

The Fine Structure of *Ceratium tripos*, a Marine Armored Dinoflagellate

II. Cytokinesis and Development of the Characteristic Cell Shape

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Cytokinesis and the subsequent development of the cell shape is examined in *Ceratium tripos*. Division is by binary fission with each daughter cell retaining approximately one-half of the parent theca. Separation of adjacent plates is along predetermined sutures. The cleavage furrow is bounded by the four unit membranes of the future cell covering which are continuous with those of the parent theca. Development of the cell shape proceeds concurrently with cytokinesis, and it is impossible to distinguish between the two processes. A distinct layer of microtubules located beneath the differentiating cell covering is apparently active in determining both the direction of division and pattern of development. The actual separation of daughter cells may occur subsequent to the completion of cell shape development. Formation of a new apical horn by one daughter cell, and the posterior horns by the other, is achieved in slightly different ways. Development of cell shape appears to result from an extension of the cell covering in a manner determined by the biosynthesis of microtubules.

INTRODUCTION

Several investigators (5, 8, 9, 13, 14) have recently focused their attention on the different mechanisms of cell division found in various organisms, especially the algae. Whether such information is of evolutionary importance is not known for certain. Pickett-Heaps (14) attempted to explain the variation in the mechanisms of cell division in terms of their evolution from more primitive systems. He, therefore, hoped that the same theory of evolution that rationalized the morphological diversity found in different species of organisms would do likewise for the diversity found at the ultrastructural level. To what extent this is profitable is questioned by Leedale (10), who argued that nuclear phenomena are of little importance as phylogenetic indicators within the algae as a whole, though such evidence may be of some significance at the generic and ordi-

dinoflagellates, diatoms, and euglenoids, which exhibit unique and possibly primitive nuclear phenomena, are considered distant from their closest ancestors and therefore locked in taxonomic isolation (10). The importance of these organisms apparently lies in the fact that certain aspects of their division processes are expected to be transitional between the prokaryotes and eukaryotes. Therefore, it is thought that highly divergent groups such as the dinoflagellates might have maintained certain primitive features which will be helpful clues in determining how present systems evolved (4, 14).

The dinoflagellate cell is unquestionably eukaryotic, though nuclear structure and the mechanism of division are believed to be primitive in comparison to most other eukaryotes. Numerous authors (4, 8, 9, 11, 14) have reviewed and discussed these characteristics. The fact that nuclear divi-

of importance as well. Research dealing with division ultrastructure has concentrated on phenomena within major phyla such as the green algae (5, 13, 14-17). In these studies investigators have concerned themselves more with the mechanism of cytokinesis than with mitosis. Particular attention has been paid to the orientation and apparent function of microtubules that participate in cytokinesis (15).

The dinoflagellates as a group divide in basically two different ways (11). The first requires that each new daughter cell synthesize an entirely new theca. This becomes necessary when the parental wall is either shed before division (ecdysis), or the daughter cells differentiate within the parent wall which is lost as the new cells emerge. The second method, which is characteristic of *Ceratium* results in each daughter cell retaining approximately one-half of the parent theca. Descriptions of *Ceratium* cell division based on light microscope observations are present in both the classical and recent literature (18, 19), and these investigators stress the distribution of the thecal plates between daughter cells. In the two major ultrastructural investigations of cell division in the dinoflagellates cytokinesis was only mentioned briefly (8, 9). In the first paper in this series the fine structure of the cell covering was examined while in this report the complicated division sequence and subsequent development of the cell shape is described.

MATERIALS AND METHODS

Ceratium tripos (O. F. M.) Nitzsch was isolated from water samples taken from the north end of the Cape Cod Canal, Bourne, Massachusetts in June, 1970. Cultures were grown in $f/2$ enriched seawater medium (6) minus silica in a regime of 16 hr light and 8 hr darkness. Cultures were maintained by Dr. Robert R. L. Guillard, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543. Cells began mitosis two hours before the end of the dark period and completed cytokinesis several hours after the beginning of the light period. Development of the vegetative cell shape proceeded for an additional 6 hr or more. Cultures were fixed at various times during

this period and individual cells were selected for sectioning depending on their state of development.

The procedure for preparation of *C. tripos* for electron microscopy was the same as described for *Polysiphonia* (20).

OBSERVATIONS

Nuclear division in *Ceratium tripos* commences during the last 2 hr of the dark period as previously reported by Jorgensen (7) and Toriumi (19). Cytokinesis and the subsequent development of the characteristic cell shape begins towards the end of nuclear division and proceeds for several hours. Each daughter cell resulting from the division retains one-half of the parent wall while reforming the missing moiety (Figs. 1-4). Separation of the parent wall during cytokinesis is along predetermined sutures (19) separating adjacent thecal plates. The splitting of the cytoplasm is by binary fission, or a gradual pinching in half of the parent cell between the recently divided nuclei. Development of the missing half of the two new daughter cells begins immediately, and is well underway before the completion of cleavage. In many cases nondisjunction following division results in chains of two or more cells which remain attached both during and after maturation (Figs. 2 and 3).

The complex cell covering which differentiates during cytokinesis and subsequent development is described in a previous paper (21). It consists of an outer continuous membrane (PM) which completely surrounds the cell. A system of large flattened vesicles underlies the PM, and it is in these vesicles that the thecal plates are synthesized. Since the plates are not always completely enclosed in the membrane vesicles, the outer and inner membranes of the vesicles are designated as being separate, the "outer plate membrane" (OPM), and "inner plate membrane" (IPM). An additional membrane, the "thecal membrane" (TM), is prominent within the vesicles during early stages of development and plate synthesis. The system of vesicles

is evident in the initial stages of development since sutures are readily recognized in the differentiating cleavage system (Figs. 5, 6, and 19).

The cleavage furrow first differentiates around the periphery of the cell just below the sutures where the eventual separation of the overlying thecal plates occurs. Cleavage proceeds with the movement of this system towards the center of the cell.

Separation of the overlying plates is gradual and appears to work its way around the circumference of the cell. The actual plate separation is a complicated process, and is accomplished sometime after the cleavage furrow is well established beneath it.

The relationship of the parent cell covering to the developing furrow is illustrated in Diagram 1. The OPM, IPM, and TM of the new system are already continuous

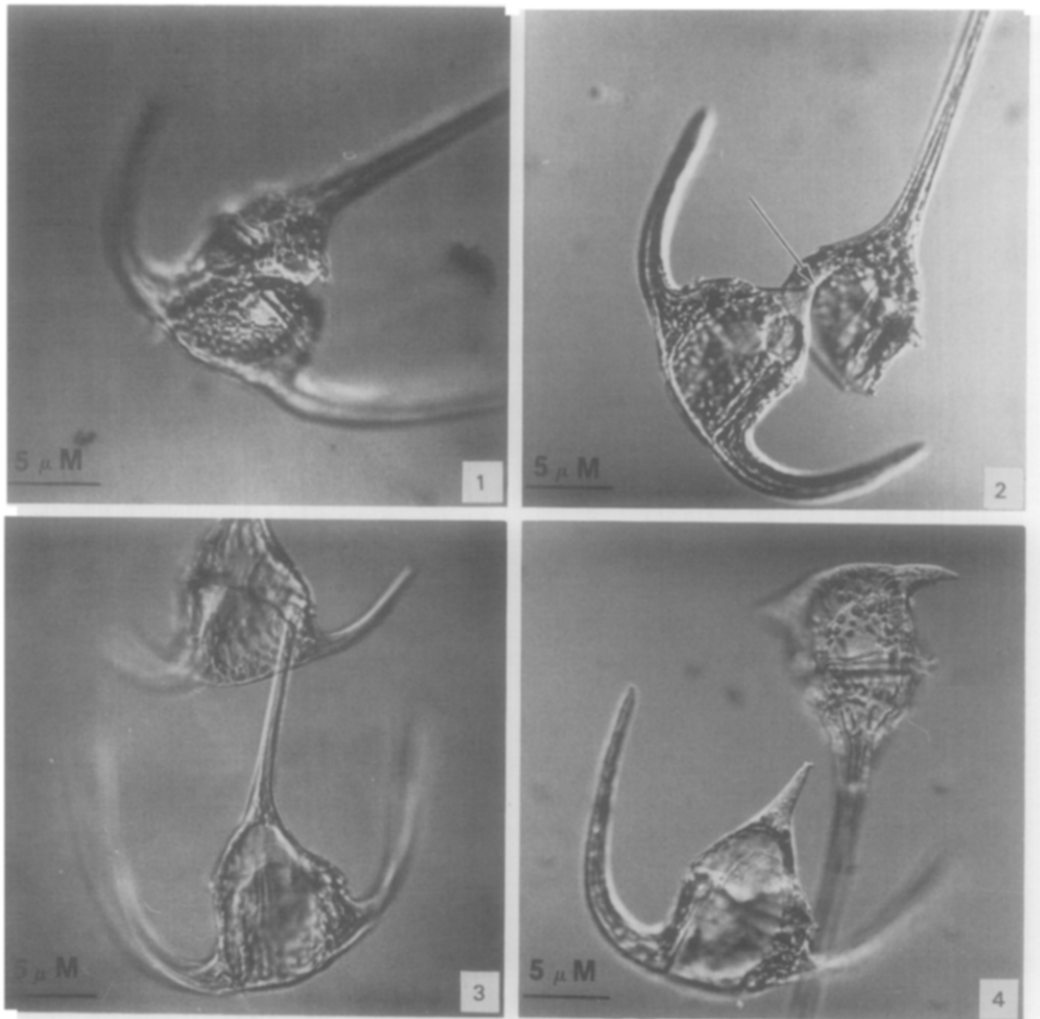
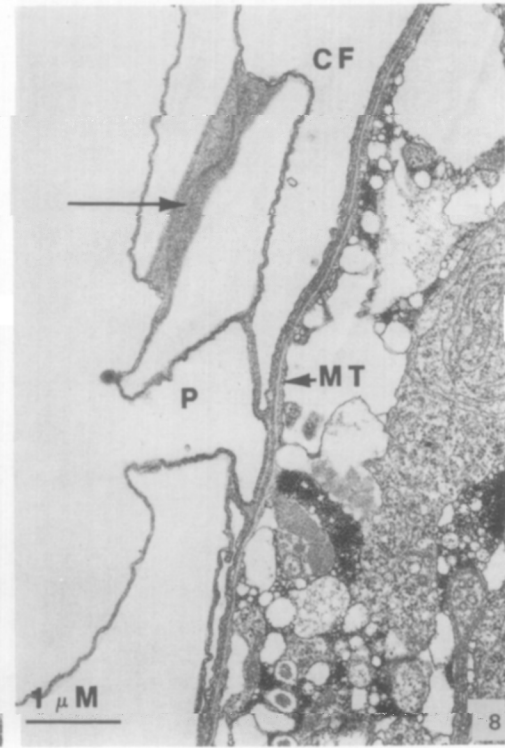
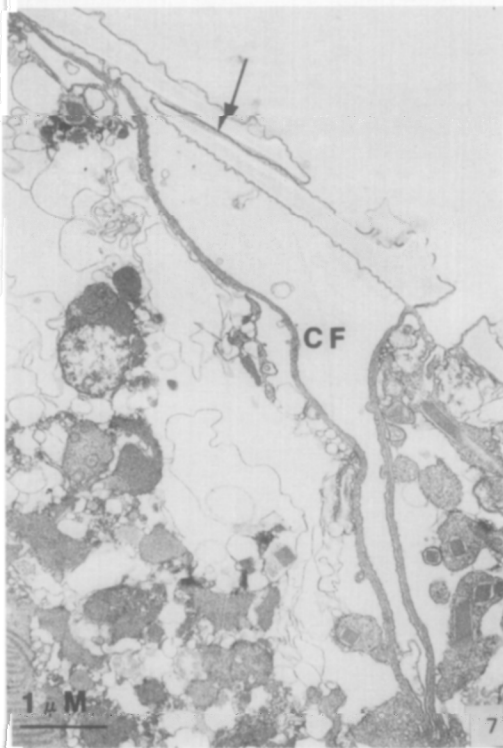
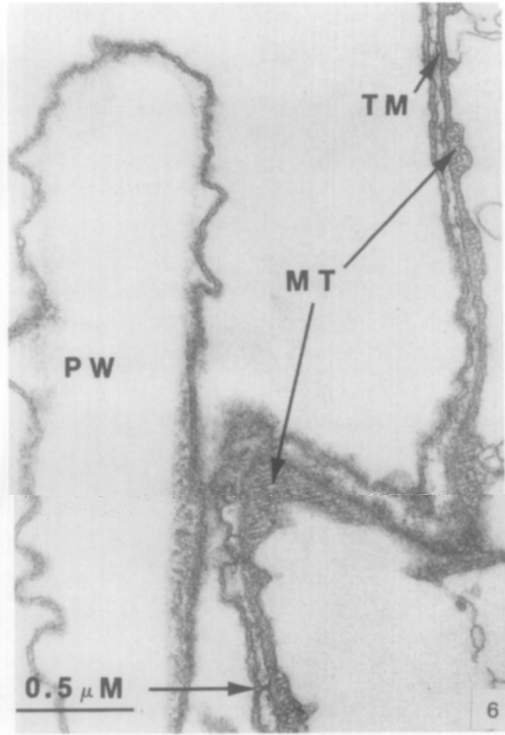
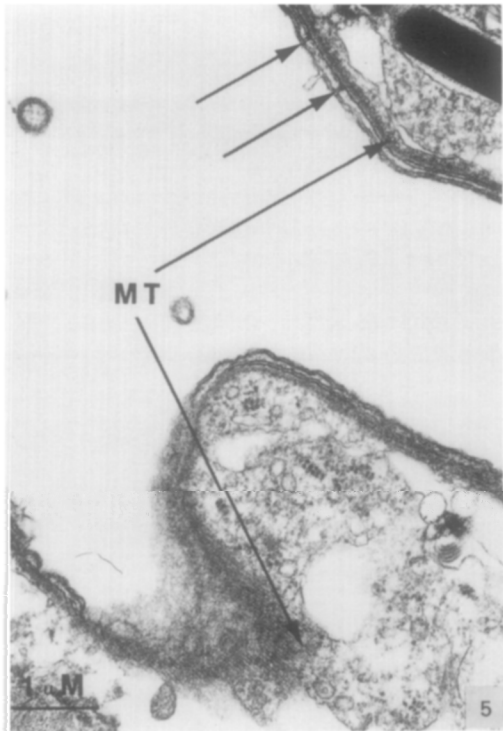


FIG. 1. *Ceratium* cell near the completion of division. The parental wall is divided between the two daughter cells. $\times 1\ 350$.

FIG. 2. Daughter cells remain attached (arrow) rather than separate near the completion of cytokinesis. $\times 1\ 125$.

FIG. 3. Often daughter cells still remain attached near the completion of cell shape development. $\times 1\ 260$.

FIG. 4. Daughter cells which have separated prior to the completion of development. $\times 1\ 440$.



with similar membranes of the parent covering. However, the outer continuous membrane of the parent theca, the PM, must establish a connection with the PM of the developing furrow. This union occurs in the region of the suture. When the two adjoining plates separate from one another, each will be surrounded by two membranes, the OPM and PM. The suture area originally contains two membranes, but becomes four just prior to the actual split (Figs. 10 and 11). The mechanism for plate separation is a complicated process. Initially the plate material immediately adjacent to the suture involved in the separation appears to break down, or change in consistency, resulting in a band of dark granular material (Figs. (7-10). Membrane-bound vesicles then appear within this band and near the former suture membranes (V, Fig. 9). The production of the two additional membranes seems to occur by vesicle coalescence along the location of the two membranes that constituted the original suture (arrow, Fig. 9). Eventually the adjacent plates separate, each surrounded by the OPM and PM (Fig. 11).

In the period of development following division, the same sutures active in separation reform anew. Figures 12 and 13 show a region of suture formation in which the area immediately surrounding the point of contact between the old and new walls is darkly stained and granular. The two membranes which comprise the new suture (arrow, Fig. 13), increase in length as wall synthesis brings the two plate regions closer together. Just as in separation, there is a change in the plate constitution in the region of membrane formation. This in-

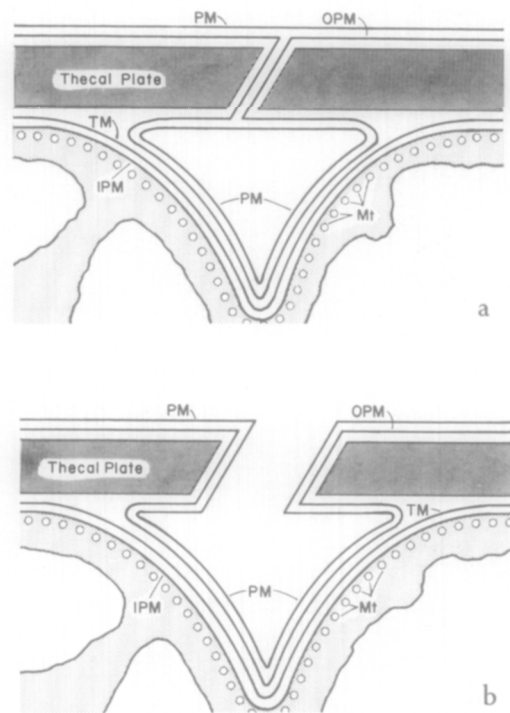


DIAGRAM 1. The association of the cleavage system of membranes with the parent membranes: (a) before the separation of the parent thecal plates and (b) following the separation of the parental plates.

tense activity at, and immediately surrounding, sutures must have additional functional significance since it is often seen at sutures which do not participate in separation. In Fig. 14 (arrows), the activity around the sutures appears similar to that observed above except that neither separation nor reformation is occurring.

Development of the characteristic cell shape is well underway before the completion of division. The microtubule-lined layer of cytoplasm which underlies the cleavage system is most prominent in areas

longer active in this region and the microtubules (MT) have become dispersed into small groups. A suture is present (arrow) and the TM has already become dispersed. $\times 36\ 450$.

FIG. 7. Cleavage furrow (CF) developing below the parent wall. Separation between adjacent plates will occur at the suture (arrow). $\times 7\ 425$.

FIG. 8. Cleavage furrow beneath the parental wall. Separation of adjacent plates at the suture has not yet occurred, though the region surrounding the suture (arrow) has undergone a change in constitution and is apparently active in separation. Note the relationship of the membranes of the parent theca to those of the cleavage system, including a trichocyst pore (P). $\times 20\ 790$.

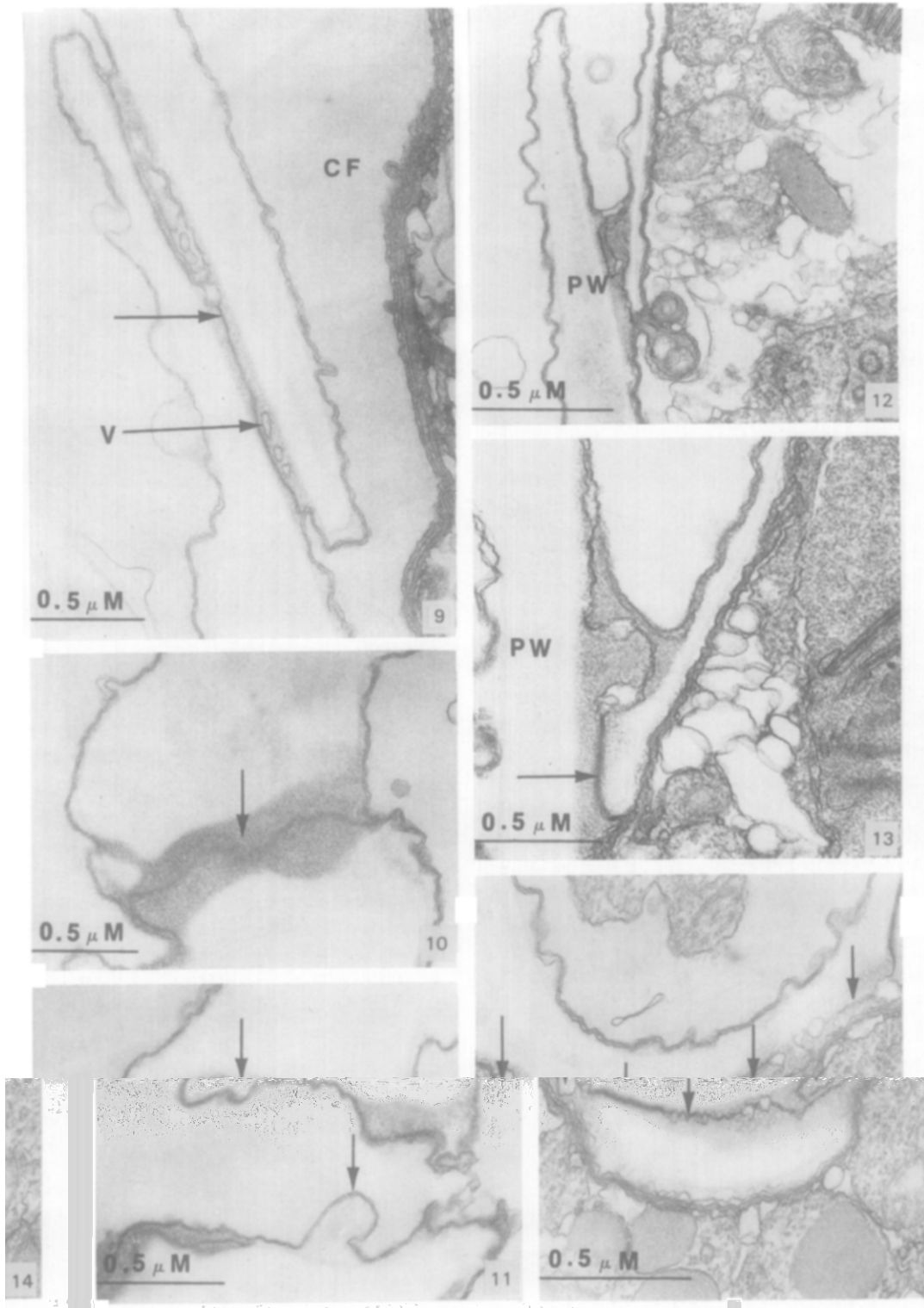


FIG. 9. The region of separation at a suture is shown above a developing cleavage furrow (CF). Membrane bound vesicles (V) differentiate within the dark band while intense activity near the former suture membranes (arrow) occurs in the separation process. $\times 36\ 000$.

FIG. 10. Suture during initial stages of separation. $\times 28\ 900$.

FIG. 11. Suture just following separation. Each plate now surrounded by two membranes (arrows). $\times 26\ 460$.

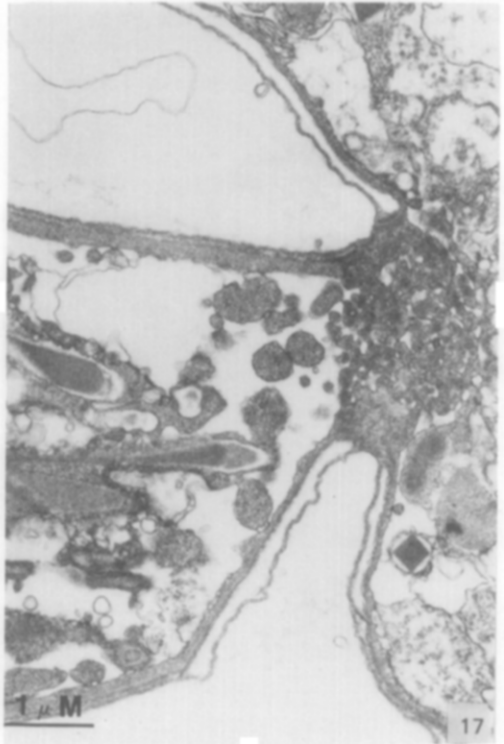
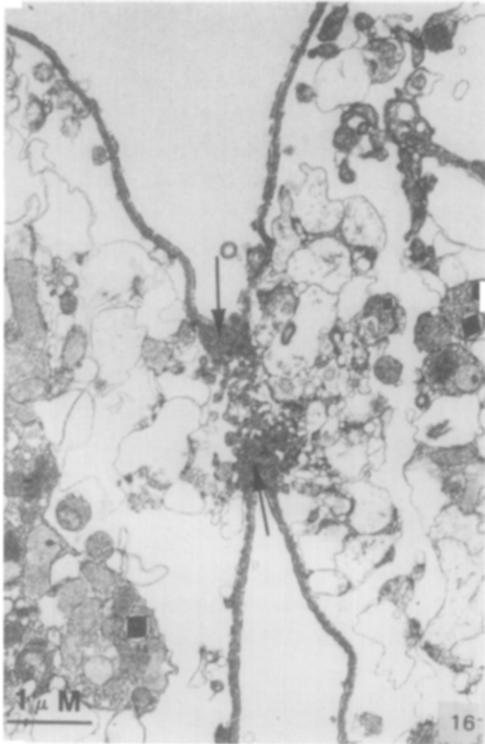
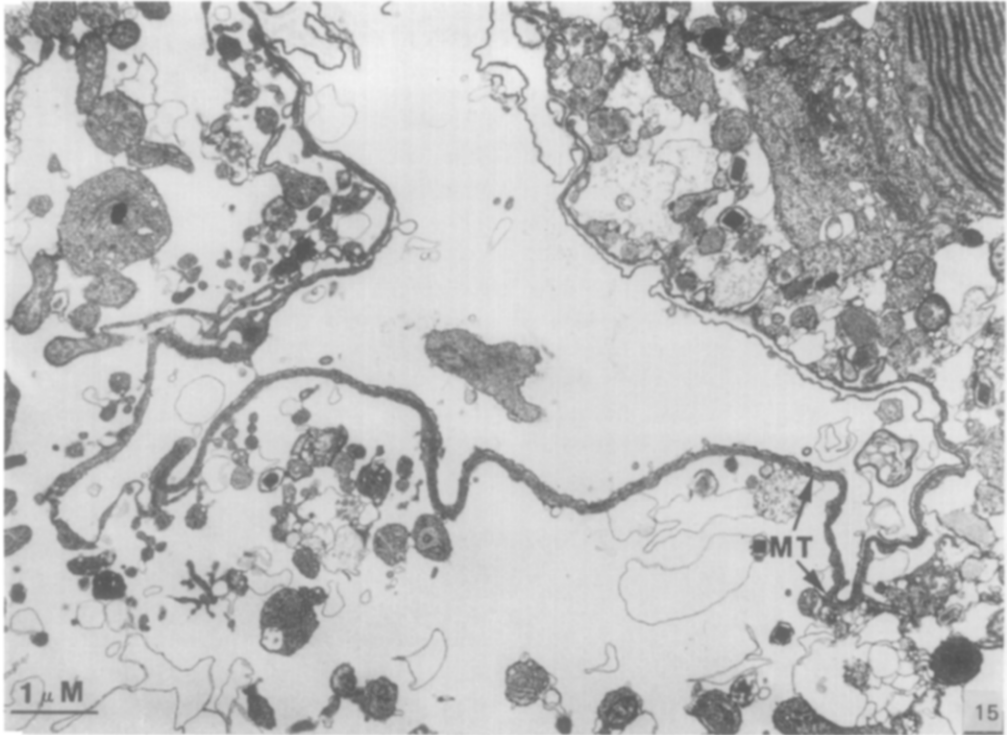
active in morphogenesis of the cell shape (Figs. 5, 15, 18-20). Regions distant from centers of active development reveal very few microtubules. Figure 15 shows a cross-section through the middle of a *Ceratium* cell during cytokinesis. It is difficult in this section to determine the leading edge of fission since the cleavage system is already invaginated in several different directions due to intense developmental activity. This activity is further indicated by the dense band of cytoplasm lined with microtubules which is observed beneath the system (MT, Fig. 15).

Occasionally, sections along the leading edge of the cleavage system reveal two diffuse regions (Fig. 16). Most sections through dividing cells do not reveal this structure. If it were continuous around the leading edge of the cleavage system, it would show in all longitudinal sections revealing the cleavage furrow. The two areas are composed of diffuse material which appears to form from the fusion of vesicles near the periphery. The microtubule containing layer of cytoplasm lying beneath the cell covering is continuous with these diffuse regions. When dividing cells fail to separate following division (Figs. 2 and 3), the final location of the combined diffuse regions becomes the point of attachment between the differentiating daughter cells (Fig. 17). Figure 17 shows the development of a new apical horn when the daughter cells are still connected whereas Fig. 18 shows similar development for a cell which has completed cytokinesis. In both cases the tip of the developing horn contains a diffuse, electron dense region. When the two cells remain attached, the substance of the

region appears to be contributed by both daughter cells, even though it only seems to be involved in the development of one of them (Fig. 17).

Formation of a new apical horn by the daughter cell which retained the posterior portion of the parental wall is somewhat different than the formation of two new posterior horns by the other daughter cell. The diffuse region observed at the apex of the growing apical horn does not appear to be present in the developing hind horns (Fig. 19). The two posterior horns grow by what appears to be the same mechanism seen during other phases of division and development. The cell covering with the accompanying layer of microtubules extends itself out in the form of the new horns. The tip represents the youngest stage and is comprised of the same system of membranes observed in the cleavage furrow. The microtubule layer is extensive just as it is in other areas of active development. Figure 19 shows a longitudinal section through a growing posterior horn and reveals the four unit membranes (including a suture) and a layer of cytoplasm which eventually becomes packed below the covering system by expanding vesicles. Figure 20 is a section through the same horn, but not exactly through the center of the tip. This micrograph reveals the cytoplasmic layer lined with microtubules which extend to the apex of the growing horn. The expansion of the covering system into the characteristic cell shape requires extensive growth by the membranes comprising the system. Since the PM lies exterior to the rest of the cell covering, materials necessary for this expanding growth may diffuse in from the cytoplasm, or may be deposited

Fig. 19. Division of suture formation between a developing thecal plate and the covering plate derived from



by large vesicles which are found to fuse with sutures during periods of active development (Fig. 22). The many interesting features of these elongate vesicles will be discussed in a subsequent paper in the series since their activity is also correlated with initial plate synthesis.

A unique feature of the developing apical horn is the presence of thecal plates just below the diffuse region, and well above the normal region of plate synthesis (Figs. 17, 18, and 21). Since the tip of the mature horn will be flattened much like the top of a cylinder, the early production of these plates may aid in the formation of this shape.

In daughter cells which do not remain attached following division, the diffuse region is present only during initial differentiation of the apical horn. The region then disappears as the tip is completely covered by plates. At this time the side walls just below this apex have the same basic system of membranes and associations seen during cleavage and development in other areas of the cell (Fig. 21). Growth appears similar to that observed for the posterior horns and originates in the sector of the cell covering just beneath the apex. Below this point active synthesis of the thecal plates begins.

DISCUSSION

In a study (8) of nuclear division in *Gyrodinium cohnii*, brief mention was made of cytoplasmic division. The authors (8) observed the cell covering to be comprised of four membranes in the region of the cleavage furrow. The two membranes closest to the surface (my PM and OPM) were both termed outer membranes, while the inner two (my TM and IPM) were said to comprise a double plasma membrane. Microtubules were reported to appear under the double PM following cytokinesis, but were not observed to be associated with the actual cleavage furrow. Division takes place within the encysted parent cell with

each daughter cell producing an entirely new theca. The complicated mechanism of plate separation observed in *C. tripos* is therefore not necessary in *G. cohnii*. The process becomes necessary in order to assure membrane continuity between the retained portion of the parental theca and the differentiating covering of the future daughter cells. As revealed in Figs. 7-9, the area of activity is isolated between plates which are surrounded by two unit membranes and overlie the cleavage furrow. The cellular constituents necessary for this operation must either be present in the suture and associated plate material, or migrate a considerable distance from the cytoplasm. This kind of activity at the sutures presumably takes place beneath the PM, although separated from the rest of the cytoplasm by the vesicle membranes. This isolation could assure the necessary specificity for the reactions observed to occur at the sutures.

Mature cells viewed with the light microscope show a noticeable increase in volume during stages of the vegetative cycle preceding division. Thus an increase in the area of the cell covering must also occur. This has been postulated by several authors (3, 11) to occur in the region of the sutures and is referred to as intercalary growth. Such growth areas are then assumed to dissolve away during, or following, division so that the dimensions of successive generations remain somewhat similar. Sutures present in the parent wall moiety retained during division often reveal activity similar to that observed during plate separation and suture reformation, though these sutures are not involved in either process. It is quite possible in such cases that the thick thecal plates produced during the previous generation time are being reduced in overall size to meet the volume requirements of the new daughter cell. The formation of intercalary growth bands could occur by a reverse process.

It is very difficult to distinguish between

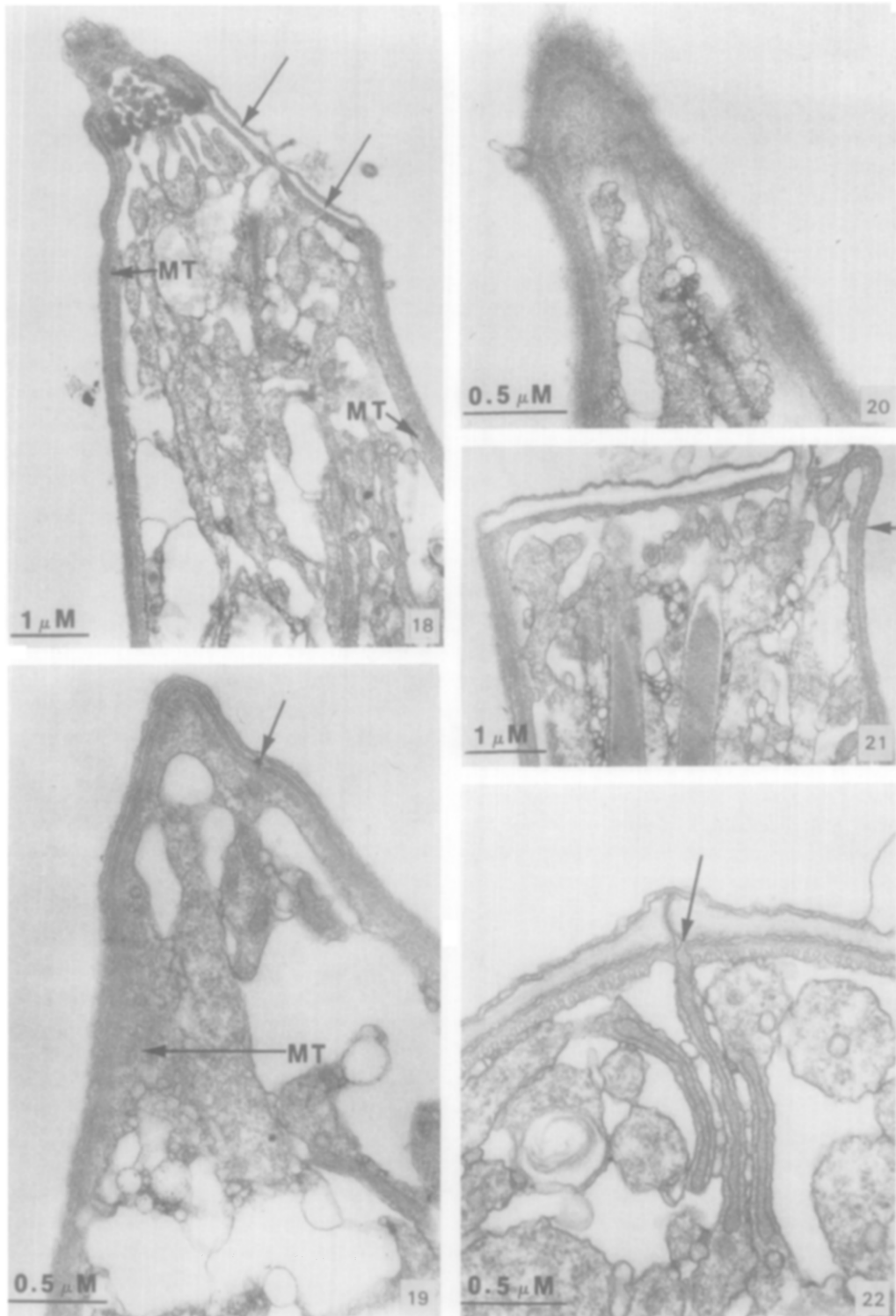


FIG. 18. Developing apical horn no longer connected to the other daughter cell. Thecal plates (arrows) are present just below the leading diffuse region while recently differentiated cell covering is seen along the sides underlain by microtubules (MT). $\times 11\ 520$.

FIG. 19. Tip of a developing posterior horn revealing the four basic membranes of the cell covering including

cytokinesis and the subsequent development of the species-specific cell shape in *C. tripos*. This is most evident in Fig. 15 where the leading edge of cleavage is not distinguishable from other areas of the system involved in development. In both cases microtubules are similarly located and could mediate the direction of cleavage as well as provide specificity to developmental patterns. Primitive cytokinetic mechanisms are believed to be characterized by a furrowing or ingrowth of the cell membrane. This has been observed in the bacteria, blue-green algae, and various higher algae (12, 16). Exactly how the growth and orientation of this membrane is achieved is not known. Various other algae show a similar cleavage, but associated with microtubules (15, 16), which are scattered in the cytoplasm directly in front of the advancing furrow and are reported to orient the furrow with respect to the nuclei and the rest of the cell. It is not clear in these organisms whether the furrow is self-generating or grows by the fusion of vesicles. In *C. tripos* cytokinesis is by the inward furrowing of four membranes. How these membranes grow is not known, though the control of their orientation probably lies with the underlying band of microtubules.

Cytokinesis is, therefore, achieved by a mechanism which appears to combine the membrane furrowing found in primitive division with the specific control provided by microtubules active during division in

and *Ochromonas* (1, 2). Cleavage and subsequent development appear to be achieved by the same mechanism in *C. tripos* since the location and orientation of the microtubules relative to the new cell covering is the same for both. The orientation of the microtubules closely parallels the initial cell form and therefore suggests that the organism's genetic construction dictates the manner in which the microtubules polymerize (1, 2). This specificity must necessarily originate in some form of organizing or nucleating center where the required information for the overall pattern of microtubules resides. Microtubule organizing centers (14), or nucleating sites for microtubule growth and distribution, have been observed to be associated with kinetosomes and centrioles (14). Distinct attachment sites for microtubules involved in the construction and maintenance of cell form in *Ochromonas* have been described by Bouck and Brown (1, 2). However, organizing centers observed in most organisms are represented by ill-defined regions rather than discrete structures (14). During development, the biogenesis of microtubules in *C. tripos* occurs within the band of cytoplasm tightly packed beneath the cell covering. The microtubule concentration increases in this region during active development and almost disappears entirely as development ceases and thecal plate synthesis becomes prominent. The microtubules would therefore appear neces-

advanced forms of cells. Their uniform presence in a precise position relative to

sary for the initial construction of the cell shape while the thickening thecal plates

which differentiate near the completion of cytokinesis and persist for some time during apical horn development is not known. Since these regions show a definite continuity with the cytoplasmic band of high microtubule concentration, it is conceivable that they act in directing the distribution of this band, and therefore the microtubules themselves. If this is indeed their function, they do not play an extensive role in the overall development of the cell. Similar structures are not seen to guide the formation of major portions of the cell shape including the posterior horns. In these regions a dark cytoplasmic band originates at the very edges of growth (Fig. 19) where a layer of cytoplasm becomes packed beneath the cell covering by large swollen vesicles and fenestrated ER. Somehow the polymers necessary for microtubule assembly accumulate in this region and generate the specific patterns required for development.

It is readily apparent that numerous sutures are formed in the covering system that differentiates during cleavage and that perhaps all the vesicles necessary for the characteristic armorment are present at this point, though not fully developed in terms of shape and size. Development of the cell shape would therefore result from the growth and extension of a cell covering completely differentiated during cleavage and directed by a concentrated layer of microtubules lying beneath the covering system.

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