DIVISION IN THE DINOFLAGELLATE *GYRODINIUM COHNII* (SCHILLER)

A New Type of Nuclear Reproduction

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ABSTRACT

Dinoflagellates are of interest because their chromosomes resemble the nucleoplasm of prokaryotes both chemically and ultrastructurally. We have studied nuclear division in the dinoflagellate *Gyrodinium cohnii* (Schiller), using cells obtained from cultures undergoing phasic growth. Electron micrographs of serial sections were used to prepare three-dimensional reconstructions of nuclei and chromosomes at various stages of nuclear division. During division, a complex process of invagination of the intact nuclear envelope takes place at one side of the nucleus and results in the formation of parallel cylindrical cytoplasmic channels through the nucleus. These invaginations contain bundles of microtubules, and each of the bundles comes to lie in the cytoplasm of a cylindrical channel. Nuclear constriction occurs perpendicular to these channels without displacement of the microtubules. There are no associations between chromosomes and the cytoplasmic microtubules. In dividing cells most chromosomes become V-shaped, and the apices of the V's make contact with the membrane surrounding cytoplasmic channels. It is proposed that the membrane surrounding cytoplasmic channels in the dividing nucleus may be involved in the separation of daughter chromosomes. Thus, dinoflagellates may resemble prokaryotes in the manner of genophore separation as well as in genophore chemistry and ultrastructure.

INTRODUCTION

The distinctive nature of the dinoflagellate nucleus was recognized as early as 1885 when Bütschli created the order Dinoflagellata by using nuclear morphology as one of the taxonomic criteria (3). Chatton further stressed the uniqueness of nuclear morphology and division in dinoflagellates and suggested the terms "dinokaryon" and "dinomitosis" to emphasize their peculiarities (4). It is only relatively recently, however, that the unusual features of this nucleus have been better understood.

The cellular organization of dinoflagellates is of the eukaryotic type, with typical membrane bounded organelles such as mitochondria, chloroplasts, and nucleus (16, 25, 28). However, organization within the nucleus is unlike that of typical eukaryotes. A striking feature is the absence of a chromosome coiling cycle. Chromosomes maintain the same appearance throughout the cell cycle and, with the aid of phase optics, are distinctly visible as rod-shaped bodies within the living interphase nucleus (12, 16). This is to be contrasted with the situation in eukaryotes where chromosomes are uncoiled during interphase and, thus, recognizable as individuals only during nuclear division. Also, the chemical com-

position of dinoflagellate chromosomes is unusual. Eukaryote chromosomes are for the most part composed of nucleohistone, the DNA together with protein forming a structural unit which in the electron microscope is recognized as 100 or 250 A fibers (for references see 33). The DNA of dinoflagellate chromosomes, on the other hand, is not associated with protein (8, 24, 32); and, at the ultrastructural level, the chromosomes appear to be made up of 25 A fibrils (14, 19, 24, 32). Thus, structurally and chemically, the dinoflagellate chromosomes display a remarkable similarity to the nucleoplasm of prokaryotes where DNA free of protein is arranged in fine fibrillar array that does not undergo a coiling cycle (12, 13, 35, 37, 40, 41).

The dinoflagellates may, therefore, be considered as eukaryotic cells which retain certain prokaryotic features within their nuclei. This view has led to the suggestion that the nucleus of dinoflagellates represents an intermediate type in the evolution of the typical eukaryote nucleus (32).

Differences in the mechanisms of division further distinguish eukaryote and prokaryote cells. While in prokaryotes replication and segregation of the single genophore is apparently accomplished without the involvement of a complex division apparatus (34), in eukaryotes an elaborate mitotic apparatus ensures the distribution of equivalent chromosome complements to daughter cells. Since in dinoflagellates, as in eukaryotes, the DNA is distributed in numerous discrete bodies, the chromosomes, one might expect that dinoflagellate nuclear division involves special mitotic organelles. As a result of numerous light microscope studies (for references see 7,39), it is generally agreed that a typical mitotic spindle is not involved. Instead, it has been suggested, an endosome or karyosome may function in chromosome separation (17, 18, 22). Leadbeater and Dodge (26) provided further evidence that the division of the dinoflagellate nucleus differs from eukaryote mitosis in essential details. Most significant in their study was the finding of extranuclear microtubules within cytoplasmic invaginations which penetrate the dividing nucleus.

Although available evidence indicates that the dinoflagellate nucleus divides in an unusual manner, the details are still obscure. In the absence of a chromosome coiling cycle, there are no gross morphological indications of the onset of nuclear division; and, as was demonstrated by Leadbeater and Dodge, significant changes may be recognizable only at the ultrastructural level.

We have made use of phasic growth in *Gyrodinium cohnii* to obtain a significant number of cells engaged in nuclear division. Serial sections through nuclei at different stages of division were used to construct three-dimensional models which facilitate the understanding of the complex structural changes occurring during nuclear division. From this study, it is apparent that dinoflagellates are unique not only in the organization of their chromosomes, but also in the mechanisms of chromosome separation and nuclear division.

MATERIALS AND METHODS

Axenic cultures of *Gyrodinium cohnii* (Schiller) were kindly provided by Dr. G. G. Holz, Jr. (Department of Microbiology, State University of New York, Upstate Medical Center, Syracuse, N. Y.). The organisms were maintained in AXM liquid medium (30) at 25–28°C. Cultures were illuminated at 20–30 fc for 14 hr of each 24-hr period. Once during the course of this investigation, it was necessary to reisolate the organism. For this purpose, several clones grown on AXM-1.5% agar plates were mixed and transferred to liquid AXM medium.

Under suitable conditions, cultures of *Gyrodinium cohnii* exhibit phasic growth (Fig. 1); most nuclear divisions occur during a 2–3 hr interval of the period in which cell number remains constant (plateau phase). (This phenomenon will be the subject of a separate communication). For observation of the process of nuclear division, cell samples were taken during the plateau phase when culture density was $1.0–2.0 \times 10^5$ cells/ml. Cytokinesis also takes place during the plateau phase. This results in the formation of daughter cells which are confined within the cyst wall. The logarithmic increase in cell number then is the result of the emergence of daughter cells from cysts. Nondividing cells were studied in samples taken during this period of logarithmic increase in cell number.

Dividing cells are nonmotile and their surface properties are such that they adhere tenaciously to Falcon Plastic Tissue Culture Plates (B-D Laboratories, Inc., Los Angeles, Calif.). To obtain young daughter cells, dividing individuals were allowed to attach to plates and nonadherent, motile cells were washed from the plate with several changes of AXM medium. Fresh medium was added to the plates, and after approximately 1 hr numerous motile individuals appeared and could be collected for fixation.

For light microscopy, cells fixed in 10% neutral formalin were stained in 0.5% aqueous methyl green

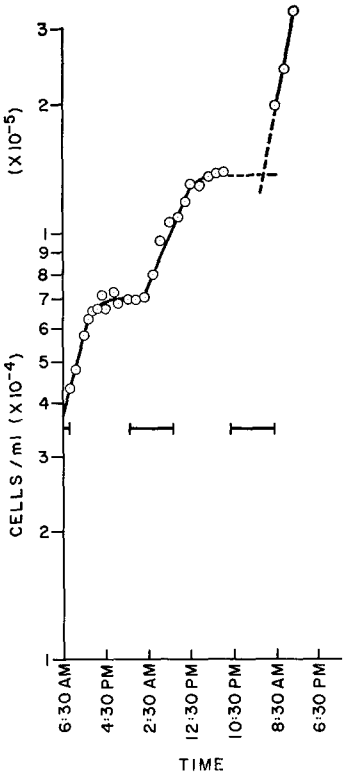


FIGURE 1 Phasic growth in a 1 liter culture of *Gyrodinium cohnii*. Formalin (0.1 ml.) was added to 0.9 ml of culture sample. Cells were counted in an AO Spencer Bright-Line Hemacytometer. The solid bars indicate dark periods.

or aceto-carmine. Encysted cells are not readily penetrated by these stains, and it was therefore necessary to heat the cells in the presence of stain at 60°C for approximately 5 min in order to render them permeable.

The periodic acid-Schiff reaction was carried out on 5μ sections of formalin-fixed, paraffin-embedded cells.

Birefringence of cytoplasmic inclusions was observed by using a microscope fitted with Polaroid discs in condenser and ocular.

For electron microscopy, cells were fixed for 1 hr at room temperature in Karnovsky's formaldehyde-glutaraldehyde fixative (23) (diluted 1:1 with 0.2 M buffer), washed several times, and left overnight in 0.1 M buffer at 4°C. They were postfixed for 1 hr in 2% osmium tetroxide in 0.1 M buffer. Phosphate buffer at pH 7.0 was used throughout. Cells were then washed in several changes of distilled water. In order to prevent clumping of chromosomal DNA fibrils, the fixed cells were treated for 2 hr with 0.5% uranyl acetate in Veronal-acetate buffer (37). In each of these steps, cells were collected by gentle centrifugation. After uranyl acetate treatment, cells were embedded in plasma clots (24), dehydrated in ethanol, and embedded in an Epon-Araldite mixture (27). In order to improve penetration of plastic into cells, capsules containing cells and unpolymerized plastic mixture were heated at 60°C for approximately 10 min, and then placed under vacuum in a vacuum desiccator for 2–3 min. The plastic was hardened at 37°C for 4–7 days.

Serial sections (thickness = 500 A as judged by interference color) were cut on an LKB Ultratome with a Du Pont diamond knife. Ribbons of sections were picked up on formvar films attached to wire loops and were transferred to single-hole grids (1 × 2 mm rectangular opening). Sections were stained for 2 hr with uranyl magnesium acetate (10) and for 10–15 min with lead citrate (31). After staining, a thin carbon layer was evaporated over the sections.

A Siemens Elmiskop I was used at 80 kv with double condenser illumination and with a 200μ condenser aperture and a 15μ or 50μ objective aperture. Micrographs were taken at an original magnification of 5500 × or 12,000 × on Kodalith LR Estar Base roll film and developed in Kodak D-19 or on Kodak Electron Image Plates and developed in Kodak HRP.

Models of nuclei at various stages of division were built by reconstruction from serial sections. The nuclear profiles seen in each micrograph were cut from cellulose acetate sheets by using a Dremel Moto-Tool (Dremel Mfg. Co., Racine, Wis.). Pieces were then glued together to form the three-dimensional model. Thickness of cellulose acetate was chosen to equal (average section thickness × magnification) so that the scale of the model would be the same in all three dimensions.

Serial section reconstruction was used also to determine the configuration and distribution of chromosomes within the nucleus. For this purpose, nuclear outlines were traced on clear cellulose acetate sheets, and the chomosome profiles were filled in with translucent colored lacquer. When these sheets were piled in sequence, it was possible to follow the course of individual chromosomes.

RESULTS

General Aspects of Cell Structure

The morphology of *Gyrodinium cohnii* was described by Schiller (38). Since the ultrastructure of this species has not been described previously, a brief discussion of the general cell organization will be presented.

The conspicuous nucleus, which in light microscope preparations appears ellipsoidal, displays

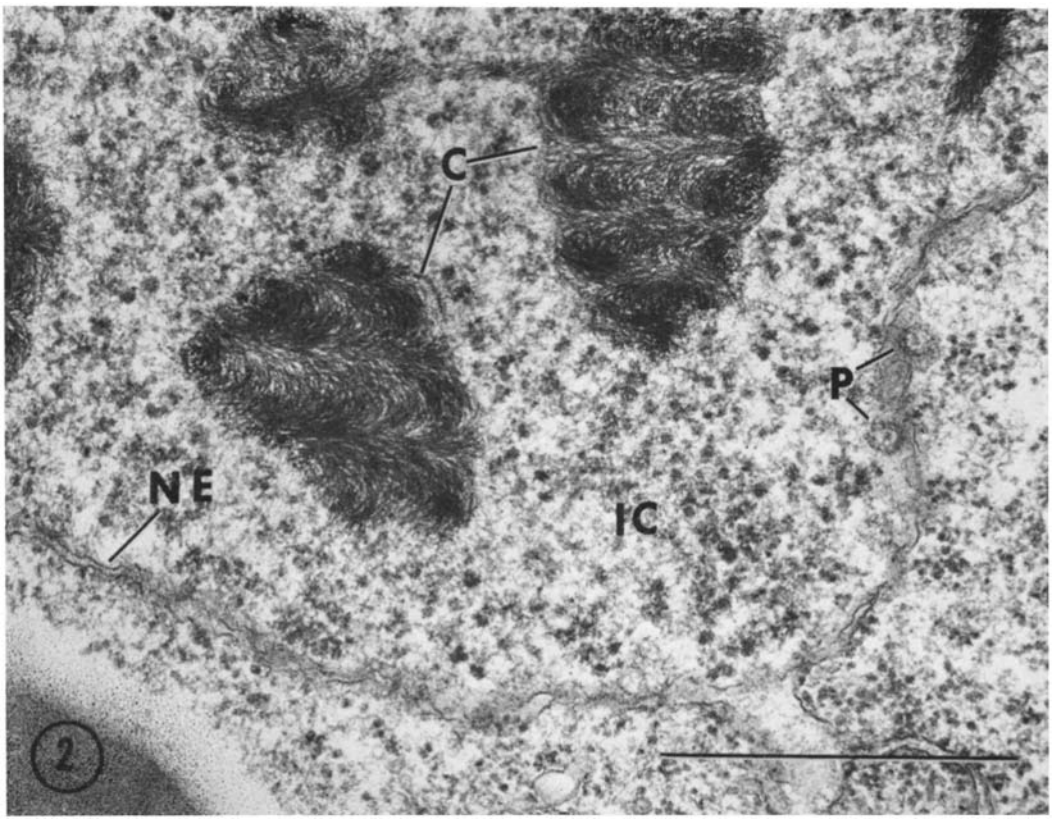
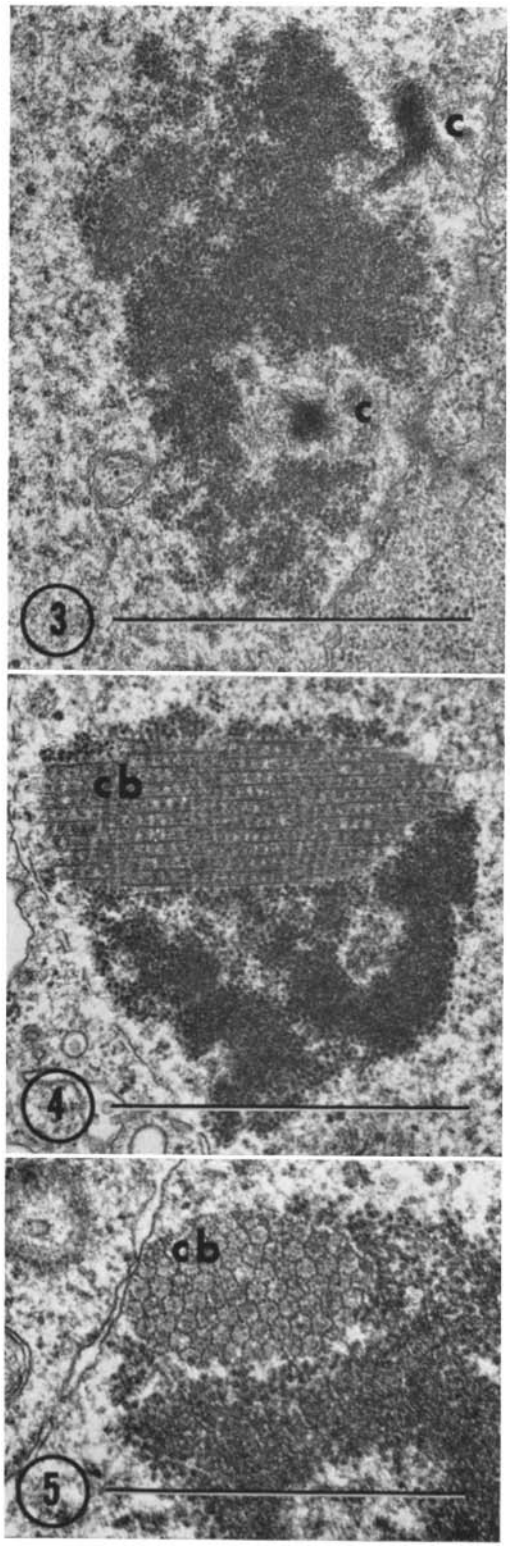


FIGURE 2 Nucleus of a nondividing cell of *Gyrodinium cohnii* showing chromosome (*C*) structure characteristic of dinoflagellates, interchromosomal material (*IC*), and typical nuclear envelope (*NE*) with annulate pores (*P*). × 47,500.

typical dinoflagellate features (Fig. 2). Chromosomes are composed of fine fibrils arranged in such a way as to impart a banded appearance to the chromosomal profiles seen in longitudinal section. Interchromosomal areas are filled with granules of various sizes and fine fibrils. A large nucleolus is present (Figs. 3–5). Like the nucleolus of typical eukaryotes, it is differentiated into an inner finely fibrillar zone and peripheral areas containing ribosome-like particles. Chromosomes may be closely associated with or embedded in the nucleolar material (Fig. 3). An unusual body of crystalline appearance occurs in the nucleolus (Figs. 4 and 5). Most often, longitudinal or slightly oblique aspects similar to that in Fig. 4 are seen. Cross-sections (Fig. 5) which are rarely encountered reveal that the crystalline body has a honeycomb structure. To our knowledge, a similar structure associated with the nucleolus has not been described previously. However, this structure has also been observed by Dr. Ivan L. Cameron and Mr. Peter M. M. Rae who are independently studying this species (personal communications). The double nuclear membrane which is continuous over the surface of the nucleus exhibits typical annulate pores (Fig. 2).

In the cytoplasm one sees mitochondria of the typical protozoan form, endoplasmic reticulum, well-defined Golgi regions, free ribosomes, trichocysts, basal bodies, and numerous starchlike granules (PAS-positive, birefringent) (Figs. 6–8). Although the cell is pigmented, plastids are not obvious; the membranous bodies shown in Fig. 8 may represent atypical plastids. In addition, a basket-like assembly of microtubules occurs in the cytoplasm of the nondividing cell (Figs. 9 and 10). In cross-section, this structure appears to be formed of partially-overlapping curved rows of microtubules which may extend over several microns in longitudinal sections. Its contents



include cigar-shaped dense bodies which resemble the shaft of trichocysts and numerous membrane-bounded vesicles.

The surface of *G. cohnii* is covered by a delicate theca which is made up of three layers (Figs. 11 and 12). The innermost layer consists of a series of separate plates composed of an amorphous material. The second layer has a unit membrane structure, and folds of this membrane penetrate between adjacent plates to form characteristic sutures. Another unit membrane forms the third, outermost layer. The two unit membranes are in close contact with each other, but approach the inner layer only at the suture region, thus forming a blister over each plate.

Beneath the theca, the surface of the cytoplasm is enclosed in a double layer composed of two unit membranes (Figs. 9 and 11). Underlying the inner of these, short rows of microtubules may be seen (Fig. 9).

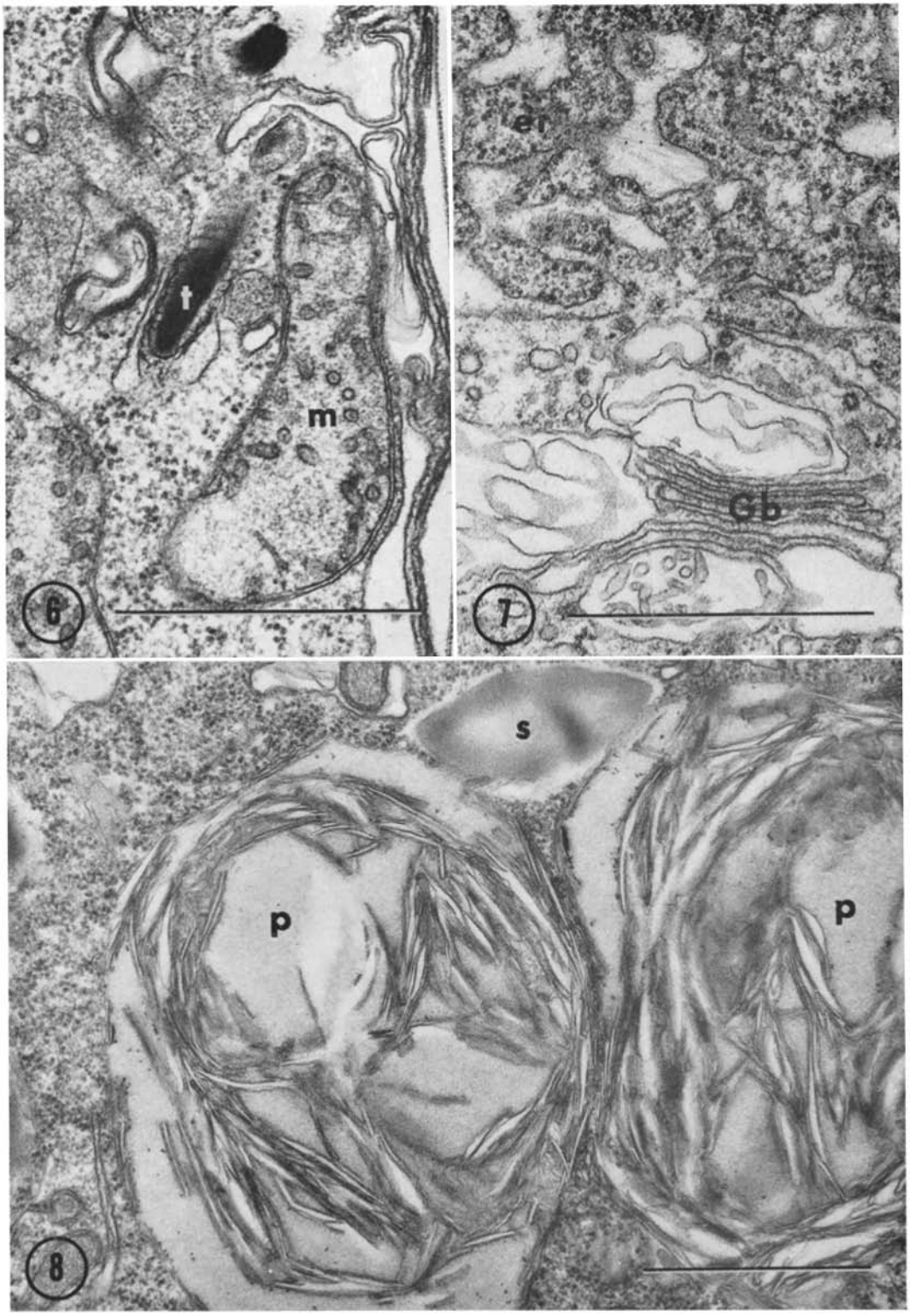
The Dividing Cell

In some dinoflagellate species, division occurs during a nonmotile phase while the individual is encased in a cyst (11). As Schiller (38) has shown, this is true also for *G. cohnii*

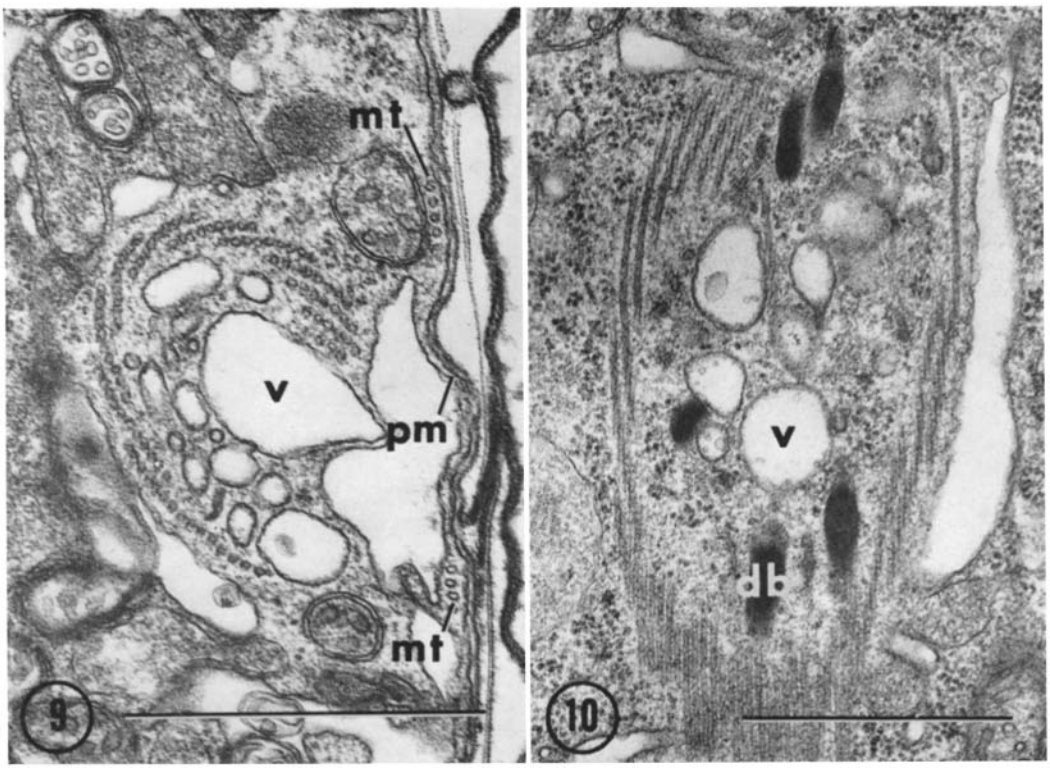
Under suitable conditions, *G. cohnii* exhibits phasic growth (Fig. 1). During the plateau phase, as many as 80% of the cells in a culture may be encysted. Encysted cells attach to the surface of Falcon Plastic Tissue Culture Plates and can be separated from the nondividing stages (see Materials and Methods). It is possible to observe these cells in the living state by phase microscopy, but, because of the large starchlike granules, it is impossible to follow the progress of nuclear division. Only late stages in which cell division has been completed are clearly identifiable. In these, one can recognize two, four, eight, or more newly formed cells which become motile while still encased by the cyst wall. The daughter cells then emerge through an opening in the wall and swim away, leaving the ruptured wall behind.

Encysted, nonmotile cells are impermeable to stains such as aceto-carmine or methyl green. However, when heated in the presence of stain,

FIGURES 3–5 Nucleoli. Fig. 3 illustrates the chromosome (*c*)-nucleolus relationship. × 47,500. Fig. 4 (slightly oblique section) and Fig. 5 (cross-section) show the unique crystalline body (*cb*) associated with the nucleolus. × 47,500.



FIGURES 6–8 Cytoplasmic structures. Mitochondrion (*m*), trichocyst (*t*), endoplasmic reticulum with ribosomes (*er*), Golgi body (*Gb*), and plastids (*p*) with starch-like material (*s*). Figs. 6 and 7 × 47,500; Fig. 8 × 35,500.



FIGURES 9 and 10 Microtubular "basket." Fig. 9: Cross-section showing curved rows of microtubules and vesiculate (*v*) contents. Groups of microtubules (*mt*) are seen below the double plasma membrane (*pm*). × 47,500. Fig. 10: Longitudinal section. Cigar-shaped dense bodies (*db*) resembling trichocyst shafts and vesicles (*v*) are contained within the "basket." × 35,500.

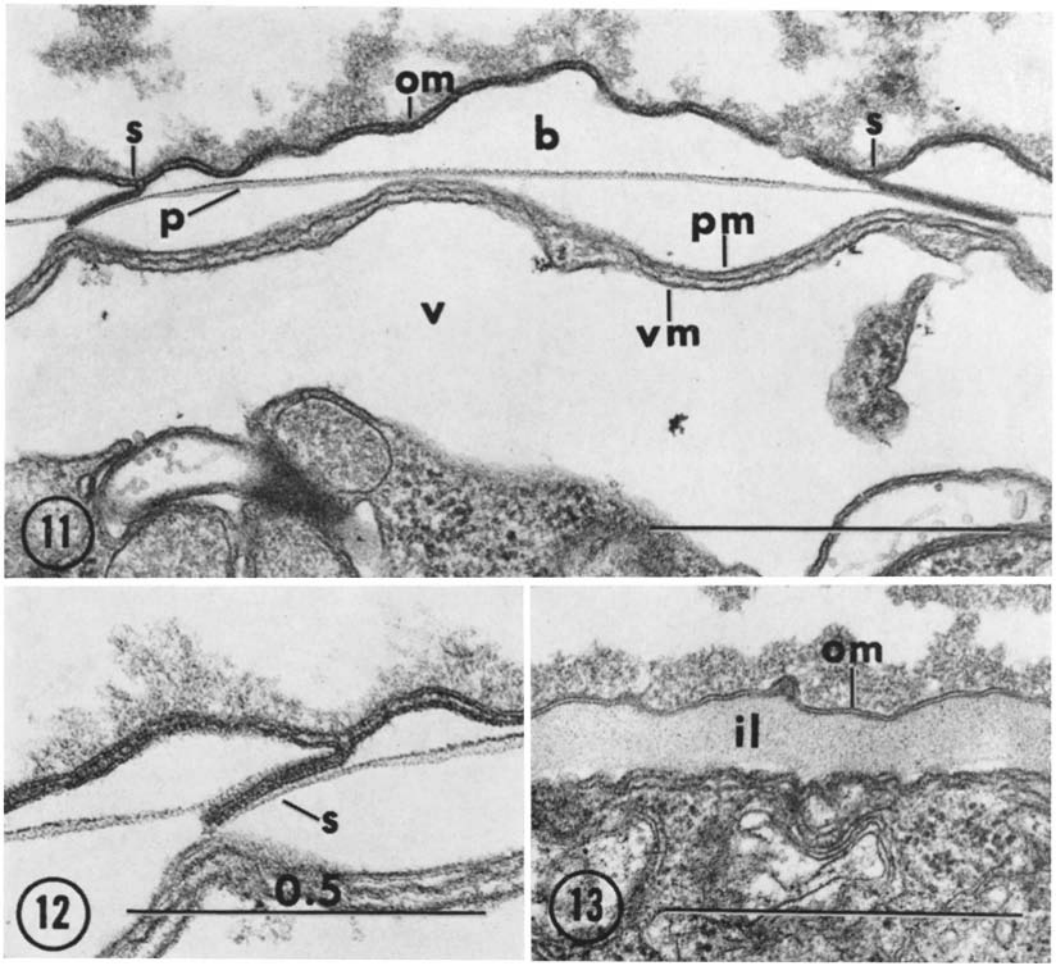
they become permeable, and it is seen that many nuclei are dumbbell-shaped or that there is more than one nucleus per cell. In the remainder of the cysts, nuclei are spherical. In contrast, the motile nondividing cells are easily stained, and the single nucleus is ellipsoidal.

Electron microscopy reveals that the division cyst wall consists of three layers (Fig. 13): a relatively thick amorphous inner layer covered by two membranes. All three layers are in close contact over the entire surface of the cell, and no interruptions or sutures have been observed. The similarity of the amorphous layer of the cyst wall and the plates of the theca suggests that the cyst wall develops through thickening and fusion of the thecal plates.

Before the first signs of nuclear division become evident, the cyst wall is completely formed and the nucleus has assumed a spherical shape.

Examination of serial sections through cells at various stages suggests that during the course of nuclear division the flagella of parent cells disappear and those of the daughter cells are later formed anew. A flagellum completely confined within the cyst wall is present during early nuclear division (Fig. 14). Later, at the time of midnuclear division, no flagella are observed although basal bodies are seen occupying a position directly beneath the plasma membrane. During late nuclear division short flagella are present (Fig. 15), but only after daughter cells have formed within the cyst do the flagella attain considerable length (Fig. 26). These findings are consistent with the observation that dividing cells are nonmotile and that daughter cells become motile while still encased in the cyst wall.

Microtubules underlying the plasma membrane are seen only at the early stage of nuclear division. In later stages, such microtubules were never seen. The basket-like microtubule array,



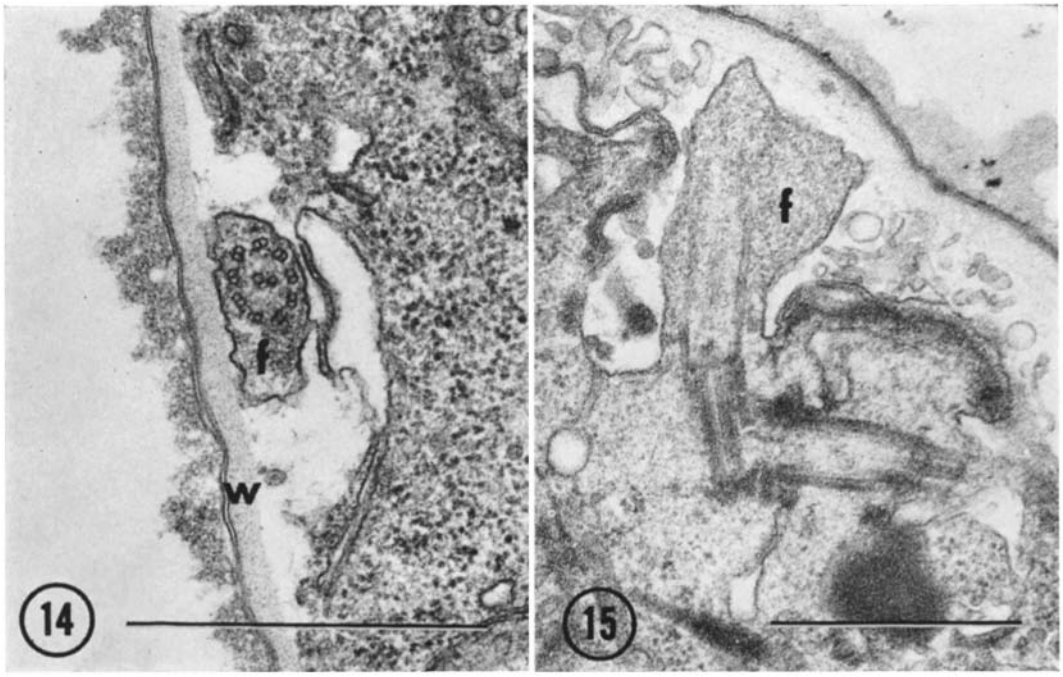
FIGURES 11–13 Cell envelopes. Fig. 11: The theca of the nondividing cell consists of two outer unit membranes (*om*) and a platelike amorphous inner layer (*p*). Sutures (*s*) and a blister (*b*) are illustrated. Beneath the double plasma membrane (*pm*) a vesicle (*v*) is enclosed by the vesicle membrane (*vm*). × 47,500. Fig. 12: Details of the suture (*s*). × 94,500. Fig. 13: Cyst wall of the dividing cell with two outer membranes (*om*) and a thickened amorphous inner layer (*il*). × 47,500. The fuzzy material on the outer surfaces of the cells is clotted plasma in which the cells were embedded.

likewise, disappears from the encysted, dividing cell.

The nucleolus persists throughout division and becomes constricted as nuclear constriction takes place. As a result, each daughter nucleus receives a nucleolus. Chromosomes continue to be associated with the nucleolus throughout this process.

Nuclear Division

In examining random sections through dividing cells, we found, in agreement with Leadbeater and Dodge (26), that the nuclei are invaded by cytoplasmic invaginations containing microtubules. However, it became obvious to us that the changes in nuclear form which occur as division progresses are so complex that only reconstructions of serial sections could reveal their exact nature. Three nuclei representing early, mid-, and late division were chosen for detailed analysis. The following descriptions are derived from a complete sequence of serial sections through each of these nuclei and from models constructed on the basis of these sections.



FIGURES 14 and 15 Flagella in dividing cells. **Fig. 14:** In early division, the flagellum (*f*) is confined beneath the cyst wall (*w*). × 47,500. **Fig. 15:** In late division, a new flagellum (*f*) is formed under the cyst wall. × 33,000.

The Early Division Nucleus

The nucleus we chose as representative of the early division stage was still spherical over-all, but was already penetrated by deep cytoplasmic invaginations containing mitochondria, vesicles, and ribosomes (Fig. 18). This leads to extensive deformation of the actual nuclear outlines (Fig. 16). The cytoplasmic invaginations assume two forms: (*a*) Cylindrical channels which traverse the entire nucleus (Fig. 16b, and 17); there are many such channels and all are parallel to each other. (*b*) Invaginations of complex and irregular form which appear to push into the nucleus in a direction perpendicular to the long axes of the cylindrical channels (Figs. 16a, and 18). The second type of invaginations occur only on one side of the nucleus, namely the side facing the large Golgi area.

Further aspects of the model constructed for this stage are illustrated diagrammatically in Fig. 16. Included in this view is a continuation between a channel of almost perfectly cylindrical configuration and an invagination of the second type (Figs. 16c and 19). Such cross-connections between the two types of invagination are seen frequently. Also, certain cylindrical channels are not continuous but are confluent with irregular invaginations (Fig. 16d). These relations indicate that cylindrical channels may be derived from the irregular invaginations.

Numerous microtubules are seen within both types of invaginations (Figs. 17–20). They traverse the entire nucleus, and all are oriented in the same direction. In irregular invaginations, microtubules occur as separate bundles lying close to the nuclear membrane (Figs. 19 and 20); and certain of these bundles can be followed from irregular invaginations into confluent cylindrical channels. If, as was suggested above, cylindrical channels originate from irregular invaginations, then each bundle of microtubules will, in the course of division, come to lie within a separate cylindrical channel.

Microtubules extend somewhat beyond the limits of the nucleus and end abruptly in the cytoplasm. The separate bundles do not converge and do not make contact with basal bodies. The

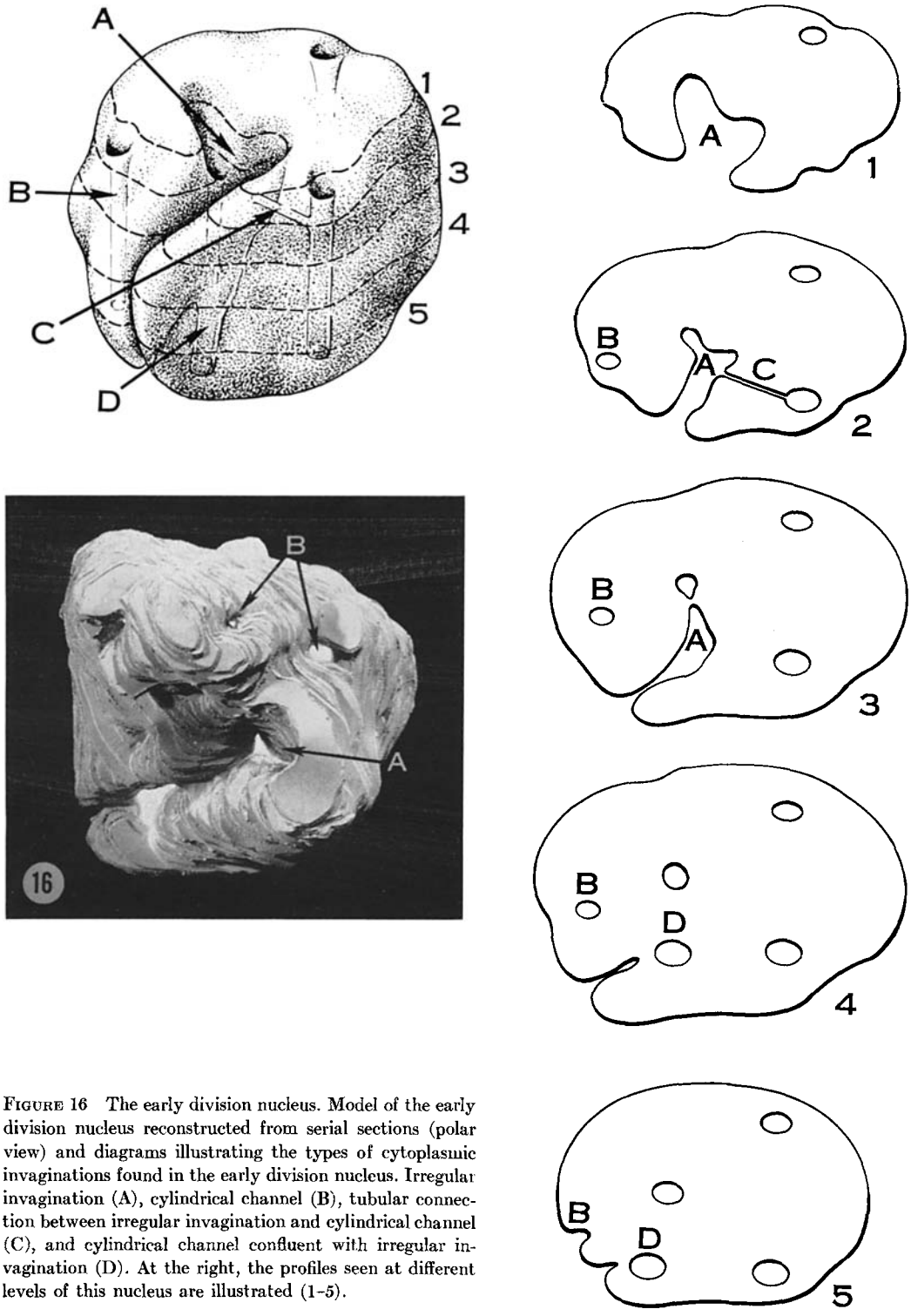


FIGURE 16 The early division nucleus. Model of the early division nucleus reconstructed from serial sections (polar view) and diagrams illustrating the types of cytoplasmic invaginations found in the early division nucleus. Irregular invagination (A), cylindrical channel (B), tubular connection between irregular invagination and cylindrical channel (C), and cylindrical channel confluent with irregular invagination (D). At the right, the profiles seen at different levels of this nucleus are illustrated (1–5).

region of cytoplasm in which microtubules terminate is highly vesiculated and contains numerous free ribosomes, few mitochondria, and no starch grains.

The nuclear membrane is intact over the entire nuclear surface (Figs. 18–20) and retains its typical morphology, including pores (Fig. 23). In both types of invaginations, therefore, the cytoplasm and nuclear contents are always separated by an intact nuclear membrane.

Thus, in the earliest division stage which we have studied in detail, we find that the nucleus has undergone extensive change in surface contours while the over-all spherical shape has been maintained. Deep cytoplasmic invaginations penetrate the nucleus from one side; and, associated with these, a new system of microtubules has appeared. The relationships between the two types of invaginations and between these and the microtubules suggest that the cylindrical channels containing bundles of microtubules originate by being pinched off from the irregular invaginations.

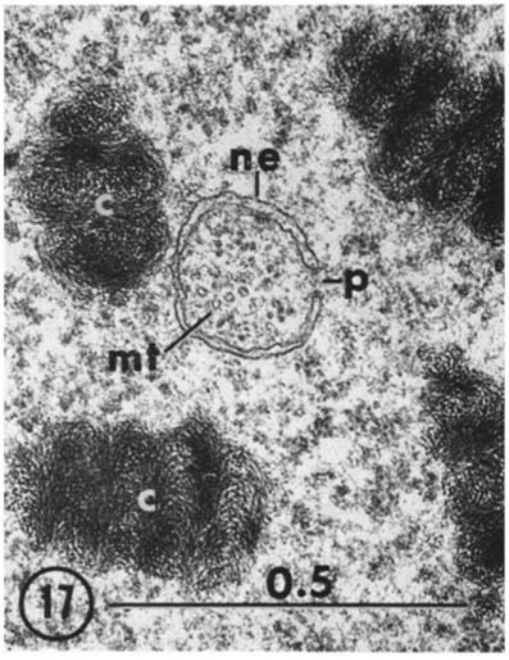


FIGURE 17 Cross-section through a cylindrical channel. The channel is bounded by a double nuclear envelope (*ne*) and contains a bundle of microtubules (*mt*). Pore in nuclear membrane (*p*). Chromosomes (*c*). × 94,500.

Mid-Division Nucleus

Light microscope observations reveal that the nucleus becomes dumbbell-shaped as division progresses. A nucleus of this form was chosen for reconstruction from serial sections as representative of an intermediate stage of nuclear division.

As demonstrated in Fig. 21, the dumbbell-shaped nucleus is penetrated by numerous cytoplasmic invaginations. In contrast to the earlier stage, however, only one type of invagination is present: cylindrical channels containing microtubules. This is taken as further evidence that the irregular invaginations seen in early division give rise to the cylindrical channels. All channels pass completely through the nucleus and run parallel to each other.

Constriction of the nucleus occurs in a plane perpendicular to the long axes of the channels; and, in the region of the constriction, the microtubule bundles pass out of a channel in one lobe of the nucleus, continue straight through the cytoplasm outside of the nucleus, and reenter a similar channel in the opposite lobe (Fig. 21, arrow). This observation suggests that the bundles of microtubules maintain their position as the mid-region of the nucleus becomes constricted.

During division, the chromosomes of *G. cohnii* are scattered throughout the nucleoplasm, and at no time do they have a regular arrangement comparable to the metaphase plate of mitotic cells. In the mid-division nucleus, chromosomes are distributed toward the poles of the nucleus, and only few are found in the region of the constriction.

As in the previous stage, the nuclear membrane remains intact and retains its typical ultrastructure.

Late Division Nucleus

The nucleus reconstructed as an example of late nuclear division is bilobed, constriction having progressed to the point where only an extremely narrow bridge connects the two lobes. Each lobe has an ellipsoidal shape similar to that of the interphase nucleus (Fig. 22).

Cylindrical cytoplasmic channels are present in each lobe (Figs. 23 and 24) and bundles of microtubules extend from a channel of one lobe, through the intervening cytoplasm (Fig. 24), and into a corresponding channel in the opposite lobe (Fig. 22). It is thus obvious that the microtubular

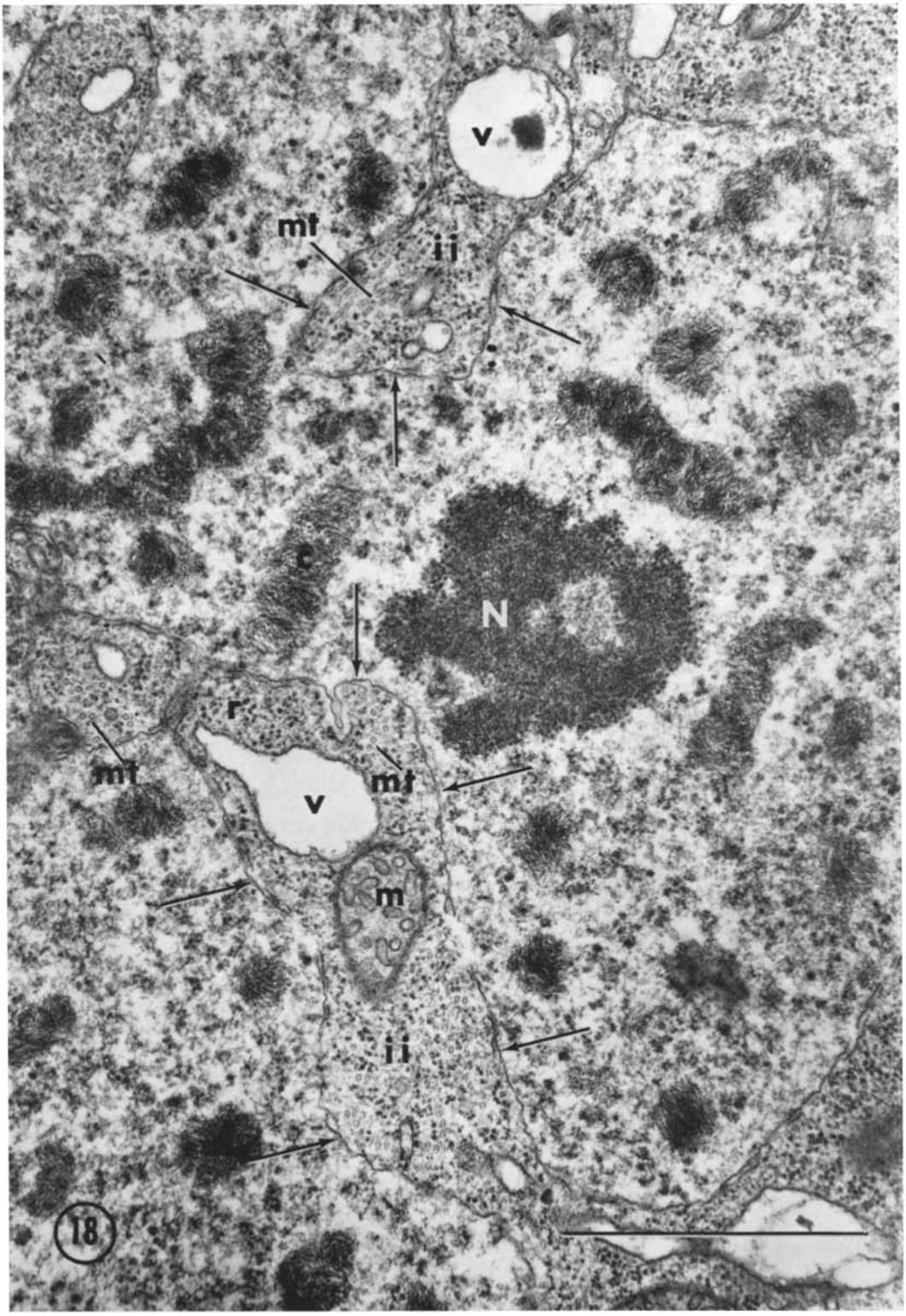
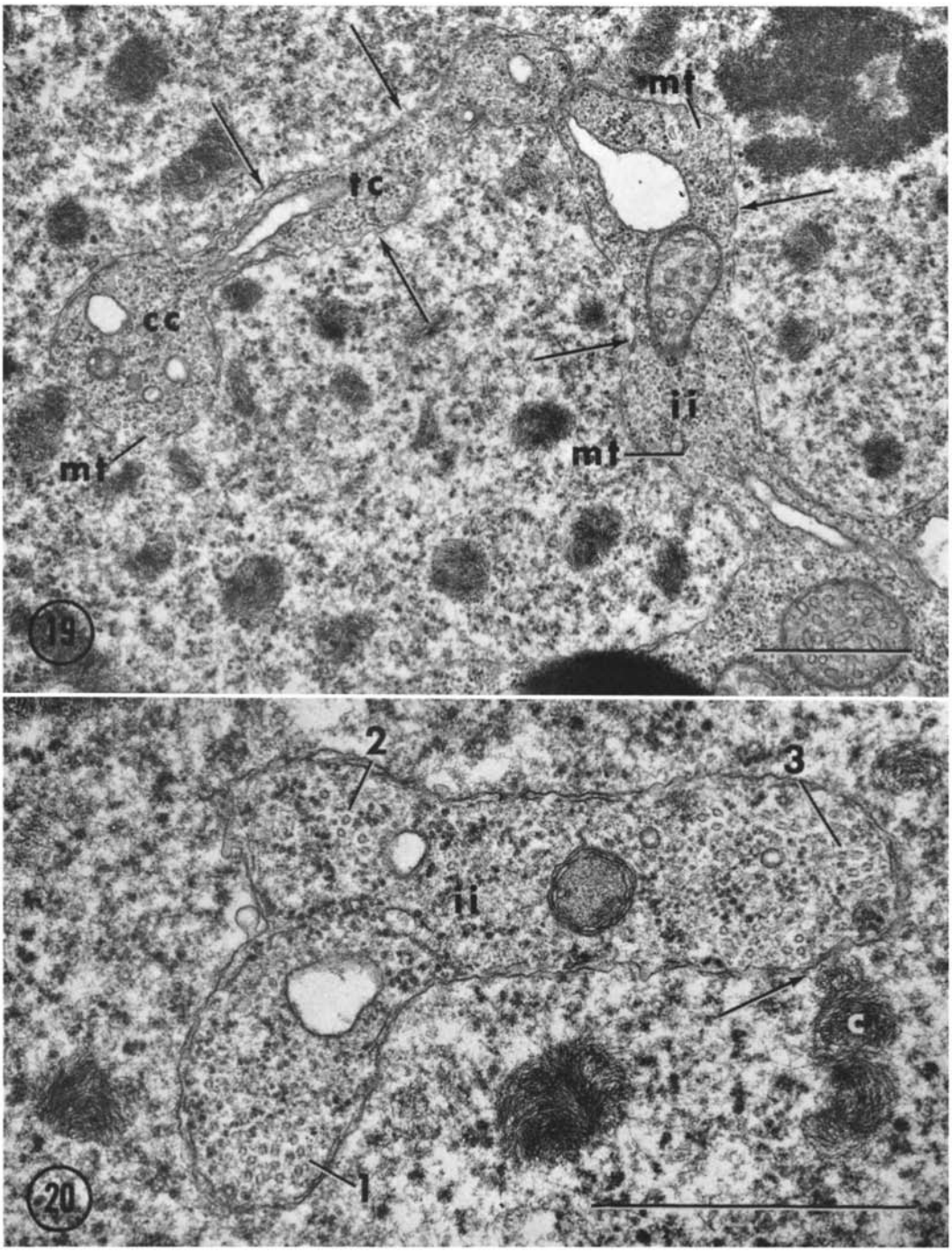


FIGURE 18 Irregular cytoplasmic invaginations (*ii*) invading the early division nucleus. They contain bundles of microtubules (*mt*), vesicles (*v*), mitochondria (*m*), and ribosomes (*r*). Nucleolus (*N*), chromosomes (*c*). The nuclear envelope is indicated by arrows. × 47,500.

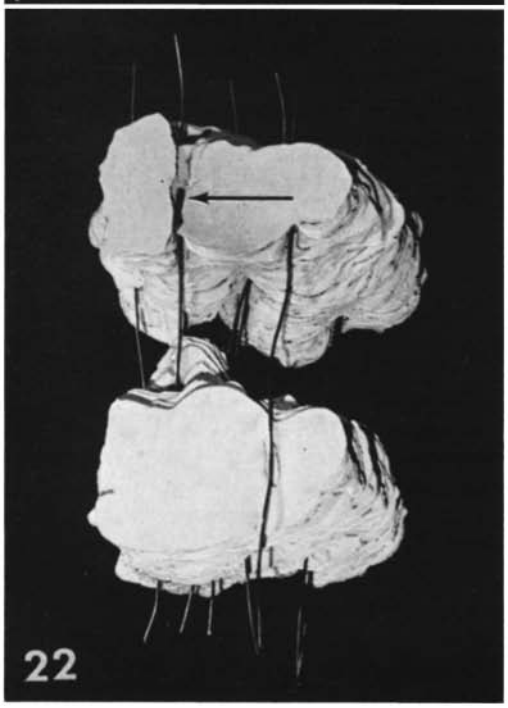


FIGURES 19 and 20 Early division nucleus. Fig. 19: Section corresponding to level 2 in diagrammatic Fig. 16. It shows an irregular invagination (*ii*), a cylindrical channel (*cc*), and the tubular connection (*tc*) between them. The nuclear envelope is indicated by arrows. Microtubules (*mt*) occur as bundles. × 23,500. Fig. 20: An irregular invagination (*ii*) containing several bundles of microtubules (1, 2, 3). In serial sections, the bundle at 1 was followed into a confluent cylindrical channel similar to D in Fig. 16. Note the intimate contact between membrane and chromosome (*c*) at arrow. Cf. Fig. 29. × 47,500.

bundles have a fixed distribution throughout the process of nuclear constriction.

Formation of Daughter Cells

After the separation of daughter nuclei, microtubule-containing cylindrical channels persist for a time in each nucleus and finally disappear completely. We have no information on the manner in which this occurs.



Even before the channels have disappeared from daughter nuclei, a membrane system begins to subdivide the cytoplasm. Fig. 25 shows an early stage of this process where a fold of two unit membranes appears to invade the cytoplasm of the dividing cells, defining a cleavage furrow. In the region of the furrow, the cell surface already consists of four unit membranes (arrow, Fig. 25). This subdivision results in the formation of separate daughter cells within the cyst wall.

Each new individual is surrounded by four unit membranes, but there are no indications of the amorphous thecal plates at the surface of these cells (Fig. 27, *om* plus *pm*). At this late stage, the flagella are of considerable length and completely confined within the cyst wall, the microtubules underlying the innermost plasma membrane have reappeared and the basket-like assembly of microtubules is present but not extensively developed. Thus, except for the thecal plates the daughter cells are completely formed while still encased within the cyst wall (Figs. 26 and 27). The amorphous layer of the cyst wall disappears, leaving only the two outer unit membranes (Fig. 27, *omc*). These membranes apparently later rupture to release the motile daughter cells.

Newly formed daughter cells collected soon after their emergence from the cyst wall are seen to have extremely thin thecal plates interposed between the two outer unit membranes and the two cytoplasm-bounding plasma membranes. In addition, sutures are again evident.

On the basis of our electron microscope observations, we can now summarize the remarkable ultrastructural changes which accompany nuclear and cell division in *Gyrodinium cohnii*. Division takes place within a cyst wall. A complex process of inviganation occurs at one side of the nucleus, re-

FIGURE 21 Model of the mid-division nucleus. Wires represent bundles of microtubules which run through cylindrical channels. At arrow, the microtubule bundle passes through the cytoplasm in the region of the nuclear constriction.

FIGURE 22 Model of the late division nucleus. Wires represent bundles of microtubules which pass from cylindrical channels in one lobe, through the cytoplasm, and into corresponding channels in the opposite lobe. A portion of the model has been removed to show a cylindrical channel (arrow).

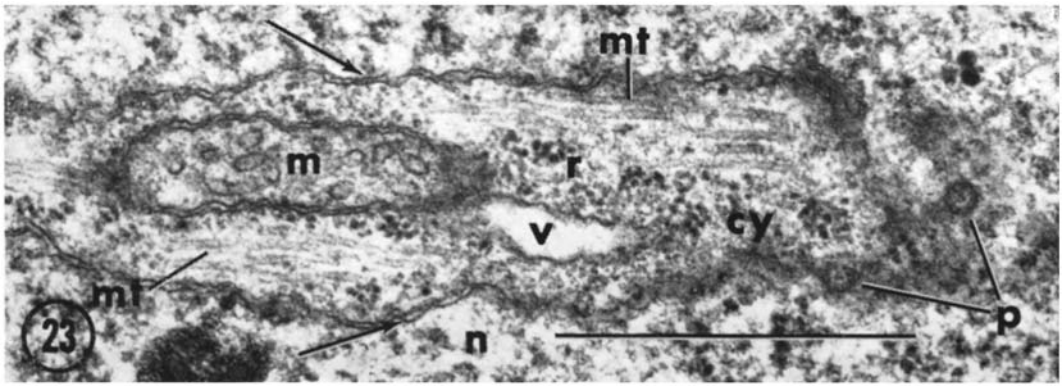


FIGURE 23 Longitudinal section through cylindrical channel in the late division nucleus. The cytoplasm (*cy*) within the channel contains mitochondria (*m*), vesicles (*v*), ribosomes (*r*), and microtubules (*mt*). The nuclear envelope bounding the channels (arrows) is a double structure with annulate pores (*p*); nucleus (*n*). × 47,500.

sulting in the formation of cylindrical channels of cytoplasm which traverse the dividing nucleus. The number of these channels is not constant; we have seen between 8 and 15 in various nuclei. In conjunction with the invagination, a new system of cytoplasmic microtubules appears, and a bundle of microtubules comes to lie in each cylindrical channel. As nuclear constriction proceeds perpendicular to the long axes of the channels, the microtubules maintain their relative positions. As a result, as the nucleus constricts, bundles of microtubules are visible in the cytoplasm between nuclear lobes, passing from a channel in one lobe to a corresponding channel in the other lobe. The microtubular bundles end a short distance beyond the nuclear limits. At no time do they converge or make contact with the basal bodies which are found at the periphery of the cell. After completion of nuclear division, the channels and microtubules disappear, and complete daughter cells are formed inside the cyst wall. The inner amorphous layer of the cyst wall disappears, and the outer cyst membranes rupture to liberate the motile daughter cells.

Chromosomes in Division

The purpose of any nuclear division process is to assure that each daughter cell receives a complete genetic complement, usually through a regular distribution of daughter chromosomes. It is not obvious how this might be accomplished in dinoflagellates. Since the nuclear membrane effectively separates chromosomes from the microtubules within the cytoplasmic channels, it is difficult to see how microtubules might be involved in moving these chromosomes in the way that spindle fibers are involved in mitotic chromosome movement.

The nuclear membrane is the only structure which appears to make direct contact with chromosomes during dinoflagellate nuclear division. In *G. cohnii*, it was noticed that often one end of a chromosome abuts on the nuclear membrane, the chromosome fibrils at this end making intimate contact with the membrane (Figs. 20 and 24, arrows). In order to determine whether such associations play a role in chromosome separation, we examined chromosome distribution and configuration by reconstruction from serial sections (Figs. 28 and 29).

In the nondividing nucleus, chromosomes are short rods; and, in 61 of the 99 chromosomes of this nucleus, one end touches the nuclear membrane.

In the early division nucleus we traced 120 complete chromosomes and found 46 rod-shaped and 74 V-shaped chromosomes. None of the rod-shaped chromosomes were associated with the nuclear membrane. However, in each V-shaped chromosome, the apex was directed toward the nuclear membrane surrounding a cylindrical channel; and, in 65 of the 74 V-shaped chromosomes, the apices made intimate contact with this membrane (Fig. 29). Such chromosome-membrane association was never found except in the region of cylindrical channels. This suggests that the chromosome-membrane association might be of significance in chromosome movement.

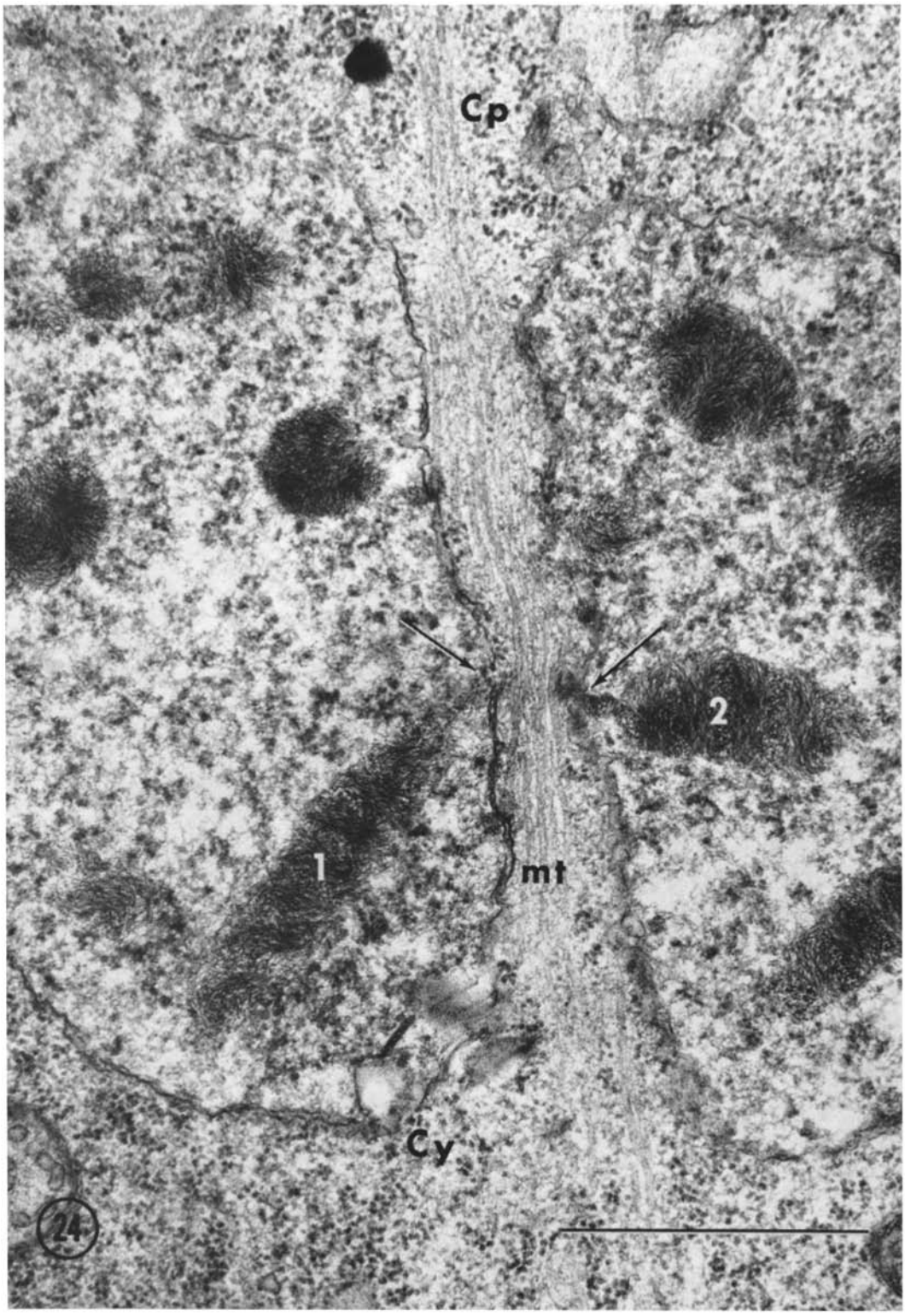
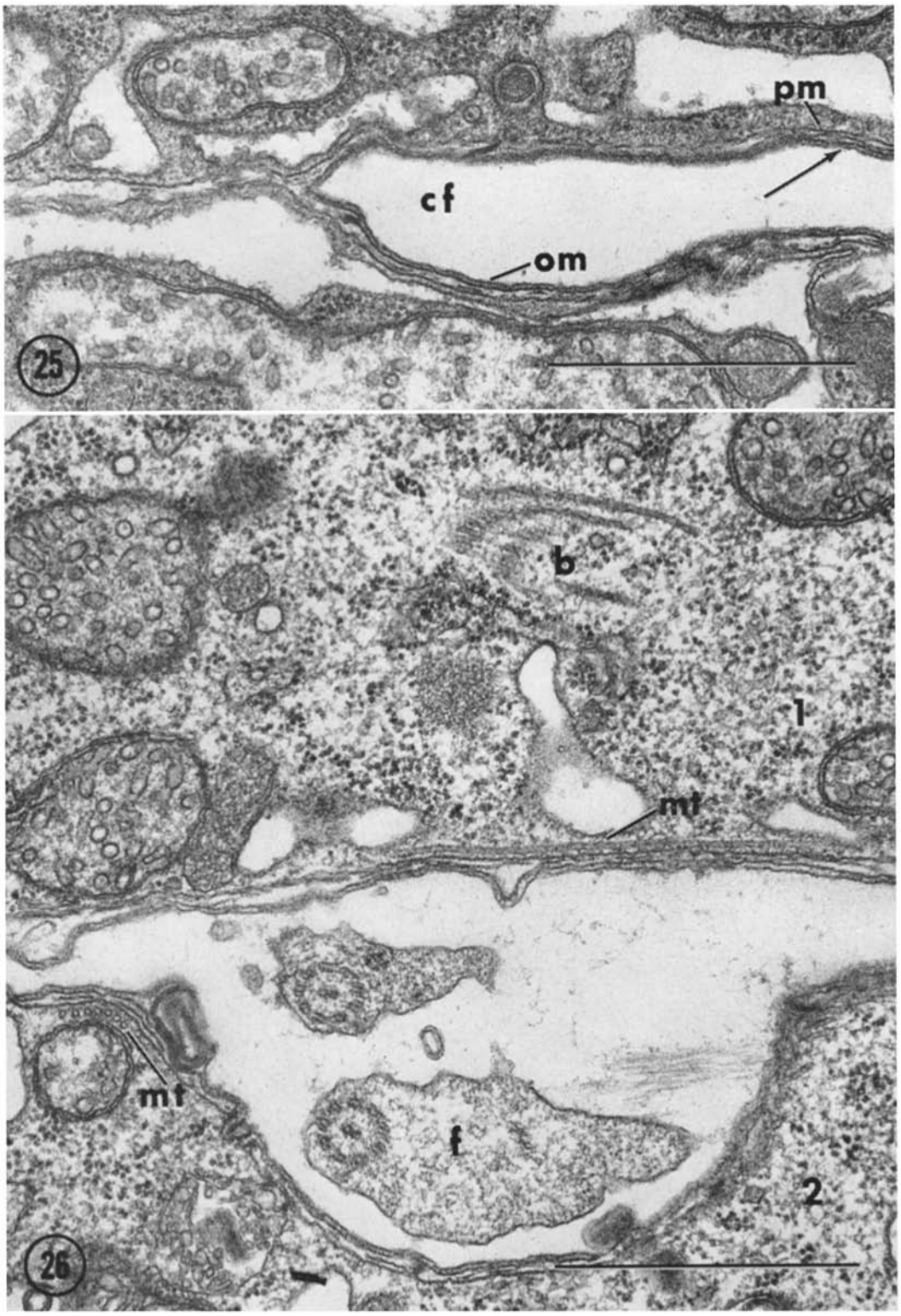


FIGURE 24 Longitudinal section through one lobe of the late division nucleus. Microtubules (*mt*) are continuous from the channel through the cytoplasm between nuclear lobes (*Cy*) and extend for a short distance into the cytoplasm at the polar end (*Cp*). Chromosomes (1, 2) make contact (arrows) with the nuclear envelope bounding the cylindrical channel. × 47,500.



FIGURES 25 and 26 Cytoplasmic division. Fig. 25: The cleavage furrow (*cf*). There are four membrane layers (arrow) in the region of the furrow, the two outer membranes (*om*) of the developing theca and the double plasma membrane (*pm*). × 47,500. Fig. 26: Two newly formed daughter cells (1, 2) within a single cyst. In the space between cells, well-developed flagella (*f*) are present. The microtubules (*mt*) underlying the plasma membrane and the "basket" microtubules (*b*) have reappeared. × 47,500.

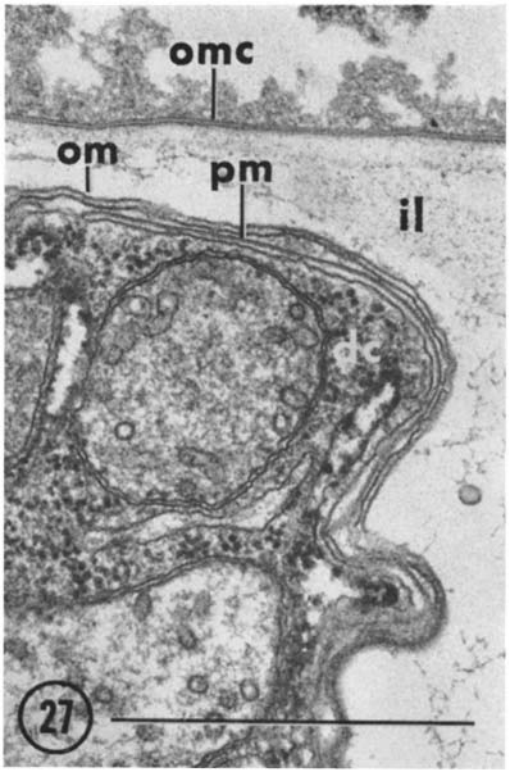


FIGURE 27 Cyst after completion of cell division. The two outer membranes of the cyst wall (*omc*) are intact although the inner amorphous layer (*il*) has dispersed. The newly formed daughter cell (*dc*) is bounded by four unit membranes, the two outer membranes (*om*) of the new theca and the double plasma membrane (*pm*). Thecal plates are not yet formed. × 47,500.

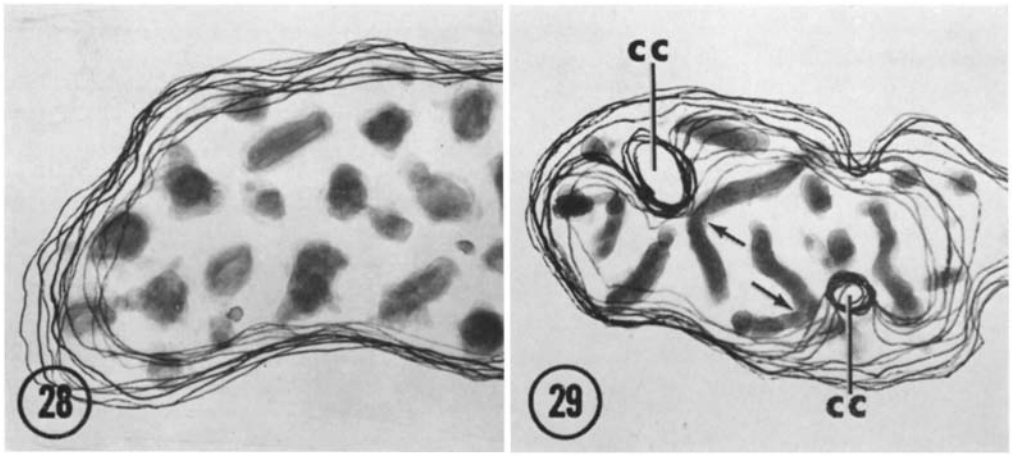
DISCUSSION

Protozoa are remarkable for the great diversity of their nuclear division processes (1, 15). Most of this variability, as Grell has pointed out, is the result of modifications in the usual mitotic organelles (15). Thus, nuclear division may be "closed" or "open," depending on whether or not the nuclear membrane persists during division, and "centriolar" or "acentriolar" on the basis of the presence or absence of centrioles (20). In all of these cases, the nuclear division must be regarded as a typical mitosis, daughter chromosomes being distributed equally to daughter nuclei with the aid of a spindle apparatus.

However, it has been recognized that in some protozoa, notably euglenoids, dinoflagellates, and radiolarians (1, 15), certain aspects of nuclear division make it difficult to consider the division simply as a mitosis with modified spindle apparatus. The observations reported in the present paper demonstrate that nuclear division in dinoflagellates differs fundamentally from typical mitosis and must be considered as a new type of nuclear division.

In itself, the persistence of the nuclear membrane throughout the dinoflagellate division is not remarkable. "Closed" nuclear divisions are found in a great variety of microorganisms (for references see 21). In most of these divisions, an intranuclear spindle is present and spindle microtubules make direct contact with the chromosomes. Even where an extranuclear mitotic apparatus is involved, as in *Barbulanympha*, the chromosomes appear to be connected to spindle elements despite the persistence of the nuclear membrane (5). In dinoflagellates, on the other hand, the microtubules which appear at the time of nuclear division are not assembled into a spindle, but instead are distributed in several cytoplasmic channels which traverse the nucleus. More remarkable is the fact that there are no indications of association between these extranuclear microtubules and the chromosomes. It is, therefore, unlikely that microtubules here play a direct role in chromosome movement comparable to their role in orthodox mitosis where spindle microtubules are attached to chromosomes.

Since microtubules are not directly involved in chromosome movement in dinoflagellates, we must search for an alternate mechanism that can distribute daughter chromosomes in a regular fashion. The V-shaped chromosome configurations seen in the dividing nuclei of dinoflagellates are throught to be formed as the result of a longitudinal separation of chromatids, the V apex representing the only remaining connection between daughter chromatids (14, 26). Thus, while the association between nuclear membrane and rod-like chromosomes in the nondividing nucleus (see also reference 2) might be fortuitous, the exclusive association between apices of V-shaped chromosomes and channel-bounding nuclear membrane in dividing cells might be directly related to chromosome separation. If, in the region of the V apex, each chromatid is individually attached to the nuclear membrane, a process of membrane growth or flow along the channels would effect the final separation and segregation of daughter chromosomes. A similar mechanism has been postulated to operate



FIGURES 28 and 29 Chromosomes reconstructed from serial sections. Fig. 28: Nondividing nucleus containing short thick chromosomes. Fig. 29: Chromosomes in the early division nucleus are longer and thinner. The apices of V-shaped chromosomes (arrows) are intimately associated with membrane surrounding cylindrical channels (*cc*). Cf. Fig. 20, arrow. Magnification is the same in both figures.

in the separation of genophores in bacteria (for discussion and references see 36).

This hypothesis requires that chromosomes be attached to the membrane for normal segregation of daughter chromosomes. Dodge and Godward (9) have concluded that dinoflagellate chromosomes do not possess such localized attachment regions. They based their conclusion on the finding that chromosome fragments produced in *Prorocentrum micans* by X-rays are always included in daughter nuclei. However, since the nuclear membrane remains intact during division (see also reference 26), any unattached chromosome fragments obviously must be included in one or the other of the daughter nuclei. Therefore, these experiments do not rule out the possibility that specialized attachment regions play a role in the *normal* segregation of daughter chromosomes.

It is generally held that a spindle apparatus is necessary to assure that daughter cells receive a complete genome except in cases where the entire genome is contained within a single genophore as is common in bacteria or where many complete genomes are contained within a single nucleus as in the ciliate macronucleus (15). However, a spindle-less, membrane-mediated segregation of genetic units could also provide the precision necessary for the equal division of a genome consisting of several genophores if there is a polarity assuring that daughter genomes move in opposite directions In bacteria which contain an episome (and, therefore, more than one genophore) the required polarity is assumed to be provided by the directional growth of the cell membrane to which genophores are attached (6). In the dividing dinoflagellate nucleus, a polarity obviously resides in the system of parallel cylindrical channels to which chromosomes are attached. If processes of membrane flow or growth along these channels move chromosomes, it is apparent that the nuclear division described by us for *G. cohnii* has the potential for the precise distribution of equivalent chromosome complements to daughter cells.

There are indications that microtubules are involved in the establishment and parallel orientation of the cytoplasmic channels which give the dividing nucleus its polarity. Microtubules are conspicuously associated with the channels as they are formed. Although we have not observed the earliest appearance of microtubules in preparation for division, Chatton's light microscope observations of the early stages of division in the *Blastodinium* trophocyte (4) (see below) suggest that the microtubules first appear as a compact parallel bundle on the surface of the nucleus and that their parallel relationship is maintained as the bundle penetrates the nucleus and is split up into the developing cylindrical channels. Thus, the orientation of the channels and, as a consequence, the polarity of the dividing nucleus would be determined by the original disposition of the microtubular bundle. Numerous examples are known

where microtubules appear to affect shape and orientation of cellular elements (for references see 29).

With few exceptions, investigators who have examined nuclear division of dinoflagellates with the light microscope concluded that a typical spindle apparatus is not present (for references see 7, 39). However, no agreement was reached as to the details of nuclear division. Chatton's description of division in the trophocyte of the parasitic dinoflagellate *Blastodinium* (4) is the only one which agrees with the results of our electron microscope investigations.

The *Blastodinium* trophocyte and its nucleus are many times larger than typical dinoflagellate cells and nuclei. This allowed Chatton to follow the detailed changes occurring during nuclear division in both living and fixed cells. At the beginning of division, a spindle-like structure appears in the cytoplasm at one side of the nucleus. This structure at first is appressed on the nuclear surface and then gradually sinks into the interior of the nucleus where it splits up, giving rise to a number of parallel cytoplasmic channels (plasmodendrites). When the nucleus divides, it constricts in a direction perpendicular to the channels.

Since he was not able to observe comparable details in the division of the smaller gonocytes or sporocytes of *Blastodinium*, Chatton did not consider the nuclear division of the trophocyte to be typical of dinoflagellates. However, the remarkable agreement between our ultrastructural findings in the free-living *G. cohnii* and Chatton's light microscope description of nuclear division in the trophocyte of *Blastodinium* indicates that Chatton discovered, without realizing it, the basic features of the unusual nuclear division characteristic of all dinoflagellates.

As was pointed out in the Introduction, dinoflagellates appear to be eukaryotes which retain certain prokaryote features of chromosomal chemistry and ultrastructure. Our findings indicate that flagellates may resemble prokaryotes also in the mechanism of segregation of daughter genophores. As in bacteria, membrane growth or flow might be involved. These findings provide further support for the suggestion that the dinoflagellate nucleus represents an intermediate type in the evolution of the typical eukaryote nucleus.

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