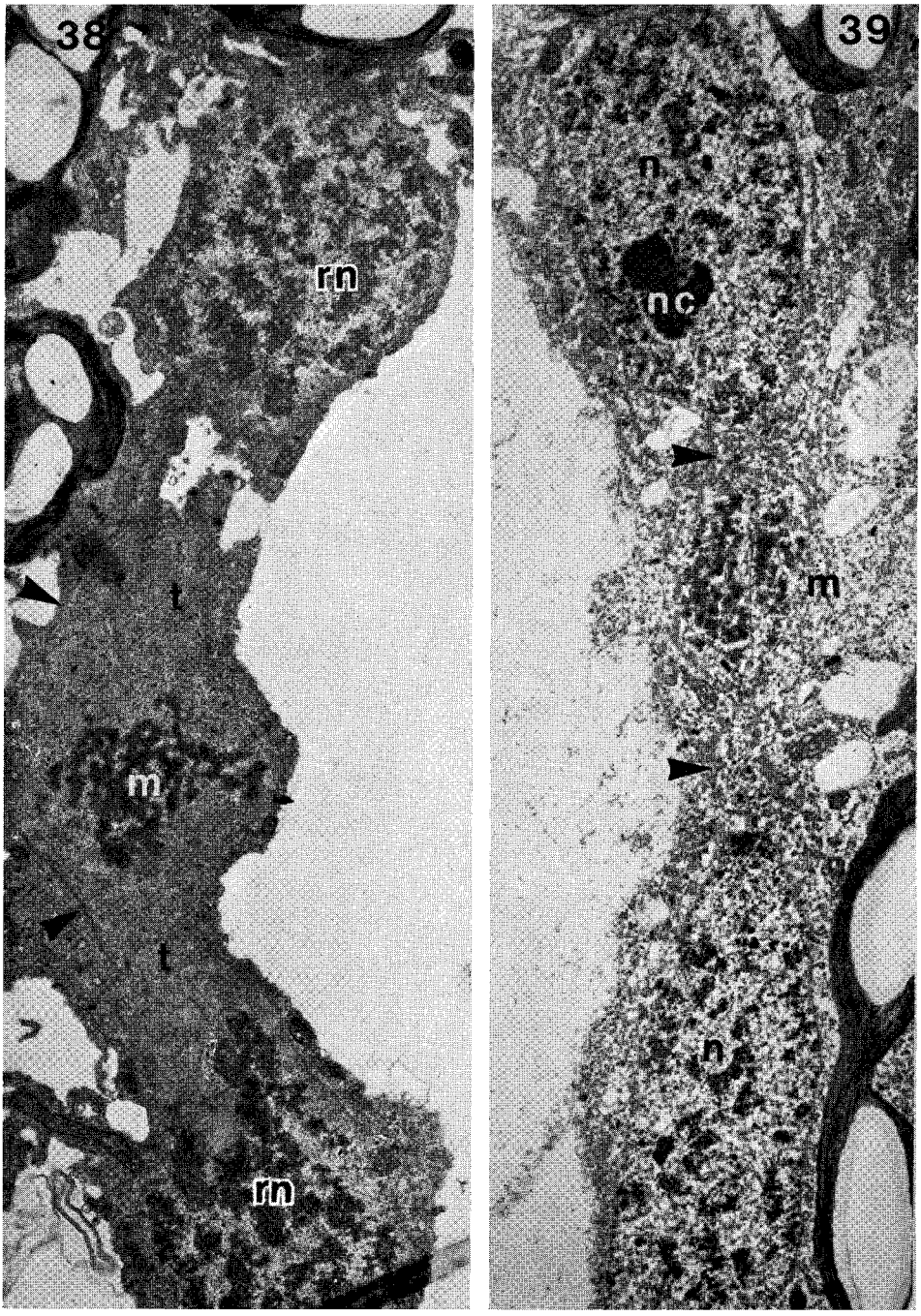


Fig. 38.—Early telophase; reforming nuclei (*rn*) at ends of very elongated spindle. Midbody (*m*), proliferating interzonal microtubules (*t*), and elements of old nuclear membrane (arrows) can be seen. ×6,800.

Fig. 39.—Telophase, later than Figure 38. Daughter nuclei formed; midbody (*m*) still present (cf. Fig. 9). Initial collection of septum components detectable at arrows. ×4,800 approx.

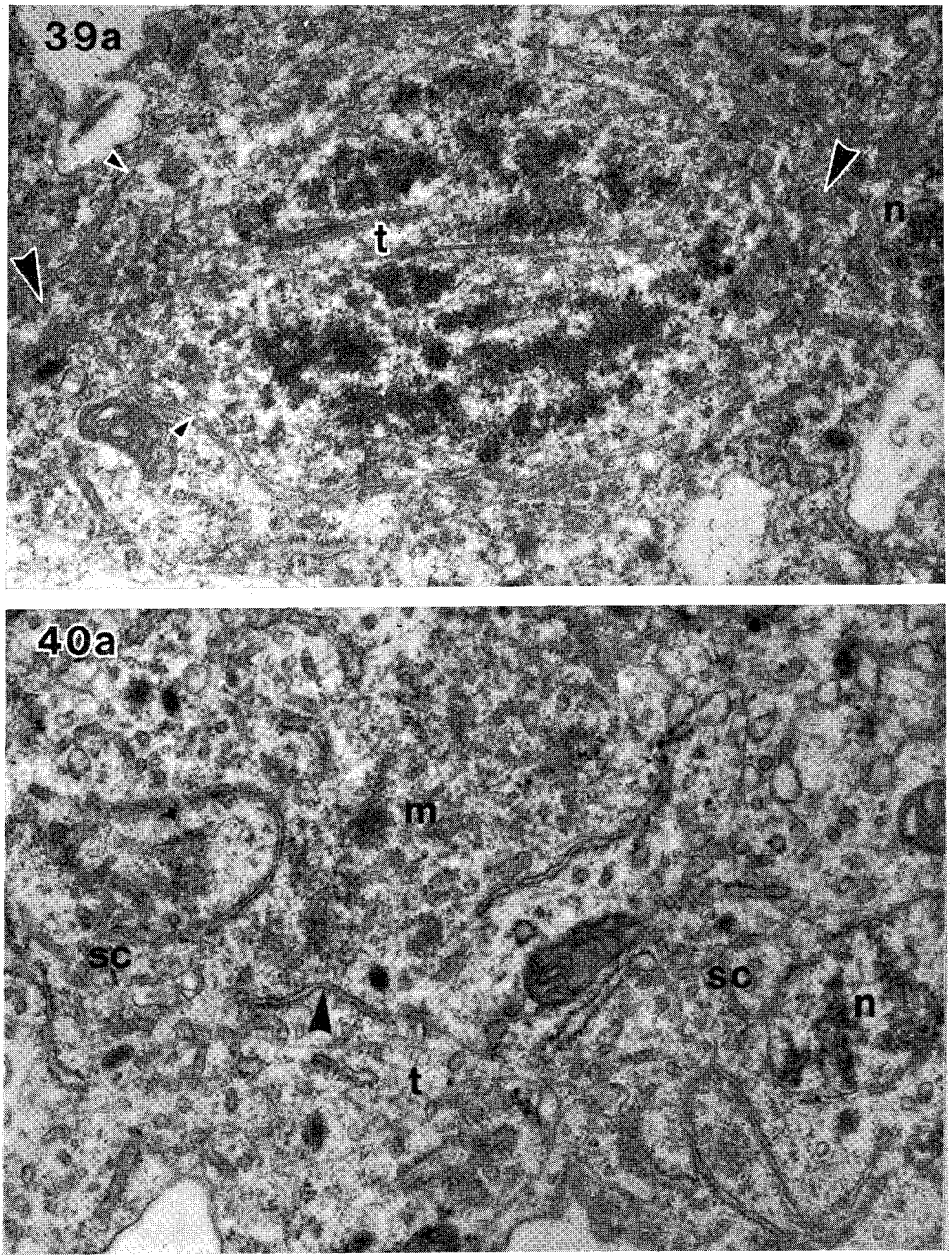


Fig. 39*a*.—Detail of midbody in Figure 39 (serial section). Septum components visible at large arrows; remains of nuclear membrane at small arrows. ×21,000.

Fig. 40*a*.—Detail of Figure 40; compare with Figure 39*a*. Midbody (*m*) and remnants of nuclear membrane (arrow) almost dispersed. Septum components (*sc*), including transversely sectioned microtubules, are seen. ×23,000.

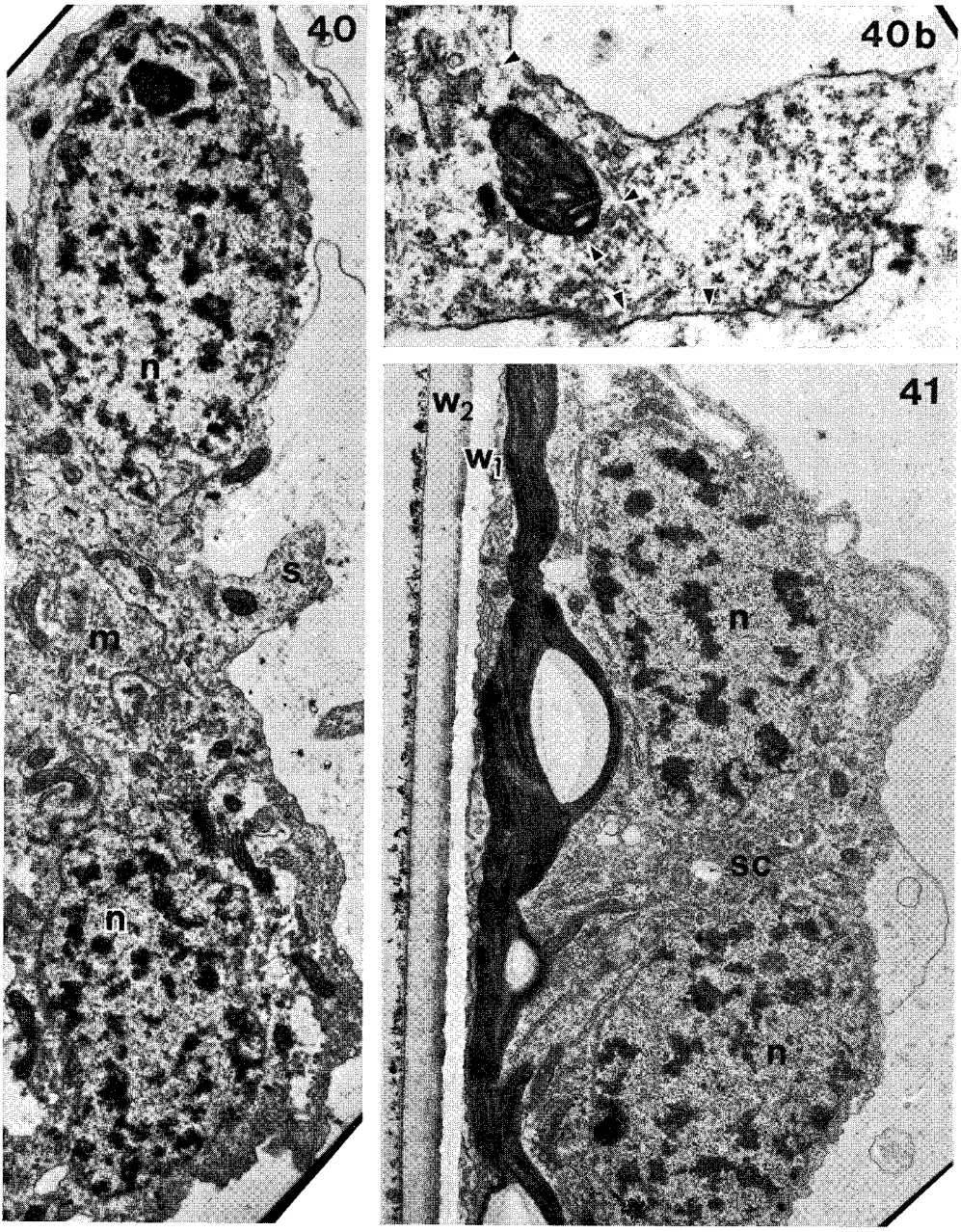
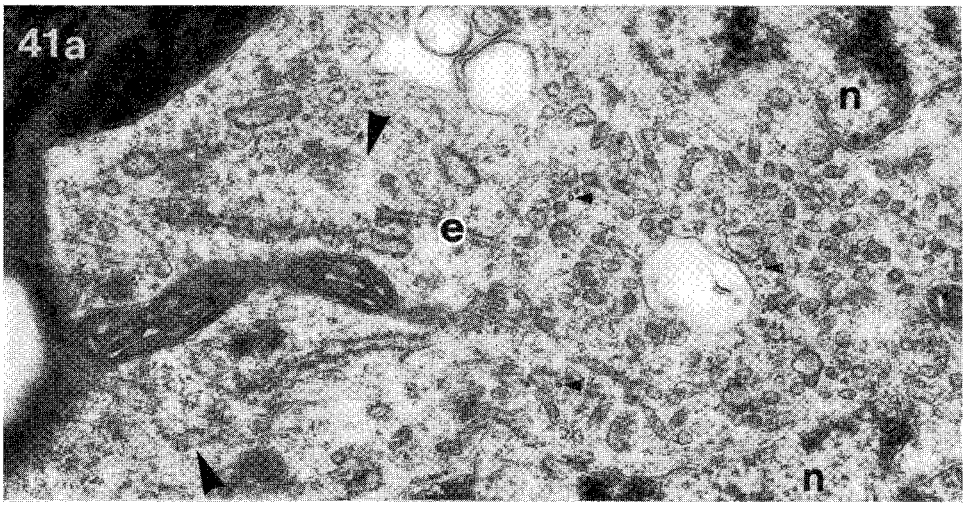


Fig. 40.—Collapse of interzonal microtubule system and dispersal of midbody almost complete. Septum is visible much earlier than normal (cf. Fig. 41). ×6,500.

Fig. 40*b*.—Detail of septum in Figure 40, showing microtubules (arrows). ×24,000.

Fig. 41.—Later telophase; nuclei close together, with gathering of septum components between them. ×6,000 approx.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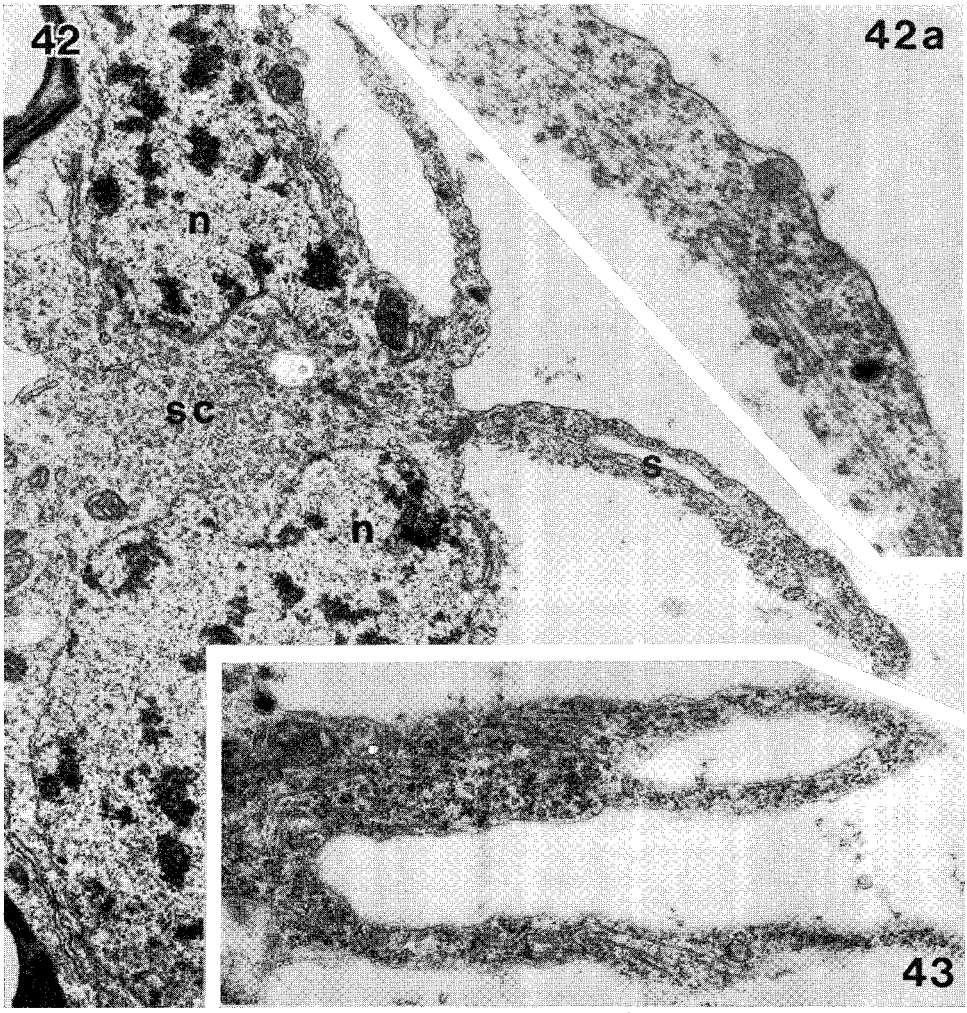
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fountain in one of the streets of Charlottesville"]. This may be convenient or unavoidable, since it is often difficult to culture algae without upsetting their complex form or behaviour (as we have found to our cost). The species described above seems to be quite typical of *Oedogonium*; the similarity at all stages of division between the light micrographs of species A and classical observations [for example, the drawings of Strasburger (1880), Tuttle (1910), Kretschmer (1930), and Ohashi (1930)] are very striking indeed. Furthermore, recent work on *O. cardiacum* and *O. foveolatum* (to be reported soon), confirms these present results in every detail so far.*

Again it is emphasized that we obtained best results with our species A when it had been fixed directly upon removal from the lake. The same species was easy to grow and keep in culture, but such material (unless very young and actively growing) gave an inferior microscopic image when similarly processed and sectioned, even though it appeared to grow well and generally showed little if any signs of ill health to our unpractised eye. Both *O. foveolatum* and *O. cardiacum* eventually tended to accumulate starch in culture; this too would strongly indicate some deficiency in or exhaustion of the culture media.

(*b*) *Cell Division*

The ultrastructure of the mitotic spindle in higher plant cells has been investigated and described in considerable detail. Likewise, mitosis in unicellular algae has received much attention. However, the considerable problems encountered in processing filamentous algae by normal methods for light and electron microscopy have meant that little useful information has been obtained on these types of plants. We consider that processing techniques have recently been developed that will enable this field to be more fully documented. Whilst some degree of obvious cytoplasmic damage is always detectable in the micrographs (e.g. plasmolysis, rupture of vacuolar membranes, etc.), the preservation achieved is sufficiently good to enable useful data to be obtained.

The results described in this paper indicate that mitosis and cytokinesis in *Oedogonium* differ in many respects from the equivalent processes in both higher plants and single-cell algae. This is also the case with *Spirogyra* (Fowke and Pickett-Heaps 1969). Thus, although *Oedogonium* (and *Spirogyra*) cannot be expected to be typical of many algae, the various similarities and dissimilarities in the mitotic structure between these and the dividing cells of other organisms may help to elucidate the fundamental processes of cell division.

* *Note added in proof.*—Hill and Machlis [*J. Phycol.* **4**, 261 (1968)] have recently described some aspects of cell division in *O. borisianum*; they have achieved excellent morphological preservation, but do not describe nuclear division. Their results will be discussed in a later paper.

Fig. 41*a*.—Detail of Figure 41, showing septum components between nuclei. Microtubules are sectioned at various angles in the plane of future septum, including some transversely (small arrows). Other tubules diverge at the cell wall (large arrows). ×22,000.

Figure. 42.—Extending septum (*s*) (cf. Figs. 12 and 13) ×9,000.

Fig. 42*a*.—Detail of septum in Figure 42 (serial section) showing microtubules. ×26,000.

Fig. 43.—Extending septum, here in two layers (cf. Fig. 13), both containing microtubules. ×21,000 approx.

The longitudinal orientation of cell wall microtubules is rather unusual for cylindrical plant cells; its significance is not known. A possibly analogous situation has been described in root hair cells by Newcomb and Bonnett (1965). The appearance of longitudinally oriented microtubules near the stretching wall material may be significant (i.e. perhaps involved in control of wall structure); however, it is equally likely that these were representative of the normal wall microtubule system re-establishing itself after division.

The lack of recognizable centrioles in vegetative cells of *Oedogonium* is perhaps not surprising although basal bodies must appear at some stage during zoospore and male gamete formation. The situation can be compared with that in *Chara*—although no centrioles could be found in vegetative cells or young cells of the antheridia (Pickett-Heaps 1967*b*, 1967*c*, 1968*a*), these structures appeared in spermatogenous filaments where they became associated with mitotic spindle structure in the normal fashion (Pickett-Heaps 1968*b*; Turner 1968). Later, they are involved in flagellum formation as would be expected. It was argued from this that they are an appendage to, and not a vital component of, the mitotic apparatus (Pickett-Heaps 1968*b*); this argument might also apply in considering the mitotic apparatus of animal cells.

It is relatively unusual for an intact nuclear membrane (Figs. 28, 30, 37, 38, and 39) to enclose the mitotic apparatus throughout chromosome separation in plant cells, although this has been observed in protozoa and ciliates (e.g. Carasso and Favard 1965; Jenkins 1967; Tucker 1967). In various algae, the open mitotic structure has been reported (Manton 1964*a*, 1966; Pickett-Heaps 1967*c*; Fowke and Pickett-Heaps 1969), but in some others, and in yeast, the membrane remains intact (Sommer and Blum 1965; Robinow and Marak 1966; Leadbeater and Dodge 1967; Johnson and Porter 1968). Ohashi (1930) incidentally says that the nuclear envelope disappears at prophase in *O. grande*.

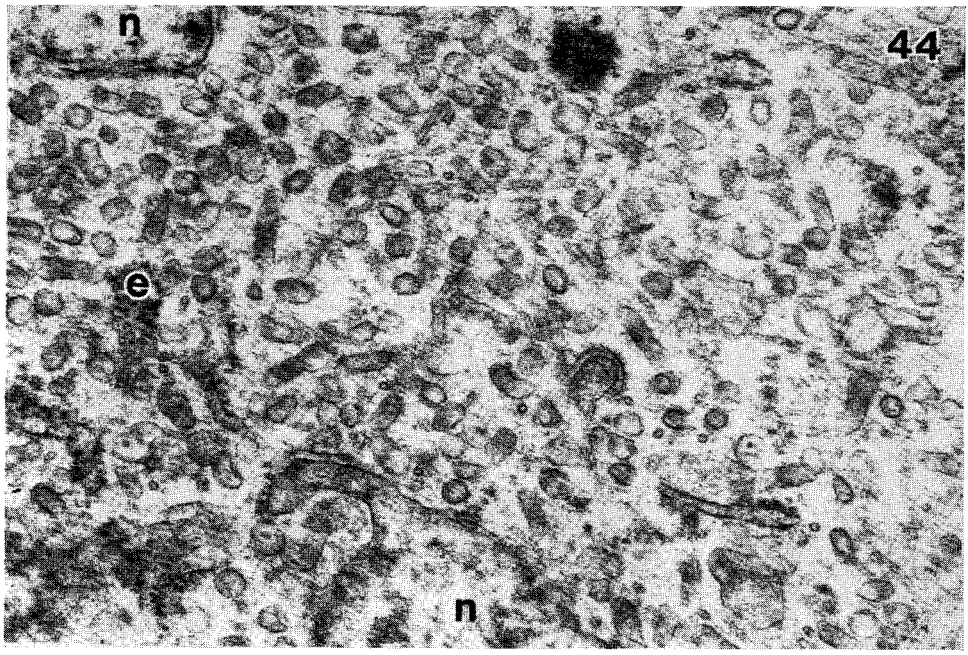
During preprophase in *Triticum* (Pickett-Heaps 1968*c*), *Commelina* (Pickett-Heaps 1969), and *Spirogyra* (Fowke and Pickett-Heaps 1969) there is strong evidence that spindle microtubules are derived directly from wall microtubules. We think that this is also true for the extranuclear microtubules of *Oedogonium* (Fig. 23*a*) but have been unable to demonstrate it clearly so far. Microtubules first appear in small numbers near the spherical nucleus as it moves up the cell before division (Fig. 22), and so they might also be associated with this characteristic premitotic movement. The subsequent change of shape of the nucleus (Figs. 2–4, 23, and 25) coincides with the appearance of the sheath of microtubules around it (Figs. 24 and 26—there are none inside it at this stage). Thus, the microtubules are likely to be the cytoplasmic agents responsible for this change in shape (cf. McIntosh and Porter 1967).

Fig. 44.—Components of the septum, interspersed with endoplasmic reticulum between daughter nuclei. × 45,000.

Fig. 45.—As for Figure 44, but with nuclei typically pressed close together. × 24,000 approx.

Fig. 46.—Septum which has commenced movement up cell after ring breakage (as in Fig. 17). × 26,000 approx.

Fig. 47.—Microtubules (arrow) of septum typically cutting through cytoplasm to wall of cells, before wall splitting. × 20,000.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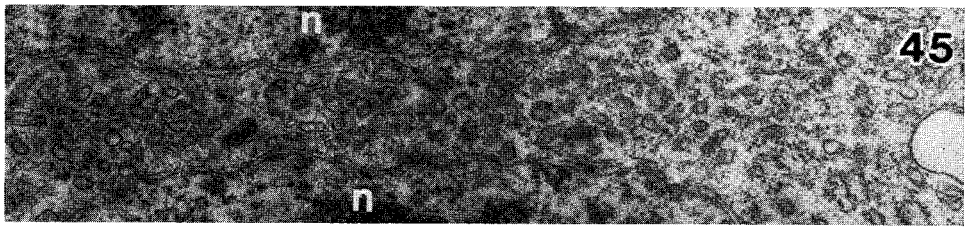
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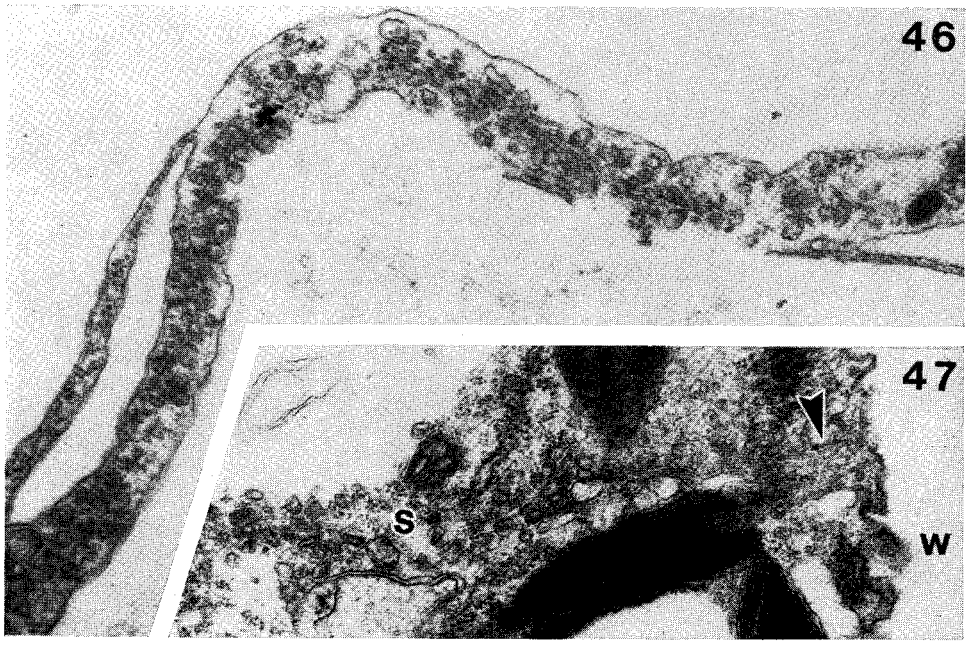
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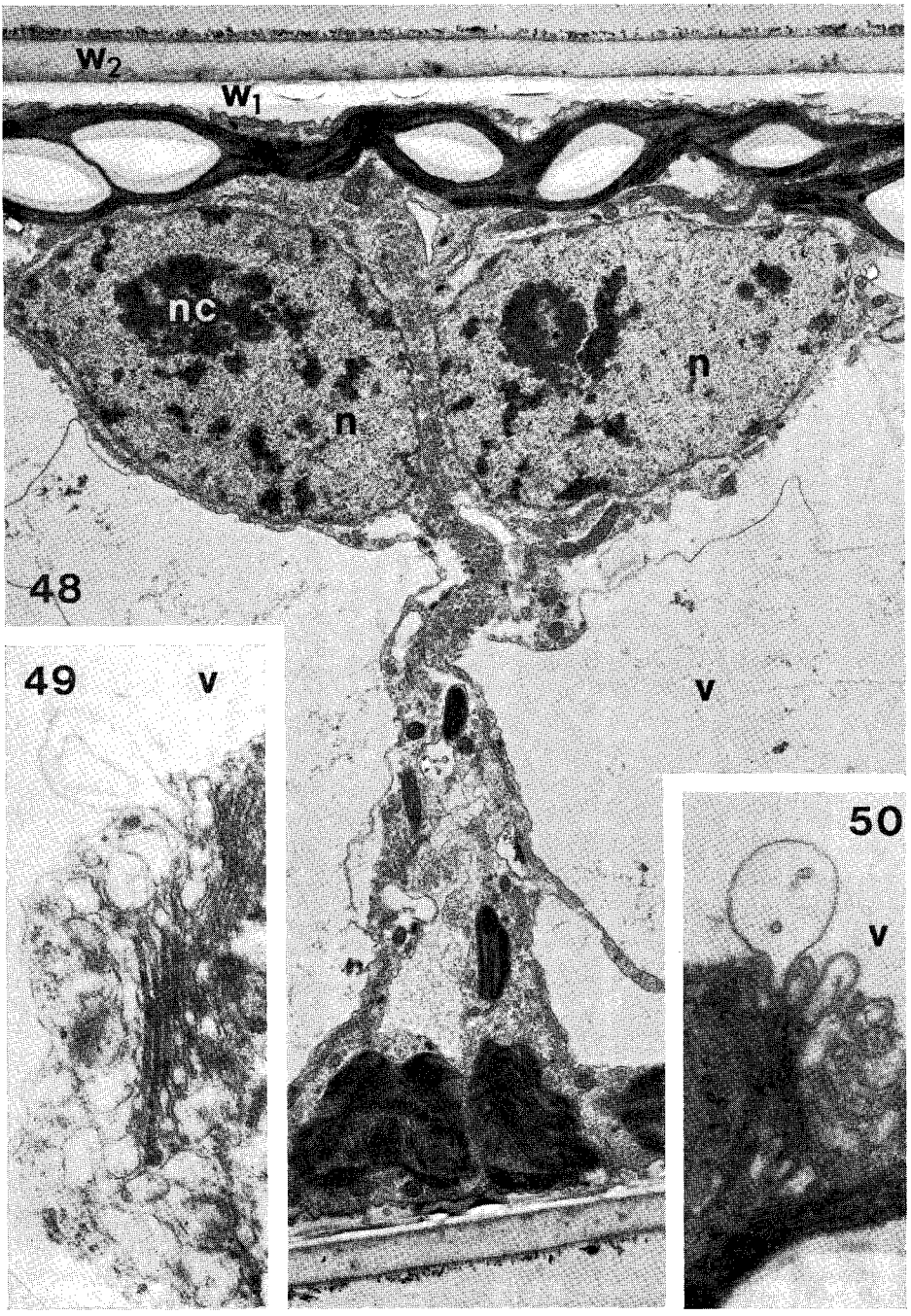
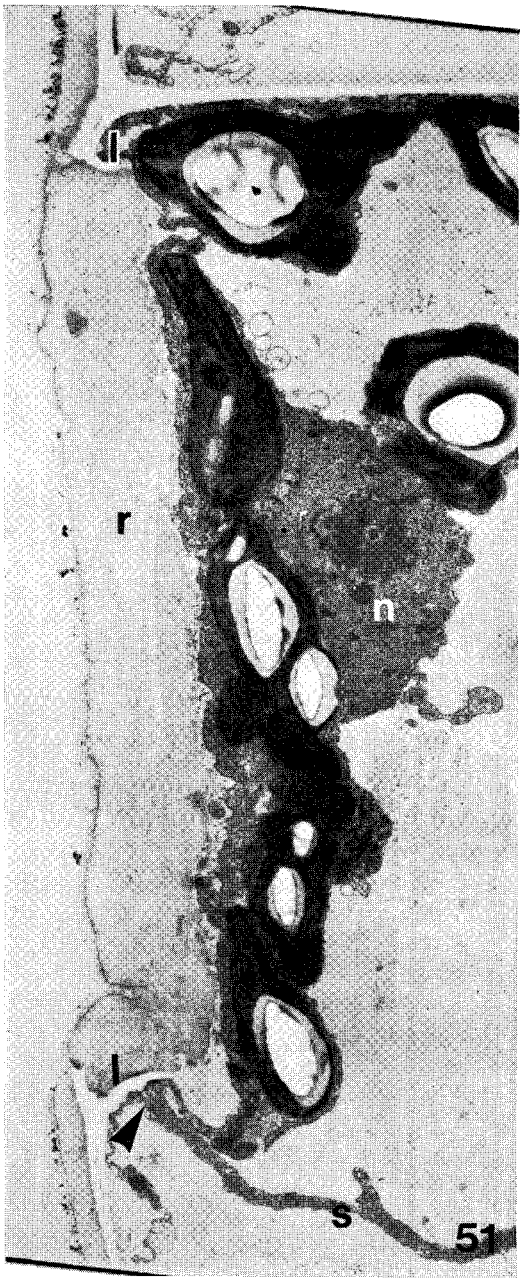
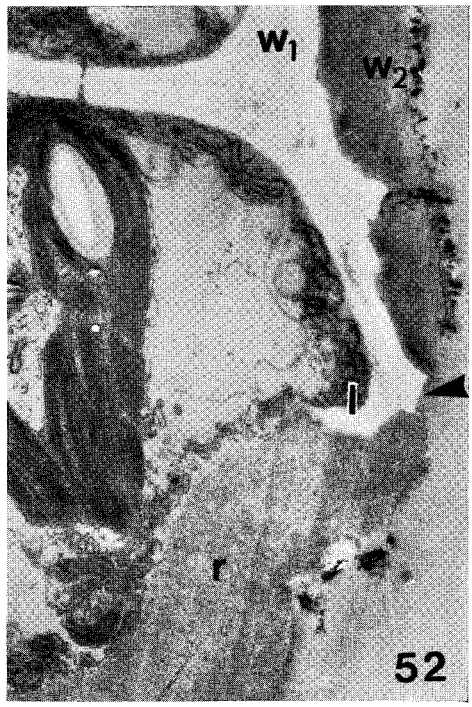
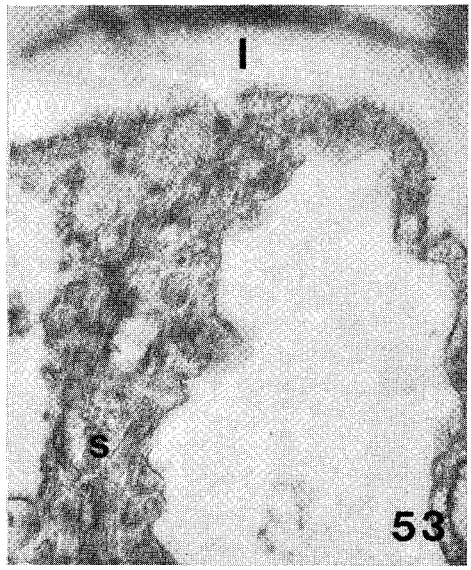


Fig. 48.—Septum fully extended across cell. Later the nuclei separate (cf. Figs. 15 and 16) before wall splitting. ×6,000.

Figs. 49 and 50.—Golgi bodies discharging (?) large vesicles into the vacuole (cf. golgi in Fig. 22). This change in appearance and apparent function occurs before wall splitting. *49*, ×33,000; *50*, ×24,000.







Figs. 51–53.—Material of the ring seen considerably extended following wall splitting (cf. Fig. 17). *51*, Septum (still composed of vesicles, tubules, etc.) has moved up cell to its final position, "caught" under the lower of the two lips (arrow) present in the ring before wall splitting (see Fig. 20). ×4,300. *52*, Breakage of inner wall layer (arrow) is forming the second cap of the cell; note lip of reinforced wall material (*l*). Note particularly the close resemblance of the outer wall layer (w_2) with the material of the extended ring (*r*). ×9,000. *53*, Septum and components caught at lip are shown. ×33,000.

In *Chlamydomonas*, the nuclear envelope contains numerous open pores, and "fenestra" are formed at the poles (Johnson and Porter 1968). This seems somewhat similar to the situation in *Oedogonium*, where the polar differentiation of the membrane in association with microtubules is most characteristic and very pronounced. On two occasions (one in *O. cardiacum*), a small polar bleb was found in the nuclear envelope of interphase cells, suggesting that some polar differentiation of the membranes exists throughout interphase. Several earlier workers (e.g. Kretschmer 1930) detected the drawn-out profile of the nucleus, and Tuttle (1910, p. 147) says that this "noteworthy" point was often neglected. It is tempting to suggest that microtubular components enter the nucleus through this part of the spindle. Recognizable intranuclear microtubules are often first seen in the polar region; later, after metaphase, they decrease

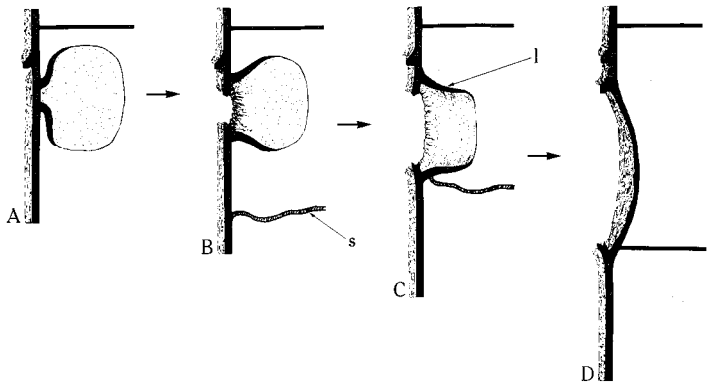


Fig. 54.—Diagram to clarify the events that occur upon wall splitting. At *A*, the two layered wall (with a cap from a previous division) has the fully formed ring with its two inner lips (exaggerated in size) of reinforced wall material. At *B*, the cell wall has split between the lips; the ring is becoming stretched and the septum (*s*) has moved a little way up the cell. At *C*, the ring is further stretched, and the lips (*l*) are forcing out the rims of the split wall into the caps; the septum has reached its final position at the lower lip. By *D*, coalescence of the vesicles in the septum has formed the new transverse wall; a new inner wall layer is appearing.

in number once the spindle has become more organized. It appears as if the outer sheath of microtubules which are formed around the nucleus during preprophase (Fig. 24, etc.) could have been depolymerized in this region, becoming visible as the

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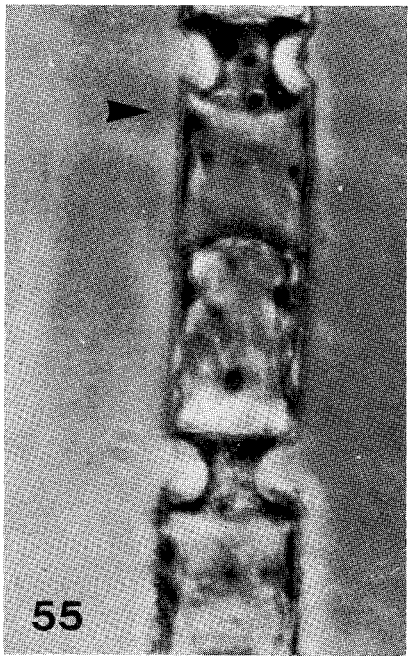
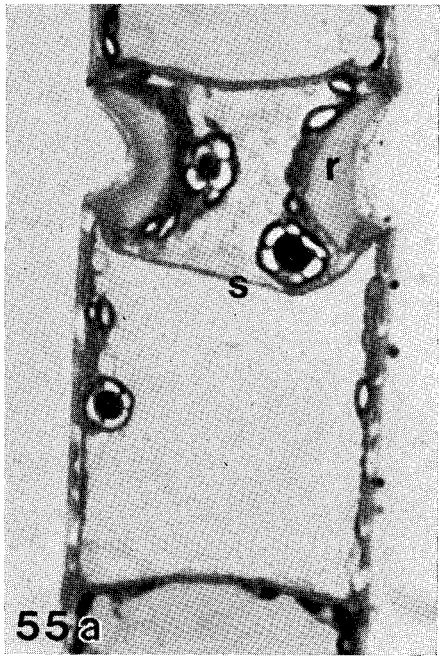
Fig. 55.—Wall splitting: fixed material photographed with phase contrast in Araldite, before sectioning (cell at arrow is shown in Figs. 55*a* and 55*b*). ×400.

Fig. 55*a*.—Light micrograph of cell after sectioning. Septum has moved to wall lips (as in Fig. 51). ×3,920.

Fig. 55*b*.—Electron micrograph of same cell as above, heavily (over) stained with lead. Lip (*l*) and inner wall layer stained heavily (cf. Figs. 51, 52, *et al.*). Septum still composed of vesicles, etc. ×10,000 approx.

Fig. 56*a*.—Same section as that shown in Figure 56*c*, showing initial coalescence of vesicles in septum (cell at arrow in Fig. 56). ×23,000.


55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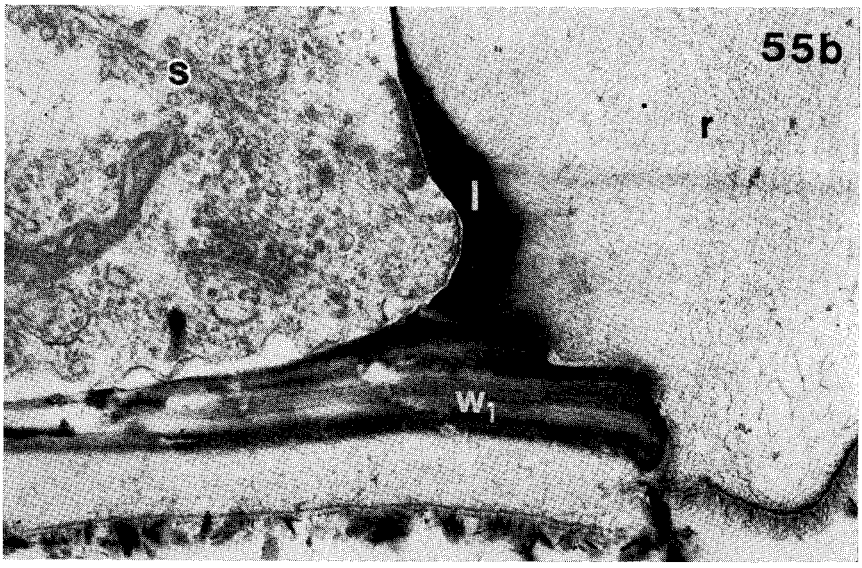
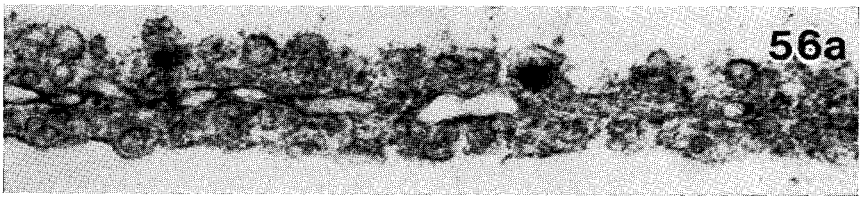
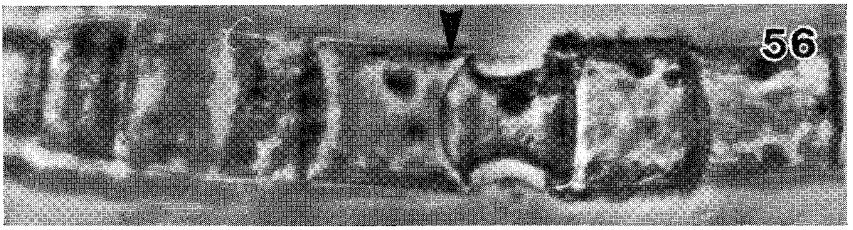
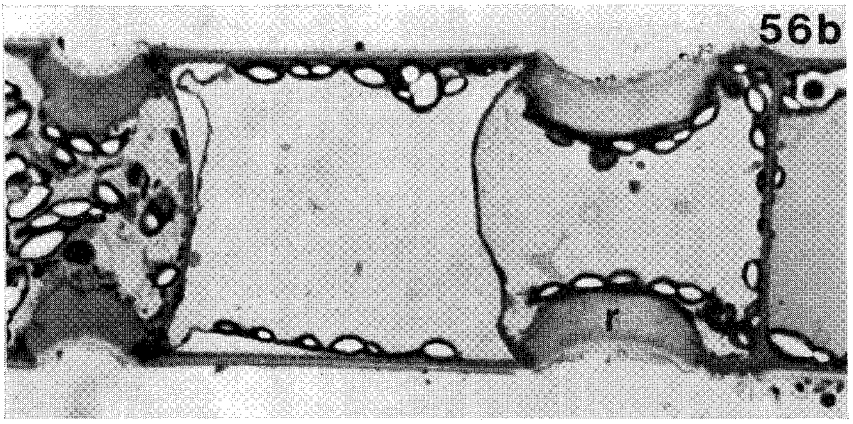
s
55a


s
55b
r
I
W1


56a


56


56b
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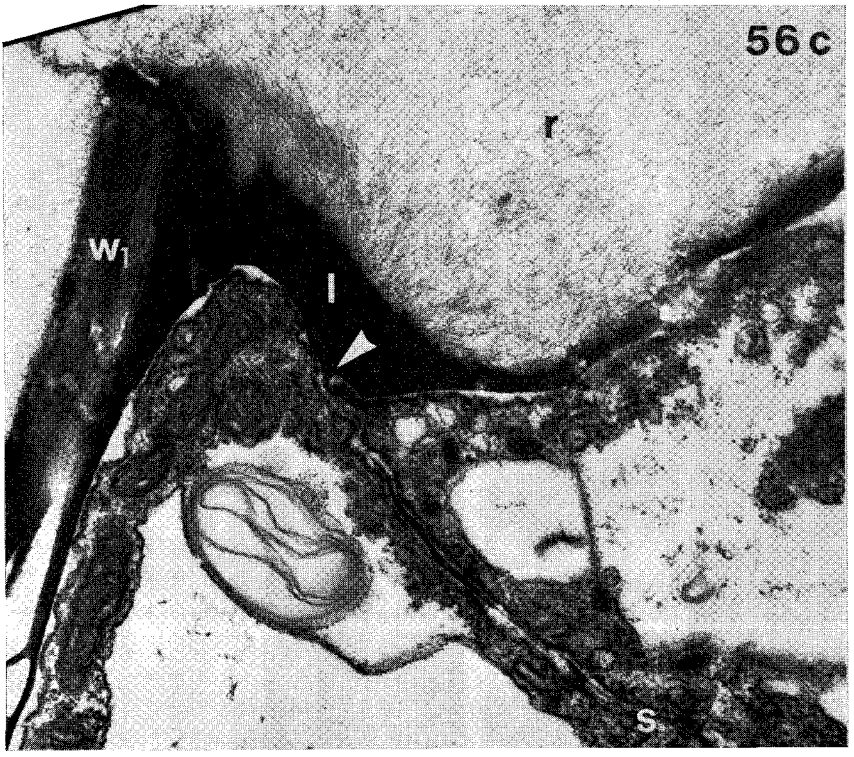
56c
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W1
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S

amorphous material (Figs. 23*b* and 35) between the membranes. However, this is highly speculative at the moment; it also appears that the number of microtubules within the dividing nucleus far exceeds the number that ensheath the nucleus earlier. Furthermore, in spite of several attempts at serial sectioning, we have been unable to reconstruct the complex three-dimensional form and organization of the polar membranes and their associated microtubules, and therefore caution is needed in interpreting the micrographs. So the source of the spindle microtubules remains obscure at this stage.

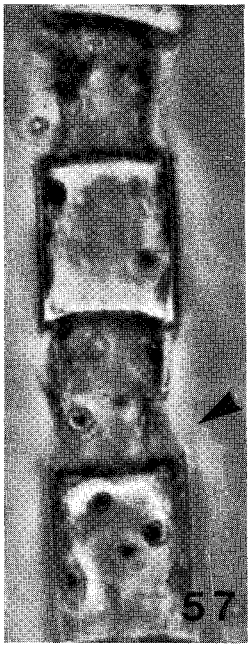
The complexity of the layered kinetochore structure (Figs. 32, 34, and 36) is surprising. To the authors' knowledge, such a complex structure has not been described before in plant cells, where the insertion of microtubules into chromosomes is generally rather structureless (e.g. Manton 1964*b*; Harris and Bajer 1965; Pickett-Heaps and Northcote 1966; Pickett-Heaps 1967*c*; Wilson 1968). Kinetochores in animal cells, however, often contain similar substructures (Brinkley and Stubblefield 1966; Jokelainen 1967). In *Oedogonium*, the kinetochores become apparent at prometaphase on the scattered chromosomes. Polymerization of microtubules in opposite directions from pairs of kinetochores into the polar zones (Fig. 30) could easily be envisaged as resulting in the metaphase plate array (Fig. 31) of chromosomes (Pickett-Heaps 1967*c*; Subirana 1968).

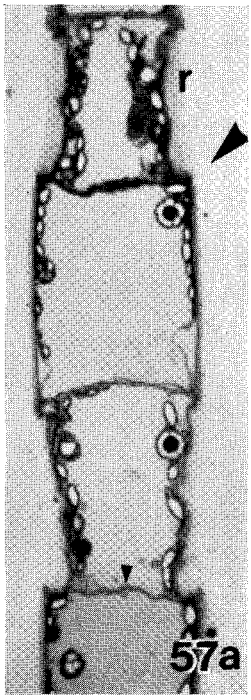
The elongation of the spindle during late anaphase (Figs. 37 and 38) is typical of many animal and unicellular plant cells (e.g. Manton 1964*a*) and seems very likely to be a result of proliferation of interzonal microtubules (Figs. 38 and 38*a*). The nuclear membrane evidentally remains intact to a large extent, enveloping the daughter nuclei as the interzonal microtubules proliferate during late anaphase. The characteristic midbody, which appears between daughter nuclei (Figs. 9, 38, and 39), could result from elimination of some component (we believe it to be of nucleolar origin) from the spindle; its derivation and significance will be discussed more fully in another paper. It was apparent in only a few of our light micrographs (Fig. 9) and was detected by Tuttle (1910). Similar (nucleolar?—compare Figs. 21 and 38) material has been observed in *Chara* (Pickett-Heaps 1967*c*) and *Spirogyra* (Fowke and Pickett-Heaps 1969). This material disappears (Figs. 39*a* and 40*a*) as the nuclei come together (Fig. 41), a movement that is probably consequent on the depolymerization or reorientation of the interzonal microtubules or both. Tuttle (1910) says that this movement is very rapid but we have managed to catch one cell at this point (Fig. 40). It is intriguing that the daughter nuclei, separated by a considerable distance at telophase, should then come so close together again prior to septum formation. In *Spirogyra*, the chromosomes cease to be identifiable during mid-anaphase (Fowke and Pickett-Heaps 1969), and a proliferation of microtubules around the spindle appears important in providing mechanical support during separation of the two chromatin–

Fig. 56.—Cell elongation, cross wall formation; fixed material photographed before sectioning (as in Fig. 55). ×420.

Fig. 56*b*.—Light micrograph of cell at arrow in Figure 56, after sectioning. ×960.

Fig. 56*c*.—Electron micrograph of cell in Figures 56 and 56*b*; section stained as in Figure 55*b*. Cross wall, forming in septum (*s*), has become attached to lip (*l*) at arrow (see also Fig. 56*a*). ×17,000.





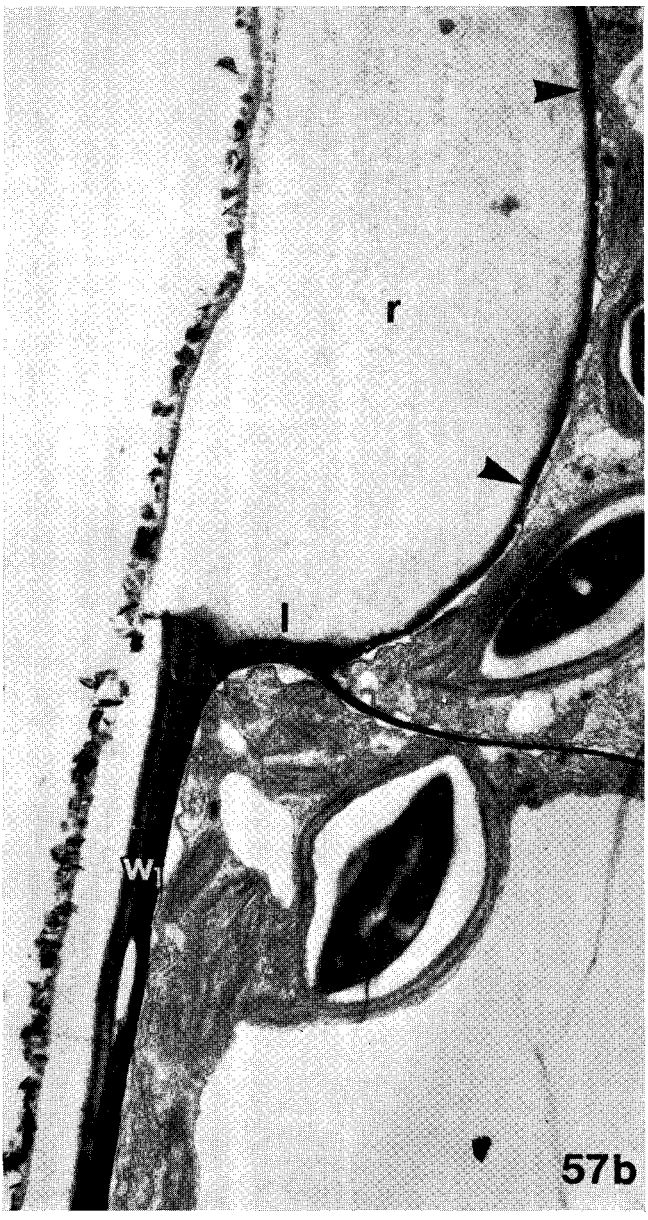


Fig. 57.—Daughter-cell elongation; whole mount as in Figures 55 and 56. ×420.
Fig. 57*a*.—Light micrograph of cell at arrow in Figure 57. Ring material (*r*) considerably drawn out. Lower cell similar; cross wall also forming in this cell (small arrow). ×520.

nucleolar complexes in the isolated spindle. In *Oedogonium*, the chromosomes seem drawn apart by the kinetochore–microtubular structure, and further separation then appears to involve the interzonal microtubules (Figs. 38 and 38*a*).

The formation of the septum (Figs. 11–14 and 41–48) is, to the authors' knowledge, unparalleled in plant cells. Recently Johnson and Porter (1968) found many microtubules in a similar orientation between daughter nuclei in *Chlamydomonas*; however, cell partitioning in that species is achieved by cleavage. Due to the peculiar mode of cell wall extension in *Oedogonium* following the splitting of the wall at the ring, these cells apparently cannot form a transverse cell wall until after cell elongation has proceeded, the latter process occurring well after nuclear division. The septum appears to be formed as microtubules are resynthesized or become reoriented transversely between the nuclei, which have come together after telophase (Fig. 41), although some reorientation is visible earlier (e.g. Figs. 39*a*, 40*a*, and 40*b*). The microtubules, in different orientations, but all in the same plane, appear to push out the tonoplast across the vacuole and so eventually partition the cell (Figs. 41–48). The complex of tubules and other cell plate components pushes through the cytoplasm as well, till it reaches the cell wall (Fig. 47). It remains coherent in structure as it moves up the cell, rather like a movable diaphragm (Figs. 16, 17, 46, 51, and 55–55*b*). The components of the future cell wall (vesicles or tubular membranes or both) fuse *after* this movement is complete (Figs. 51, 52, and 55–56*c*). [Strasburger (1880) is evidentally incorrect in maintaining that wall formation is completed immediately after cytokinesis.] Thus, the formation of the transverse cell wall in *Oedogonium* is similar to, but also different from, the equivalent process in higher plants and another more complex filamentous alga, *Chara* (Pickett-Heaps 1967*c*). More specifically, it is similar because microtubules appear to be the important cytoskeletal structures organizing the future cell wall components, but different in that the orientation and consequently the spatial formation of these microtubules is entirely different.

The septum appears to achieve its final location following movement up the cell when it becomes caught against the rim of reinforced wall material of the stretched ring (Figs. 51 and 55*b*). As a result, the new cross wall is formed precisely at the location of the bottom cap (Figs. 56*c* and 57*b*) as is typical of *Oedogonium*. This is shown in Figure 54. The mechanism by which the synchronized but delayed fusion of cell wall vesicles is achieved is not apparent from our micrographs (cf. Figs. 55*b*, 56*a*, 56*c*, and 57*b*). The lips of reinforcing material appear to be quite firmly connected to the older wall. Thus, as the cell elongates at telophase and the ring material is stretched into the new wall (Figs. 17 and 51–57*b*), the rims of the older broken wall are forced outwards to form the upper and lower caps. The older wall between these lips of reinforced material could be weakened to permit the clean split that occurs. In this regard, it is interesting to recall that zoospore release is also preceded by a similar circumferential wall splitting, but without any wall thickening being present. Since wall splitting is very abrupt, we doubt that enzymic dissolution of some wall

Fig. 57*b*.—Electron micrograph of same cell as that arrowed in Figures 57 and 57*a*. Cross wall, attached to lip, is now thickening; note new inner wall layer (arrows) equivalent to w_1 appearing along inner edge of drawn-out ring material (*r*), which seems destined to form the outer wall layer (cf. w_2 in Figs. 20, 52, *et al.*). ×4,100 approx.

component is primarily and specifically involved in achieving the breakage, although such a mechanism could obviously prepare the wall for this event. There is clear evidence of a weakened wall region between the lips of the ring in *O. cardiacum*.

The process of ring splitting is quite explosive and fascinating to watch *in vivo* (Figs. 18.1–18.5) since the filaments twist and jerk as each ring breaks. This explains why we have seldom found cells with walls that have split by only a small amount. Smith (1938) says that cell elongation is often rapid (i.e. can be completed within 15 min) but otherwise, we have found little mention of this particular aspect of behaviour in the literature. The older wall often tears unevenly, bending the filament abruptly; this was responsible for the change in orientation of the live filament seen in Figure 18.5. Generally the filament straightens as the tear is completed. For several minutes, further expansion is detectable by eye. Such a process is consistent with the idea that a build-up of turgor pressure inside the cell is the prime cause of wall rupture and elongation. In any event, rapid uptake of water into the cell must occur to prevent collapse during such rapid elongation. Our observations suggest that perhaps large, electron-transparent vesicles derived from golgi bodies may be more actively discharged into the vacuole at this time (Figs. 49 and 50) and so may be associated in some way with the possible build-up of turgor pressure (e.g. by pumping liquid or at least solute into the vacuole). The results described above are merely suggestive at this stage, as better preservation of the tonoplast, for example, is needed to be more sure of the observations. An apparent discharge of such large golgi-derived vesicles into the vacuole or contractile vacuole has been described in *Glaucocystis* (Schnepf and Koch 1966*a*), *Vacuolaria* (Schnepf and Koch 1966*b*), *Chara* (Pickett-Heaps 1967*b*), *Ulva* (West and Pitman 1967), and possibly *Prymnesium* (Manton and Leedale 1963); a similar process may occur in some higher plant cells (Pickett-Heaps 1967*d*). In other algae (*Euglena* and *Stigeoclonium*) smaller, "coated" vesicles appear to be thus involved (Manton 1964*c*; Leedale, Meeuse, and Pringsheim 1965).

The characteristic and virtually invariable association between the golgi bodies and differentiated elements of endoplasmic reticulum (as in Fig. 22) is considered very significant; it can be compared to that in a diatom, *Pinnularia* (Drum 1966). Mollenhauer (1965) considers that in higher plants the endoplasmic reticulum could represent a possible coordinating system between golgi bodies, for instance using a vesicular bridge (see also Mollenhauer and Morré 1966). In *Botrydium* (Falk 1967) and *Tribonema* (Falk and Kleinig 1968) there is evidence of a vesicular transfer direct from the nuclear envelope to the golgi bodies. In *Oedogonium*, the golgi bodies are quite small during interphase; they become markedly larger and more active during division and then apparently contribute some material to the vacuole (see above). The spatial association of endoplasmic reticulum with such golgi bodies is considered equivalent to that suggested by Mollenhauer (1965). As a rough analogy, the golgi bodies could be small synthetic or packaging assemblies "programmed" by the small vesicles derived from the endoplasmic reticulum (a movement of the vesicles in the reverse direction seems unlikely).

As far as we can tell, the material derived from the wall ring becomes the outer layer of cell wall material (Figs. 51, 55*b*, 57*a*, and 57*b*). This does *not* seem to represent the rigid cell wall, which appears to be the inner layer (Figs. 20, 51, etc.). Following

extension of the ring material into the form of the new cell, it appears as if a new layer of the inner wall is laid down (see Fig. 57*b*), although this inner wall might also be derived directly from the outer layer by some chemical change. The outer layer must be quite soft (since it is extensible and the filaments bend very easily at the regions of wall elongation before the inner layer appears), and could be mucilagenous. Thus the formation of the ring, so characteristic of mitosis in *Oedogonium*, might represent an adaptation of a more usual mucilage secretion process that occurs in many other algae.

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