FINE STRUCTURE OF CELL DIVISION IN *CHLAMYDOMONAS REINHARDI*

Basal Bodies and Microtubules

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ABSTRACT

Cell division in log-phase cultures of the unicellular, biflagellate alga, *Chlamydomonas reinhardi*, has been studied with the electron microscope. The two basal bodies of the cell replicate prior to cytokinesis; stages in basal body formation are presented. At the time of cell division, the original basal bodies detach from the flagella, and the four basal bodies appear to be involved in the orientation of the plane of the cleavage furrow. Four sets of microtubules participate in cell division. Spindle microtubules are involved in a mitosis that is marked by the presence of an intact nuclear envelope. A band of microtubules arcs over the mitotic nucleus, indicating the future cleavage plane. A third set of microtubules appears between the daughter nuclei at telophase, and microtubules comprising the "cleavage apparatus" radiate from the basal bodies and extend along both sides of the cleavage furrow during cytokinesis. Features of cell division in *C. reinhardi* are discussed and related to cell division in other organisms. It is proposed that microtubules participate in the formation of the cleavage furrow in *C. reinhardi*.

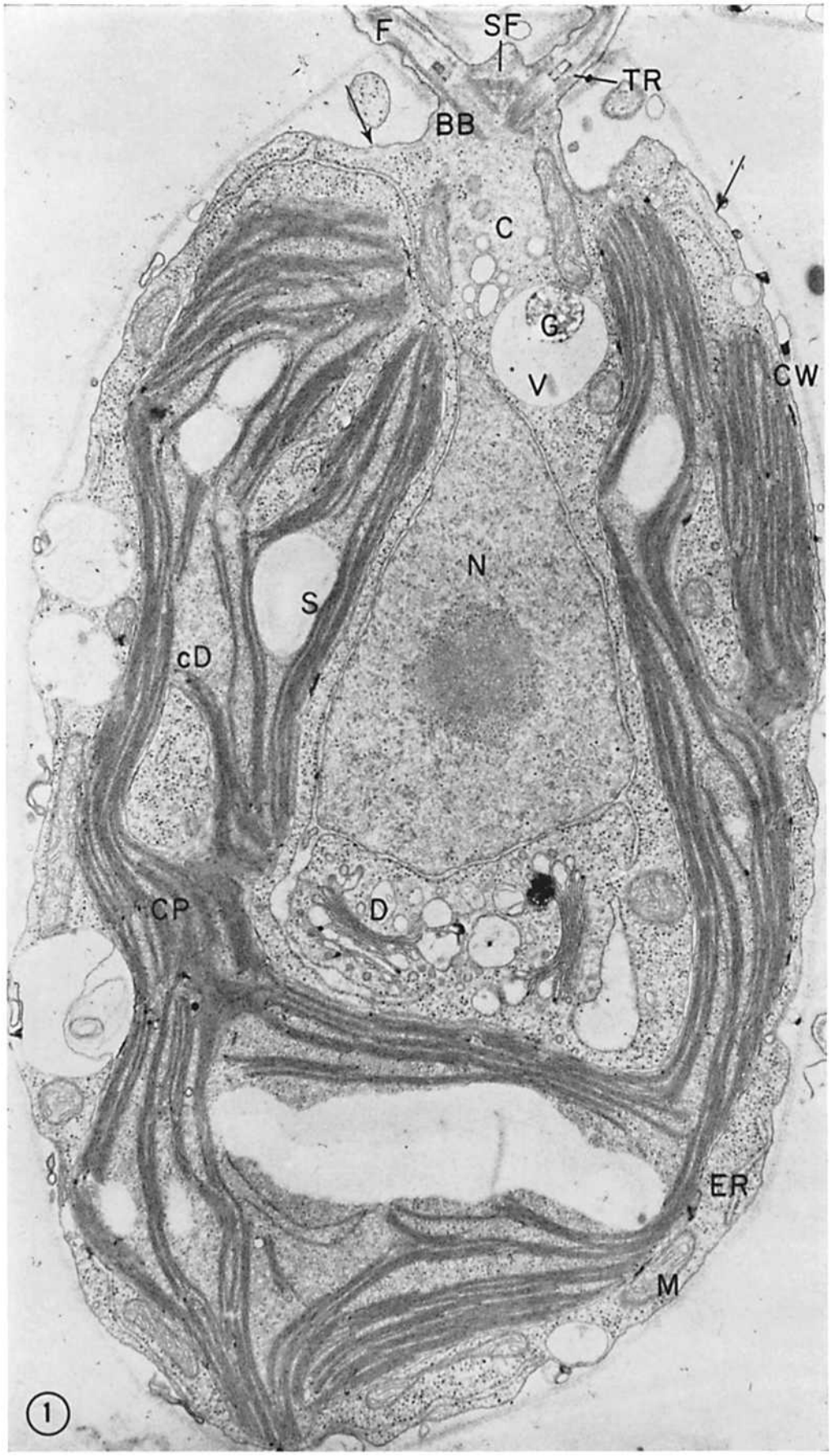
INTRODUCTION

Chlamydomonas reinhardi (Fig. 1) is a green, unicellular, biflagellate alga whose genetics and biochemistry are being investigated in a number of laboratories (7, 11, 12, 38). The interphase cell has been extensively studied with the electron microscope. Sager and Palade (39) and Ohad et al. (22) have described its general morphology, and Ringo (30) has presented a detailed description of its flagellar apparatus. The present study focuses on the fine structure of *C. reinhardi* during the course of mitosis and cell cleavage. It has become apparent that *C. reinhardi* exhibits patterns of nuclear division, basal body replication, and cytokinesis that are pertinent to a better understanding of these phenomena as they occur in other organisms.

MATERIALS AND METHODS

Cultures of wild-type *C. reinhardi* (strain 137c, mating-type minus) were grown on minimal medium (44) supplemented with 2% acetate in continuous light of 4000 lux on rotary shakers. They were harvested in the log phase of growth, 36 hr after inoculation.

Cells were centrifuged and resuspended in 5 ml of medium at room temperature. 5 ml of cold (~4°C) 4% gluaraldehyde, buffered in 0.05 M collidine, pH 7.5, were added to give a final glutaraldehyde concentration of 2%. The fixation vial was then cooled to 0°C in an ice bucket, and the ensuing fixation and dehydration steps were carried out at this temperature. Since microtubules are found to be well preserved after this procedure, it would seem that they are either insensitive to low temperatures in *C. reinhardi*, or that they are stabilized by the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glutaraldehyde while the temperature of the fixative is relatively warm so that the subsequent cooling does not depolymerize them (46).

After 2 hr in glutaraldehyde, cells were rinsed for 2 hr in several changes of the collidine buffer, postfixed in 1% OsO_4 in the same buffer for 1½ hr, rapidly dehydrated in an ethanol series and propylene oxide, and placed in an embedding mixture (modified after Mollenhauer, reference 20) containing 19.5% Araldite 6005, 29.5% Epon 812, and 51% dodecenyl succinic anhydride (DDSA) by weight, to which 1.5% DMP-30 is added. The material was left in DMP-containing monomer for a week to ensure adequate infiltration, with frequent changes to fresh monomer. It was then placed under vacuum at 80°C for 1 hr and polymerized in an 80°C oven for 2 days. Preservation of the cells is judged satisfactory with two reservations: the osmiophilic contents of chloroplast granules and the eyespot are extracted, and the membrane which surrounds vacuoles is often damaged.

Silver-gray sections were cut on a Porter-Blum MT-2 microtome with a diamond knife, placed on parlodion-covered 200-mesh grids, stained with uranyl acetate and lead citrate, and examined with an RCA EMU-3F or a Hitachi HU 11C electron microscope. Over 300 micrographs of dividing log-phase cells have been studied.

THE GENERAL FEATURES OF CELL DIVISION

At the onset of mitosis in *C. reinhardi*, the two original, flagella-associated basal bodies are found to be adjacent to two new basal bodies. The two original basal bodies lose their connections with the flagella, and the flagella degenerate. The four basal bodies are then distributed equally to the daughter cells at cytokinesis.

Four distinct sets of microtubules are involved in *C. reinhardi* cell division. The first set, termed the "metaphase band," lies just beneath the cell membrane and arcs over the surface of the mitotic nucleus in the region of the metaphase plate. The other three sets of microtubules, located in the cell interior, can be considered to lie roughly at right angles to one another. Spindle microtubules are found within the nucleoplasm of the dividing nucleus. At telophase, the spindle microtubules disappear and "internuclear microtubules," oriented at right angles to the spindle axis, appear between the daughter nuclei. "Cleavage microtubules" are found on both sides of the cleavage furrow that penetrates through the middle of the cell at cytokinesis. They lie at right angles to both the internuclear microtubules and the spindle microtubules.

After the original cell has divided in two, the process is usually repeated so that the two cells divide to give four cells. These four may, in turn, divide to give eight cells. All of these divisions involve similar arrays of microtubules, and all occur within the original mother wall. During the maturation stage that follows, each daughter cell forms new flagella and a cell wall. Finally, the small, motile cells break out of the mother wall.

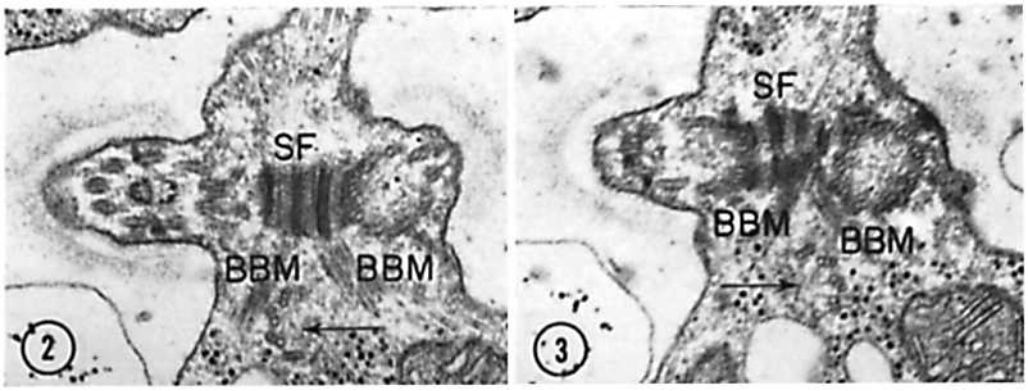
These events will be described in detail in the sections that follow, and will be discussed and interpreted at the conclusion of the paper.

OBSERVATIONS

Basal Body Replication

BASAL BODY REPLICATION DURING INTERPHASE: The basal apparatus of interphase *C. reinhardi*, as described by Ringo (30), is composed of two basal

FIGURE 1 Interphase *C. reinhardi* cell. The centrally located nucleus (*N*) contains a nucleolus. A large, single, cup-shaped chloroplast (*CP*) surrounds the nucleus. Regions of DNA (*cD*), starch grains (*S*), and ribosomes lie in the chloroplast stroma. The large starch plate at the basal end of the chloroplast surrounds a pyrenoid which is not included in this section. The endoplasmic reticulum (*ER*) is continuous at several points with the nuclear envelope and entends to the cell membrane (top of cell) and to dictyosomes (*D*). Ribosomes are attached to the ER except on the surface facing the dictyosomes, and they are abundant in the cytoplasm. Vacuoles (*V*), often containing dense granules (*G*), are also present in the cytoplasm. Mitochondria (*M*) are frequently adjacent to the chloroplast envelope. Elements of the contractile vacuole system appear at *C*. Arrows point to basal-body band microtubules that approach the two basal bodies (*BB*) at the anterior end of the cell. A striated fiber (*SF*) connects the basal bodies, and above the basal bodies are the platelike transition regions (*TR*). Two flagella (*F*) emerge through channels in the cell wall (*CW*). × 20,000.



FIGURES 2 and 3 Serial sections through the anterior end of an interphase cell. Two mature basal bodies are connected by a striated fiber (*SF*). The third basal body, lying between two basal-body bands of microtubules (*BBM*), is indicated by arrows. × 48,700.

bodies that are joined by striated fibers (Figs. 1–3, *SF*), and a system of 16 microtubules, grouped into four, four-membered bands, that forms a network beneath the cell surface and radiates from the basal bodies (Fig. 1, arrows; Figs. 2 and 3, *BBM*). These groups of microtubules will be referred to as "basal-body bands" in this paper.

Basal-body replication in *C. reinhardi* can apparently occur during interphase. Ringo (30) has studied *C. reinhardi* from synchronized cultures that are fixed during a period when cell division does not occur, and he notes the occasional appearance of a third "additional" basal body lying adjacent to the two flagella-associated basal bodies. He reports that he has not found four basal bodies in a cell. In the present study, four basal bodies are seen only in cells that are in the process of division.

Immature third basal bodies appear to be much shorter than mature basal bodies, for a single section ($\sim$ 50 mμ thick) often does not include all of their triplet arrays of microtubules. An example of this point is given in Figs. 2 and 3, which show serial sections of the anterior end of an interphase cell. The two mature basal bodies are seen connected by a striated fiber (*SF*); the third basal body is represented by a crescent of triplets lying between two basal-body bands of microtubules (*BBM*). In Fig. 2, four well defined triplets of the third basal body are seen; these are absent in Fig. 3, whereas four other triplets are present. Presumably, then, the entire structure extends only over a few sections. It is possible that the crescent-shaped intracellular axonemes described by Outka and Kluss (23) were also produced by oblique sections of short, circular structures.

Stages in the assembly of third basal bodies have not been observed. However, it is likely that the pattern resembles the formation of new basal bodies in daughter cells, a process that is described in detail in a later section.

BASAL BODY REPLICATION AT THE TIME OF MITOTIC DIVISION: Two daughter basal bodies are found to be associated with the original basal bodies at the time of mitotic division. One of these daughters is perhaps the third basal body formed during interphase, but it has now increased in length so that it has the dimensions of a mature basal body. The other appears to be formed at the time of mitosis. Possibly, the assembly of the fourth basal body is an initiating event in mitosis, but no evidence supports this speculation.

The four basal bodies are grouped into two pairs (Fig. 4). It is not known whether each pair consists of an original and a daughter or whether the two original basal bodies remain associated and the two daughters become associated; however, by analogy with patterns of centriole and basal body replication in other organisms, the former hypothesis is considered more probable. The longitudinal axes of the two basal bodies in each pair lie at a slight tilt with respect to one another, such that when one is sectioned transversely the other is seen in oblique profile. This tilted orientation is also exhibited by interphase basal bodies (30).

Dissociation of Basal Bodies from Flagella

At the time of mitotic division, the basal bodies lose their association with their flagella. Fig. 5 depicts a flagellum in a divided cell. When Fig. 5 is compared with Fig. 1, it is clear that the region

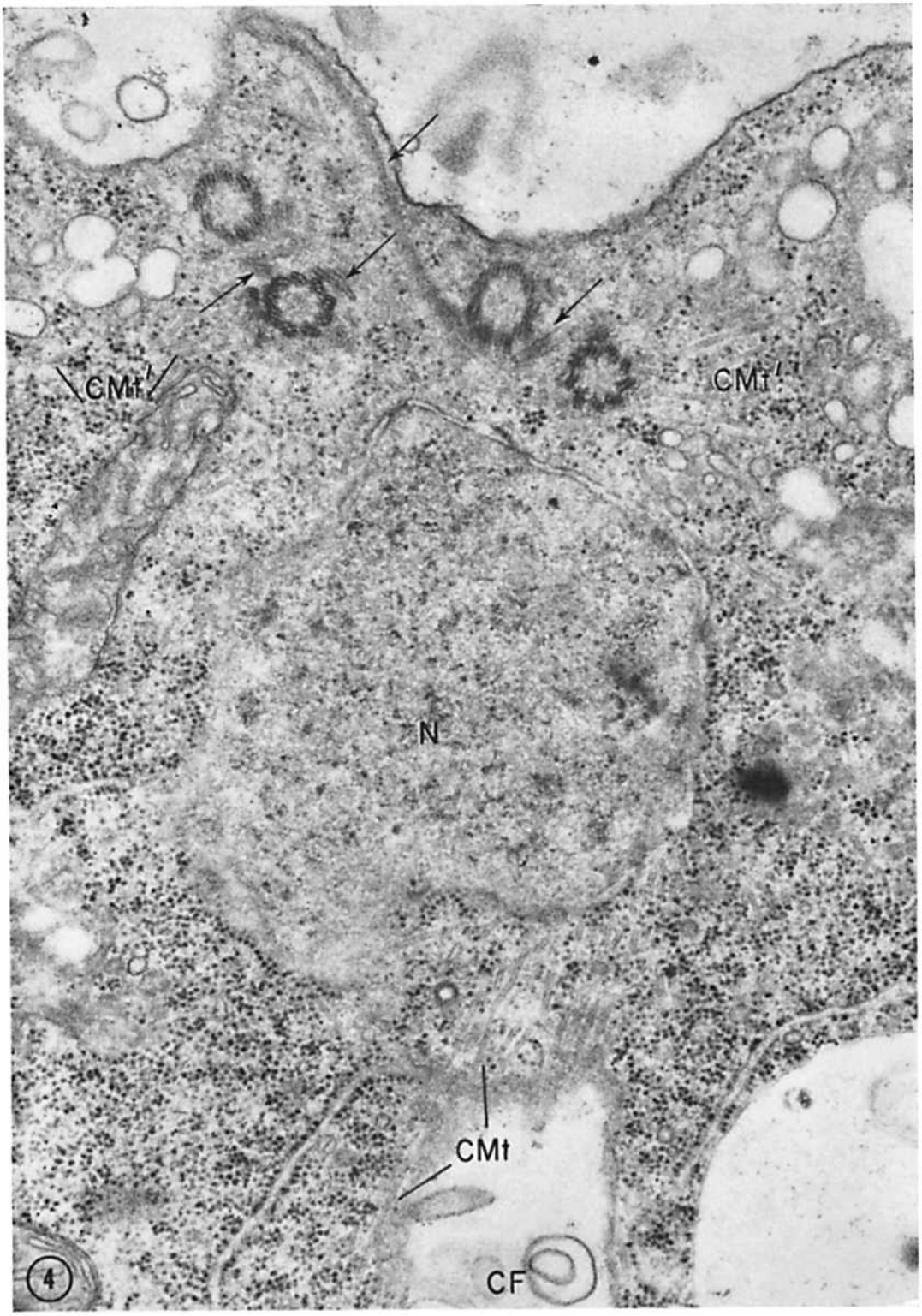


FIGURE 4 Tangential section through the anterior portion of a dividing cell. Four basal bodies lie above a nucleus (*N*) which is cut in grazing section. Arrows point to basal-body band microtubules. Other microtubules (*CMt* and *CMt'*) form part of the cell's cleavage apparatus (see text). Cleavage furrow is at *CF*. × 51,000.

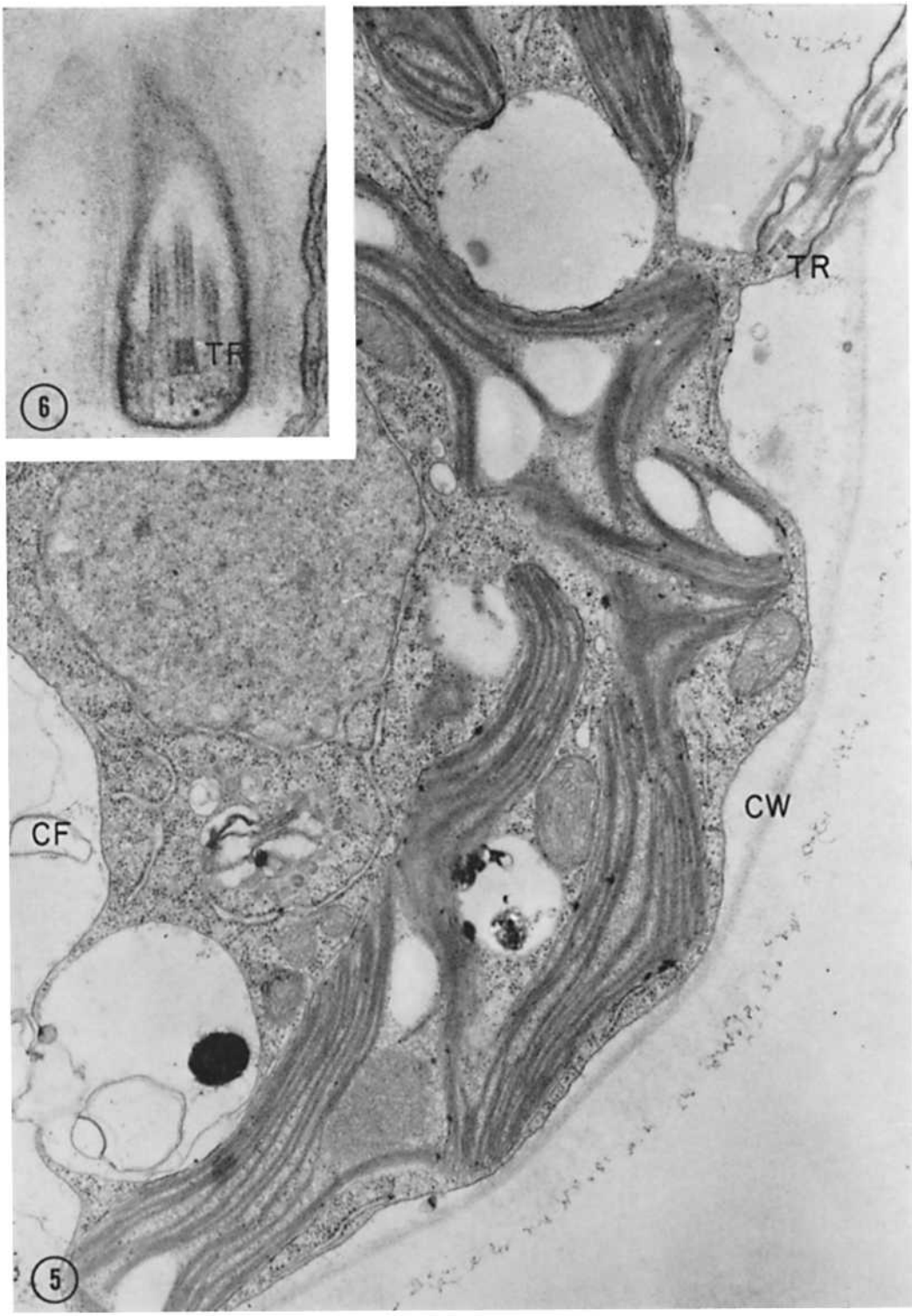


FIGURE 5 Daughter cell lying next to cleavage furrow (*CF*) and within mother wall (*CW*). A flagellum dissociated from its basal body lies within a channel in the cell wall and exhibits a transition region (*TR*). The chloroplast has rotated across the base of the flagellum. × 22,600.

FIGURE 6 Flagellum dissociated from its basal body and lying within a cell-wall channel. The flagellar membrane extends across the base of the transition region (*TR*), isolating the flagellum from the cytoplasm of the dividing cell. × 68,500.

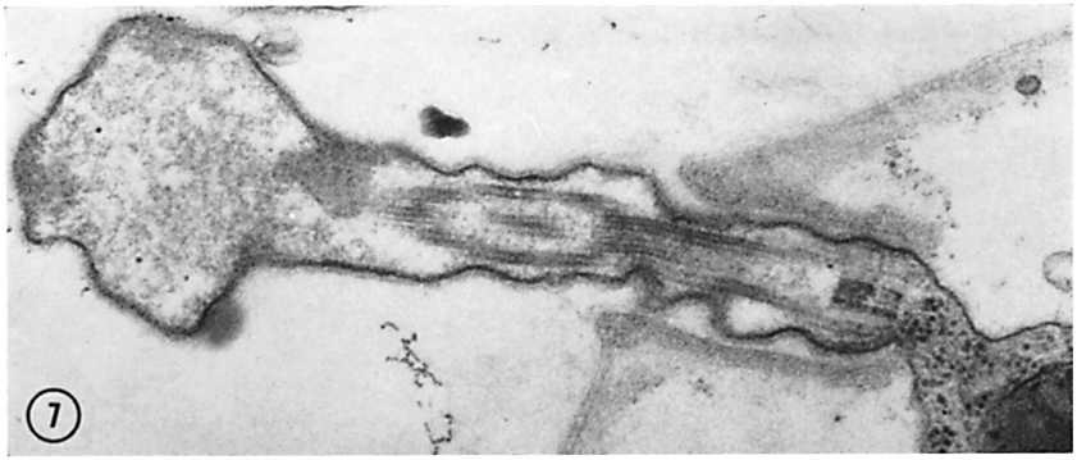


FIGURE 7 Higher magnification of the flagellum seen in Fig. 5, showing its degenerating end. × 47,200.

formerly occupied by basal bodies now contains cytoplasmic ground substance and ribosomes; such images are not produced when interphase flagella are sectioned at comparable angles.

Flagella that are not associated with basal bodies are often sealed off from the cytoplasm by a membrane (Fig. 6) and eventually discarded. In other cases, the flagellar membrane is seen in continuity with the cell membrane, and this continuity may persist throughout the course of cell division (Fig. 5). Fig. 7 shows at higher magnification the flagellum seen in Fig. 5. Although the flagellum is still in contact with the cytoplasm, it evidently is in the process of degenerating: its distal end is dilated and contains a mass of fibrous material that may represent depolymerized microtubules (45). It is known that the flagella of interphase *C. reinhardi* may be broken or removed by mechanical shearing and that they regenerate immediately; in such experiments, however, the basal bodies themselves are not damaged (31). Such observations suggest that a basal body is essential for the maintenance of a flagellum, for in the presence of a basal body a broken flagellum is regenerated, whereas in the absence of a basal body an intact flagellum degenerates or is discarded.

Dividing *C. reinhardi* cells are immotile (5). Present observations do not confirm a direct relationship between the presence of a basal body and flagellar motility, however, for the degenerate flagella may only secondarily become immotile.

It is of interest that a basal body detaches from its flagellum at the proximal end of the platelike transition region (Figs. 5–7, *TR*; cf. Fig. 1, *TR*), a point where the triplet-to-doublet transition occurs (30). This implies that the transition-zone structures are not integral components of a basal body and that they are assembled at the time a basal body gives rise to a flagellum.

Once the flagellar connections are lost, the four basal bodies are apparently free to move in the cytoplasm, although the two basal bodies comprising a pair maintain their defined, tilted orientation with respect to one another and move together, each pair connected by striated fibers. This movement is evident by comparing Figs. 2 and 4: in the interphase cell (Fig. 2) the third basal body lies close to the two flagella-associated basal bodies while in the dividing cell (Fig. 4) the two basal body pairs are seen to be much farther apart.

The basal bodies, devoid of any transition structures, are indistinguishable from centrioles. However, they are not found at the poles of the mitotic apparatus as are the centrioles of animal and certain plant cells. Rather, they are invariably found to lie next to the cleavage furrow at the anterior end of the dividing cell, one basal-body pair lying on either side of the furrow. Before the behavior of the basal bodies during cell division can be considered further, however, it is necessary to describe nuclear division and cytokinesis.

Nuclear Division

Mitosis in *C. reinhardi* has been studied with the light microscope by Buffaloe (5), who observed the classical stages of prophase, metaphase, anaphase, and telophase. The present study will focus

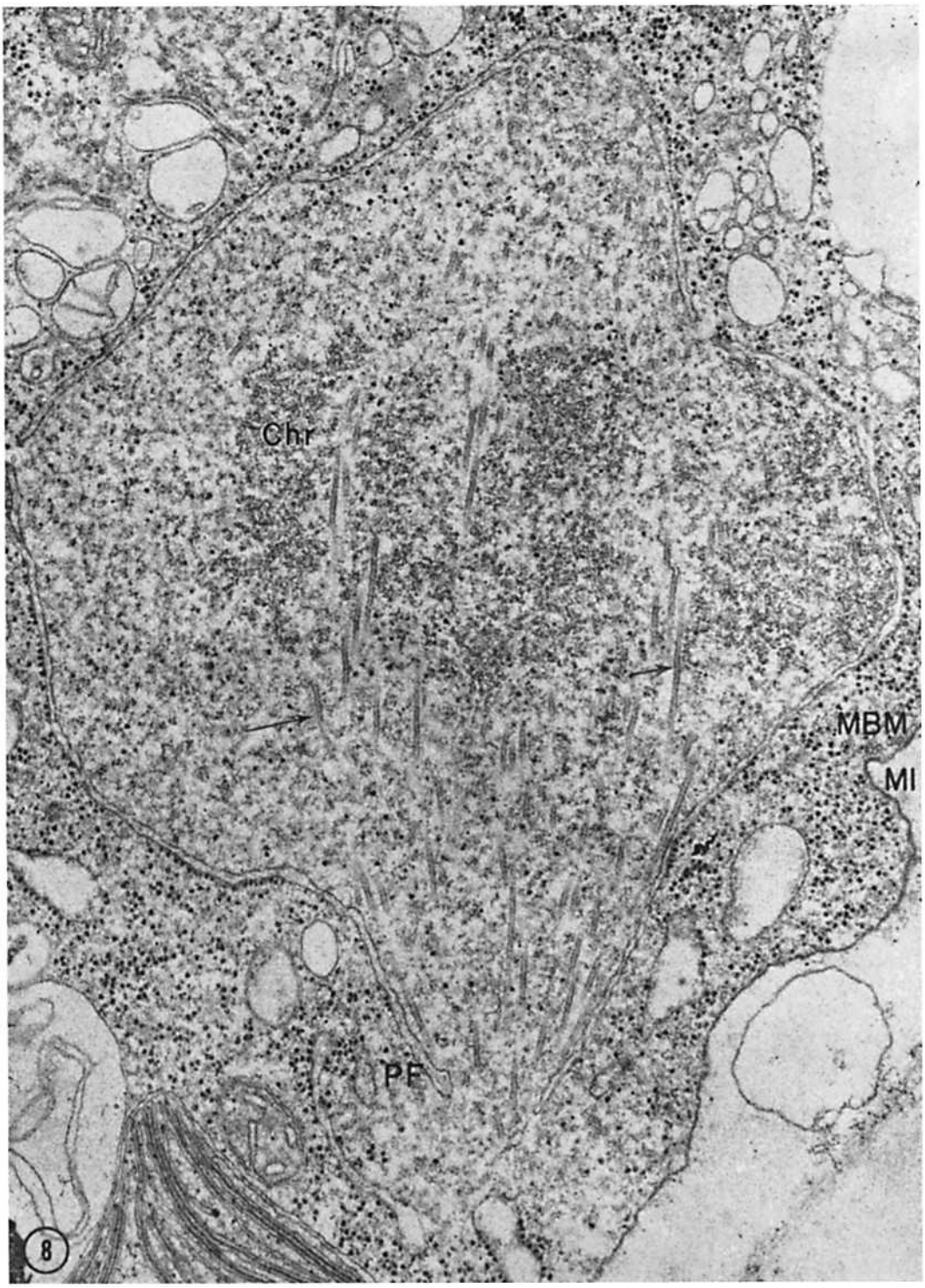


FIGURE 8 Late metaphase–early anaphase nucleus. Spindle microtubules (arrows) extend toward chromosomes (*Chr*) and toward a polar fenestra (*PF*). A membrane invagination (*MI*) and associated metaphase band microtubules (*MBM*) are seen. × 42,400.

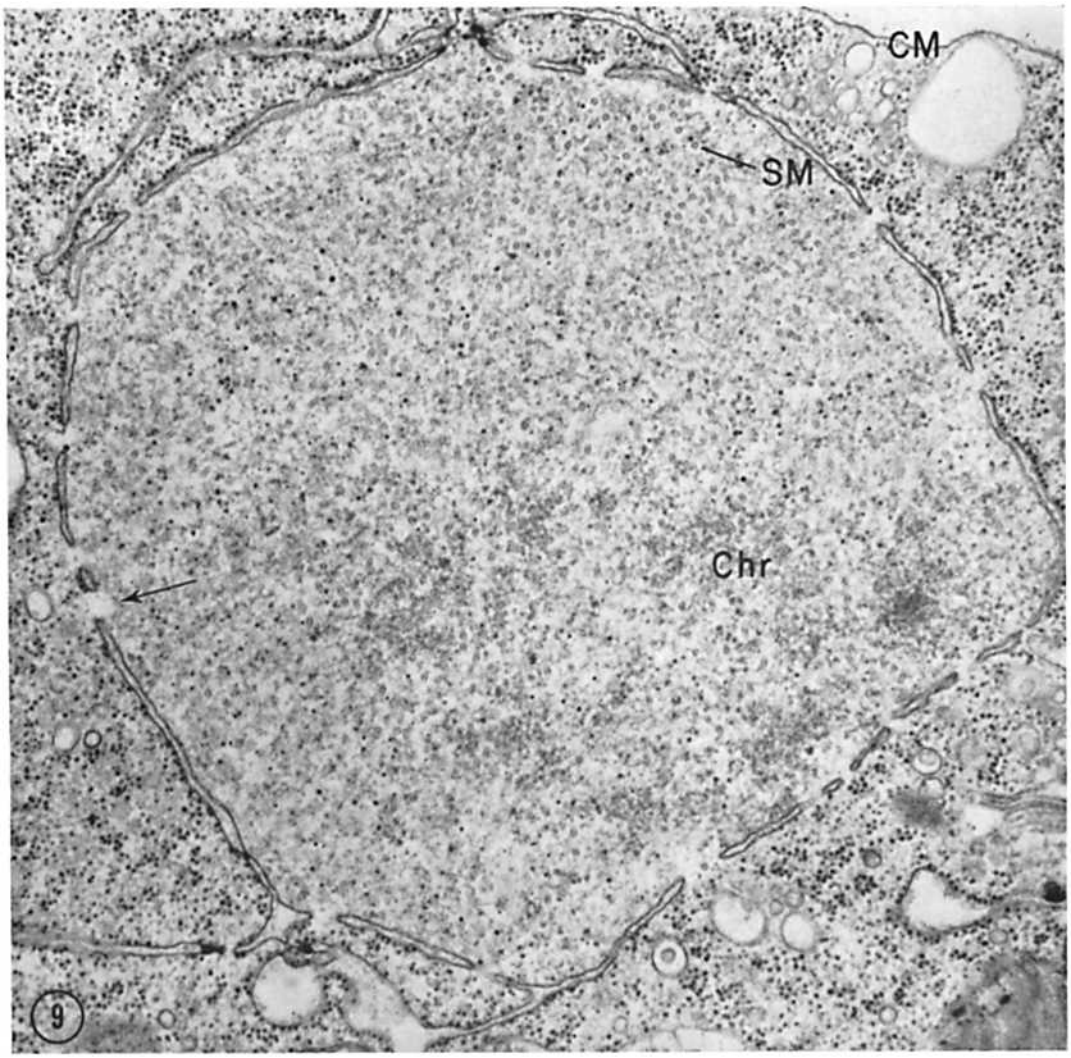


FIGURE 9 Cross-section of a dividing nucleus showing spindle microtubules in cross-section (*SM*) and chromosomes (*Chr*). Nuclear pores lack septa and are associated with clear regions of nucleoplasm and cytoplasm (arrow). The nucleus lies near the cell membrane (*CM*). × 36,300.

on aspects of nuclear division that are not seen with the light microscope.

Figs. 8–10 show, respectively, longitudinal, transverse, and oblique sections of dividing nuclei, to be compared with the interphase nucleus (Fig. 1). Several characteristics identify a dividing nucleus. The prominent nucleolus of the interphase cell has disappeared, and granules that resemble nucleolar components are dispersed throughout the nucleoplasm (Fig. 10, *Gr*). The nucleus assumes an irregular spindle shape at metaphase and early anaphase (Figs. 8 and 10): the condensed chromosomes lie within a large median bulge which tapers at either end. Spindle microtubules traverse the nucleoplasm and are oriented toward the poles. Within the nucleoplasm, they are directed toward the chromosomes (Figs. 8 and 10, *Chr*). No specialized kinetochore regions have been recognized in the chromosomes, and none were noted by Buffaloe (5).

The nuclear envelope does not break down during mitotic division. However, very large fenestrae

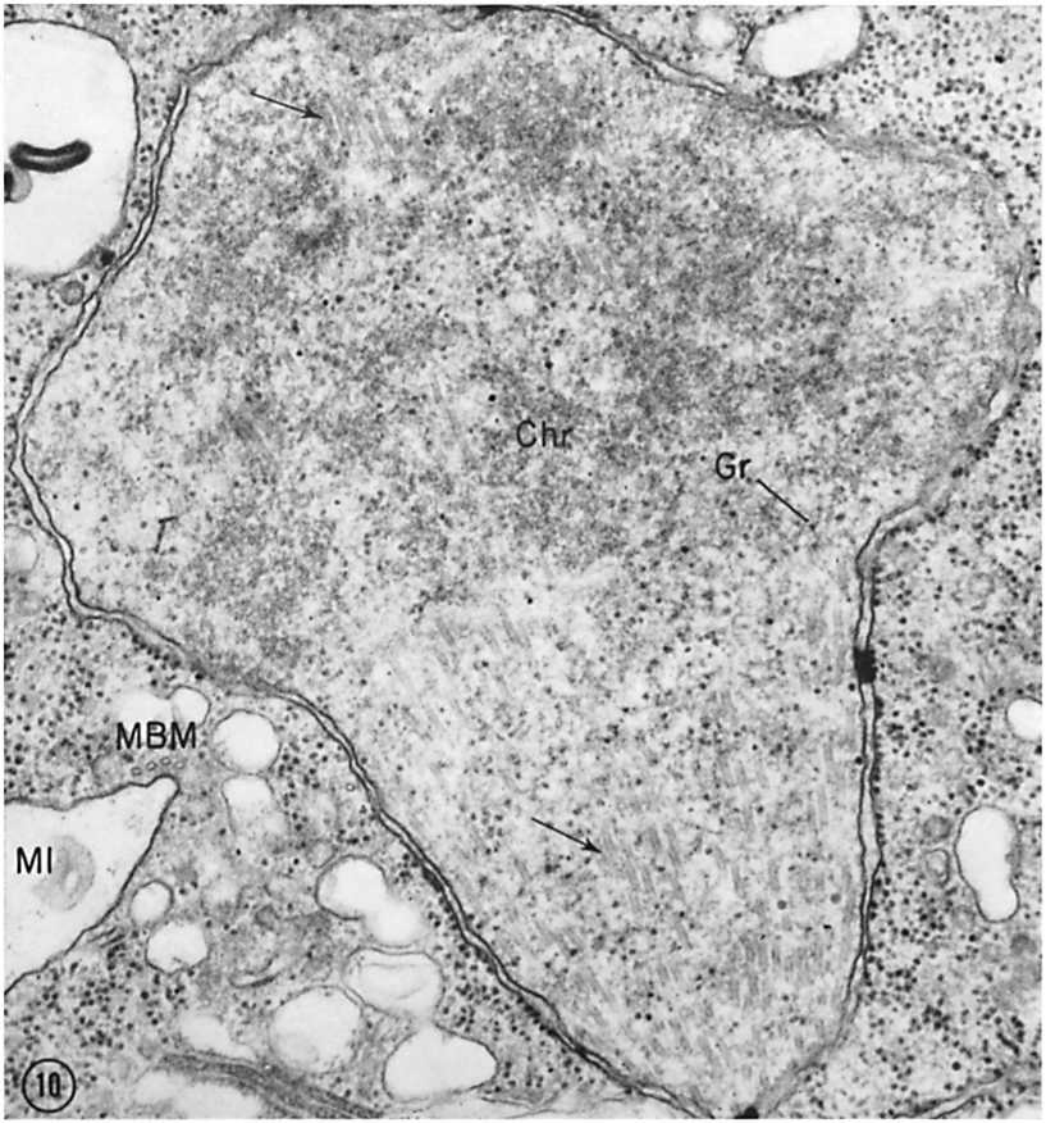


FIGURE 10 Oblique section of a dividing nucleus. Spindle microtubules (arrow) extend toward chromosomes (*Chr*). Granules (*Gr*) resembling nucleolar material are dispersed in the nucleoplasm. A membrane invagination (*MI*) and four metaphase band microtubules (*MBM*) lie parallel to the nuclear midline. × 53,500.

(300–500 mμ in diameter, as compared with the 60–80 mμ diameter of interphase nuclear pores) appear at both poles in a plane passing medially through the long axis of the nucleus. One such polar fenestra (*PF*) is seen in Fig. 8; since the section is slightly oblique with respect to the spindle axis, the opening at the opposite end of the nucleus is not visible in this micrograph, but two polar fenestrae have been seen in other dividing nuclei.

A specialized region of the cytoplasm surrounds the polar fenestrae. It is characteristically free of ribosomes and larger structures so that its electron opacity appears lower than that of the surrounding cytoplasm. Spindle microtubules appear to originate from (or terminate in) this specialized area, but we have encountered no evidence of any structure that might participate in spindle organization (cf. 33, 47). Dictyosomes and profiles of

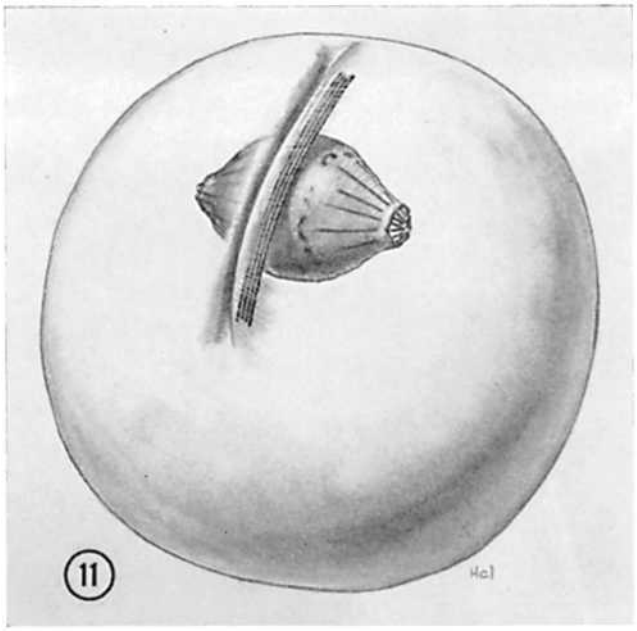


FIGURE 11 Diagram showing metaphase band microtubules, the associated surface invagination of the cell membrane, and their relationship to the mitotic nucleus.

endoplasmic reticulum lie just outside the area (Fig. 8) but, since similar membrane elements are disposed around the entire perimeter of the dividing nucleus, their association with the fenestrae cannot be regarded as significant.

The nuclear pores that are not located at the poles of the spindle retain an average 80 mμ diameter during mitosis. However, they, too, are modified (Fig. 9), for they lack the well defined septum that extends across the pores of nondividing nuclei (Figs. 1, 13, 14). The open pores usually appear electron-translucent compared with the adjacent nucleoplasm and cytoplasm (Fig. 9, arrow), suggesting that they may contain a distinct material in the living cell, possibly a material which is extracted during the fixation process.

One surface of the dividing nucleus is always found very close to the cell membrane (Figs. 8–10). Since the chloroplast or contractile vacuole system always lies between the nucleus and the cell membrane in any section of a mature interphase cell (Fig. 1), it follows that the nucleus must move toward the cell surface at the time of mitosis, and that the other organelles must somehow change their positions at this time.

The Metaphase Band

In any longitudinal (Fig. 8) or oblique (Fig. 10) section of a dividing nucleus, an invagination of the cell membrane (*MI*) is located near the nuclear surface and parallel to the nuclear midline, often just beneath the bulge in the spindle-shaped nucleus. A band of microtubules, usually a row of four, lies on one surface of this invagination (Figs. 8 and 10, *MBM*), and will be referred to as the "metaphase band."

In micrographs that include a longitudinal or oblique section of a mitotic nucleus, metaphase-band microtubules are seen either in transverse or in oblique section; when the microtubules are cut obliquely, their long axis lies roughly perpendicular to the spindle axis. These relationships suggest that the microtubules form an arc over the nucleus in the region in which the nuclear surface lies next to the cell surface, as drawn in Fig. 11.

Separation of the Daughter Nuclei

The nucleus elongates as the daughter chromosomes separate at anaphase and by late anaphase the chromosomes are appressed against the envelope at either end of the nucleus. The next stage we have found is shown in Fig. 12. Two daughter nuclei (N_1 and N_2) are present. Each still contains condensed chromatin (*Chr*), but septa (*S*) are now seen across the nuclear pores, and spindle microtubules have disappeared from the nucleoplasm.

A complex of rough-surfaced endoplasmic retic-

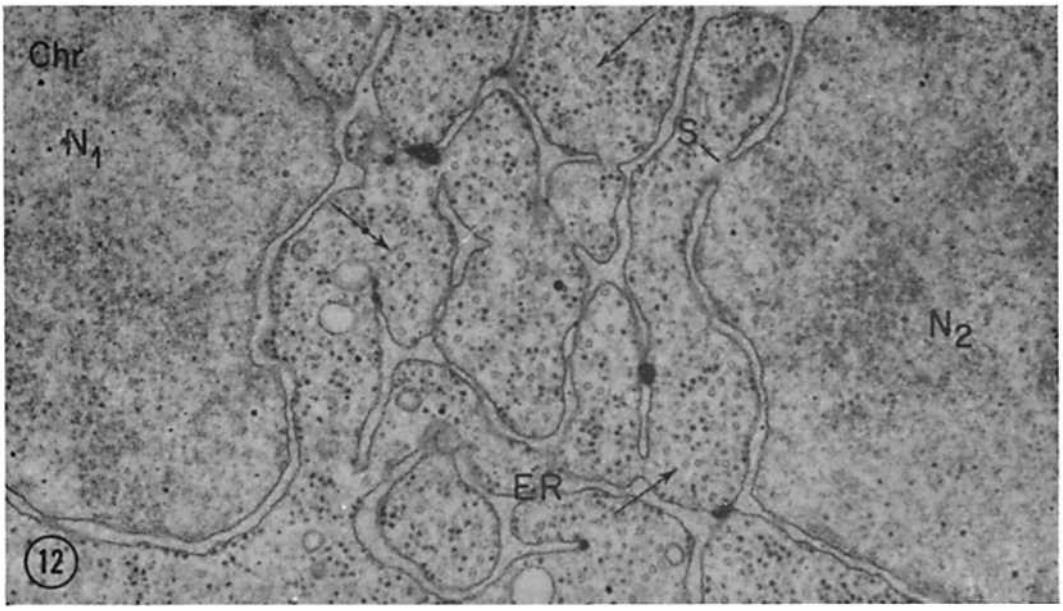


FIGURE 12 Telophase. Daughter nuclei (N_1 and N_2), containing condensed chromatin (*Chr*), are connected by a network of endoplasmic reticulum (*ER*). Internuclear microtubules (arrows) are seen in cross-section between the nuclei. Septa (*S*) now cover nuclear pores. × 43,200.

ulum (*ER*) connects the envelopes of the two daughter nuclei shown in Fig. 12. Serial sections of this cell have confirmed that the membranes are continuous from one nuclear envelope to the other. The possiblity has been considered that these ER connections result from a reduction in surface area of the two telophase nuclei relative to the anaphase nucleus such that there is a surplus of nuclear envelope at telophase. This does not appear to be the case, however, for the estimated surface area of a telophase nucleus is at least half the estimated surface area of an anaphase nucleus in the limited number of micrographs available. Rather, it appears that the nuclear envelope expands and infolds over the two separated sets of daughter chromosomes to form the two daughter nuclei, and that, as the two daughter nuclei move apart, this elaborated nuclear envelope appears in the cytoplasm in the form of endoplasmic reticulum.

A parallel array of microtubules, designated "internuclear microtubules," appears in the cytoplasm between the two daughter nuclei at telophase (Fig. 12, arrows). These microtubules are found only between the separating surfaces of the nuclei, and they are seen only in sections of dividing cells that contain nuclei, indicating that they occupy a restricted region of the cytoplasm and do not extend across the cell. They lie at right angles to the spindle axis; it is, therefore, unlikely that they are remnants of the spindle, i.e. comparable to a midbody or phragmoplast.

Cell Cleavage

ORIENTATION OF THE CLEAVAGE PLANE: Attempts to observe the cleavage process in living *C. reinhardi* cells have not been successful, for the cells rapidly become moribund under the high light intensities required to follow the cleavage of a 10-μ cell. The determination of the cleavage-plane orientation, therefore, has involved the study of numerous electron micrographs of cells sectioned at many angles. Since it is not feasible to reproduce all of these micrographs in this paper, many of the statements made in the following paragraphs will not be accompanied by illustrations.

When the cleavage furrow first appears, it is oriented in a plane that passes between the two flagella and through the anterior and posterior poles of the cell. Cleavage is apparently initiated in the region of the membrane invagination that lies next to the metaphase plate and that is associated with the metaphase band of micro-

tubules (Figs. 8 and 10). This invaginated region of the cell surface may lie to one side of the anterior end of the cell, as drawn in Fig. 11, so that furrowing is not necessarily initiated at the anterior pole. The furrow then grows in two directions: it extends laterally over the anterior surface and along the sides of the cell, and it penetrates medially into the interior of the cell. The lateral furrowing evidently occurs more rapidly than the medial furrowing, so that by a late stage in cytokinesis the furrow is seen extending into the cell interior from all sides, as drawn in Fig. 15.

The above paragraph describes the orientation of the cleavage plane at the onset of cytokinesis. However, it appears that during the course of cytokinesis there is a rotation of the cytoplasm so that the final orientation of the furrow with respect to the cell axis is roughly at right angles to its original orientation (5). One may see this rotation by following the position of certain organelles during cytokinesis. The nucleus lies next to the cell surface during mitotic division (Figs. 8–10), but by the end of cytokinesis it is located deep in the cell interior (Fig. 5). The original position of the flagella at the anterior end of the cell is marked by flagellar channels in the cell wall (Fig. 1) or by a degenerating flagellum (Fig. 5); these structures are found to lie ~45° from the final furrow separating the two daughter cells (Fig. 5), whereas originally the cleavage plane passes between the two flagella. In Fig. 5, the chloroplast is seen to extend across the base of the flagellum, an image that is never encountered in sections of interphase cells (Fig. 1), indicating that the cup-shaped chloroplast also rotates during the course of cleavage. The process of furrowing, then, appears to involve an extensive movement of cytoplasm.

In the sections that follow, the term "anterior end of the furrow" will refer to the margin of the cleavage furrow that exhibits the four basal bodies. It should be borne in mind that because of the cytoplasmic rotation the anterior end of the furrow will not necessarily coincide with the anterior, flagella-bearing pole of the interphase cell, except at early stages in the cleavage process.

CLEAVAGE MICROTUBULES: An array of microtubules appears to be associated with the furrowing process, in that it is located on either side of the plane of cleavage. These will, therefore, be referred to as "cleavage microtubules," and the body of microtubules will be denoted the "cleavage apparatus." Figs. 13 and 14 depict roughly median longitudinal sections normal to the cleavage plane. In Fig. 13, an early stage in cytokinesis, internuclear microtubules (arrows) are seen in cross-section between the daughter nuclei. The cleavage microtubules (*CMt*) generally lie at right angles to the internuclear microtubules but in the same plane, i.e. the plane of the cleavage furrow. They line the nascent cleavage furrow (*CF*) and extend into the cytoplasm, apparently passing between the internuclear microtubules. It should be noted that the pattern of cell cleavage, manifested by the cleavage microtubules, is established well before the furrow penetrates into the cytoplasm.

A more advanced stage of furrowing is seen in Fig. 14. The daughter nuclei, now containing well formed nucleoli, are divided between the two daughter cells, but a few internuclear microtubules (*IMt*) persist. The length of the cleavage microtubules (*CMt*) is evident in this micrograph; it is possible that a single microtubule extends the full length of the furrow.

ORIENTATION OF THE CLEAVAGE APPARATUS: When cleavage microtubules first appear along the cleavage plane their orientation with respect to one another appears to be somewhat irregular (Fig. 13), but as furrowing proceeds they become aligned along the surface of the furrow, such that in longitudinal sections normal to the cleavage furrow (Fig. 14) they lie nearly parallel to the furrow membrane. However, in cross-sections through the cleavage furrow itself (i.e., sections through the middle of a dividing cell which include both faces of the furrow), it is seen that the microtubules are not all parallel to one another. Rather, they appear to radiate, so that when the microtubules that line the furrow in the central region of the cell are cut in cross-section, the microtubules on either side are sectioned obliquely. The radiating microtubules converge toward the anterior end of the furrow.

As noted earlier, four basal-body bands of microtubules extend beneath the cell membrane in interphase cells. Ringo (30) has also noted the infrequent appearance of "additional" microtubules in the anterior region of the interphase cell which are not members of the four basal-body bands. In the cleaving cell, many such additional microtubules are found beneath the cell's anterior

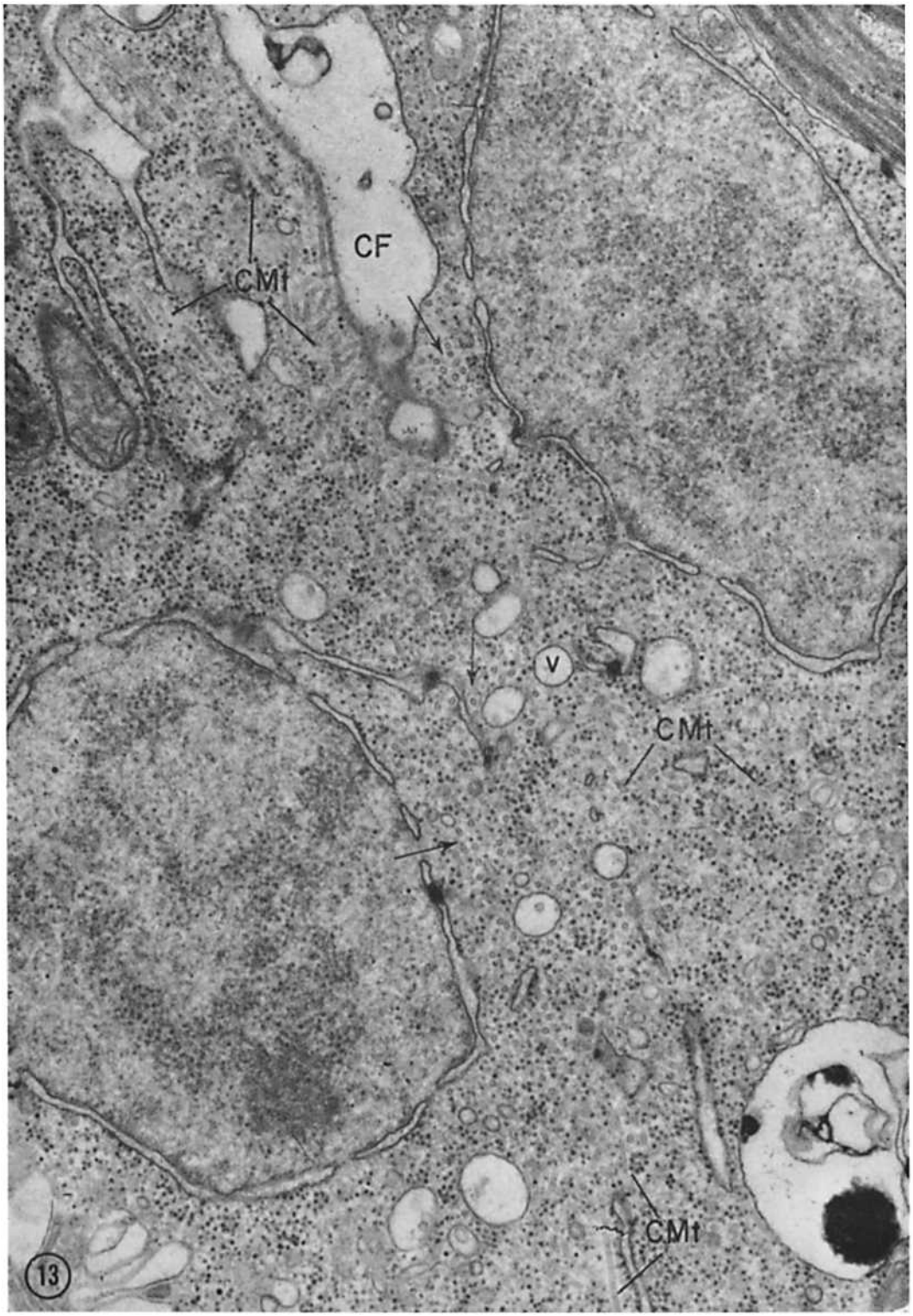


FIGURE 13 Early cleavage. Two daughter nuclei are separated by an array of internuclear microtubules (arrows), sectioned transversely. The nascent cleavage furrow (*CF*) is lined by cleavage microtubules (*CMt*) that extend deep into the cytoplasm. Small vesicles (*v*) lie along the plane of cleavage. × 41,000.

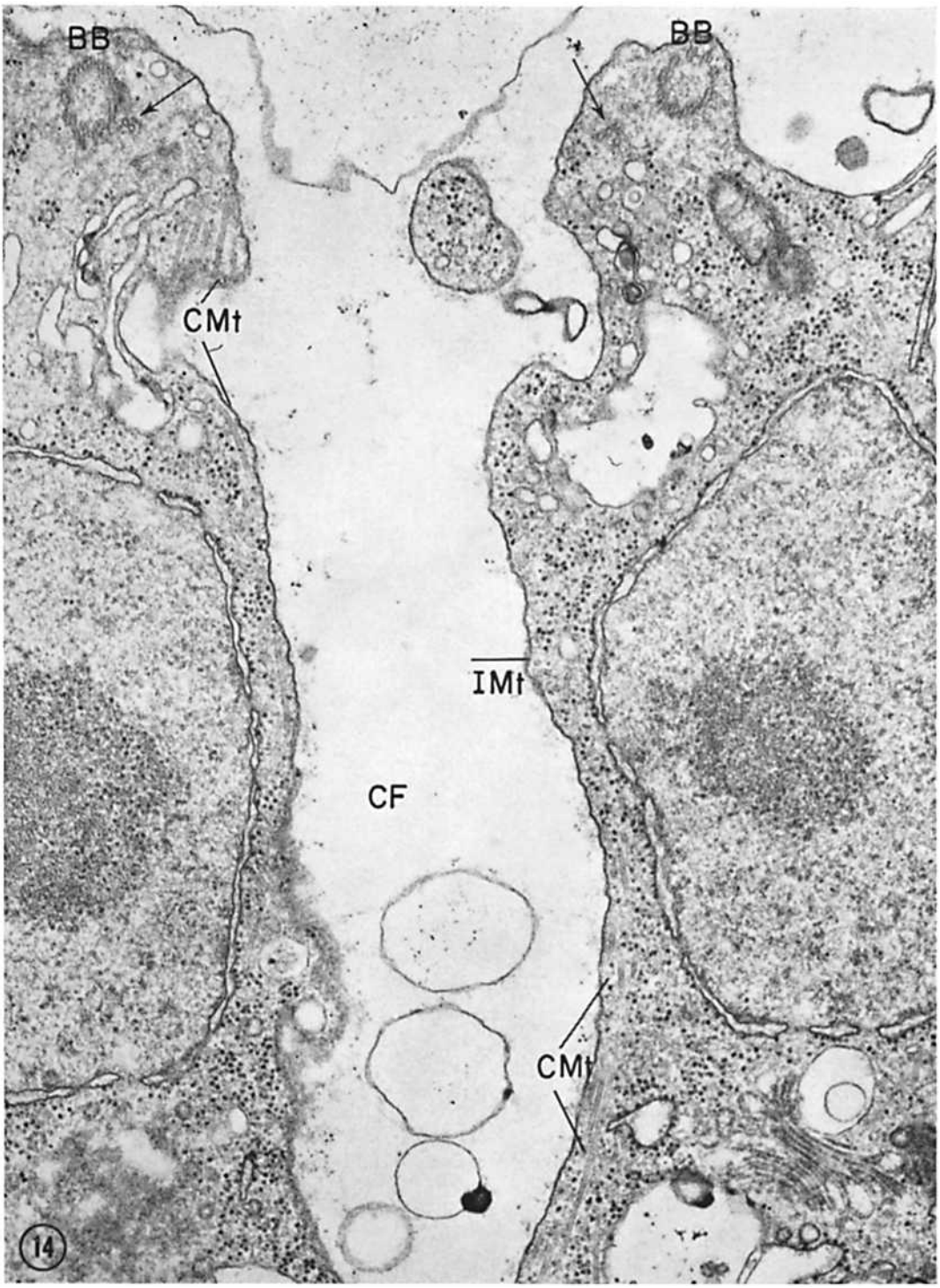


FIGURE 14 Mid-cleavage. Daughter nuclei now contain well formed nucleoli. A few internuclear microtubules (*IMt*) persist at this stage. Cleavage microtubules (*CMt*) line the cleavage furrow (*CF*). Two basal bodies (*BB*) lie at the anterior end of the cleavage furrow (the other two basal bodies, not included in this section, have been located in adjacent serial sections of this cell). Arrows point to basal-body band microtubules. × 52,000.

surface, and these are considered to be a part of the cell's cleavage apparatus.

The cleavage apparatus, then, consists of an array of microtubules that radiates beneath the cell membrane and converges toward the anterior end of the cleavage furrow, and a second array of microtubules that also converges toward the anterior end of the furrow but that radiates along either side of it. These relationships are drawn diagrammatically in Fig. 15.

BASAL BODIES AND CELL CLEAVAGE: The focal point of both sets of radiating cleavage microtubules appears to be the four basal bodies (Fig. 15) which, as noted earlier, lie in pairs on either side of the cleavage furrow and at the anterior end of the dividing cell. In Fig. 14, two of the four basal bodies are seen in this position (the other two appear in adjacent sections of this cell) and the cleavage microtubules are seen to be directed toward them.

The radiating orientation of the cleavage microtubules with respect to the basal bodies is apparent only when the cell is sectioned at certain angles. Figs. 4 and 16 depict two such cases. Fig. 4 shows a tangential section of the anterior end of a dividing cell. The four basal bodies lie at the anterior pole, and the cleavage furrow (*CF*), penetrating the cell from the side, has not yet passed between them. Cleavage microtubules that line the furrow (*CMt*) and cleavage microtubules that radiate beneath the cell surface (*CMt'*) are directed toward the basal body pairs. In Fig. 16, the plane of section coincides with the plane of cleavage so that one side of the furrow is seen in face view. Cleavage microtubules (*CMt*) clearly radiate toward the basal-body pair.

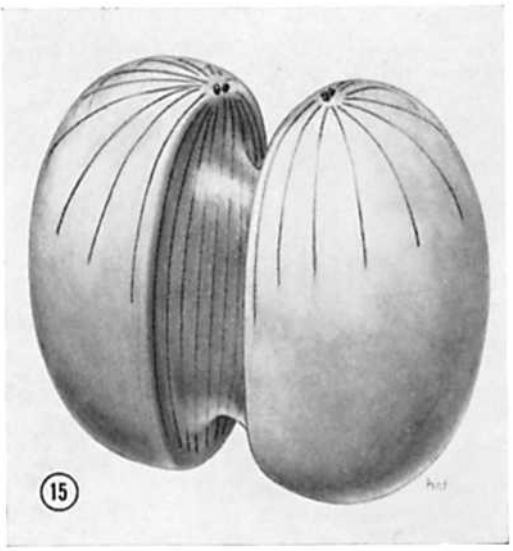


FIGURE 15 Diagram of the disposition of the microtubules in the cleavage apparatus during first cleavage. Four basal bodies are drawn at the anterior end of the furrow. The furrow is shown penetrating into the cell interior from all sides.

The basal-body bands associated with the basal apparatus during interphase retain this association even when the basal bodies are free of flagella and associated with the cleavage microtubules. The basal-body bands can be distinguished from cleavage microtubules both by their greater electron opacity and by their characteristic configuration as they approach the basal bodies, as described by Ringo (30). In Figs. 4 and 16, the basal-body bands (arrows) appear denser than the cleavage microtubules; in Fig. 14, they are recognized as a dense pair of microtubules with a flat plate of material above them and as a group of four microtubules in a three-over-one configuration (arrows). Since the bands are associated with each of the two basal body pairs in a dividing cell, they are presumably duplicated at the time the basal bodies are duplicated.

THE SOURCE OF MEMBRANE FOR CELL CLEAVAGE: The sum of the volumes of the two daughter cells is evidently the same as the volume of the original cell, for the two daughters fill the confines of the original mother wall at the end of division. The three-dimensional shape of *C. reinhardi* is roughly that of an ellipsoid (Fig. 15). It is evident that two ellipsoids having the same total volume as one ellipsoid will have a greater surface area. Therefore, new cell membrane must be added to the surface as furrowing progresses.

The source of new membrane in dividing *C. reinhardi* has not been clearly established. Small vesicles that resemble those formed in the dictyosomes are clearly brought into and aligned along the cleavage plane in some cells (Fig. 13, *v*), and in others ER cisternae are also present and exhibit a similar alignment (cf 14, 28). In many micrographs of cleaving cells, no clear ordering of membrane precursors is evident.

Division from Two to Four Cells

Nuclei undergoing a second division move to the cell surface adjacent to the mother wall. Again, a membrane invagination forms, a meta-

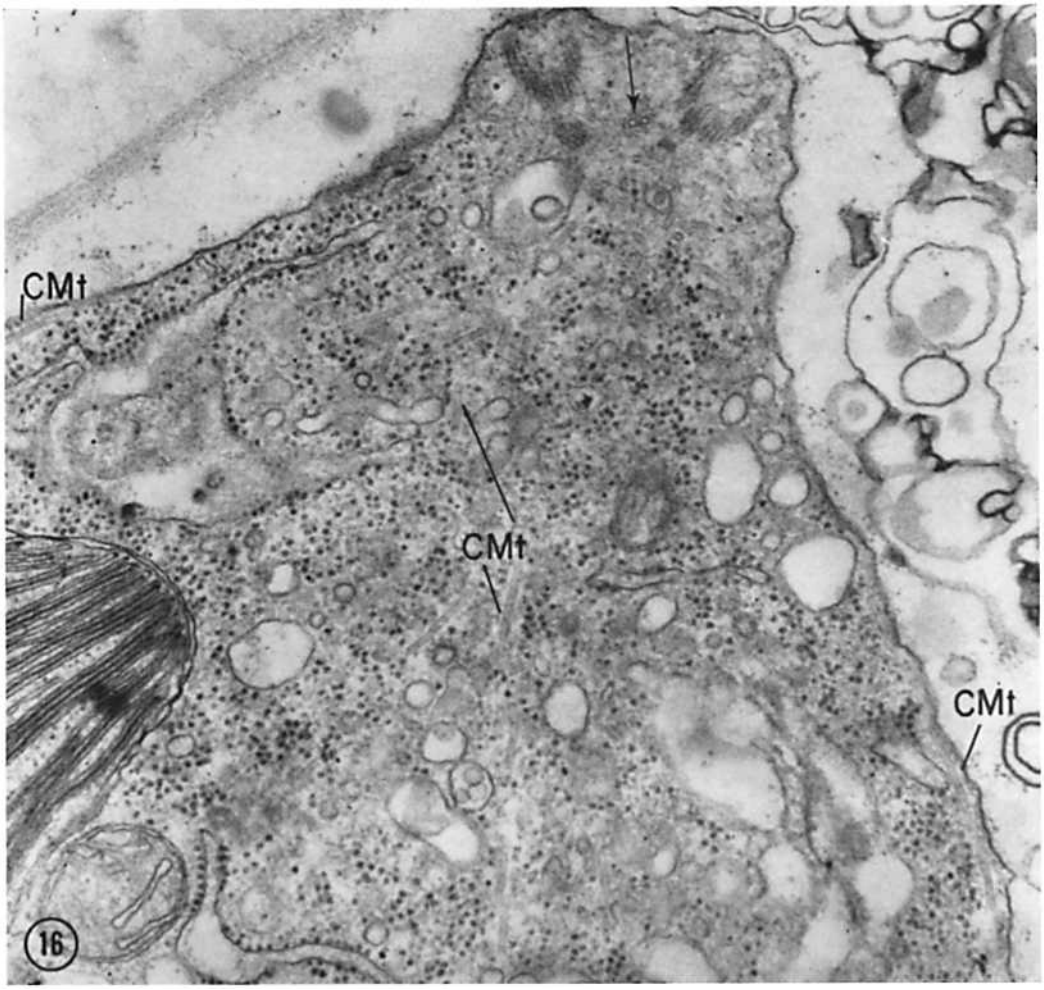


FIGURE 16 Section in the plane of the cleavage furrow. Cleavage microtubules (*CMt*) are directed toward a basal-body pair. Arrow indicates basal-body band microtubules. × 48,200.

phase band arcs over the nuclear midline, and nuclear separation and cleavage microtubules participate in cell division.

The plane of the second division appears to be generally at right angles to the first (5) so that effectively the original mother cell is divided into quarters. There are two distinct orientations to the second cleavage apparatus, however. In some micrographs, the second-cleavage microtubules that line the median furrow lie parallel to the first cleavage microtubules, but the second-cleavage plane is at right angles to the first; in this case, then, the daughter half-cells are divided longitudinally. In other micrographs, the second-cleavage microtubules lie at right angles to the first-cleavage microtubules, and the second-cleavage plane is also at right angles to the first; in this case, the daughter half-cells are divided equatorially. Fig. 17 illustrates these two alternatives.

The existence of these two classes of cleavage-microtubule and furrow orientations may reflect the fact that, once a cell has divided into two, there are two further possibilities: it can divide into four, or it can divide into four and then into eight. Possibly one orientation represents the plane of cleavage in a daughter half-cell that will undergo only one further division, while the other orientation is taken by daughter half-cells that will undergo two further divisions.

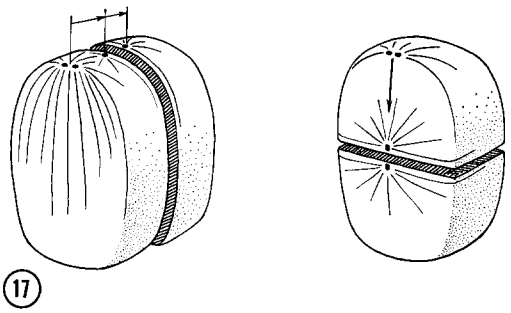


FIGURE 17 Diagram of the two observed planes of cleavage, longitudinal and equatorial, in division from two to four cells. Only one of the two daughter half-cells is shown in each case; the plane of the first-cleavage furrow is seen in face view. Arrows indicate the direction of migration of the basal bodies.

In both cases, the basal bodies lie in their characteristic focal position with respect to the cleavage apparatus. It is evident that the basal bodies must migrate to their new positions (Fig. 17, arrows) and that, in cases in which the daughter half-cell divides equatorially, the basal bodies must migrate a considerable distance. Cells have been fixed at stages when the basal bodies are indeed located between their position at one end of the first-cleavage furrow and their second-cleavage position in the middle of the daughter half-cell.

In some micrographs of dividing half-cells, two basal bodies are seen on either side of the furrow. Other micrographs indicate that only one basal body will go to each daughter, as we have drawn in Fig. 17. Serial sections of the latter micrographs would be required to demonstrate that a second basal body does not lie in an adjacent portion of the cell. However, numerous examples of developing basal bodies in mitotic tetrads have been encountered, and it is concluded that, at least in some cases, division from two to four cells precedes basal body replication and that a second basal body is assembled in the maturing daughter cells.

Basal Body Formation in Daughter Cells

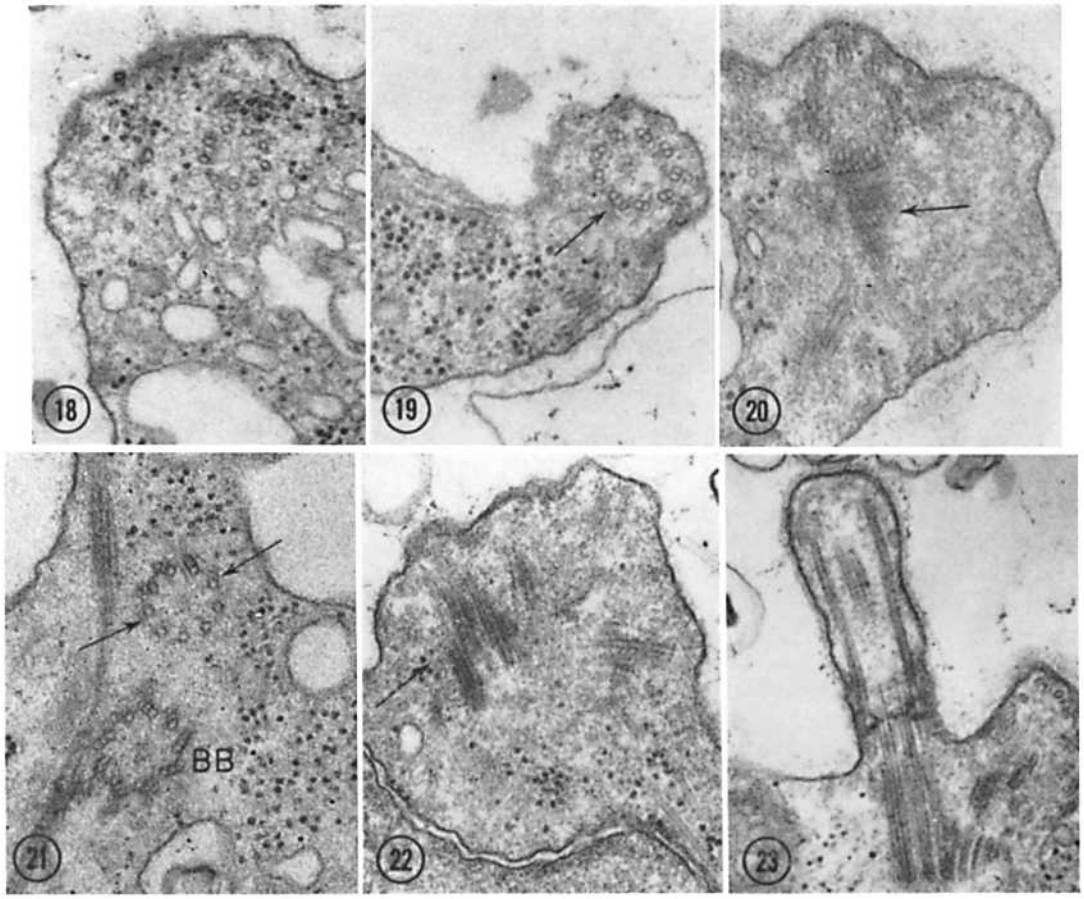
Stages in the formation of new basal bodies in daughter cells (Figs. 18–21) parallel those observed by Dippell (10) in the ciliary rows of *Paramecium*. The earliest stage we have encountered is a ring of nine singlet microtubules (Fig. 18) that are connected to "spokes" radiating from the center of the ring. A ring of nine singlets has also been observed in *C. reinhardi* daughter cells and zygotes by T. Cavalier-Smith (personal communication).

Additional microtubules appear to be added to this primary structure in an irregular manner. In Fig. 21, four singlets, four triplets, and a possible doublet form a ring of microtubules. In Fig. 19, eight doublets and a triplet are seen; it is unlikely that this is a section of a mature transition region since transitional filaments (30) are absent. Fig. 20 is interpreted to be a micrograph of a developing striated fiber. When Fig. 20 is compared with Fig. 2, the cross-striations appear to be much less compact. All of these new structures lie in a ribosome-free region of the cytoplasm. The nucleus has not been observed to be involved in any of the developmental stages (cf 23).

The diameter of a ring of nine singlets, when measured from the inner walls of its component microtubules, is ~135 mμ (Fig. 18). The inner diameter of developing (Fig. 19) and mature basal bodies is also found to be ~135 mμ in this species. However, the outer diameter, as measured from the outermost walls of the microtubules, increases from ~185 mμ in a ring of singlets to ~225 mμ in a mature basal body. Hence, the second and third microtubules of the triplet appear to be added to the outside of the "primary" singlet ring.

In Fig. 21, the microtubules of the developing and mature basal bodies are nearly parallel to one another. Third basal bodies in interphase cells also lie parallel to one of the mature basal bodies (30). These observations would suggest that one mature basal body somehow orients a new one, and that the tilted orientation of basal body pairs develops at a later time. In other daugther cells (Figs. 18–20), however, new basal bodies do not appear to be associated with any mature structures. If there is any one patterned relationship between old and new basal bodies in *C. reinhardi*, then, it is not apparent from this study.

Mature and developing basal bodies lie within a cytoplasmic bulge at the anterior end of the daughter cell (Figs. 18–21). They are eventually oriented toward the cell membrane (Fig. 22), and new daughter flagella are formed (Fig. 23). The flagellar membrane closely surrounds the growing axoneme; a "ballooning" of the mem-



FIGURES 18–23 Stages in the development of basal bodies and flagella.

FIGURE 18 Ring of nine singlets. × 59,200.

FIGURE 19 Ring of eight doublets and a triplet (arrow). × 60,000.

FIGURE 20 Developing striated fiber (arrow). × 60,500.

FIGURE 21 Ring of singlets, triplets, and possible doublets (arrows) adjacent to a mature basal body (*BB*). × 60,500.

FIGURE 22 Young basal body (arrow) oriented toward the cell membrane. × 52,200.

FIGURE 23 New daughter flagellum. × 51,200.

brane, as observed in *Tetramitis* (23), has not been encountered in *C. reinhardi*.

DISCUSSION

Nuclear Division

Electron microscope studies have shown that an intranuclear or "closed" (15) mitotic apparatus exists in certain Ciliates (6, 15, 36, 47), Euglenoids (41), parasitic protozoa (13, 48), amebae (35), Myxomycetes (1, 9, 40), Phycomycetes (16), Ascomycetes (33), and flagellated Chlorophytes (the present study). The presence of a "closed" mitotic apparatus does not appear to be a characteristic of all unicellular organisms, however, for an "open" mitotic apparatus has been demonstrated with the electron microscope in unicellular, biflagellate Chrysophytes (17, 18) and in the giant ameba (34).

It is apparent in the present study that the

existence of an "intact" or "dispersed" nuclear envelope may be a structural and not a functional distinction, for the spindle microtubules in *C. reinhardi* appear to extend into the cytoplasm through large dilations of the nuclear envelope. A similar picture is seen in *Plasmodium* (13). Some investigators (40, 48) have reported that the spindle microtubules in "closed" systems terminate on an intact inner nuclear membrane, but it is possible that oblique sections, similar to Fig. 10 in the present study, have given such an impression. Jenkins (15) emphasizes that the nuclear envelope remains intact during micronuclear division in *Blepharisma*, but, since the micronucleus reportedly does not exhibit pores during interphase in this organism, it may represent a special case.

A "mixoplasm" is classically thought to form when the nuclear envelope disperses at mitotic division (34). It is, of course, difficult to judge from electron micrographs the degree of nuclear-cytoplasmic interchange that occurs in dividing *C. reinhardi*, but it does not appear to be extensive. Septa disappear from the nuclear pores and large dilations appear at the poles, but the cytoplasm in contact with these nuclear openings is differentiated from the surrounding cytoplasm.

The Metaphase Band

The metaphase band is formed at the time of mitotic division and indicates the future plane of cleavage. It is thus analagous to the pre-prophase band of microtubules in dividing wheat cells (24, 25), although the metaphase band in *C. reinhardi* differs from this pre-prophase band in several ways: it consists of fewer microtubules; it does not completely encircle the cell; it is associated with a surface invagination of the cell membrane; and it persists throughout mitosis.

Since the metaphase band consists of four microtubules, the possiblity has been considered that the metaphase band is identical to one of the four-membered basal-body bands described by Ringo (30). However, they do not appear to be the same structures. The microtubules in a basal-body band diverge from each other as they leave the vicinity of the basal bodies, whereas the microtubules in the metaphase band are closely associated as they pass over the nuclear surface. Moreover, in daughter half-cells undergoing a second division, the metaphase band lies beneath the cell surface that faces the mother cell wall, whereas the basal bodies are often located at the opposite side of the cell (Fig. 17), i.e. at the cell surface created by the first-division cleavage furrow.

It has not been possible to determine the fate of the metaphase band at the conclusion of nuclear division, for at this time cleavage microtubules appear in the plane of the metaphase band, and the two sets of microtubules cannot be distinguished with any certainty.

Nuclear Separation

The fine structure of the telophase that follows a "closed" mitosis has been described in two Ciliates. In *Blepharisma*, a new micronuclear envelope is formed within the old envelope, and the old envelope is eventually shed (15). A different picture is seen in *Nassula* (47): the inner membrane of the envelope first closes around the two sets of daughter chromosomes, and the resultant daughter nuclei remain connected by their outer nuclear membranes, i.e. by endoplasmic reticulum. Telophase in *C. reinhardi* essentially parallels that in *Nassula*.

It is of interest that when *C. reinhardi* is mated the two nuclei in the zygote make initial contact by interconnections of the endoplasmic reticulum and subsequently fuse (U. G. Johnson, unpublished observations, and T. Cavalier-Smith, personal communication). Nuclear separation in this species thus appears to be the reverse of nuclear fusion, in this respect.

The internuclear microtubules that appear between daughter nuclei at telophase have not, to our knowledge, been observed in other organisms. It is possible that these microtubules represent a distinct region of the cleavage apparatus which is aligned at right angles to the remaining cleavage microtubules. However, their specific association with separating nuclei suggests that they may play a role in the separation process, perhaps by effecting a gelation of the intervening cytoplasm so that the nuclei are moved apart. The participation of microtubules in gel formation is discussed in more detail at the conclusion of this paper.

Basal Bodies

During the course of cell division, the basal bodies are free of flagella, exhibit no transition structures, and migrate in the cytoplasm. In these respects, they may be termed centrioles.

Centrioles have often been seen to give rise to flagella (i.e. to become basal bodies) (8, 16, 29, 37, 42, 43), and basal bodies possibly act as mitotic division centers (i.e. as centrioles) in certain organisms (17, 41). In *C. reinhardi*, the basal bodies acquire the morphology of centrioles during mitosis but do not seem to participate in the division of the nucleus. They do appear to be directly involved in orienting the plane of cell division, however, for the cleavage microtubules radiate toward them during cytokinesis.

The basal bodies maintain their interphase orientation with respect to one another and are associated with their characteristic bands of microtubules even when they are free from flagella and migrating in the cytoplasm. It appears, then, that these are inherent properties of the organelles themselves and the cytoplasmic system they represent, and are not properties that are imposed upon them when they have taken their "functional" positions at the bases of flagella.

Cytokinesis

Cleavage patterns of two general types have been described with the electron microscope. In the first type, groups of small vesicles are aligned along the cleavage plane and then appear to fuse (4, 21, 24, 25, 27, 28, 37, 40). The second type involves the formation of a furrow, often associated with a dense region of underlying cytoplasm, which appears to constrict the cell in two (2, 3, 32, 49). A third cleavage pattern is seen in the present study: an array of microtubules is first oriented along the cleavage plane, and a furrow then passes between them. It is possible that the few "circular profiles" noted by Allenspach and Roth (2) at the edge of a chick mesenchymal cleavage furrow may also be microtubules, but they are visible in only one of those authors' micrographs.

Since cells commonly appear to divide without comparable arrays of microtubules, the presence of these microtubules in *C. reinhardi* has some special significance. In higher plant cells, microtubules extend into the cytoplasm at telophase and are thought to direct membrane to the cell plate (14, 26). While it is possible that the cleavage microtubules in *C. reinhardi* have a similar function, their disposition in the dividing cell does not favor this hypothesis since they are confined to a narrow region of cytoplasm along the cell midline (Fig. 15). It seems more likely that their presence effects an alteration of the cytoplasm in the cleavage-plane region so that furrowing is somehow facilitated. Since the presence of microtubules can be linked to structural rigidity in a variety of cells (26), they may, in this instance, facilitate cytokinesis by providing a rigid framework on the opposing sides of the furrow against which cytoplasmic furrowing (and perhaps nuclear separation) can take place.

A series of experiments by Marsland and his collaborators (19, 50) has shown that low temperatures and high pressures, agents that produce a gel-sol transformation, inhibit mitosis and the subsequent formation of a cleavage furrow in sea urchin eggs. Such agents have also been shown to disrupt cytoplasmic microtubules in *Actinosphaerium* (45, 46). Tilney et al. (45) have presented evidence for a close relationship between the development of gel strength and the presence of microtubules. In dividing *C. reinhardi* this relationship is explicit: microtubules appear within the cytoplasm at a time when the formation of a gel is classically thought to produce the forces required for the formation of a cleavage furrow.

The disposition of the microtubules in the cleavage apparatus, diagrammed in Fig. 15, also suggests that they may exert a directive force during the cleavage process. The creation of a furrow would seem to require a flow of cytoplasm, and a flow of cytoplasm along the lines defined by the microtubule pattern could result in the formation of a furrow through the cell midline. Indeed, evidence for considerable cytoplasmic movement during the course of cell division in *C. reinhardi* is presented in this paper. It is not yet possible to determine whether the microtubules have an active role in such cytoplasmic movement or whether the presence of the rigid microtubules directs the more fluid cytoplasm in the appropriate directions to create a furrow.

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