

# ULTRASTRUCTURE AND DIFFERENTIATION IN *CHARA* SP.

## I. VEGETATIVE CELLS

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### *Summary*

The ultrastructure of young growing cells of *Chara* is described. The cells showed many features typical of plant cells. The nuclei of larger cells invariably contained groups of close-packed, heavily staining microtubular elements. Typical wall microtubules were always found, and large internodal cells contained filaments possibly involved in cytoplasmic streaming. Vacuolation in young internodes apparently commenced by extensive dilation of elements of the endoplasmic reticulum. In other cells, close apposition of endoplasmic reticulum to cytoplasmic inclusions indicated possible secretion of material into the organelles had been occurring. Golgi bodies with intercisternal elements were often grouped together, sometimes with interconnected cisternae. Isolated reticulate membrane systems, similar to those found at the reticulate face of golgi bodies, were sometimes seen in vacuolated cells. The golgi bodies might have been involved in vacuolation; they were also seen in association with coated vesicles that appeared to be involved in wall deposition. A large number of different cytoplasmic inclusions were found, whose nature and function is obscure. These sometimes contained crystal-like bodies.

The results are discussed in terms of the possible functions of the organelles, particularly with respect to morphogenesis and the patterns of cells formed during growth.

## I. INTRODUCTION

The Charales are algae which exhibit considerable complexity both in their vegetative organization, and in the structure of their sexual reproductive organs, a remarkable feature being the repeated patterns of cells formed during growth (see Figs. 1 and 2). In the basically filamentous stem of these plants, huge vacuolated internodal cells alternate with small flat nodal cells, the pattern being further repeated in the laterals of more limited growth, which arise at the nodes. In *Chara*, the cells forming the main stem are surrounded externally by a layer of cortical cells, which also divide during growth to form a related pattern with an almost mathematical precision; some of these cells later elongate to become stipular outgrowth. For further structural details of the Charales, see Fritsch (1935). The oogonia and antheridia are formed at the nodes of the laterals. Growth occurs initially at a dome-shaped apical cell which cuts off segments parallel to its base; each segment then divides to form a young node and internode cell. The nodal cell then divides further, and a whole series of mitoses give rise to both the laterals of limited growth, and to the pattern of cortical cells surrounding the internodal cells. The ultrastructure of mitotic cells will be discussed in a forthcoming paper.

Little work has been presented on the ultrastructure of the Charales. This is somewhat surprising since they appear eminently suitable for studies on morpho-

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genesis. The huge internodes have been used extensively already for investigating protoplasmic streaming, ion transport, etc. in single cells. Chambers and Mercer (1964) described the protoplast of *C. australis*; their somewhat severe methods of fixation (6–24 hr in 2% osmium tetroxide) appear to have given some artefacts (e.g. apparent absence of plasmalemma—p. 376). Nagai and Rebhun (1966) have recently used glutaraldehyde fixation to give good preservation of *Nitella*. Other authors have presented some brief observations on Charales; these will be mentioned later.

The author has undertaken extensive investigations into several aspects of differentiation in *Chara*. This paper deals with the ultrastructure of vegetative cells in the meristematic region of the plant, where cell division and rapid growth occurs.

## II. METHODS AND MATERIALS

Several named species of Charales (including *C. australis*) were originally supplied by the Botany Department, Cambridge University. These, however, were not entirely suitable for the work undertaken, being somewhat quiescent. Later an unknown species of *Chara* was very kindly made available by Mr. R. R. Willing, Department of Botany, Australian National University; this dioecious variety exhibits vigorous growth and extensive development of oogonia and antheridia, and so was used for all the work described below. The ultrastructure of this tissue appeared in every way comparable to that observed using *C. australis*.

Fixation and embedding of *Chara* and *Nitella* has proved difficult. Slight plasmolysis has been unavoidable so far; Nagai and Rebhun (1966) in their study of *Nitella* also found that "detaching of the plasma membrane from the cell wall could not be prevented in any of the fixatives" (of which they list ten). Other work here on similar algae has also met with this difficulty. However, although the plasmalemma was stretched, and occasionally showed peculiar (probably artefactual) forms, the rest of the cytoplasm was generally very well preserved. For fixation, a solution of glutaraldehyde (6%) was buffered to pH 7 with 0.025M phosphate buffer, with added calcium chloride (see Pickett-Heaps and Northcote 1966a). The stock fixative was diluted 1 : 2 or 1 : 4 and the specimens were fixed, at room temperature for 0.5–2 hr. After washing in buffer (Ledbetter and Porter 1963) the specimens were post-fixed in veronal-buffered 1% osmium tetroxide for 1 hr. They were then thoroughly washed, dehydrated in an ethanol series, and transferred to propylene oxide. They were embedded in Araldite; this was difficult, since air bubbles always appeared in larger cells (and particularly in the outer shield cells of the antheridia). No means has yet been found of preventing this but it constituted only a minor nuisance. Gold and silver sections were cut on glass knives using a Porter-Blum or Reichert microtome. They were mounted on bare grids or grids coated with Parlodion-carbon, stained with uranium and lead (Millonig 1961), and examined in a Philips EM200 electron microscope at 60 kV. Osmium fixation alone yielded poor results.

For light microscopy thick sections (green-red) were cut as above, expanded, and transferred to a glass slide on to which they were dried. A drop of 1% toluidine blue (in 1% sodium tetraborate solution) was placed on the sections, and the slide was warmed till this almost boiled. The excess stain was sluiced away, the slide was gently dried, and the sections were mounted with Depex and a coverslip for photography.

### III. RESULTS

#### (a) *The Cell Wall*

All cells were surrounded by a typical fibrous wall, which stained heavily with lead, and with toluidine blue (Figs. 3, 4, etc.). In several cases, wall deposition appeared to be occurring by vesicular incorporation (see below).

#### (b) *Plasmalemma*

A typical unit membrane enclosed the cytoplasm of all cells (Fig. 3). Plasmadesmata were very numerous between the cells and the pores were lined with the plasmalemma (Figs. 3 and 4). The plasmadesmata were generally seen to contain some central structure except in the region where stretching (due to plasmolysis) had occurred; an intricate pattern of wall fibres existed between the plasmadesmata (Fig. 4). The degrees of plasmolysis varied, this apparently leading to distortion (occasionally gross) and peculiar forms of the membrane. Membranous structures similar to those described by Crawley (1965) were also found close to the plasmalemma (Fig. 13).

#### (c) *Nucleus*

Younger cells contained a single, large nucleus (Figs. 1, 2, 17, etc.) whilst sections of the large internodal cells often contained several very large nuclei. Most conspicuous in the nucleus was one or more large, densely staining (with both osmium and toluidine blue), generally circular bodies (Figs. 2, 17, etc.). Their behaviour during mitosis indicated that these were nucleoli. The chromatin, which did not stain heavily, appeared as a very finely divided granular material, which condensed into more obvious chromosomes during mitosis. The nuclei of younger cells also generally contained a few other bodies of indeterminate nature, and irregularities in the texture of the nucleoplasm were always present. The typical nuclear envelope containing prominent pores was frequently continuous with the endoplasmic reticulum.

In older, vacuolated cells (particularly in internodal cells), the nuclei showed interesting changes. Amitosis presumably occurs to give the multinucleate condition of these cells; constricted nuclei were sometimes found but whether or not these were undergoing amitosis is debatable. The densely staining nucleolar material always became more dispersed in these larger nuclei, appearing as larger or smaller irregular lumps (e.g. in Fig. 2).

A most striking feature of all the large nuclei was the appearance of densely staining structures apparently composed of close-packed microtubular elements (Figs. 5-10). The outside diameter of individual tubules measured approximately 280 Å (Fig. 9). In transverse section, the bundles of tubules varied in number between about 3 and 15; in longitudinal section, they often extended over considerable distances within the nucleus and sometimes were intimately associated with the nucleolar material (Figs. 5-7). They were frequently found near the nuclear envelope (Figs. 6 and 8) often within small extrusions of the membrane. The groups of tubules were not necessarily straight or parallel to each other (Fig. 10).

*(d) Plastids and Mitochondria*

Chloroplasts were found in all cells, these being generally distributed along the lateral walls [e.g. densely stained organelles near the walls in Figs. 2, 14 (inset), 17, etc.]. Their general structure was similar to that found in other plant cells. In younger cells, the thylakoid system was poorly developed, and many of the organelles were apparently dividing by constriction (Fig. 11). In many plastids, small circular inclusions were present [Fig. 11—compare with the similar “globuli” in chloroplasts of brown algae (Bouck 1965)]. Very prominent starch grains appeared in the plastids of most older cells (Figs. 15, 16) and also in developing oogonia and spermatozoids. The mitochondria were generally small and numerous in the cytoplasm. Elements of the endoplasmic reticulum have never been found continuous with mitochondrial membranes (cf. Chambers and Mercer 1964, Fig. 1).

*(e) Microtubules*

Microtubules were invariably present near the cell wall (Figs. 3, 12, 13, 14, etc.). Their distribution was apparently not so highly organized as in higher plants; often some were found at different angles and at different levels in the cytoplasm to the majority of typical wall microtubules. Their orientation often mirrored that of the wall microfibrils (Fig. 12). Their distribution around the wall of elongating cells (e.g. stipular outgrowths; see Fritsch 1935, p.450) is of interest. Typical wall microtubules were generally seen only at regions of the wall where more rapid expansion must have been occurring (e.g. Figs. 13, 14). In internodal, and even in the flat nodal cells, they always appeared grouped along the side walls (Fig. 13). In elongating stipular outgrowths (i.e. epidermal hairs), they ran transversely around the cell, congregated mostly close to the main stem of the plant, but they were seldom found along the region of the wall shared with adjacent cells in these cases (Fig. 14). A similar disposition was noted in the rounded cells on the laterals (as in Fig. 1), which were to develop into antheridia (Fig. 13).

Some other microtubules were always found distributed throughout the cytoplasm of the cells (particularly in younger cells), these being most frequent close to the nucleus. Such isolated tubules lacked obvious patterns of organization.

*(f) Cytoplasmic Filaments*

In the large internodes and other vacuolated cells, bunches of fine cytoplasmic filaments were frequently encountered (Figs. 15, 16). These appeared exactly equivalent to those described by Nagai and Rebhun (1966).

*(g) Endoplasmic Reticulum*

Profiles of endoplasmic reticulum were always very conspicuous in the cells, being clearly visible even under the light microscope (Fig. 2). These had typical ribosomes attached to some regions of the membranes. In some cells, the lumen of the endoplasmic reticulum contained staining material (e.g. Fig. 22).

In very young, vacuolating internodal cells (Figs. 18, 30), the lumen of many elements of the endoplasmic reticulum was enormously distended; other such distensions were indistinguishable from small prevacuolar bodies (Fig. 17).

In several cells, a very intimate association of the endoplasmic reticulum with cell inclusions has been frequently observed (Figs. 21, 22, 24). For example, in the apical cell, the apposition of endoplasmic reticulum to a small vacuole-like body was seen, and the latter contained a rim of staining material (Fig. 24). (In developing cells of antheridia and oogonia, a similar association of endoplasmic reticulum with spherical and more irregular, lipid-like inclusions was invariably found.) In other regions of the cytoplasm elements of endoplasmic reticulum were closely apposed together and a dense-staining material was found in the cytoplasm between such cisternae; the significance of this is unknown (Fig. 23).

#### (h) Golgi Apparatus

All cells contained a large number of golgi bodies. Almost invariably, at one face of the golgi apparatus, the cisternae were highly reticulate (Fig. 31); occasionally continuous with such cisternae were rough-coated vesicles (Fig. 25). Vesicular components of a very similar appearance were invariably found in all young cells near to the wall, and also continuous with the plasmalemma (Figs. 12, 16, etc.). This occurred also immediately after the formation of the cell plate, when the wall between two cells was very thin (Fig. 28). Around the rest of the golgi apparatus, numbers of typical smooth vesicular components were apparent. These were occasionally large in vacuolating cells (Fig. 30).

Intercisternal elements were visible between the cisternae of most golgi bodies; these either appeared as a row of dots or lines (e.g. Fig. 30). Glancing sections showed fibrous striations on the cisternae, confirming the observations of Turner and Whaley (1965) on golgi bodies in *Nitella*. In some golgi bodies the groups of intercisternal elements were not all oriented the same way (Fig. 26).

The golgi bodies were frequently present in clusters (Fig. 27). Non-reticulate cisternae were sometimes seen to be continuous across several of the golgi bodies (Figs. 27, 29). The tubular elements of the reticulate face of the golgi bodies also often closely intermingled; it is difficult to judge whether the tubular cisternae were likewise continuous, interconnecting golgi bodies, but this seems possible (Figs. 27, 31). Similar isolated reticulate patterns of membranes were occasionally seen in large vacuolated cells (Figs. 19, 20).

#### (i) Vacuole

The vacuole was limited by a typical tonoplast. In internodal cells, the cytoplasm often appeared "spongy" at the corners of the cell. In some vacuolating cells, larger vacuolar bodies apparently enclosed a large number of smaller vesicles (Figs. 32, 33). The appearance in the section was that of a mass of bubbles, and these appeared to be coalescing; other similar bubbles were associated with the golgi apparatus (Fig. 32).

(j) *Other Cellular Inclusions*

A remarkable feature of *Chara* cells was the presence of large numbers of other inclusions whose nature was generally obscure. Dense osmiophilic inclusions (probably lipid droplets) were often found, and these could be very large in internodal cells (Fig. 35). Smaller, spherical, densely staining bodies enclosed in a membrane were always very numerous and these often tended to cluster together (Fig. 34); while generally spherical, the densely staining contents were sometimes crystalline in shape (Fig. 14). There were vacuole-like inclusions of various sizes, the membrane enclosing an aggregated, densely staining material (Figs. 17, 34, 36, etc.). These inclusions also evidently contained crystalline material in some cases; the crystals did not usually section properly, and left characteristic holes in the section (Figs. 17, 34, etc.). Other inclusions often showed a very extended sheet-like form (Figs. 35, 36), the staining material exhibiting a layered structure (Fig. 35, inset). Other crystal-containing bodies (more typical of higher plant cells) were also found (Fig. 37). Elsewhere in the cytoplasm, ill-defined lumps of osmiophilic material were often found. Chambers and Mercer (1964) note the frequent occurrence of structureless bodies in *C. australis*, some of these apparently corresponding to a ninhydrin-positive staining inclusion present *in vivo*.

#### IV. DISCUSSION

In the foregoing study on *Chara*, the ultimate aim has been primarily to try to relate ultrastructure and function, with particular reference to differentiation and morphogenesis. This will be the main object of the discussion.

Microtubules in vegetative *Chara* cells appear equivalent to their counterparts in higher plant cells in many ways. The typical correspondence between the orientation of wall microfibrils and most wall microtubules (Fig. 12) parallels the observation of Ledbetter and Porter (1963) and others. Previous work by the author (Pickett-Heaps 1966a; Pickett-Heaps and Northcote 1966a) on wall differentiation in xylem cells of wheat, however, suggests that the microtubules are not directly involved in microfibril synthesis, a conclusion considered also by Newcomb and Bonnett (1965). A more ill-defined but highly important role in morphogenesis generally seemed indicated (Pickett-Heaps 1966a) since these organelles are not only involved in the structure of the spindle, but also (in the wheat seedling) apparently in predetermining the plane of division of pre-prophase cells (Pickett-Heaps and Northcote 1966b, 1966c). The general disposition of microtubules in *Chara* might support such a view (and also the hypothesis that they are associated with microfibril synthesis); their association with pre-prophase nuclei, and particularly their organization within young complexes of antheridia and oogonia, is complex and as yet unclarified.

The disposition of wall microtubules within young cells of *Chara* is interesting and possibly significant since their arrangement in the cell could be influenced by the position of adjacent cells (see Figs. 13, 14). A similar conclusion is difficult to avoid, for example, regarding the disposition of microtubules in premitotic leaf epidermal cells, in which assymetric divisions give rise to the subsidiary cells of stomatal complexes (Pickett-Heaps and Northcote 1966c). Interactions between

plant cells must occur to maintain patterns of growth and differentiation; one manifestation of such interactions might be evident in the distribution of microtubules, which appear to be involved in the control of wall synthesis (e.g. in xylem—Pickett-Heaps 1966a) and perhaps of the plane of cell division. Furthermore, during development of the shields of the antheridia in *Chara*, the cytoplasm of the shield cells become compartmentalized by the inward growth of the wall [described by Fritsch (1935) as a “characteristic infolding of their membranes”]. Many microtubules were associated with this localized region of wall development (Pickett-Heaps, unpublished data); the situation closely resembled that found during the later development of xylem wall thickenings (Hepler and Newcomb 1964; Wooding and Northcote 1964; Cronshaw and Bouck 1965; Pickett-Heaps and Northcote 1966a).

Other microtubules could almost be found in the cytoplasm of young cells; they were most frequently seen near the nuclei, but little can be surmised as to their function. The general occurrence of cytoplasmic filaments in such large, vacuolated cells (Figs. 15, 16) confirms the work of Nagai and Rebhun (1966); the author would support their contention that these filaments (and not the microtubules) are probably a functional part of the mechanism of cytoplasmic streaming. This view is strengthened by experiments involving exposure of the plants to colchicine (Pickett-Heaps, unpublished data); such a treatment has important effects on the shape and size of the cells (see Green 1962). After an 8-day treatment, cytoplasmic streaming was apparently unaffected; sections of such tissue prepared for microscopy revealed the presence of cytoplasmic filaments, but very few microtubules.

The existence of packed microtubular structures within the large nuclei of vacuolated cells (Figs. 5–10) has not, to the author's knowledge, been previously described in plant cells. These tubules appear to be cytoskeletal in function, perhaps maintaining the overall shape and volume of the nuclei as they are rapidly swirled around the cell. The tubules may also be involved in amitosis; no evidence can be offered in this regard. Their intimate association with nucleolar material (Figs. 5, 7) is likewise unexplained but this may be related to the dispersion of the large nucleoli into smaller units as the nuclei enlarge in vacuolated cells. No such tubules have ever been found in the nuclei of younger cells that would need to divide further. For example, in the formation of the antheridia, these tubules appear in the nuclei of outer shield cells only after they have completed all divisions necessary to form the outer structure of the antheridia (Pickett-Heaps, unpublished data). These tubular structures therefore apparently are products of differentiation, being only found in the nucleus when the cell loses its potential for true mitosis and cytokinesis.

The golgi bodies in the cells sometimes appeared as if they formed a complex interconnected system within the cell (e.g. Figs. 27, 29, 31). Cunningham, Morr , and Mollenhauer (1966) have described the extensive proliferation of tubular elements at the reticulate face of isolated plant golgi bodies; Mollenhauer and Morr  (1966) have also found interconnections between golgi bodies and secretion vesicles. The results described above support their observations. Cunningham, Morr , and Mollenhauer (1966) also showed that two distinct vesicular components were associated with the golgi bodies they examined, and Pickett-Heaps (1966b) has made

similar observations in sectioned material of the wheat seedling. One of these components sometimes appears to be a "coated" vesicle (Cunningham, Morré, and Mollenhauer 1966), apparently attached to the reticulate region of the cisternal stack. In *Chara*, similar coated vesicles appear to be involved in wall deposition; it is difficult to suggest another reason why they should be found apposed to walls in all cells (particularly to very thin walls), although a role in osmoregulation (see below) could not be excluded. Reports of coated vesicles are becoming more frequent; for example, Newcomb and Bonnett (1965) found them in the cytoplasm of elongating root hairs; Leedale, Meeuse, and Pringsheim (1964) described their association with the contractile vacuole of *Euglena* (where they might be concerned with osmoregulation); Roth and Porter (1964) have demonstrated a possible role of these vesicles in protein uptake in mosquito oocytes. The function of the coat on these vesicles in *Chara* cells is not known.

The golgi apparatus might also function in the process of vacuolation. Pickett-Heaps (1966b) has offered some evidence that vesicles derived from the golgi bodies coalesce with the vacuole in developing stomatal guard cells. The same process could conceivably occur in *Chara* (Fig. 30); these vesicles could also give rise to the froth-like condition occasionally observed in internodal cells (e.g. in the laterals — Figs. 32, 33). It is conceivable that the golgi complex and the endoplasmic reticulum both react to a stimulus for vacuolation (compare Fig. 30 with Pickett-Heaps 1966b, Figs. 21, 22).

A function of the endoplasmic reticulum in vacuolation (Figs. 18, 30) appears equivalent to that described in other plant cells by Poux (1962) and Buvat and Mousseau (1960). In *Chara*, continuity of ribosome-coated cisternae of endoplasmic reticulum and the membranes of a typical prevacuolar body was clearly demonstrable in almost all young (and therefore vacuolating) internodal cells (Figs. 17, 18). Close examination of subapical daughter cell pairs (after telophase — Fig. 2) that were going to differentiate into two cells of entirely different potential (i.e. nodal and internodal cells), indicated that no readily discernible differences existed initially between these two cells. The impression gained from examining slightly older pairs of cells was that this process of vacuolation in the future internodal cells had become greatly stimulated. With wall deposition also stimulated, possibly via the golgi apparatus, and cell elongation and growth controlled by the disposition of the microtubules, the internodal cell of *Chara* might exemplify in a simplified system, the processes of controlled cell expansion observed in higher plant cells.

There seems little doubt that the endoplasmic reticulum is very intimately concerned with some aspect of secretion of components within the cytoplasm (Fig. 24). In young antheridia and oogonia, the invariable close apposition of elements of the endoplasmic reticulum to various-shaped inclusions coincides with the proliferation of these inclusions within the pregamete cells (Pickett-Heaps, unpublished data). Preliminary histochemical work has failed to establish the nature of such inclusions, but they are probably lipid or protein. In some other young cells, a similar association of the endoplasmic reticulum with vacuolar components leads one to suspect that secretion of particular material into a vacuolar body might be occurring (Figs. 21, 22). Wooding and Northcote (1965) have recorded other cases where close

application of the endoplasmic reticulum to cell organelles (plastids in particular) may have functional significance.

The cytoplasm of *Chara* invariably contains many inclusions whose nature is unknown. There were always present large numbers of vacuole-like components with contents varying from aggregated material to various larger or smaller crystalline bodies. These cannot be purely artefactual, as the streaming cytoplasm of *Chara in vivo* always contains large numbers of components of similar appearance. Crystal-containing bodies are, of course, by no means uncommon in plant cells (e.g. see Cronshaw 1964; Crawley 1964; Thornton and Thimann 1964; Bouck 1965; etc.). The significance of such inclusions is not clear. In the gamete cells, some inclusions almost certainly represent storage organelles; this also seems likely in the vegetative cells.

Crawley (1965) and Barton (1965) have reported the occurrence of a mass of interconnected tubules in a sack of membranes, near the wall; similar structures have been seen in the course of the investigations described above (Fig. 13), and also in the cytoplasm of many cells in the wheat seedling. In view of the marked distortion apparently suffered by the plasmalemma in many such cells, the author is at present unwilling to comment on this interesting phenomenon.

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#### EXPLANATION OF PLATES 1-9

The following abbreviations are used in the figures in Plates 1-9:

<i>a</i> antheridia	<i>i</i> internodal cell	<i>nd</i> nodal cell
<i>ap</i> apical cell	<i>l</i> laterals	<i>ne</i> nuclear envelope
<i>c</i> cortical cells (surrounding internode)	<i>li</i> inclusion (probably lipid)	<i>st</i> starch
<i>ch</i> chloroplast	<i>m</i> membranous inclusion (near cell wall)	<i>s</i> stipular outgrowths
<i>er</i> endoplasmic reticulum	<i>n</i> nucleus	<i>t</i> microtubules
<i>g</i> golgi bodies	<i>nc</i> nucleolus	<i>v</i> vacuole
		<i>w</i> cell wall

#### PLATE 1

Fig. 1.—Light micrograph, longitudinal section, of apical region of *Chara*. Internodal cells (*i*) in various stages of enlargement alternate with thin nodal cells. Antheridia (*a*) at different stages of development arise at the nodes of the laterals (*l*). Chloroplasts line the inside of the larger internodes; a layer of cortical cells (*c*), including some stipular outgrowths (*s*), surrounds the internodes externally. Axillary shoots form at the nodes (large arrows, see Fig. 2). Figure 13 was taken from a region equivalent to that indicated by the small arrow.

Fig. 2.—As for Figure 1. The nucleus of the apical cell (*ap*) is typical, containing a prominent nucleolus and heterogeneous nucleoplasm. The subapical pair of cells was at late telophase; these daughter cells have totally different potential (becoming nodes and internodes), but no differences between them have been discerned at this stage. The next youngest internode (containing a large nucleus and dispersed nucleoli) was vacuolating rapidly; the ultrastructure of such cells is shown in Figures 17 and 18. Elements of the endoplasmic reticulum and other organelles are clearly visible even at this magnification, in the larger internodes. For more structural details, compare Figures 1 and 2 with Fritsch (1939, p.450, 454).

## PLATE 2

Fig. 3.—Plasmadesmata penetrating the wall (*w*) were lined with a membrane continuous with the plasmalemma. Microtubules (*t*) were always present near the wall. Transverse sections of the pores are shown in Figure 4.

Fig. 4.—Section of wall at right angles to that in Figure 3. At *x* (cf. Fig. 3), the pores appeared "hollow", probably due to the membranes being stretched following slight plasmolysis. At *y* (cf. Fig. 3), the unit membrane enclosed some internal structure. The pattern of wall fibres between the pores was complex.

Fig. 5.—Packed tubular elements closely associated with nucleolar material in a large nucleus.

Fig. 6.—As for Figure 5. The packed tubular elements often extended over considerable distances within the large nucleus (*n*), frequently close to the nuclear envelope (*ne*).

Fig. 7.—As for Figures 5 and 6. In this case, the nucleolar material had an extended form, in association with the packed tubular components; part of a second group of these are visible (large arrow).

## PLATE 3

Fig. 8.—The section had cut the nucleus tangentially and shows three groups of the tubules lying close to the nuclear envelope (*ne*).

Fig. 9.—A group of the tubules cut in transverse section.

Fig. 10.—The groups of close-packed tubules were not always straight, and frequently were seen at different angles to each other.

Fig. 11.—Mitochondria and chloroplasts in a young antheridial cell. The chloroplasts in younger cells generally had poorly developed thylakoid systems and also contained dense inclusions; these chloroplasts were apparently dividing by annular constriction.

Fig. 12.—Section tangential to wall of young cell. Typical microtubules are shown, these being oriented parallel to the underlying fibres of the wall. "Coated" vesicles (arrowed) were invariably found near growing walls.

## PLATE 4

Fig. 13.—Two potential antheridial cells separated by the node on a lateral (i.e. equivalent in position to the small arrow, Fig. 1). Wall microtubules were most numerous along the bulging part of the pro-antheridial cell wall, the number often decreasing markedly where the wall was shared with the node. In all nodes wall microtubules were always concentrated along the side (i.e. the lateral) walls. A membranous inclusion (*m*) very near the wall is also shown (cf. Crawley 1965; Barton 1965).

Fig. 14.—Stipular outgrowth, shown in low power in insert. Wall microtubules were most evident along the region of wall *a-b-c* in inset (being concentrated in *a* and *b*); very few were found in the region *a-d-c*. The higher magnification picture shows how the concentration changes at region *a* in the inset. Note also, the crystalline form of the inclusions (arrow).

## PLATE 5

- Fig. 15.—Longitudinal section of large vacuolated internodal cell. Chloroplasts lined the walls. Long strands of microfibrillar structures were found above the chloroplasts, in the peripheral cytoplasm that exhibits rapid cytoplasmic steaming *in vivo*.
- Fig. 16.—Detail of Figure 15; the chloroplasts (*ch*) in older cells generally contained prominent starch grains (*st*). The texture of the microfibrillar structure is more clearly shown here (cf. Nagai and Rebhun 1966). Microtubules (*t*) and “coated” vesicles (arrowed) were present near the wall.
- Fig. 17.—Longitudinal section of vacuolating internodal cell (cf. Fig. 2), between two nodes (*nd*) which contained typical nuclei (*n*) and nucleoli (*nc*). The large, prevacuolar bodies (which often contained aggregated material) in the internode can be compared to the much smaller inclusions in the nodes. In all cells, such membrane-bounded organelles had often apparently contained some harder, probably crystalline material, which left holes in the sections (arrows).

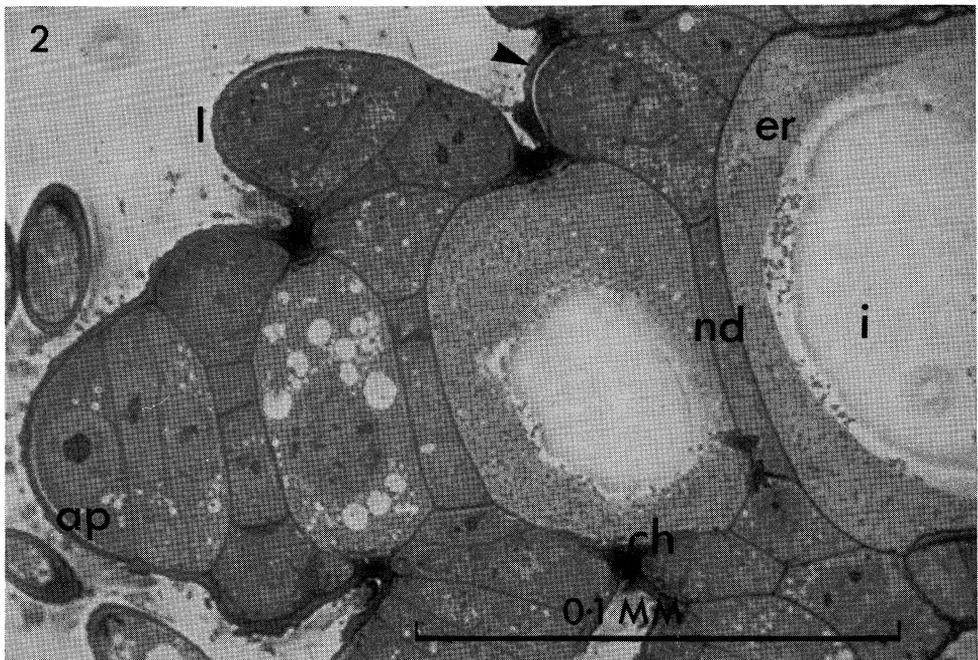
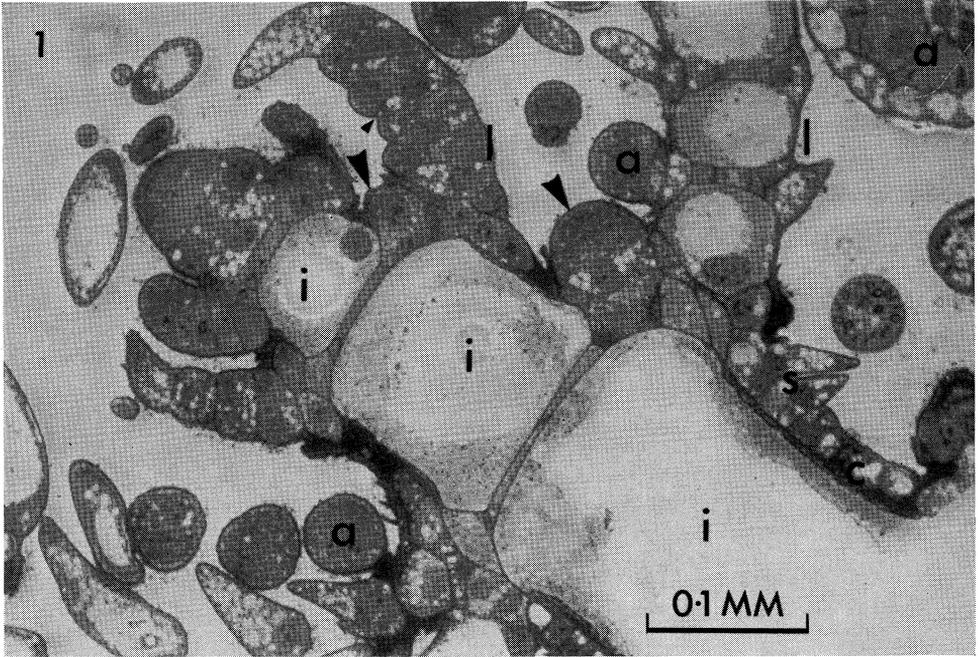
## PLATE 6

- Fig. 18.—Same internodal cell as that in Figure 17. In young nodes, prevacuolar bodies were apparently formed as a result of extensive dilation of the lumen of the endoplasmic reticulum.
- Figs. 19 and 20.—Reticulate membrane structures sometimes seen in large vacuolated (e.g. internodal) cells. Compare with the reticulation of membranes seen in golgi bodies (Fig. 31).
- Fig. 21.—Young internode of a lateral. Elements of the endoplasmic reticulum are shown closely apposed to vacuole-like components (arrowed).
- Fig. 22.—Detail from Figure 21. The lumen of the endoplasmic reticulum (*er*) in this cell contained some staining material. Elements of the endoplasmic reticulum apposed to the vacuole-like inclusions would appear to have been involved in the secretion of some material into the latter.

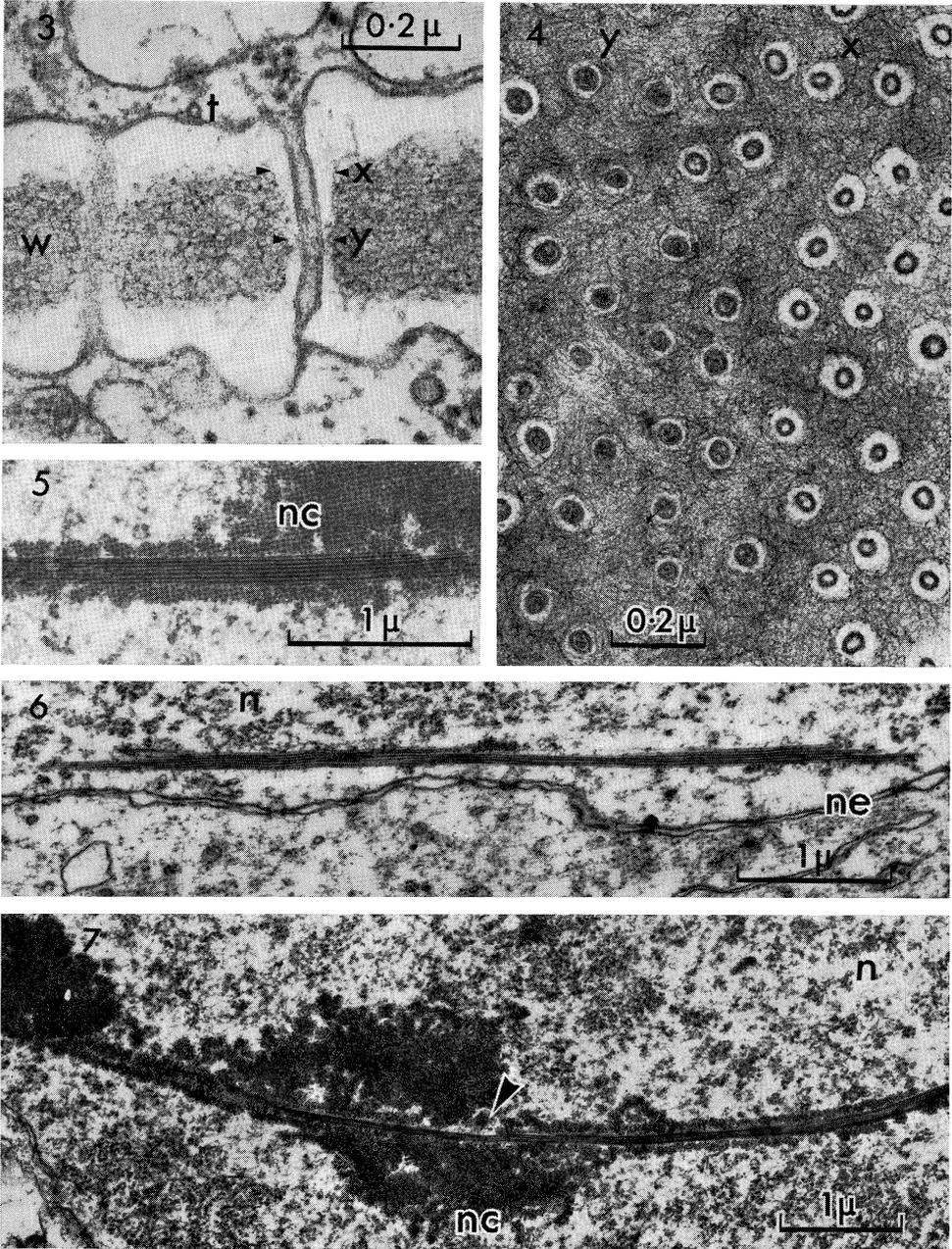
## PLATE 7

- Fig. 23.—Elements of endoplasmic reticulum in the apical cell almost always aggregated in some small areas and a stainable material was detected between such profiles of the membranes.
- Fig. 24.—Frequently in the apical cell (and invariably in younger gamete cells), elements of the endoplasmic reticulum were closely apposed to spherical inclusions (cf. Fig. 22).
- Fig. 25.—“Coated” vesicles near the reticulate face of a golgi apparatus; the membrane of one coated vesicle was continuous with the membrane of the golgi cisternae.
- Fig. 26.—Intercisternal elements in a golgi apparatus. The row of dots and the thin lines (arrowed) apparently represent the elements sectioned in two directions in the same organelle.
- Fig. 27.—Golgi bodies in young lateral node. Continuity between cisternae in adjacent stacks is obvious at one place (arrowed); continuity between other more reticulate cisternae seems quite possible at other regions.
- Fig. 28.—Longitudinal section showing telophase in lateral. Cell-plate microtubules (*t*) were still present, though the wall had been completed across the daughter cells. Many “coated” vesicles (arrowed) were apparently being incorporated into the cell plate.
- Fig. 29.—Golgi bodies interconnected by one cisternae.

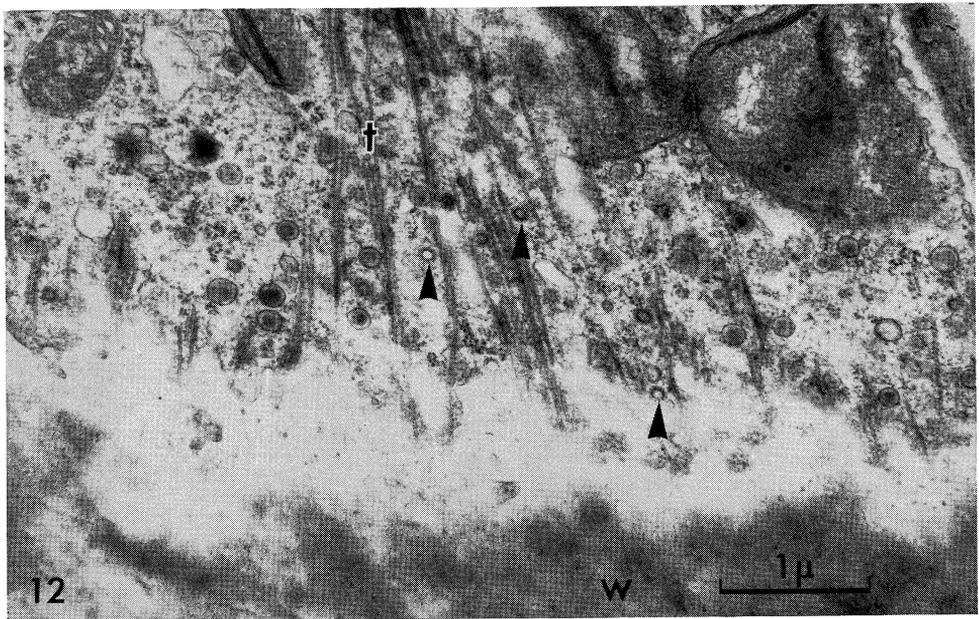
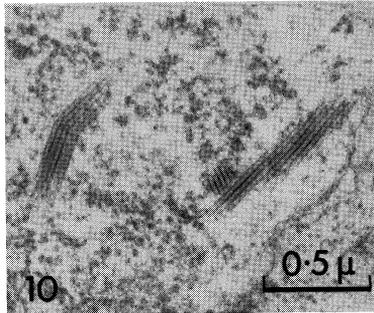
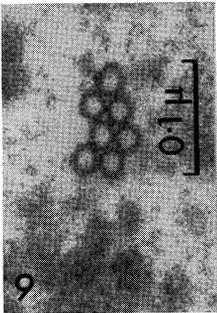
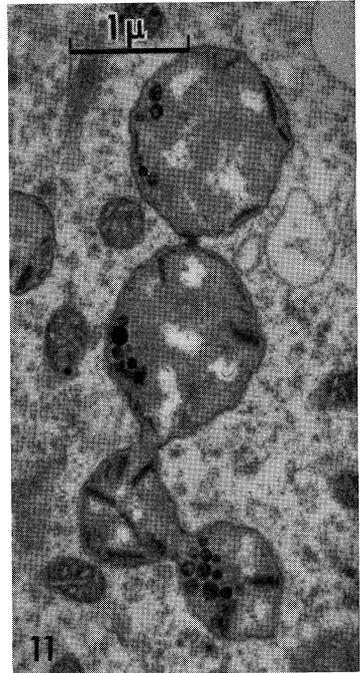
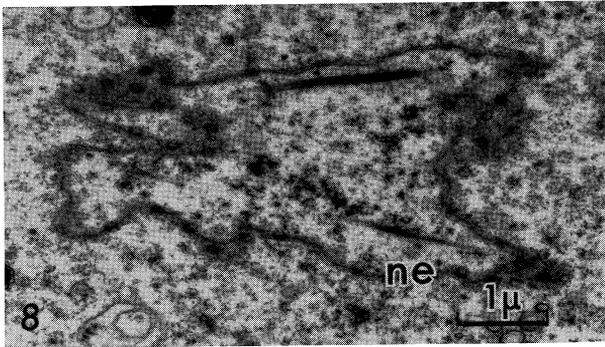
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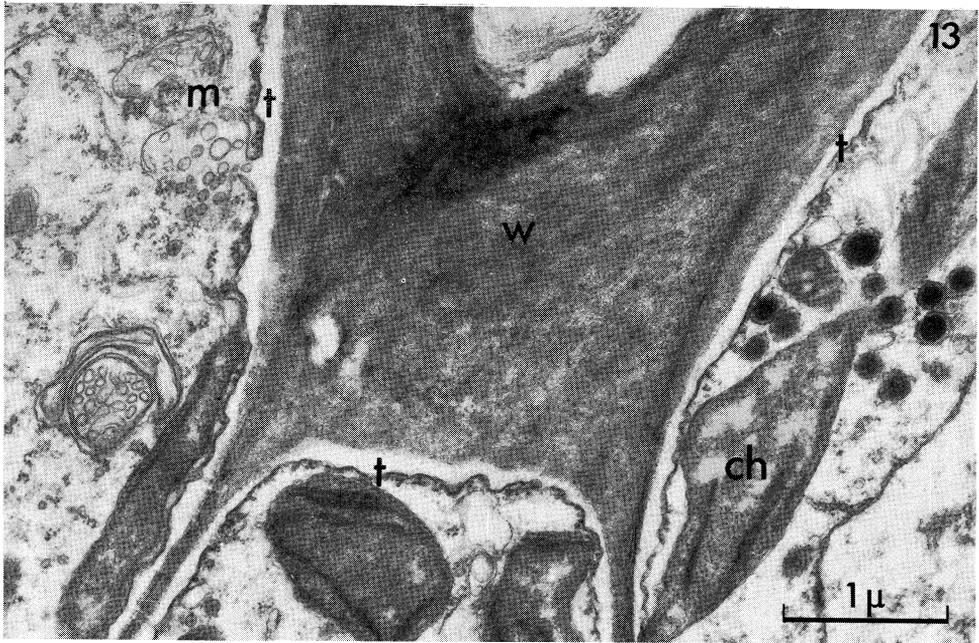
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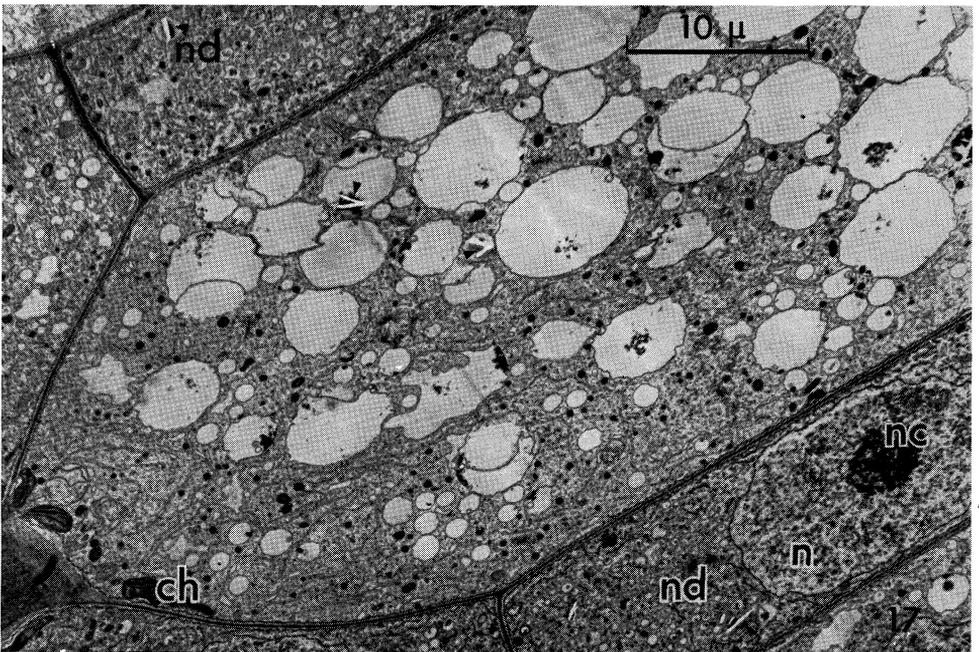
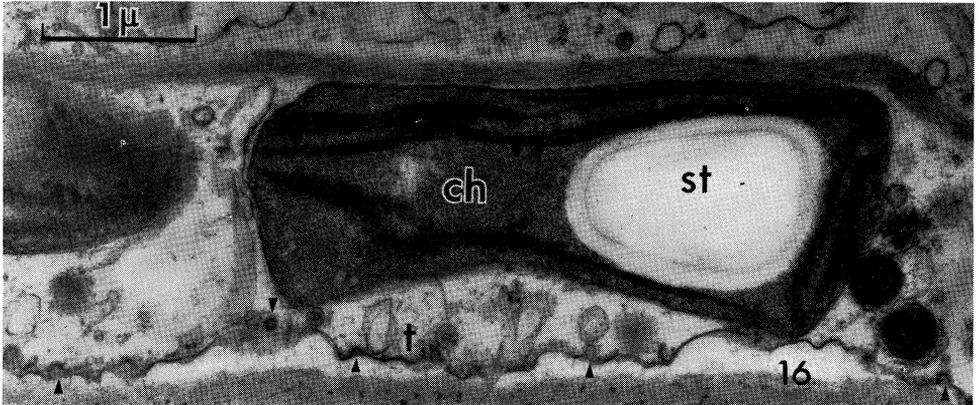
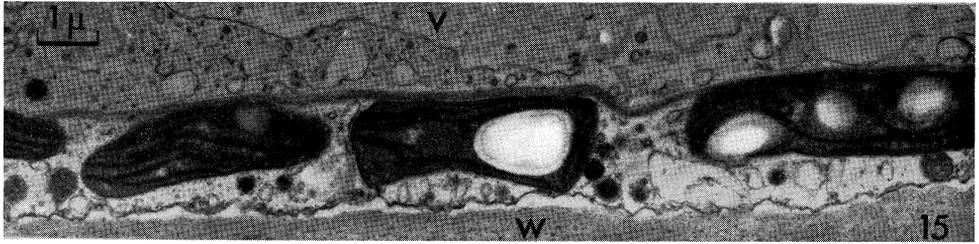
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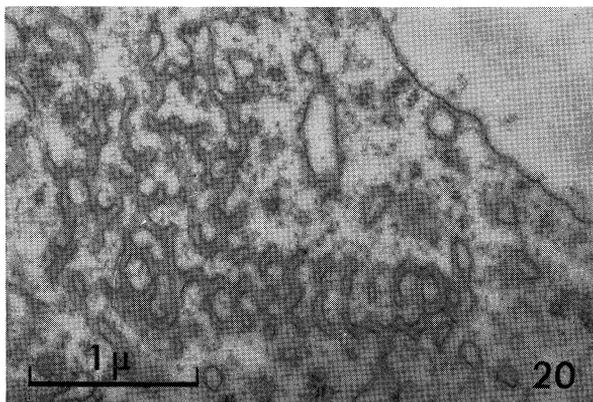
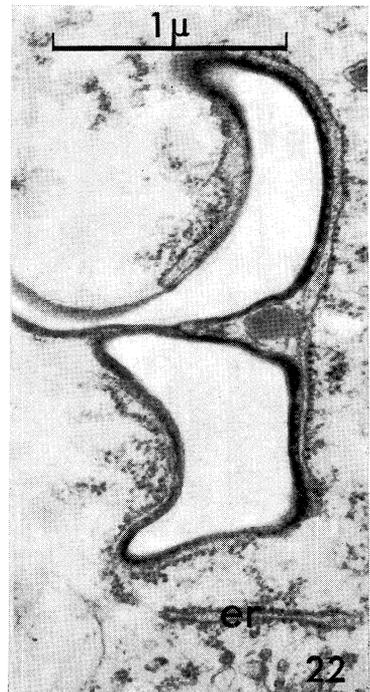
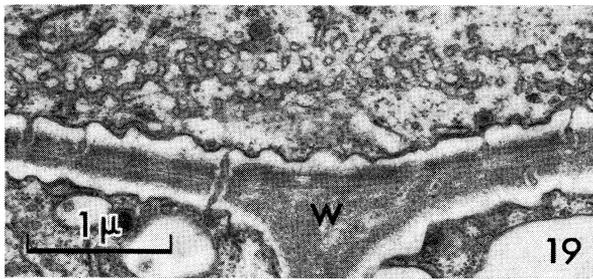
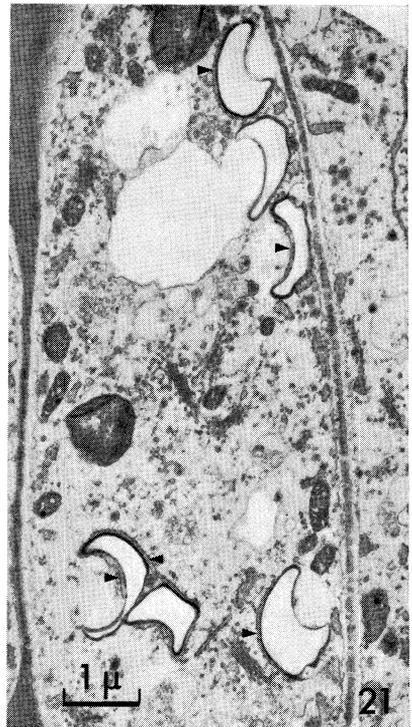
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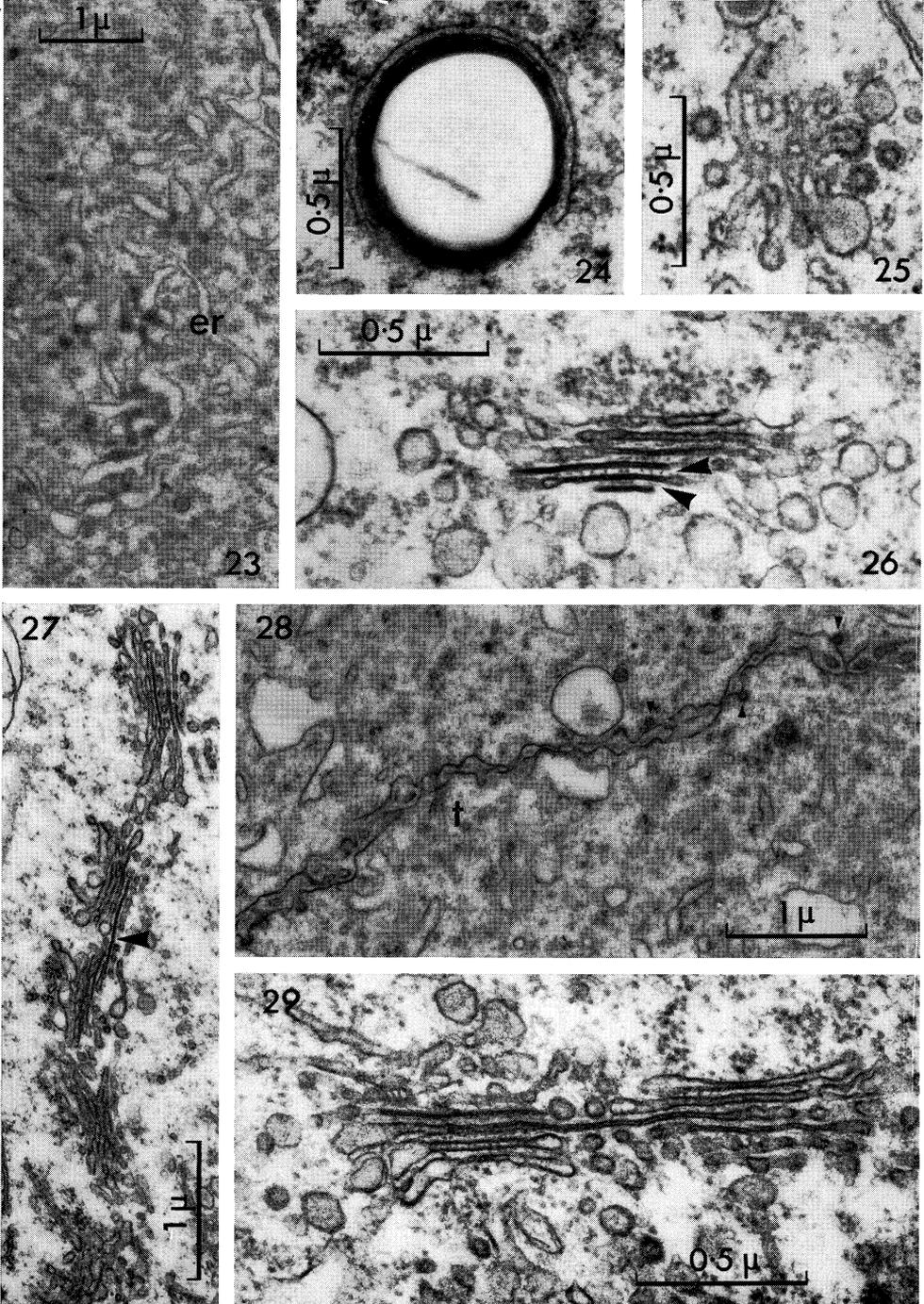
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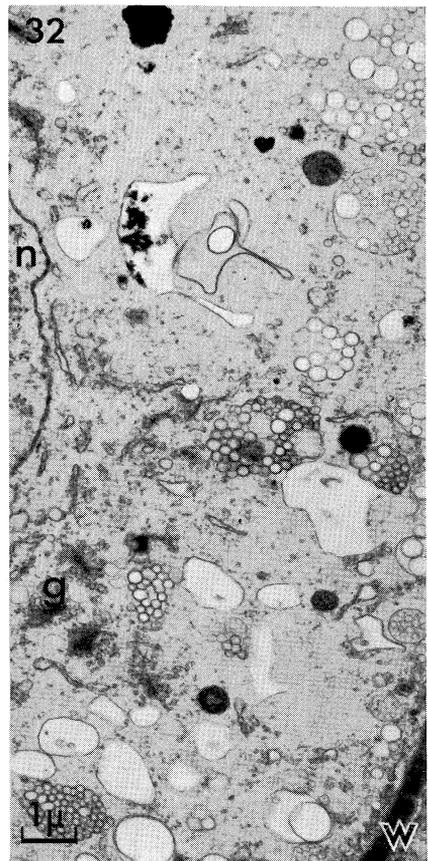
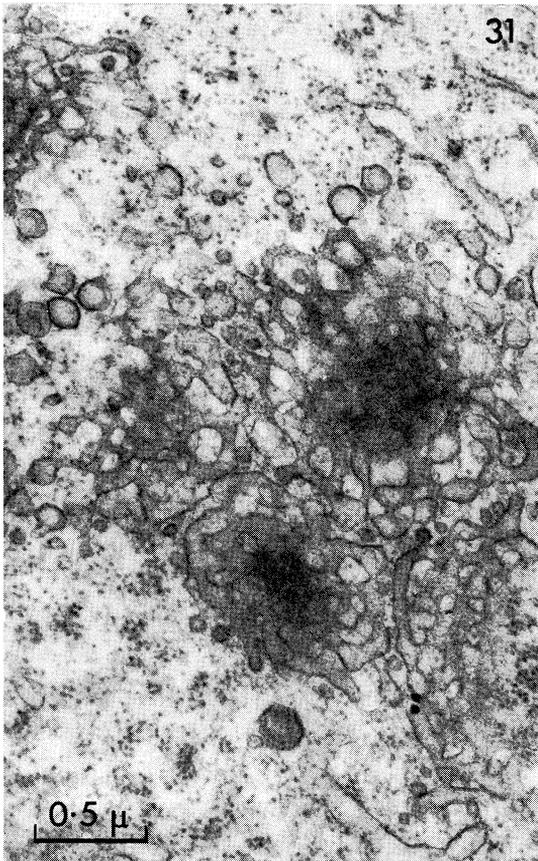
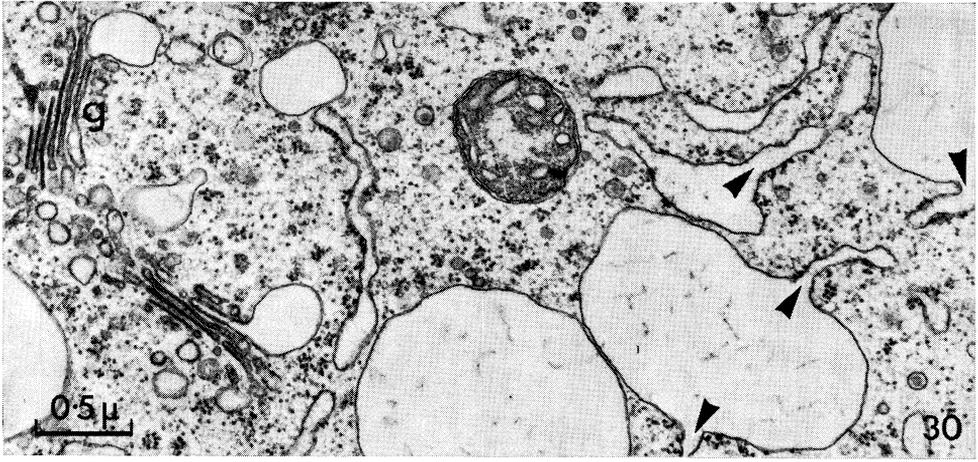
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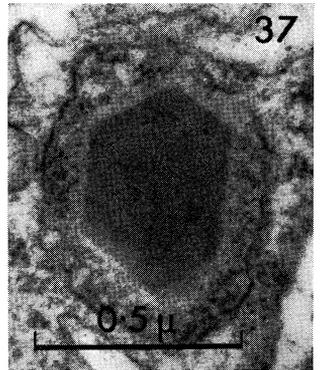
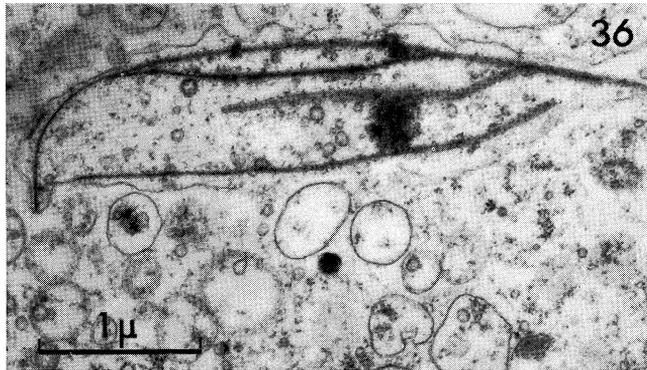
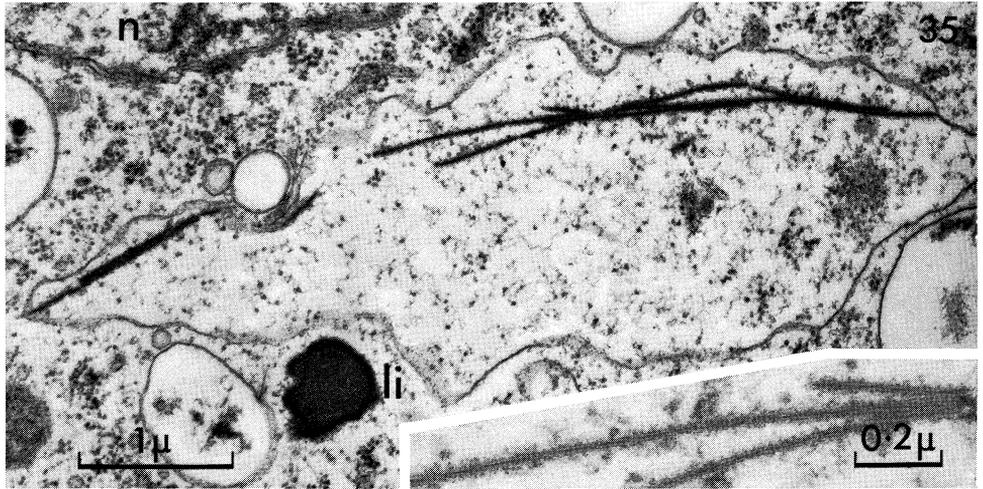
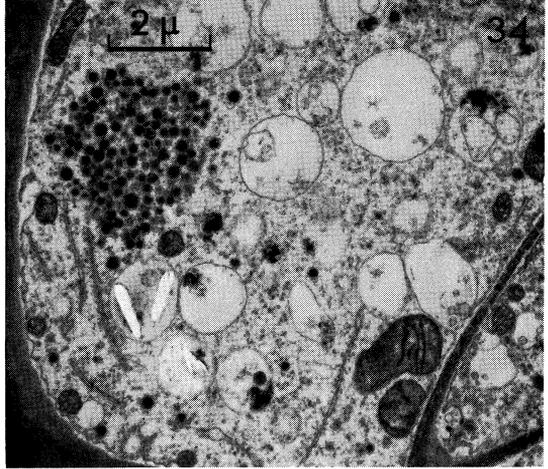
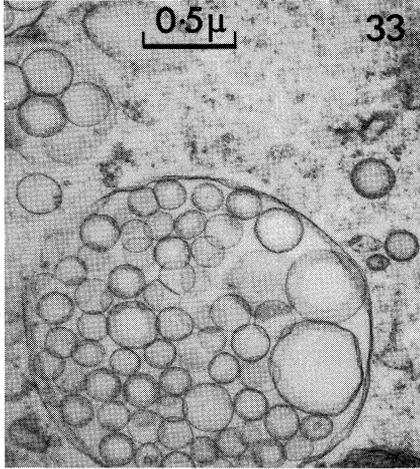
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## PLATE 8

- Fig. 30.—Golgi bodies apparently producing two types of vesicles, in vacuolating internode cell. Note the dilation of nearby elements of endoplasmic reticulum (arrows; cf. Fig. 18).
- Fig. 31.—Reticulate cisternae of several golgi bodies. It appears quite likely that these might be interconnected. Compare the pattern in the membranes with that shown in Figures 19 and 20.
- Fig. 32.—Longitudinal section of internode in young lateral. Occasionally, cells were seen which contained aggregates of vesicular components, giving the appearance of "froth" in the cytoplasm. Similar isolated vesicular components were sometimes associated with the golgi bodies (*g*).

## PLATE 9

- Fig. 33.—Detail from Figure 32, showing a collection of vesicles contained within a membrane; other similar isolated vesicles were present in the cytoplasm.
- Fig. 34.—Transverse section of lateral internode showing cluster of spherosome-like components. The densely staining contents occasionally exhibited crystalline profiles (see in Fig. 14). Other inclusions are typical, including the holes torn in the section probably due to a hard, crystalline substance (cf. Fig. 17).
- Fig. 35.—Densely staining lamellae (?) enclosed in a membrane-bounded inclusion in a transverse section of a young internode. Inset shows detail observed in ultra-thin serial section of this inclusion. Another typical heavily staining inclusion is also shown (*li*); it was probably lipoid in nature.
- Fig. 36.—As for Figure 35, this inclusion being found in a young antheridial cell.
- Fig. 37.—Inclusion in the form of a crystal, enclosed in a unit membrane.

