ULTRASTRUCTURE AND DIFFERENTIATION IN *CHARA* SP.

III.* FORMATION OF THE ANTHERIDIUM

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Summary

The formation of the antheridium from an enlarged nodal cell is described. After a basal cell has been cut off, precisely oriented mitoses divide the antheridial cell initially into octants; then follow two periclinal divisions in each octant. The outer layer of cells become the shield cells, the middle form the manubria, and the inner capitula cells divide further to produce secondary capitula and ultimately the spermatogenous threads. The shield cells become compartmentalized during enlargement by ingrowths in the wall which finally form a very distinctive pattern. Microtubules are associated with the regions of wall deposition. Plastids in the shields accumulate large numbers of globuli, and this is probably associated with the orange pigmentation they acquire. Large amounts of material seem to be secreted into the antheridia by the manubria; this material is formed within large vesicles which are apparently discharged through the plasmalemma. In the young capitula, a characteristic, highly organized grouping of many cytoplasmic microtubules forms a band which appears to wind through the cytoplasm; its significance and functions are obscure. Presumptive "spherosomes" are present in large numbers in these and spermatogenous cells. The spherosomes and lipid-like inclusions are very frequently coated with membranes of the endoplasmic reticulum.

I. INTRODUCTION

The author has already described some aspects of ultrastructure (Pickett-Heaps 1967*a*) and cell division (Pickett-Heaps 1967*b*) in *Chara*.‡ The Charales form beautiful and remarkably regular patterns of cell organization during growth and development. This is evident in the gross vegetative structure of the organism, but the alga's remarkable capability for complex but precise morphogenesis is perhaps even more obvious during sexual differentiation. The development of both oogonia and antheridia follows a very complex series of events after the division of a nodal cell situated in the laterals. Oogenesis will be described in a further paper.

The antheridia *in vivo* are beautiful objects, bright orange in colour. The outer shield consists of an interwoven layer of cells which form a very distinctive pattern; the shield encloses a mass of spermatogenous threads which are attached to central mother cells. At maturation, the shield opens out to form an intricate orange rosette, and the spermatozoids are released from the spermatogenous threads into the water. Some ultrastructural aspects of the developmental process that forms the antheridial structure will be described.

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‡ The *Chara* sp. used in this and the previous two papers in the series has now been identified as *C. fibrosa*.

II. Methods and Materials

These are exactly as those described previously (Pickett-Heaps 1967*a*, 1967*b*). In view of the importance of illumination in promoting growth and formation of reproductive organs in *Chara* (Karling 1924), the cultures were illuminated constantly by a 15-W fluorescent light. Formation of oogonia on the dioecious organism used was profuse but male plants were far less frequent.

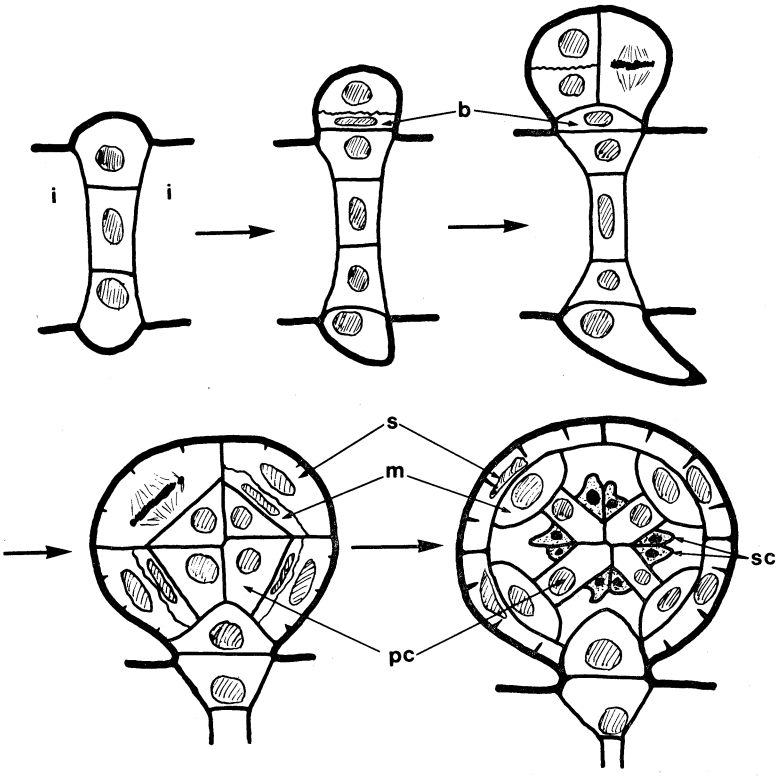

Fig. 1.—Diagrammatic representation of the formation of antheridium, *not* to scale. During further development, the secondary capitula (*sc*) give rise to spermatogenous threads, which eventually fill the antheridium as a tangled mass. Compare with Figures 2–5. The meaning of other abbreviations used in this figure, and in Figures 2–34 (pp. 263–74) are as follows: *ax*, axillary shoot; *b*, basal cell; *c*, chloroplast (plastid); *ch*, chromosome; *er*, endoplasmic reticulum; *f*, cytoplasmic fibrils; *g*, golgi bodies; *i*, internode; *n*, nucleus; *nd*, node; *m*, manubrium; *p*, polar zone (mitotic spindle); *pc*, primary capitulum; *ps*, polysomes; *s*, spermatogenous cell; *t*, microtubules; *w*, cell wall.

III. Observations

The sequence of events that lead up to the formation of the antheridial structure is shown diagramatically in Figure 1. The nodal cell in the lateral destined to form an antheridium became swollen and divided twice, forming a thin basal cell between the future antheridium and the node. In Figure 3, the younger antheridial cell is unusual,

in that it appears to have cut off two basal cells. The much larger, outer cell divided twice longitudinally and then once laterally, to produce eight octants. Each octant underwent a further two periclinal mitoses (Figs. 2 and 3). The three resultant cells in each octant then differentiated to form three totally different components of the antheridium (Figs. 3–5). A continuous and considerable increase in size accompanied all these changes.

At the ultrastructural level, these mitoses were all examined in great detail (Fig. 6). The ultrastructure of dividing cells was comparable in every way to that described previously (Figs. 6 and 7; cf. Pickett-Heaps 1967*b*). The remarkable precision of the mitoses was always apparent. In particular, the metaphase plate (Figs. 6 and 7) was exactly aligned in the plane of the future cell wall. At no stage, however, was any distribution of the cytoplasmic organelles discerned before division that could be significantly related to the future plane of division. Rather, a generalized cytoplasmic asymmetry appeared to be gradually built up as the nucleus went into prophase, the nucleus itself occupying the appropriate region of the cytoplasm.

(*a*) *Development of the Shield Cells*

Following the second periclinal division, the outer shield cells grew steadily with the increasing size of the antheridium. Soon after the final division, the nucleus enlarged and intranuclear microtubules appeared (Fig. 15; cf. Pickett-Heaps 1967*a*). The cells also vacuolated rapidly as they enlarged; plastids moved during growth of the shield to the inner walls of the cells (Figs. 7–10).

At this stage, a striking differentiation of the cell wall occurred, described by Fritsch (1935) as "a characteristic infolding of their (i.e. *the cells*) membranes". At regularly spaced intervals, cell-wall material was deposited to form evaginations of the wall into the cytoplasm (Fig. 8). As growth proceeded, these ingrowths compartmentalized the cells in a complex but regular pattern (Figs. 9 and 10; cf. Figs. 2, 4, and 5). The overall pattern of the growth led to the final beautiful structure of the shield cells of older antheridia. At all stages, microtubules were associated with these ingrowths, as well as the older wall (Fig. 11). The disposition of the microtubules was complex; they were not always parallel to one another (Fig. 14) or to the nearby microfibrils of the wall (Fig. 16). Figure 14 perhaps shows a cytoplasmic microtubule in the process of being laid down near two others. Such a deduction would be far-fetched, except that a similar phenomenon is not uncommon in young wheat xylem cells (e.g. see Fig. 15, Pickett-Heaps and Northcote 1966*c*), when microtubule synthesis and redistribution must also occur. The association in many ways resembled that observed with xylem wall thickenings; however, the wall pattern being a complex one in three dimensions, it was difficult to decide whether a specific grouping of microtubules preceded wall differentiation, as is the case with wheat xylem cells (Pickett-Heaps 1967*c*, 1967*d*).

Globuli were present in most plastids of *Chara*. However, the number present in plastids of shield cells increased markedly during further development (Figs. 12 and 13). The appearance of these globules was probably related to the strong orange colour, characteristic of maturing antheridia. When fully developed the intricately compartmentalized cells were very highly vacuolate, containing only a very thin

peripheral layer of cytoplasm, and chloroplasts were confined to the inner walls of the cells.

(*b*) *Manubrium*

The manubrium initially was a very thin cell, sandwiched between the shield and the capitulum cells (Figs. 3, 8, and 23). Very soon after formation they apparently commenced to secrete large amounts of material through their plasmalemma. Initially, when there was little extracellular space inside the antheridium, the vesicles collected at the corners of the cells (Fig. 17). There was less tendency for this to happen during later development (Fig. 19). In older antheridia, the internal components were immersed in a diffuse material. It is suspected that this was a mucilaginous material and the micrographs suggest that it was secreted into the extracellular space inside the antheridia via vesicles produced within the manubria (Figs. 17, 19, and 21). The origin of the vesicles was not absolutely clear; however, they appear to have been formed from small golgi vesicles which had become very swollen before being discharged through the plasmalemma (Fig. 18). In these, as in all other cells of *Chara*, "coated" vesicles were abundant. It appeared that they were produced from tubular (golgi) components and were subsequently discharged into the cell wall (Fig. 22; see Pickett-Heaps 1967*a*). The reverse sequence of events, however, cannot be ruled out.

In most of the component cells of the young antheridia, many plastids were apparently dividing by constriction. At the point of constriction there was often a ring of darker-staining material. An example of this is shown in Figure 20, which was taken from a young manubrium cell. Similar observations were also frequently made in the young developing oogonia.

(*c*) *Capitula*

The primary capitula underwent a series of divisions during further growth of the antheridia, forming secondary capitula (Figs. 3–5). The actual number of the secondary capitula produced was impossible to decide from thin sections [Fritsch (1935) and Karling (1927) say about six are usual.] The spermatogenous threads arise from these secondary capitula by a further large number of mitoses (Fig. 5).

The cytoplasm of many (not all) primary capitulum cells were found to contain a large number of closely packed microtubules, oriented longitudinally into a band which apparently wound through the cell (Figs. 23, 24*A*–24*D*). The author has found an identical phenomenon in the young developing oogonia, but nowhere else in the plant. Following the discovery of this characteristic band of microtubules in many specimens, the author "lost" them during a subsequent winter period. However, antheridia and oogonia appearing the following spring and summer, were found to contain identical microtubule bands again. It appears therefore, that these bands are found only in antheridia and oogonia which are rapidly growing. In oogonia (Pickett-Heaps, unpublished data), the grouping appeared early in cell development and remained for some time as the oogonial cell enlarged. However, in capitula, they seemed to appear and disappear more quickly; they were never found in dividing capitular cells but were apparently present in daughter cells following the first one

or two mitoses that form the secondary capitula. After this stage the bands were not seen again. The organization of the microtubules seen when the band was cut transversely was very characteristic (Figs. 24*C*, 24*D*, and 26), and the band was often quite long (Fig. 25). The way this band was formed and dispersed has not so far been discovered and its significance is not understood.

In other respects, the cells and all cytoplasmic components of the capitulum appeared quite normal (cf. Pickett-Heaps 1967*a*). During mitoses, the metaphase plate of chromosomes as usual was precisely oriented across the cell with perpendicular spindle microtubules extending up into the polar zones. Pronounced "polarization" (i.e. unequal distribution of cytoplasmic organelles) accompanied and consequently resulted from these divisions (Figs. 4 and 27). Clear "vacuolar" components collected at one end of the cell and the other (the spermatogenous end) became very osmiophilic and densely staining (Fig. 27).

In a previous paper (Pickett-Heaps 1967*a*) the author has described an intimate association between elements of the endoplasmic reticulum and several different types of inclusions. The inclusions could be enveloped to varying extents by the membranes (Figs. 29–31), and this association was very frequent in the primary and secondary capitular cells, and young cells of the spermatogenous threads (Figs. 28–34). Two distinct forms of the larger inclusions were usual. The first were irregularly shaped osmiophilic objects of varying size (Fig. 32) and the second were smaller with a circular or oval profile, probably spherosomes (Figs. 29–31). These two inclusions (particularly the latter) became very frequent in the cytoplasm of young spermatogenous cells. A limiting membrane around the inclusion itself was never obvious, although a layer of some sort was seen at the surface of the inclusions (Fig. 31).

An association of membranes (probably endoplasmic reticulum) and smaller vesicular components was found in almost all cells of the capitula and young spermatogenous threads (Figs. 28, 33, and 34). This often resulted in quite complex inclusions being formed, but little can be surmised as to their function or significance. One often gained the impression that the vesicles enclosed might have been derived from the golgi bodies (see Fig. 33) but this is only conjecture at the moment.

IV. Discussion

The cytologist cannot help being impressed at the precision which *Chara* displays in morphogenesis. Yet at the ultrastructural level, there is only little to indicate how morphogenesis might be controlled by cell organelles. The author has already given at some length reasons for implicating microtubules in several important aspects of morphogenesis in plant cells; they appear significant not only as structural components of the mitotic spindle (Ledbetter and Porter 1963; Esau and Gill 1965; Harris and Bajer 1965; Pickett-Heaps and Northcote 1966*a*, 1966*b*), but also in predetermining the plane of division of wheat cells (Pickett-Heaps and Northcote 1966*a*, 1966*b*). These organelles are also found near cell walls (Ledbetter and Porter 1963 and others), and a commonly reiterated hypothesis suggests, without much supporting evidence, that they are involved in microfibril deposition. Newcomb and Bonnett (1965) showed that they were associated with asymmetric wall growth in root hairs (and not necessarily the orientation of wall microfibrils). Their observa-

tions are apparently similar to those in some cells of *Chara* (e.g. Fig. 16) where again wall microtubules are not necessarily oriented parallel to wall microfibrils. Recent experiments observing the effects of colchicine on xylem wall deposition suggest quite strongly that the organelles are involved in the spatial control of wall synthesis (Pickett-Heaps 1967*d*). More recently still, Preston (1967) has described the presence of microtubules near the cell surface of naked protoplasts (*Cladophora* swarmers) up to the time that a cell wall is deposited, but not thereafter; he therefore also concludes that microtubules are not involved in cellulose deposition. In animal cells, there is a growing volume of literature which suggests that the organelles are involved in formation and maintenance of cell asymmetry, morphogenesis, and movement within the cytoplasm (e.g. see Porter 1966) among other possible functions.

In *Chara*, their relationship to cell walls and their involvement in the structure of the spindle has been described (Pickett-Heaps 1967*a*, 1967*b*). No evidence has yet been obtained that positively associated them with the predetermination of the plane of cell division. Polarization, or asymmetric organization of the cytoplasm, preceded most of the divisions that form the antheridia and the metaphase plate was always precisely aligned parallel to the future cell wall (Figs. 6 and 7). In the formation of the secondary capitula, the mitotic spindle occupied one considerably more osmiophilic end of the cell and most of the small vacuolar inclusions were seen at the other (Fig. 27). In the shield cells of the antheridia, the constant association of microtubules with the ingrowths in the cell wall (Fig. 11) reminds one of the similar situation seen with xylem wall formation in higher plants (Hepler and Newcomb 1964; Wooding and Northcote 1964; Cronshaw and Bouck 1965; Pickett-Heaps and Northcote 1966*c*). Thus, by analogy with the results reported previously (Pickett-Heaps 1967*d*) they might be involved in control over the sites of wall formation, and ultimately therefore the regular pattern of the shield cells. Preliminary experiments on growing antheridia indicate as expected that the patterns of wall growth cannot develop in the presence of colchicine.

The occurrence of bands of highly organized microtubules in the primary and some secondary capitula cells (Figs. 23–26) was entirely unexpected and as yet no satisfying hypothesis as to the import of this observation can be offered. However, it is almost certainly highly significant that an apparently identical phenomenon has been encountered only in the growing oogonium cell. The author has found only two reports of somewhat similar structures, in insect nuclei (Smith and Smith 1965) and in some insect blood cells (Hoffman 1966), and would be interested to hear of any similar observations in plant cells.

The secretion of material into the interior of the antheridium by the manubria seems somewhat similar to that frequently described in root cap cells (Mollenhauer, Whaley, and Leech 1961; Northcote and Pickett-Heaps 1966). The source of the vesicles is not clear, but they were probably derived from the golgi bodies and became swollen before being discharged through the plasmalemma. Coated vesicles were also frequently seen in similar circumstances, as in all other cells of *Chara* (Pickett-Heaps 1967*a*).

The invariable finding of an association of elements of the endoplasmic reticulum with many of the lipoid or spherosome-like inclusions (Figs. 29–32) or both

suggests that some aspect of their formation or functions is influenced by the membranes. Similar spatial juxtapositioning of the endoplasmic reticulum and cell organelles is not uncommon in plant cells. For example, Wooding and Northcote (1965) have described an intimate, probably functional, association of the endoplasmic reticulum and plastids in *Pinus* and *Acer*. Bouck's (1965) work on brown algae, suggests that the endoplasmic reticulum, because of its close apposition to the pyrenoid and chloroplast could serve a transport and perhaps storage function. Mollenhauer, Morré, and Kelley (1966) described "single-membrane-bounded organelles characterized by a physical association with the endoplasmic reticulum" in a wide variety of cell types (including algae). However, the organelles, called cytosomes, were not associated with rough (i.e. ribosome-coated) regions of the endoplasmic reticulum (Mollenhauer, Morré, and Kelley 1966, p. 46), and this association often survived tissue homogenization and centrifugation. In *Chara*, the author considers the term "spherosome" more appropriate to the spherical or ovoid inclusions (compare Figs. 29–31 with the micrographs published by Sorokin and Sorokin 1966); these were consistently enveloped by rough endoplasmic reticulum at the time when one suspects their number to be rapidly increasing. Sorokin and Sorokin (1966), however, state that the spherosomes described in their paper were limited by a membrane; in *Chara* a membrane was never obvious although some limiting layer was present (Fig. 31). Whether these presumptive spherosomes were derived originally from blebs of the endoplasmic reticulum (cf. Frey-Wyssling, Grieshaber, and Mühlethaler 1963; Matile *et al.* 1965) has not been established.

The presence of some convoluted membranes (probably the endoplasmic reticulum) enclosing vesicular objects (Figs. 28, 33, and 34) is almost invariable in older capitula and spermatogenous cells. No explanation of this phenomenon can yet be offered. Likewise, the frequent appearance of fibrous components near the mitotic spindle (generally at the poles, e.g. Fig. 31) is unexplained (cf. Pickett-Heaps 1967*b*).

The existence of "globuli" in the chloroplasts of vegetative *Chara* has already been described (Pickett-Heaps 1967*a*) and this phenomenon is frequently encountered in micrographs of many other plant chloroplasts, including algae (Bouck 1965). The antheridia of *Chara* take on a very strong orange colour during growth; at maturation, the chloroplasts of the opened antheridia *in vivo* are bright orange. The development of this pigmentation is probably associated with the accumulation of globuli in the chloroplasts (Figs. 12 and 13).

V. Acknowledgment

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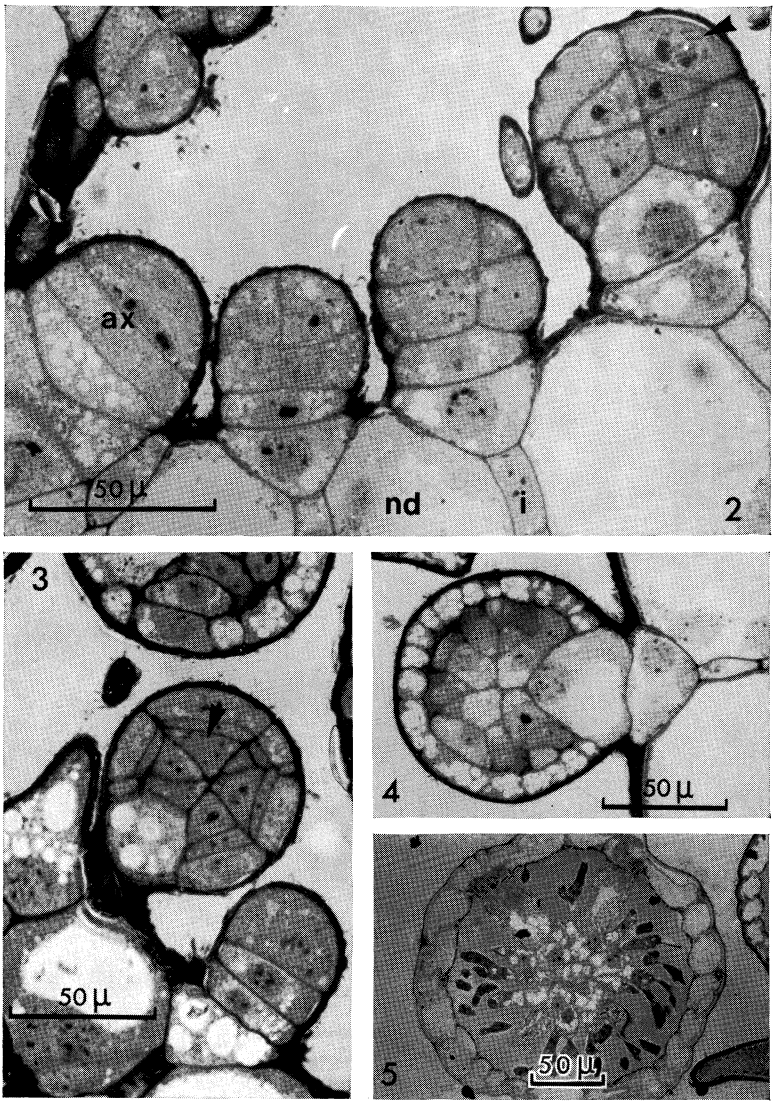


Figs. 2–5.—Light micrographs of thick Araldite sections, stained with toluidine blue: *2*, typical sequence of very young antheridia, growing at internodes (*i*) of laterals; an axillary shoot (*ax*) is also present adjacent to the stem of the plant. An outer cell (arrow) is at early prophase. *3*, Youngest antheridium here is unusual in that it appears to have cut off two basal cells; the nucleus is in prometaphase. The other antheridium is forming secondary capitula—one cell (arrow) is at late anaphase. *4*, Formation of secondary capitula has commenced—notice the polarization of the primary capitula (cf. Fig. 27). Compartmentalization of outer shield cells is well under way, and the interior space of antheridium is filled with a staining material (mucilage?). *5*, Formation of spermatogenous threads from secondary capitula has been initiated. Chloroplasts have moved to the inner edge of the shield cells (cf. Fig. 10); these outer cells are very difficult to embed properly when older and often collapse on sectioning.

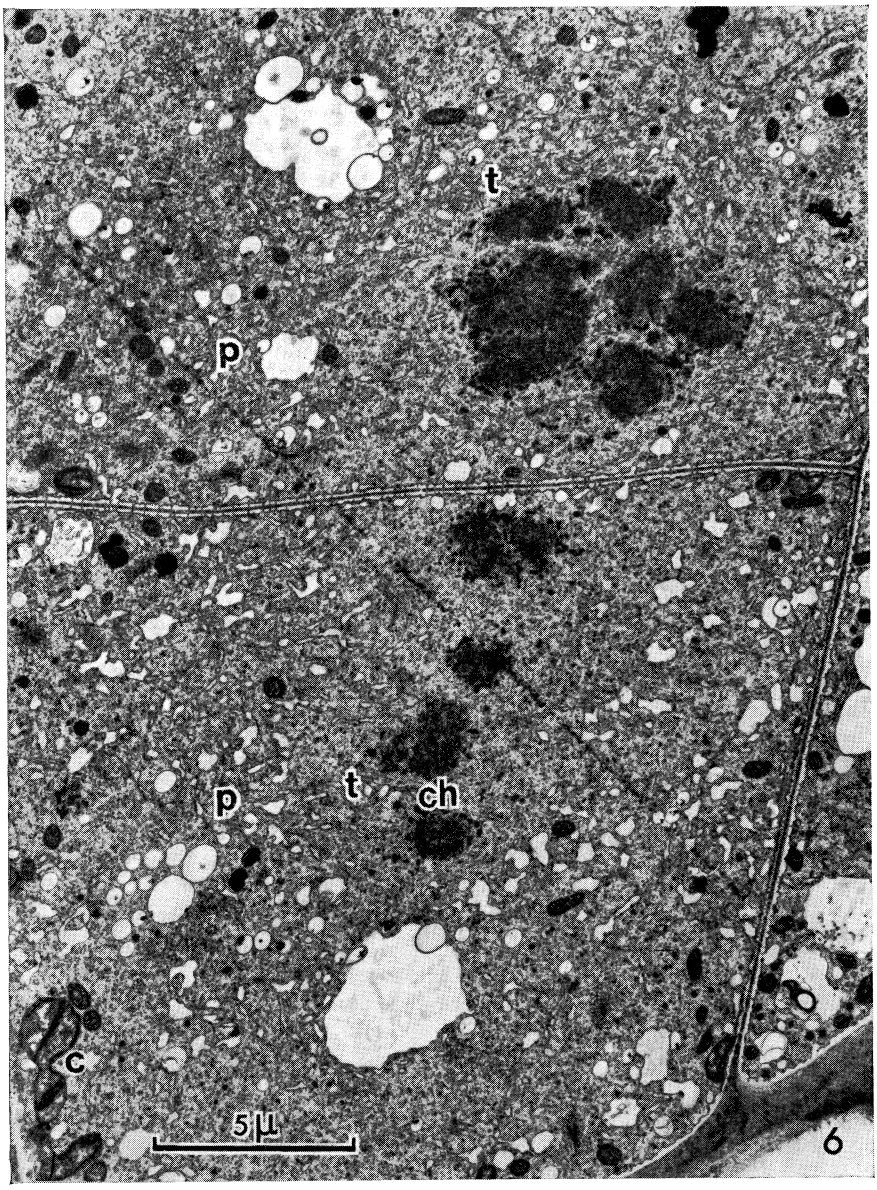


Fig. 6.—Prophase and metaphase, transverse division in antheridium. Polar regions (*p*) show typical concentration of endoplasmic reticulum, with spindle microtubules (*t*) penetrating into it. Chromosomes are coated with presumed nucleolar material (cf. Pickett-Heaps 1967*b*).

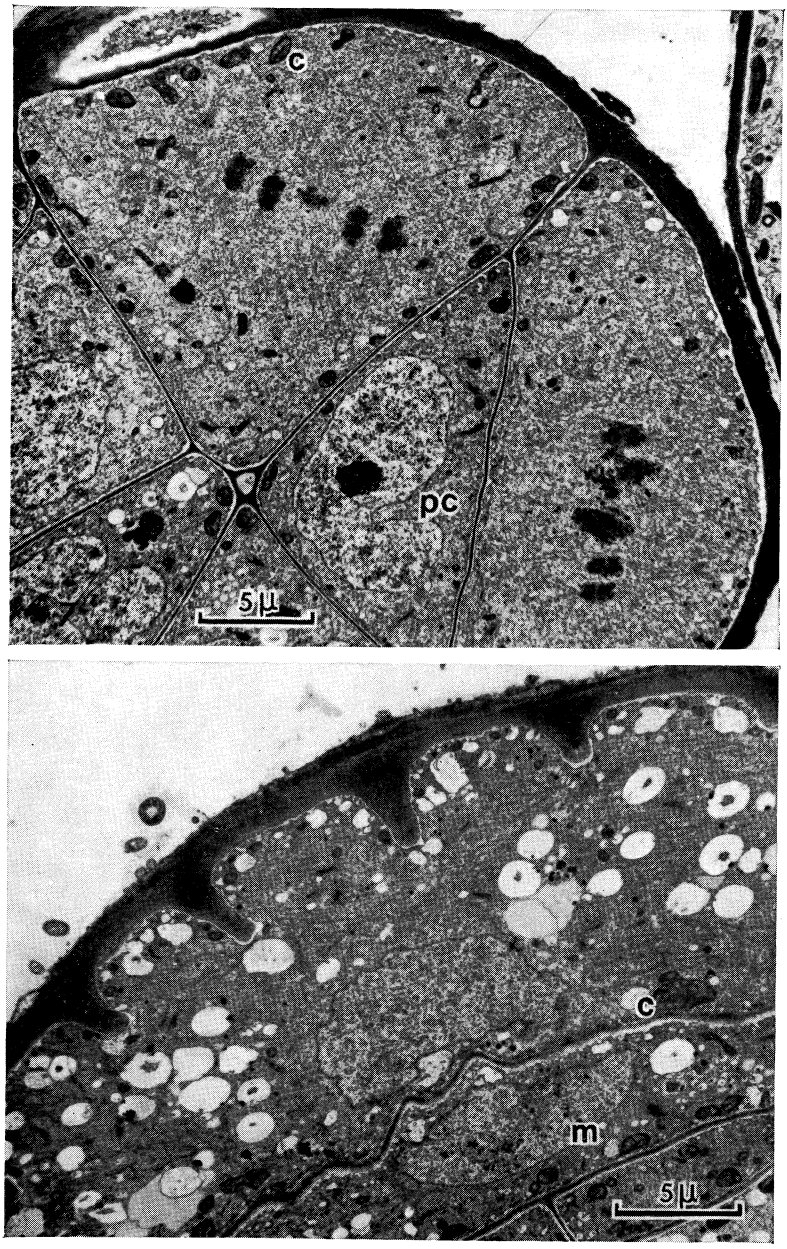


Fig. 7.—Two precisely aligned metaphase plates. The top division is forming the primary capitula (*pc*); the division on the right-hand side is forming the manubrium and outer shield cell. Chloroplasts (*c*) in the latter are found around most of the wall at this stage.

Fig. 8.—Differentiation of the outer shield cell after formation of the thin manubrium (*m*). Chloroplasts (*c*) move inwards. Ingrowths in the wall begin to divide the cell. Some micrographs show these as regularly spaced protuberances, but they form a complex pattern in surface view (cf. Fig. 9).

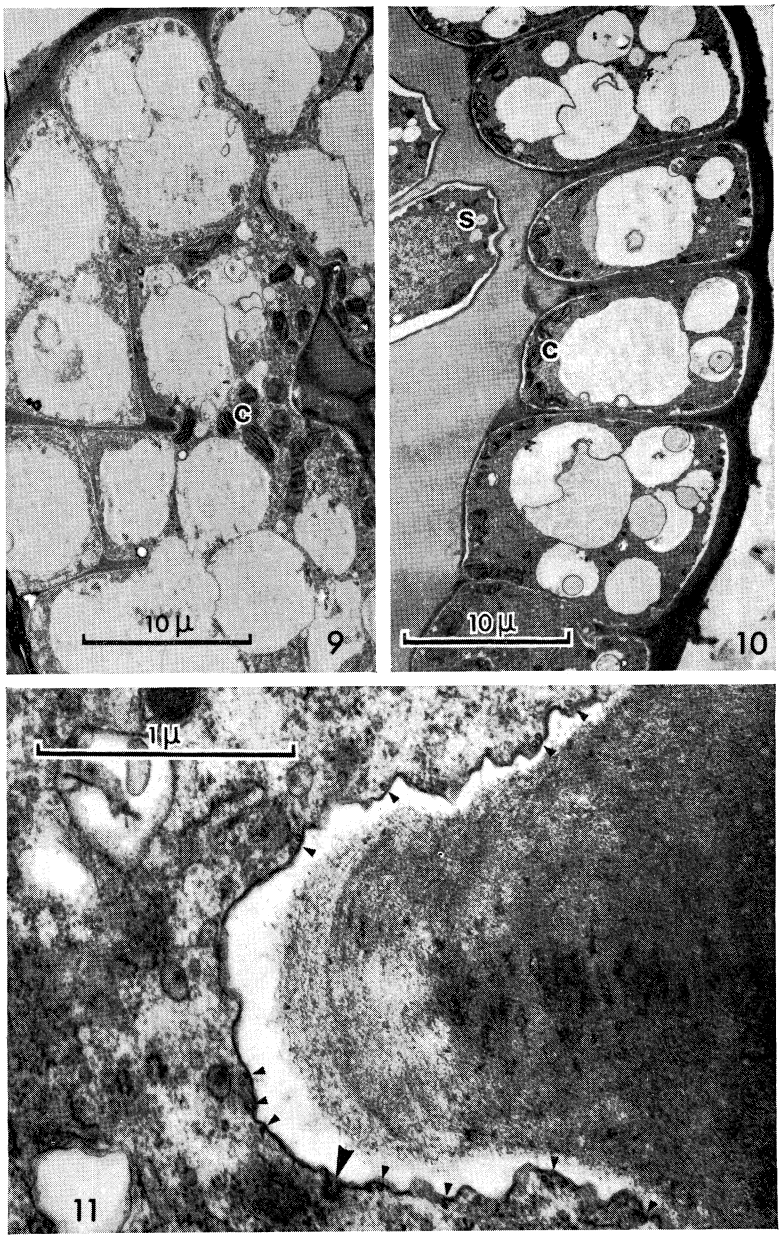


Fig. 9.—Tangential section. A young shield cell, with chloroplasts (*c*) concentrated at the inner surfaces. This vacuolating cell shows the complexity of the wall pattern being developed by the ingrowths.

Fig. 10.—Older shield cell; section shows the compartmentalization developed. Portions of two very young spermatogenous cells are visible, surrounded by a diffuse (mucilaginous?) matrix material. Chloroplasts have moved to inside edge of the cell.

Fig. 11.—Part of Figure 8 at higher magnification. Microtubules are seen above the wall ingrowth (small arrows). A "coated" vesicle (large arrow) appears to be discharging its contents into the wall (cf. Fig. 22).

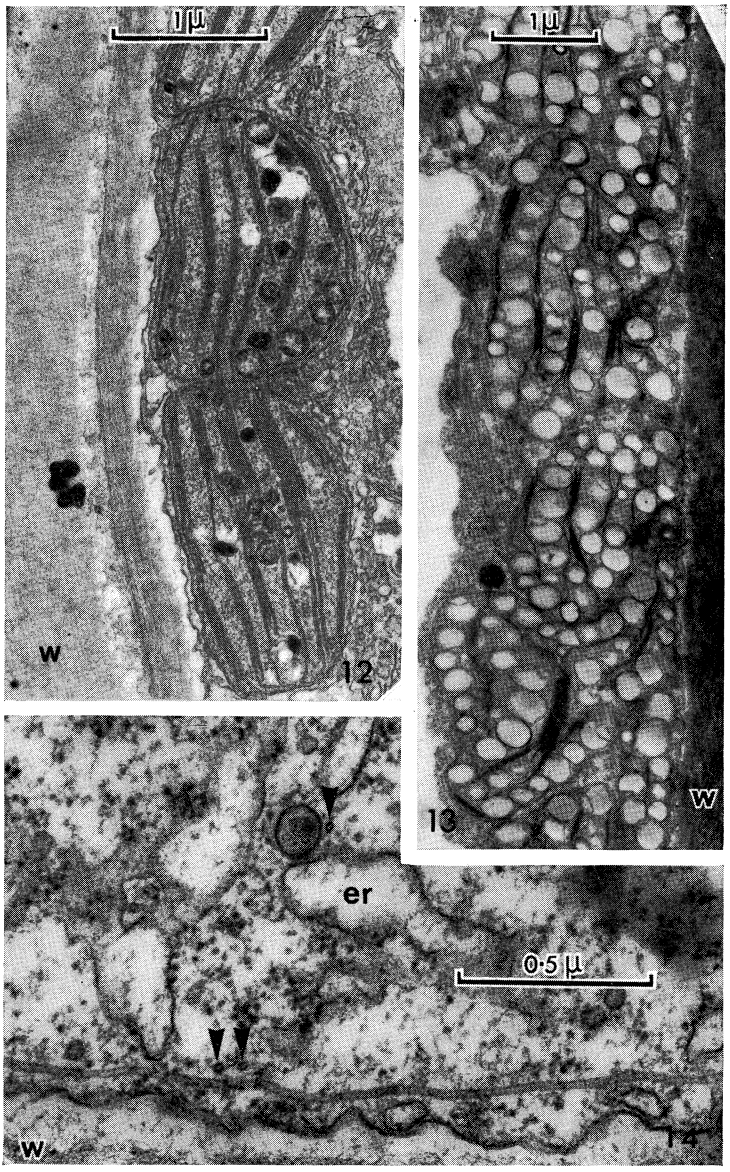


Fig. 12.—Chloroplasts of a *young* shield cell. A few globuli are present in these, as in most chloroplasts in other cells of *Chara*.

Fig. 13.—Chloroplasts in a *much older* shield cell; the number of globuli present has increased considerably. The chloroplasts of the shield cells become coloured bright orange *in vivo*.

Fig. 14.—Very young shield cell; some wall microtubules (double arrows) are perpendicular to others. Further in the cytoplasm, another tubule (single arrow) has the same orientation (in this section at least) as the previous two, suggesting the possibility that it is in the process of being laid down.

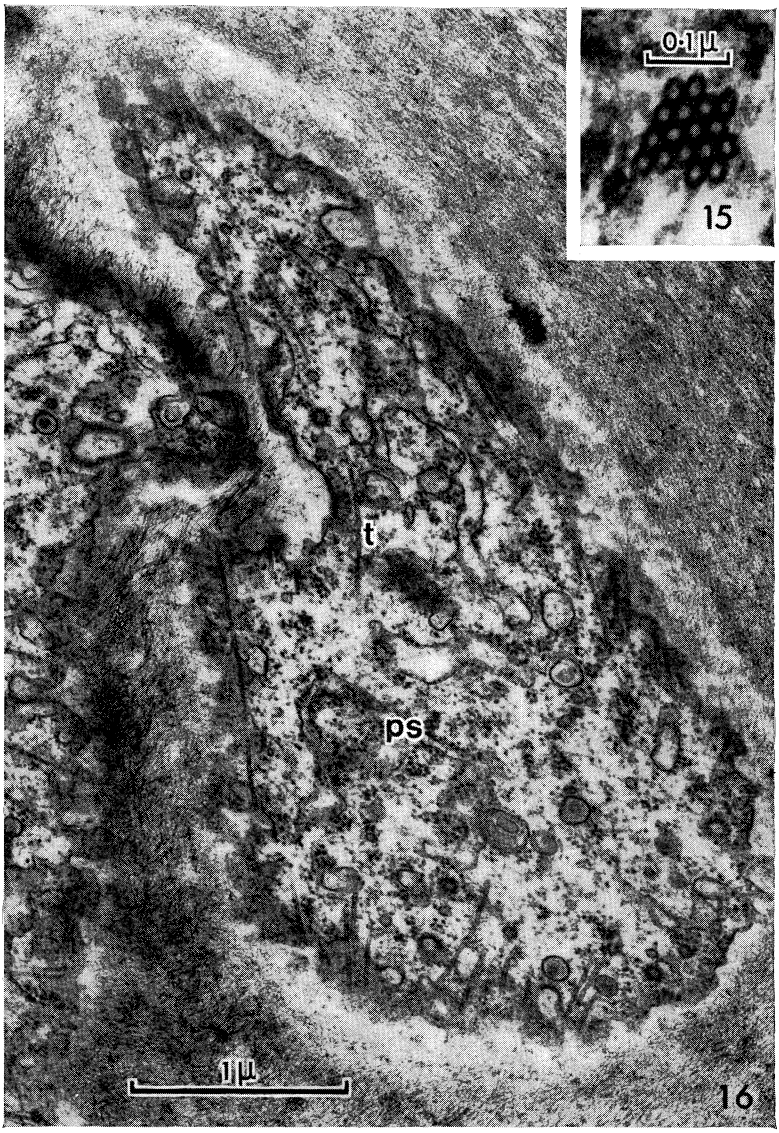


Fig. 15.—The close-packed intranuclear microtubules found in the greatly enlarged nucleus of an older shield cell (cf. Pickett-Heaps 1967*a*).

Fig. 16.—Section tangential to a very young shield cell. Microtubules are not all parallel (cf. Fig. 14). Orientation of wall microfibrils immediately under the plasmalemma is complex, and often not parallel to the wall microtubules.

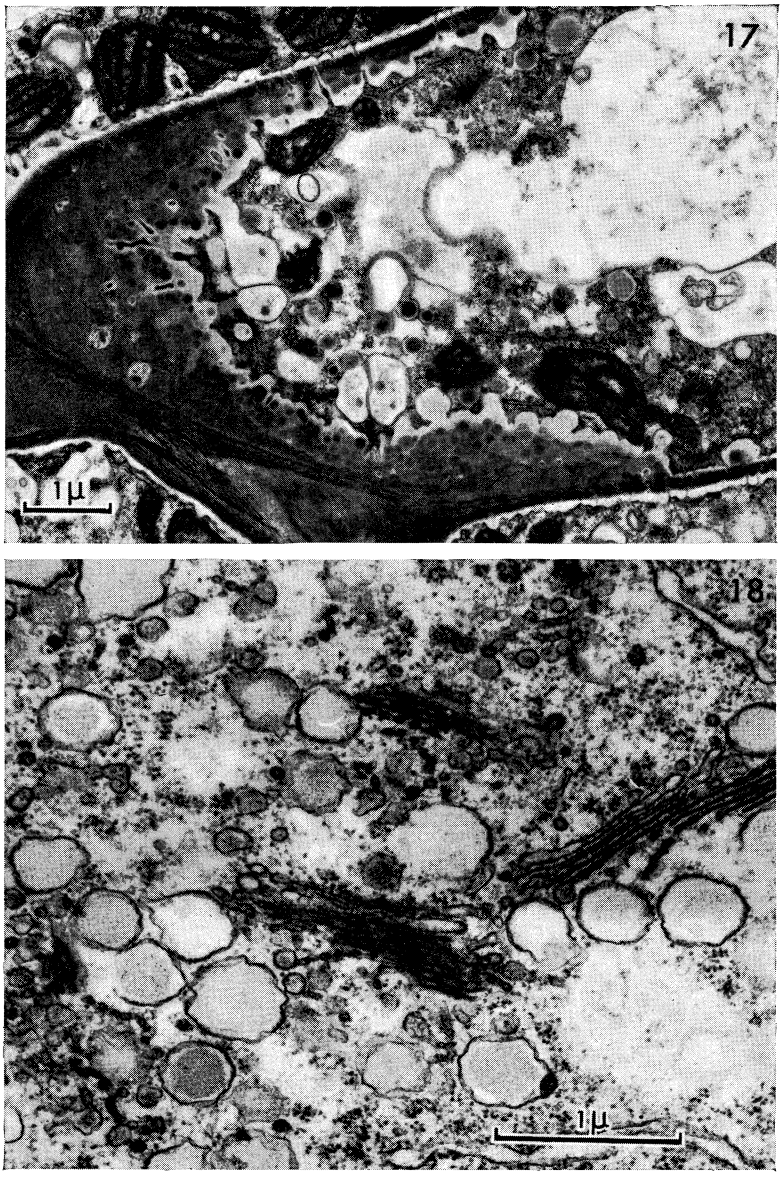


Fig. 17.—Corner of young, thin manubrium cell. Accumulation of vesicular components under the plasmalemma is evident. At this stage of development, there is virtually no extracellular space inside the antheridium.

Fig. 18.—Golgi bodies in a young manubrium cell. The large vesicles evidentally discharged through the plasmalemma (cf. Figs. 17 and 19) are not derived from the golgi bodies directly; it appears as if the smaller vesicles swell considerably before being secreted.

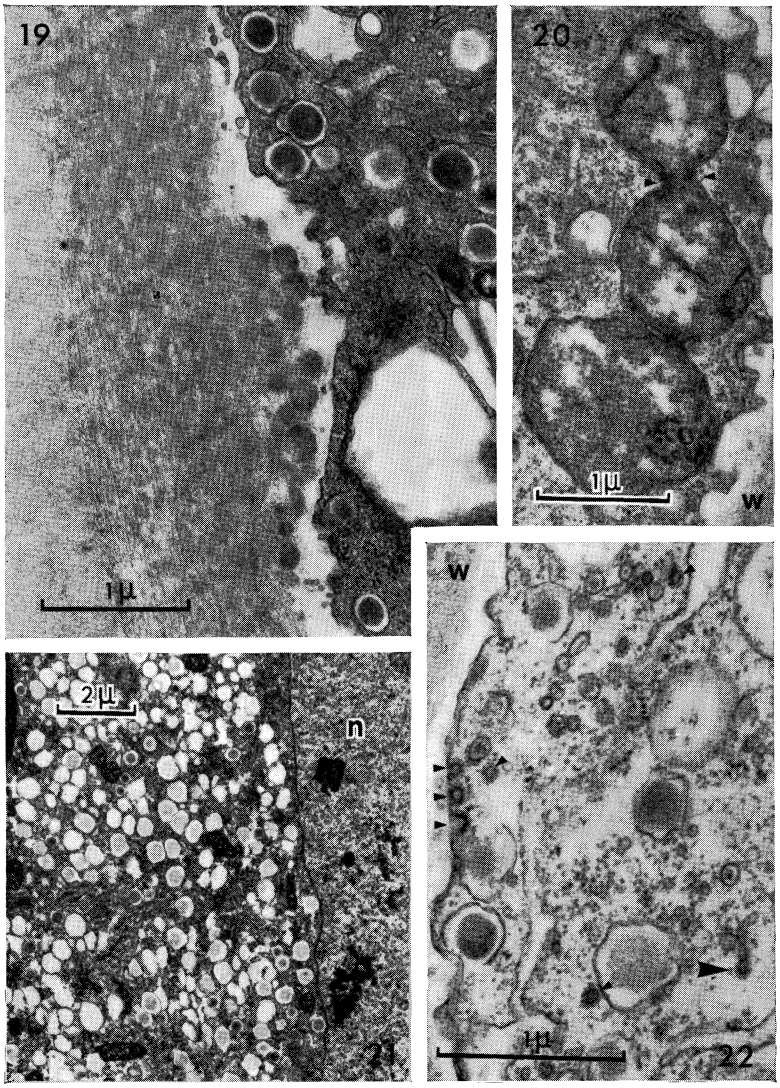
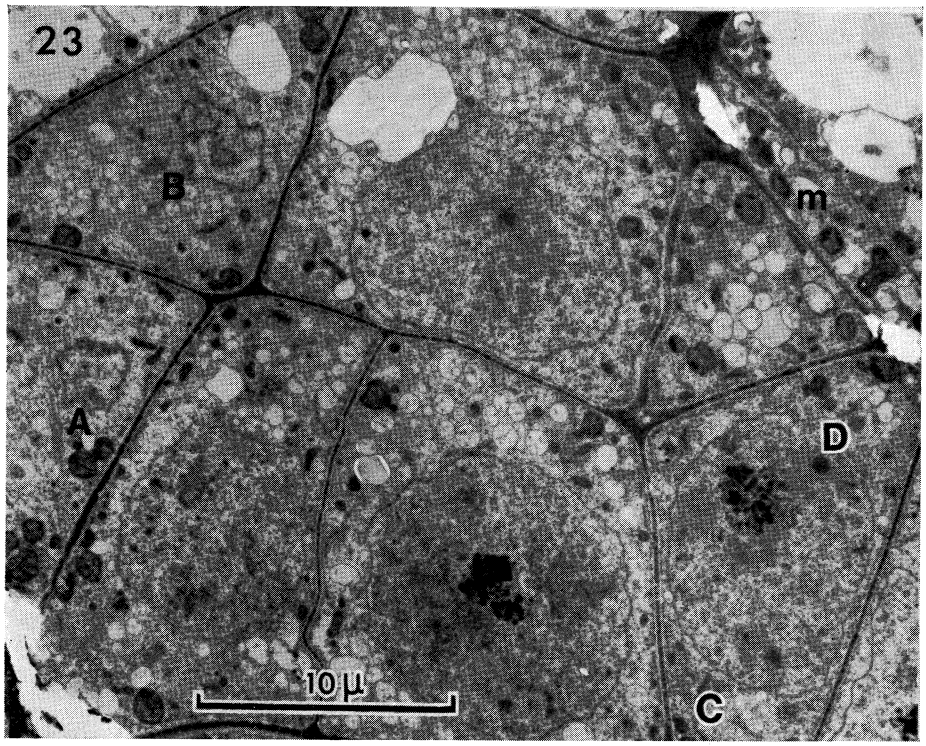


Fig. 19.—Older manubrium. Extracellular space inside the antheridium is filled with diffuse material apparently derived from vesicles present in the manubria. Note the discontinuity between the cell wall and diffuse contents of the antheridium.

Fig. 20.—Plastids dividing by constriction (in this case, in the manubrium) often show a dark-staining "collar" (arrows) at the region of constriction.

Fig. 21.—Cytoplasm of a greatly enlarged manubrium cell (i.e. as in Fig. 5). The nucleus has enlarged enormously, and contains packed microtubules (cf. Fig. 15). The cytoplasm contains numerous vesicles (cf. Figs. 17–19) apparently destined for discharge through the plasmalemma.

Fig. 22.—The manubrium also contains many coated vesicles, some continuous with the plasmalemma (arrows) and others occasionally continuous with tubular (Golgi?) membranes (large arrow). This is typical of all other cells of *Chara*.



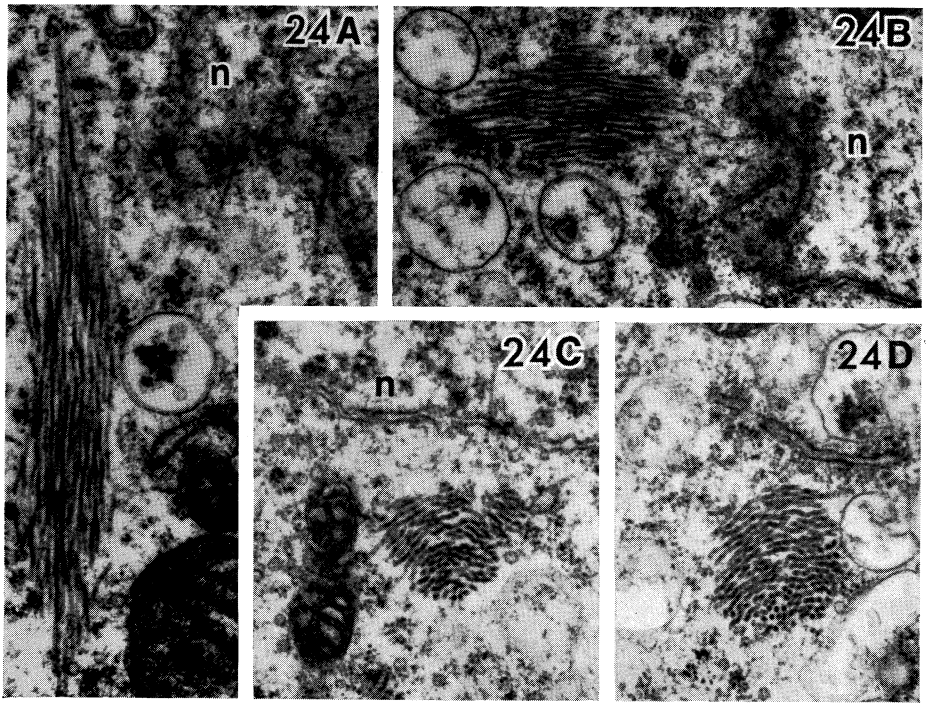


Fig. 23.—Group of primary and secondary capitula cells inside a young antheridium (note the thin manubrium, *m*, at this stage). Many of the capitula cells at these early stages of development contain microtubules packed in a highly characteristic band, generally close to the nucleus.

Figs. 24*A*–24*D*.—Sections of such bands visible at *A*, *B*, *C*, and *D* (Fig. 23) are shown respectively at higher magnification.

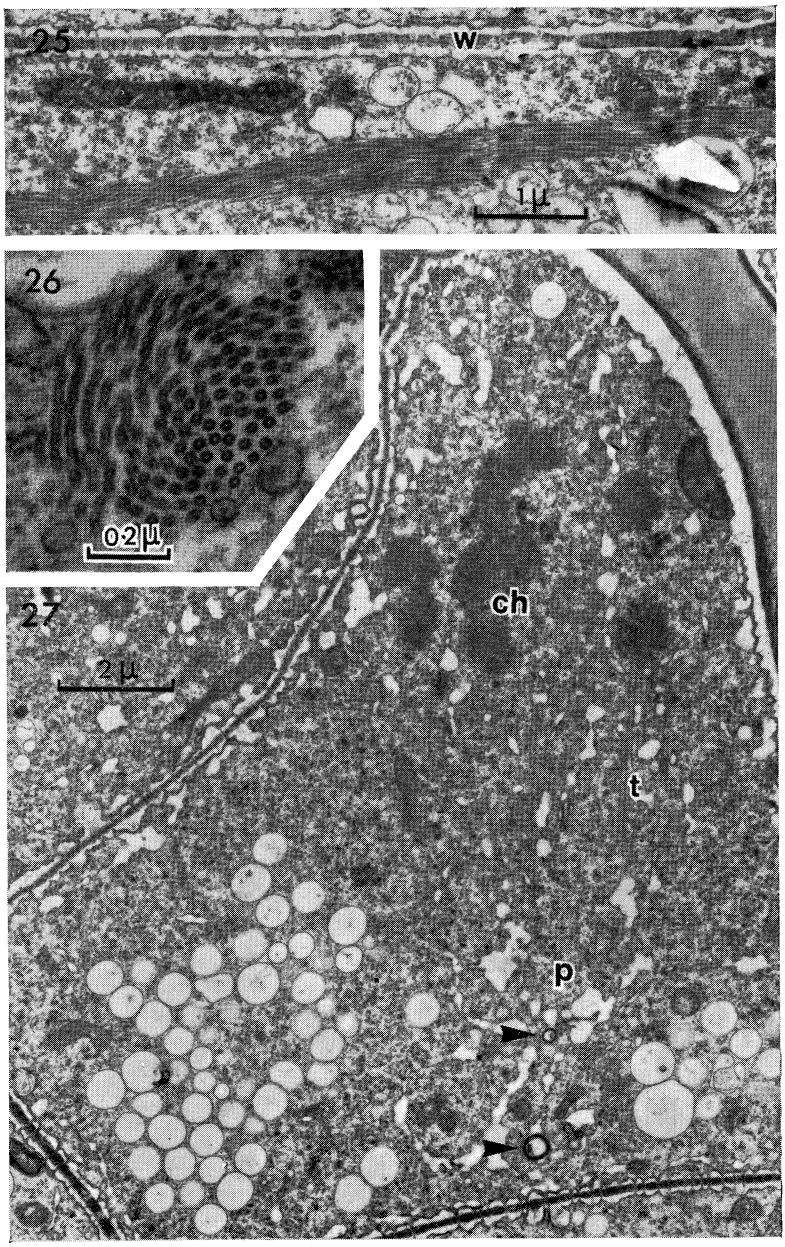


Fig. 25.—As for Figure 23, showing the length of this band often seen in sections.

Fig. 26.—Transverse section of the band at high magnification. This spatial organization of the tubules is very characteristic.

Fig. 27.—Highly polarized anaphase in the formation of a secondary capitulum cell. Polar zone (*p*) contains typical inclusions (arrows) seen at higher magnification in Figure 28. Note the asymmetric concentration of small vacuolar bodies (visible also in Figs. 4 and 5).

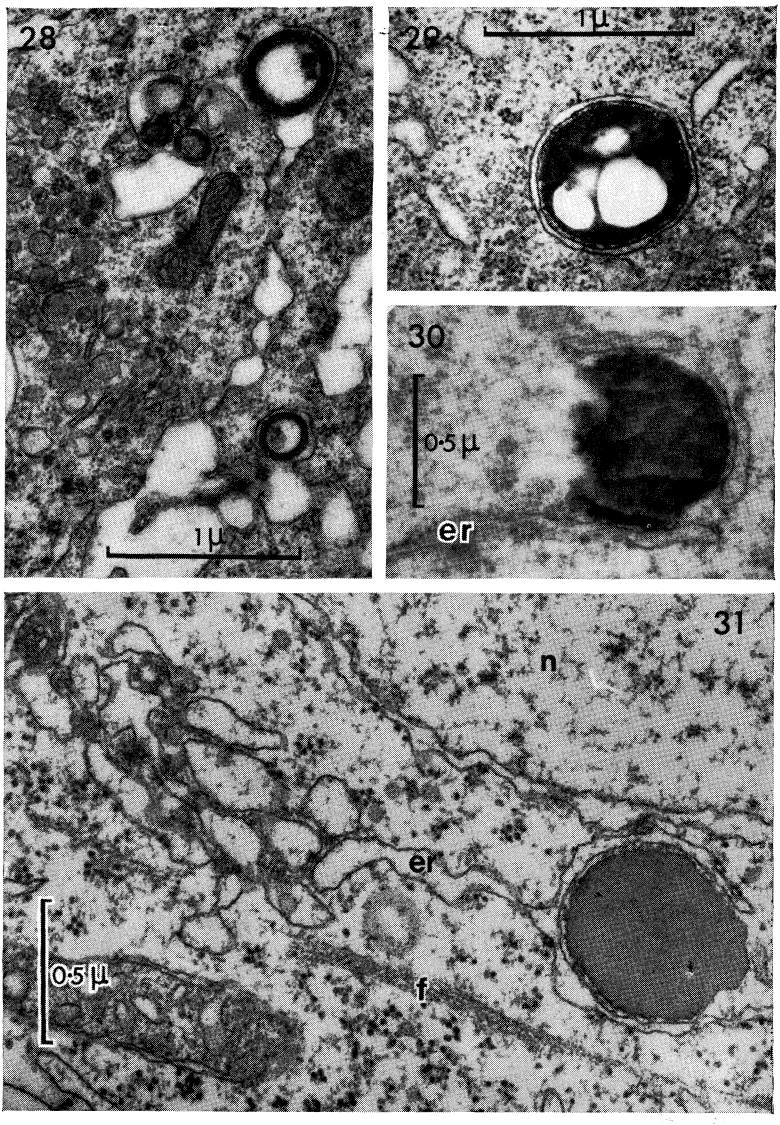


Fig. 28.—Detail of Figure 27. Membranes of the endoplasmic reticulum often appear to envelop small vesicles (cf. Figs. 33 and 34) as well as "spherosomes" (Figs. 29–31).

Fig. 29.—Typical spherosome-like inclusion enclosed by membrane of the endoplasmic reticulum.

Fig. 30.—As for Figure 29, but this inclusion appears to be only partly formed (or being broken up).

Fig. 31.—Very thin section. Spherosome-like object, partly enveloped by the endoplasmic reticulum, shows little evidence of a limiting membrane. Aggregation of the endoplasmic reticulum is also frequently observed, as shown here, and cytoplasmic fibrils (*f*) are often found.

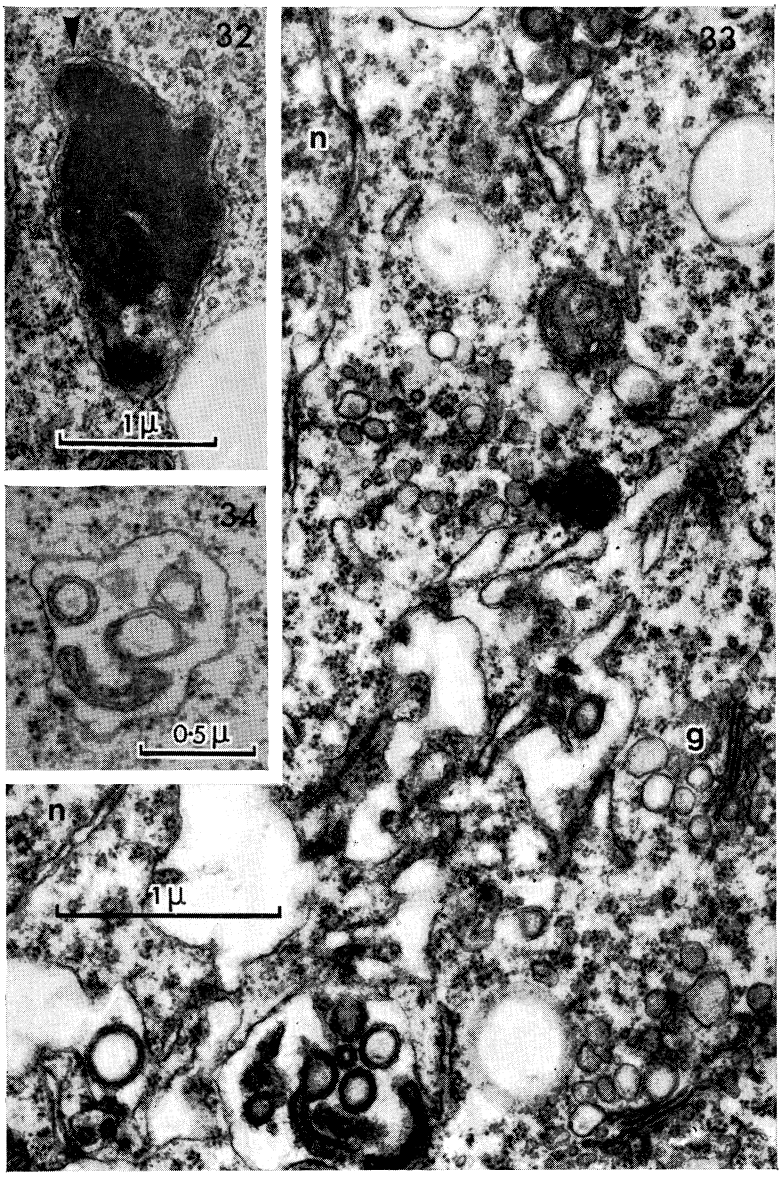


Fig. 32.—Irregularly shaped inclusion (lipoidal?) enveloped by endoplasmic reticulum (note ribosomes at arrow). These are also common in young oogonial cells.

Fig. 33.—Typical association of membranes and vesicles found in capitular and young spermatogenous cells. The vesicles might possibly have been derived originally from the golgi bodies (*g*).

Fig. 34.—Very thin section of vesicle and membrane aggregation, as in Figure 33.