

# ULTRASTRUCTURE OF MITOSIS AND CYTOKINESIS IN THE MULTINUCLEATE GREEN ALGA *ACROSIPHONIA*

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## ABSTRACT

The processes of mitosis and cytokinesis in the multinucleate green alga *Acrosiphonia* have been examined in the light and electron microscopes. The course of events in division includes thickening of the chloroplast and migration of numerous nuclei and other cytoplasmic inclusions to form a band in which mitosis occurs, while other nuclei in the same cell but not in the band do not divide. Centrioles and microtubules are associated with migrated and dividing nuclei but not with nonmigrated, nondividing nuclei. Cytokinesis is accomplished in the region of the band, by means of an annular furrow which is preceded by a hoop of microtubules. No other microtubules are associated with the furrow. Characteristics of nuclear and cell division in *Acrosiphonia* are compared with those of other multinucleate cells and with those of other green algae.

## INTRODUCTION

In multinucleate cells, nuclear division may occur synchronously, asynchronously, or in a wave spreading from one part of the cell to another (for a general discussion, see Agrell, 1964; Grell, 1964; Erickson, 1964). Cytokinesis may or may not be associated with nuclear division (Grell, 1964; Jónsson, 1962; Kornmann, 1965, 1966; Schussnig, 1931, 1954; Lewis, 1909). In the multinucleate green alga *Acrosiphonia*, an unusual pattern of nuclear and cell division occurs (Jónsson, 1962; Kornmann, 1965): some of the nuclei of each multinucleate cell gather to form a band of nuclei located in the region where cytokinesis will later occur. Next the nuclei in the band undergo synchronous division, forming two bands of daughter nuclei that move away from one another during ingrowth of an annular septum in the region of the bands. Meanwhile, nuclei that are not part of the

band remain scattered in the cytoplasm at some distance from the band and do not participate in mitosis. The recently divided nuclei soon scatter into the cytoplasm. Thus, as in uninucleate cells, nuclear and cell division in *Acrosiphonia* are closely coordinated spatially and temporally, but in the multinucleate *Acrosiphonia*, a substantial portion of the nuclei in a dividing cell do not participate in the synchronous nuclear divisions which occur in the band. This paper presents the results of observations which shed new light on this unusual pattern of nuclear and cell division. We have studied this process by means of sequential photomicrography of living, dividing cells and have examined the process at the ultrastructural level. We have also compared this process with nuclear and cell division as it occurs in other multinucleate cell types. In addition, we have

compared our results with those reported for other green algae, since nuclear and cell division patterns appear to be of importance in considering the phylogeny of these organisms (Pickett-Heaps, 1972 *b*; Pickett-Heaps and Marchant, 1972; Stewart et al., 1973).

## MATERIALS AND METHODS

The isolate of *Acrosiphonia spinescens* (Kütz.) Kjellm. which has been used in this study was originally obtained from zooids released from green unicellular endophytes of the foliose red alga *Schizymenia* sp. (Wynne no. 1620) collected 12 May 1968 on the east side of Foulweather Bluff, Skunk Bay, Hansville, Wash., by Dr. Michael J. Wynne. Since that time it has been grown in unialgal culture at 10°C in Provasoli's Enriched Seawater medium (PES) (Provasoli, 1968) in Pyrex no. 3250 storage dishes. Illumination at about 1,200 lx is provided by cool-white fluorescent lights, on a 16-h light/8-h dark photoregime.

We have followed the recommendation of Wille (1900) that multinucleate, branched members of the Acrosiphoniaceae be called *Acrosiphonia* and uninucleate branched ones be called *Spongomorpha*. The taxonomic and nomenclatural problems in this group are rather involved and will not be dealt with here.

The details of the growth and life history of this alga correspond in general to those described by Kornmann (1965), Jónsson (1962), and Chihara (1969) for *A. spinescens* (Kütz.) Kjellm.

In obtaining material for the cytological and ultrastructural study of nuclear and cell division, we have taken advantage of the fact that cells of *Acrosiphonia* plants grown in alternating cycles of light and dark undergo most nuclear and cell divisions after entering the dark cycle (Jónsson, 1962; Kornmann, 1965). Cells at the tips of filaments divide within about 1 h after entering the dark cycle; the process occurs somewhat later and less frequently in intercalary cells. Tip cells have been used in this study because it is easy to obtain large numbers of dividing tip cells at one time, and because, in preparing cells for electron microscopy, we have greater success with tip cells than with intercalary cells.

### Light Microscopy

Living cells in division were studied by placing short filaments in PES on glass slides and making a thick mount by supporting the coverglass with cover glass fragments. The thick preparation was placed on a heating-cooling stage (Leitz model 80, E. Leitz, Inc., Rockleigh, N. J.) maintained at 10°C. Condensation on the top of the cold cover slip was prevented by applying a thin film of Spray-Kleen (American Optical Corp., Research Div., Framingham Center, Mass.) (H. H. Heunert, personal communication) to the upper face of

the cover slip before placing it over the alga. With a change of medium approximately once per hour, many of the tip cells completed nuclear and cell division within a few hours, the time required depending mainly on the stage of the process of division in which we found the cells when we put them on the stage. It is easy to identify a cell in which the division processes have begun, since the presence of the band of aligned nuclei in tip cells is evident to the unaided eye. Micrographs were taken mostly with bright-field optics to reduce the problems created by the high refractivity of the cell walls and the numerous pyrenoids.

A modification of Buffaloe's fixative (Buffaloe, 1958; Kapraun, 1969) was used before acetocarmine staining of plants for squash preparations.

Sections of material prepared for electron microscopy (see below) were cut at 1  $\mu$ m thickness with glass knives and stained with Richardson's stain (Richardson et al., 1960) for subsequent light microscope examination.

### Electron Microscopy

Several different techniques were used in attempting to find a satisfactory method of preparing *Acrosiphonia* for ultrastructural studies. The large, highly vacuolate cells were quite susceptible to collapse and plasmolysis. Best results were obtained when we used phosphate-buffered 2.5% glutaraldehyde followed by postfixation in 1% phosphate-buffered osmium tetroxide, with osmolalities of the fixing and rinsing solutions adjusted to approximately that of sea water by the addition of NaCl (Cloney and Florey, 1968). After OsO<sub>4</sub> postfixation the material was rinsed in buffer solutions with gradual stepwise decreases in osmolality as recommended by Burr and West (1970) and then embedded in agar and passed through a series of water-methyl Cellosolve solutions to absolute methyl Cellosolve before ethanol dehydration, as recommended by Fowke and Pickett-Heaps (1969 *a*). The material was rinsed in propylene oxide and infiltrated and embedded in Epon 812 (Luft, 1961).

Sections were cut with a diamond knife and stained with uranyl acetate (omitted when in block staining was done using 1% uranyl nitrate in 70% ethanol overnight) and lead citrate (Reynolds, 1963). They were examined with a Zeiss 9S-2 electron microscope.

## RESULTS

### *Thallus Structure and Interphase Cell Structure*

*Acrosiphonia* is a uniseriate, filamentous branched green alga which is a characteristic member of many marine algal communities in cool-temperate and cold regions and is quite common in Puget Sound. Each plant is differentiated into an upright and rhizoidal portion. The

upright, branched shoot system consists of cells about 100  $\mu\text{m}$  in diameter; the rhizoidal system is made up of branched filaments 30–40  $\mu\text{m}$  in diameter that can terminate in small adherent disks composed of repeatedly branched filaments of cells 8–10  $\mu\text{m}$  in diameter. In the upright portion of the plant, the cell length is typically one to three times the diameter but may reach a millimeter or more in rapidly growing tip cells.

Young cells of the upright part of the plant each contain a peripheral layer of cytoplasm averaging about 5  $\mu\text{m}$  in thickness, appressed to the cell wall by the large central vacuole. The single large reticulate chloroplast containing numerous pyrenoids is the most striking component of the cells when they are viewed at low magnification (Figs. 5, 6). Smaller organelles and inclusions are obscured by the chloroplast when it over- or underlies them, but they are more easily seen when they occur in the cytoplasm filling the perforations in the chloroplast (Fig. 1).

Ultrastructurally, the cells of *Acrosiphonia* are like those of many other green algae. The chloroplast contains lamellae characteristically consisting of two thylakoids each (Fig. 10) which appear to enlarge and protrude as finger-like projections into the polypyramidal pyrenoids (Chadefaud, 1941). Numerous mitochondria about  $0.5 \times 1\text{--}5 \mu\text{m}$  in longitudinal section, dictyosomes, lengths of endoplasmic reticulum, vesicles, and a number of nuclei 3–5  $\mu\text{m}$  in diameter containing one prominent nucleolus each, are scattered apparently at random in the cytoplasm. The number of nuclei per cell varies according to the size of the cell and the volume of its cytoplasm, ranging from two or three nuclei in tiny rhizoid cells, to over a thousand nuclei in large tip cells. The difference between nuclei which are going to divide and those which are not is not evident structurally in interphase cells. We have not observed microtubules or

centrioles in association with interphase nuclei either in dividing or nondividing cells. Microtubules, though sparse, seem to be consistently oriented parallel to the longitudinal axis of the interphase cell. The walls of young tip cells may be less than 1  $\mu\text{m}$  in thickness (Fig. 3), whereas the walls of older cells may reach 10  $\mu\text{m}$  in thickness, and appear to be composed of several layers (Fig. 2). Ultrastructurally these layers are made up of fibrillar material, which in section presents a herringbone effect at least in the innermost layers (Fig. 9). A somewhat more electron-dense nonfibrillar outer wall layer about 35 nm thick is present in some cells (Fig. 9); it sometimes is lost in preparation. This wall thickening with advancing age appears to be a consequence of continued wall deposition combined with minimal elongation growth of intercalary cells.

### Mitosis and Cytokinesis

**BAND FORMATION:** Our light microscope observations on nuclear and cell division agree with those of Jónsson (1962) and to some extent with those of Kornmann (1965) on *A. spinescens*. We have found that the same process takes place in *A. coalita*, another species common in cool marine waters along the Pacific coast of North America.

At low magnifications in the living tip cells, the nuclei cannot be observed as they undergo division within the band because of the highly refractile chloroplast and pyrenoids nearby, and because of the thickness of the specimen. In the thinner preparations necessary for observations at higher magnifications, the refraction problems are quite severe, and the cells do not survive for the length of time necessary to complete division. A preliminary attempt to bleach a "window" in the cytoplasm (Kamitsubo, 1972) in the area in which nuclear

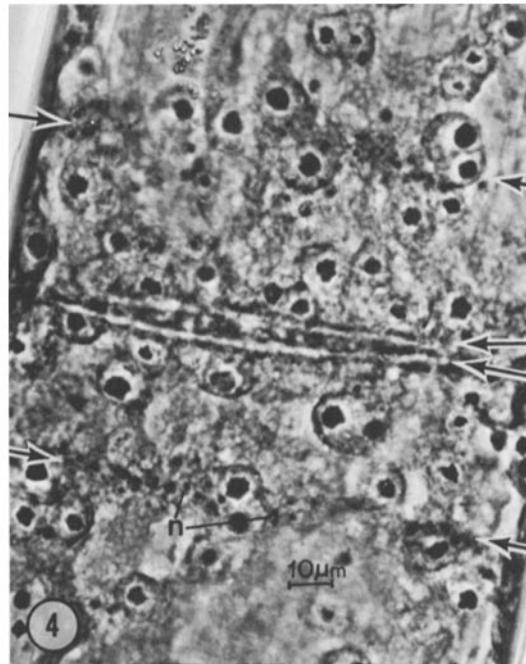
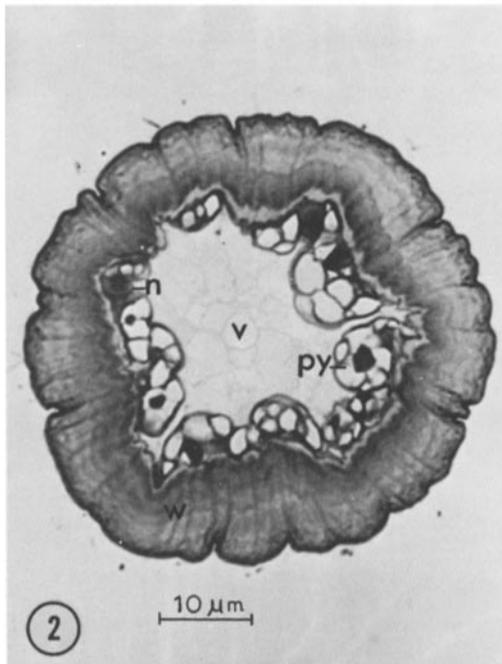
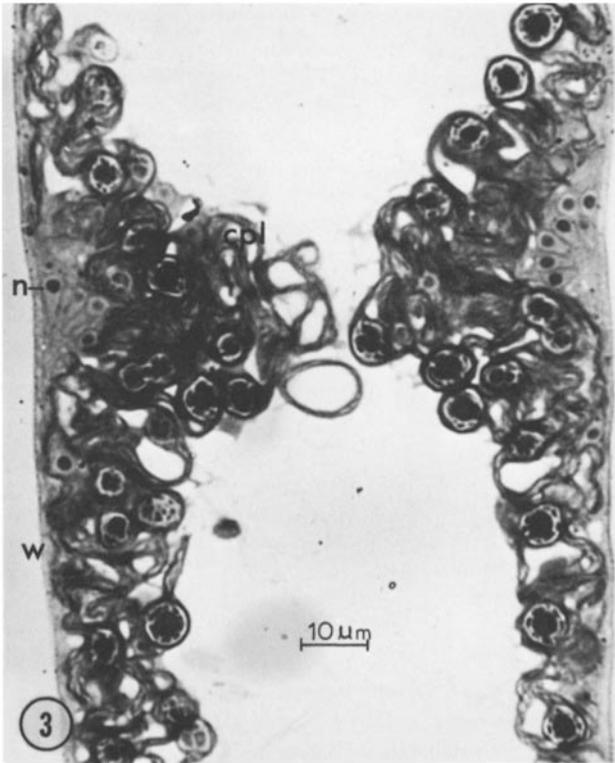
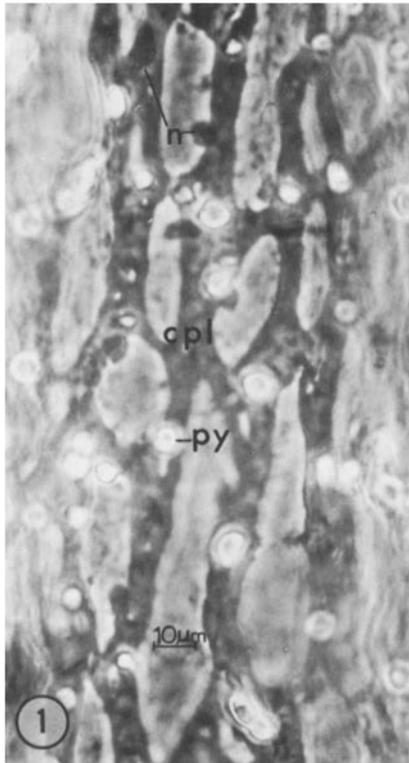
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FIGURE 1 Phase-contrast longitudinal surface view of interphase cell. Note reticulate chloroplast (*cpl*) with refractile pyrenoids (*py*), and numerous small nuclei (*n*).  $\times 545$ .

FIGURE 2 Thick cross section of old cell, showing thick, layered wall (*w*), nuclei (*n*), pyrenoids (*py*), and large central vacuole (*v*).  $\times 1,200$ .

FIGURE 3 Thick longitudinal section of cell with aligned premitotic nuclei. Note elongate, teardrop-shaped nuclei (*n*) with nucleoli, thickened chloroplast (*cpl*), thin wall (*w*) which is characteristic of young cells.  $\times 860$ .

FIGURE 4 Postdivision migration of bands of nuclei (*n*) in intercalary cell. Single arrows mark nuclear bands, double arrow marks septum. Pyrenoids are also stained by acetocarmine technique.  $\times 530$ .



band formation and cell division could be predicted to occur bleached the chloroplast but killed the cell. In our work we have attempted to relate the changes in band shape which we observed in living tip cells to the band shapes which appear to be characteristic of the stages in nuclear division which we have observed in fixed and sectioned material.

While cytoplasmic streaming is not evident in living cells of *Acrosiphonia*, rearrangements of nuclei and of local regions of the chloroplast and some other organelles do precede nuclear and cell division.

The first visible event in the division process is a local thickening of the chloroplast, which indicates the region of the cell in which nuclear and cell division will occur (Fig. 5 *a-c*). Within the next 30 min, a bright colorless band forms at the periphery of the thickening chloroplast, which is thus excluded to regions above, below, and inward from the bright band (Fig. 5 *d*). Numerous nuclei, in association with a particular arrangement of some other organelles (discussed below), constitute the band (Figs. 3, 7).

Comparison of the division process in tip cells with that in intercalary cells reveals some differences in location of the band of nuclei, number of nuclei contained in the band, partitioning of nuclei into daughter cells, size of daughter cells formed, and frequency of division. In tip cells, the band forms at a distance about  $\frac{1}{4}$  to  $\frac{1}{2}$  the length of the cell back from its tip, and is up to six nuclei in height and four nuclei in thickness. The band of nuclei undergoes changes in shape during the division process, and three to five times as many divided nuclei are partitioned into the smaller, newly formed tip cell as into the larger, new subapical cell. The new tip cell then elongates rapidly during the 12–24-h period preceding the next division, increasing its postdivision volume by a factor of three to five, while the new subapical cell elongates only a few micrometers, if at all, during the same period. In contrast, the band in dividing intercalary cells is characteristically only one nucleus in height and thickness, and is symmetrical throughout division, with equal numbers of daughter nuclei partitioned into daughter cells of equal size (Fig. 4). Although they continue to deposit cell wall material, intercalary cells do not elongate significantly, and instead add to the thickness of their walls. In addition, they divide less frequently than do tip cells.

When first formed, the bright band in tip cells is only a few micrometers in height, and appears longitudinally symmetrical. As it increases in size, however, it extends and thickens asymmetrically, so that its thickest region is near its base, and its upper portion tapers out somewhat toward the cell tip (Figs. 5 *e-g*, 6 *a, b*). The arrangement of the components of a band of this shape is unusual. The nuclei, which constitute the greater part of the volume of the band, form its center. They are teardrop-shaped in longitudinal section, their narrow ends appearing to focus on a region at the base of the band (Figs. 3, 7). At this stage the nucleoli are quite apparent as large, dark-staining bodies at the larger ends of the nuclei. Centrioles are located at the narrow ends of the elongated nuclei (Figs. 7, 8) and a few microtubules oriented approximately parallel to the longitudinal axis of the cell interpolate between nuclei or between the outermost nuclei and the plasmalemma, near the narrowed tips of the nuclei (Fig. 8). Several layers of endoplasmic reticulum form the upper and lower boundaries of the band, and some endoplasmic reticulum also is present between nuclei within the band (Fig. 7). Vesicles and dictyosomes appear throughout the band but are most concentrated toward its base, where they are located in the area that constitutes the apparent focal point for the elongated nuclei. The vesicles in the band are of two types which can be distinguished by their contents: the majority of them have electron-lucent contents and the others have electron-dense, granular contents (Figs. 7, 7 *a*, 12–16). We have not observed intermediates between these two types. Apparently the vesicles with electron-dense contents are formed from smooth endoplasmic reticulum (Fig. 12) and in addition their size (0.5–3.0  $\mu\text{m}$  diameter) and their electron-dense contents agree with those described for the general class of organelles identified as microbodies (Newcomb and Frederick, 1971; Graves et al., 1971). We have not yet performed cytochemical tests to determine whether they contain enzymes commonly found in microbodies. The origin of the vesicles with electron-lucent contents is not clear. The vesicles with electron-dense contents are grouped at the focal point of the nuclei, while the more numerous vesicles with electron-lucent contents are scattered throughout the base of the band (Figs. 7, 7 *a*, 13).

**MITOSIS:** About 1.5–2 h after the first slight thickening of the chloroplast is observed, the

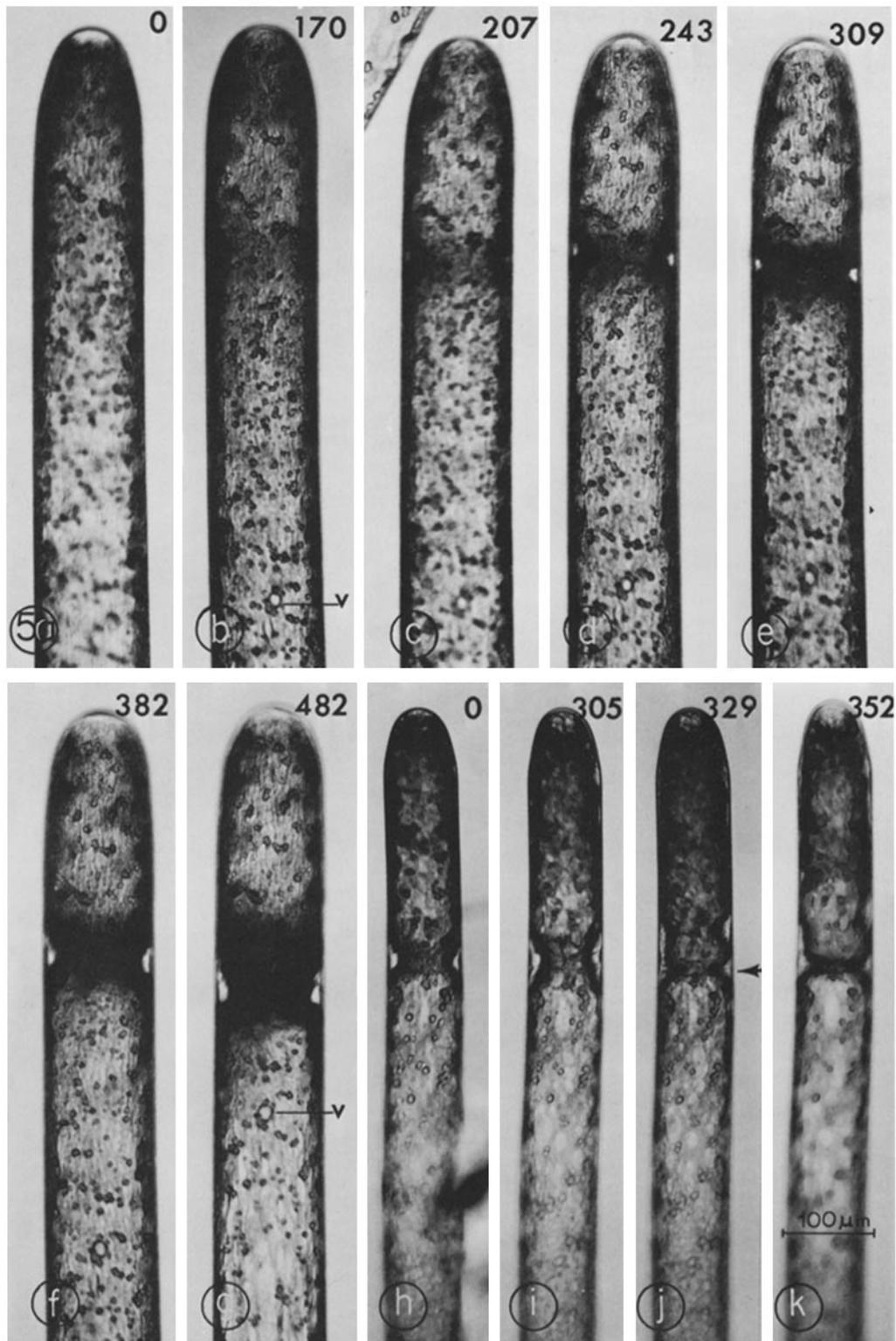


FIGURE 5 Division in living tip cells. Cell 1 (*a-g*) shows formation of the band. Cell 2 (*h-k*) shows septation at the base of the band. Time is given in minutes from the start of observation. Arrow in (*j*) marks septum. Note appearance of band at (*c*) and (*d*), change of shape in (*e-f*) and (*g-j*). A vesicle (*v*) has moved during the observation period for cell 1. The time for division appears to be much longer when observed on the microscope stage in this way than under usual culture conditions.  $\times 140$ .

begins to change in shape, apparently by a shift in the contents from the base of the band toward its center (Figs. 5 *g, h*; 6 *b, c*), so that it becomes elongate and symmetrical. Longitudinal sections through such an elongate symmetrical band reveal nuclei in mitosis.

In thick sections of cells in which nuclei are in mitosis, the band appears homogeneous because the nucleoli are no longer present and the chromosomes are not apparent in the small nuclei. However, in acetocarmine squash preparations the chromosomes are quite evident and it is clear that the nuclei in the band divide synchronously. The spindle axes are not oriented with respect to the longitudinal axis of the cell but they are parallel to the cell surface, so that the metaphase plates are seen edge-on unless the preparations have been crumpled or the nuclei have been squeezed out of a broken cell. During division of nuclei in the band, the other nuclei in the cell remain undivided. These observations agree with those of Jónsson (1962).

We have seen a condition which may represent

spindle formation in only one instance, in which the nucleolus is still present but is displaced toward one end of the nucleus, and centrioles with radiating microtubules are located at approximately right angles in sectional view (Fig. 9). Whether this represents migration of centrioles with accompanying spindle formation, or an aberrant situation, we do not know.

During mitosis, the nuclear envelope does not break down, but at each pole a large perforation permits spindle microtubules to extend from the spindle into a region of fine, fuzzy-appearing material which contains the paired centrioles (Fig. 10). No distinct kinetochores are evident. Endoplasmic reticulum is usually associated with the band of nuclei as a whole and often interposes between nuclei but does not form a discrete envelope around each nucleus. The nuclei elongate into dumbbell shapes at anaphase (Fig. 11) and separate.

The slight asynchrony of division of nuclei within the band which was noted by Jónsson (1962)

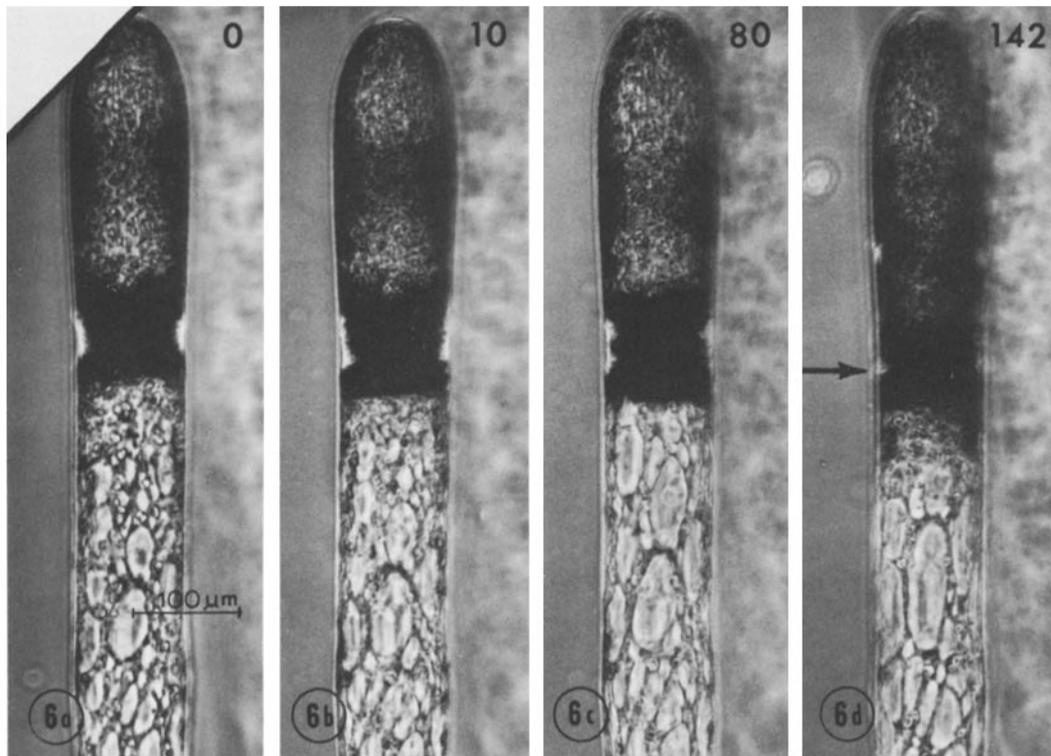


FIGURE 6 Division in living tip cell. Note band shape change. Time is given in minutes from beginning of observations. (a) asymmetrical band, (b) lengthening of asymmetrical band, (c) symmetrical band, (d) septation (arrow) and apical spreading of band.  $\times 140$ .

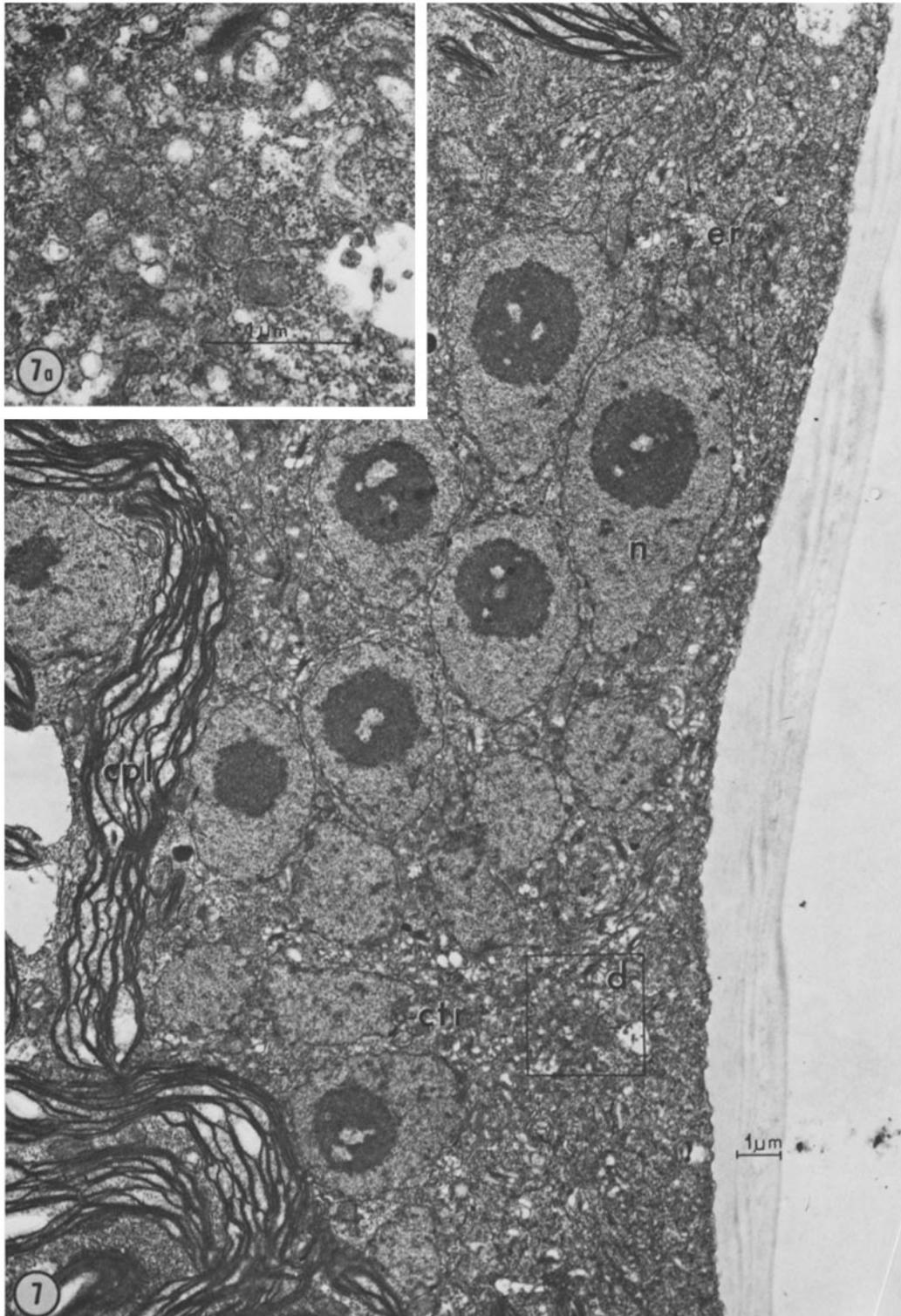


FIGURE 7 Longitudinal thin section through asymmetrical band with elongate premitotic nuclei (*n*) accompanied by endoplasmic reticulum (*er*) and vesicles, with the chloroplast (*cpl*) excluded to the edges of the band. Numerous dictyosomes (*d*) are located in the base of the band at the focal point for the nuclei. Enlargement of focal point, Fig. 7 a, shows vesicles with dense contents. Centrioles (*ctr*) are at narrow ends of nuclei. Fig. 7,  $\times 6,300$ ; Fig. 7 a,  $\times 24,000$ .

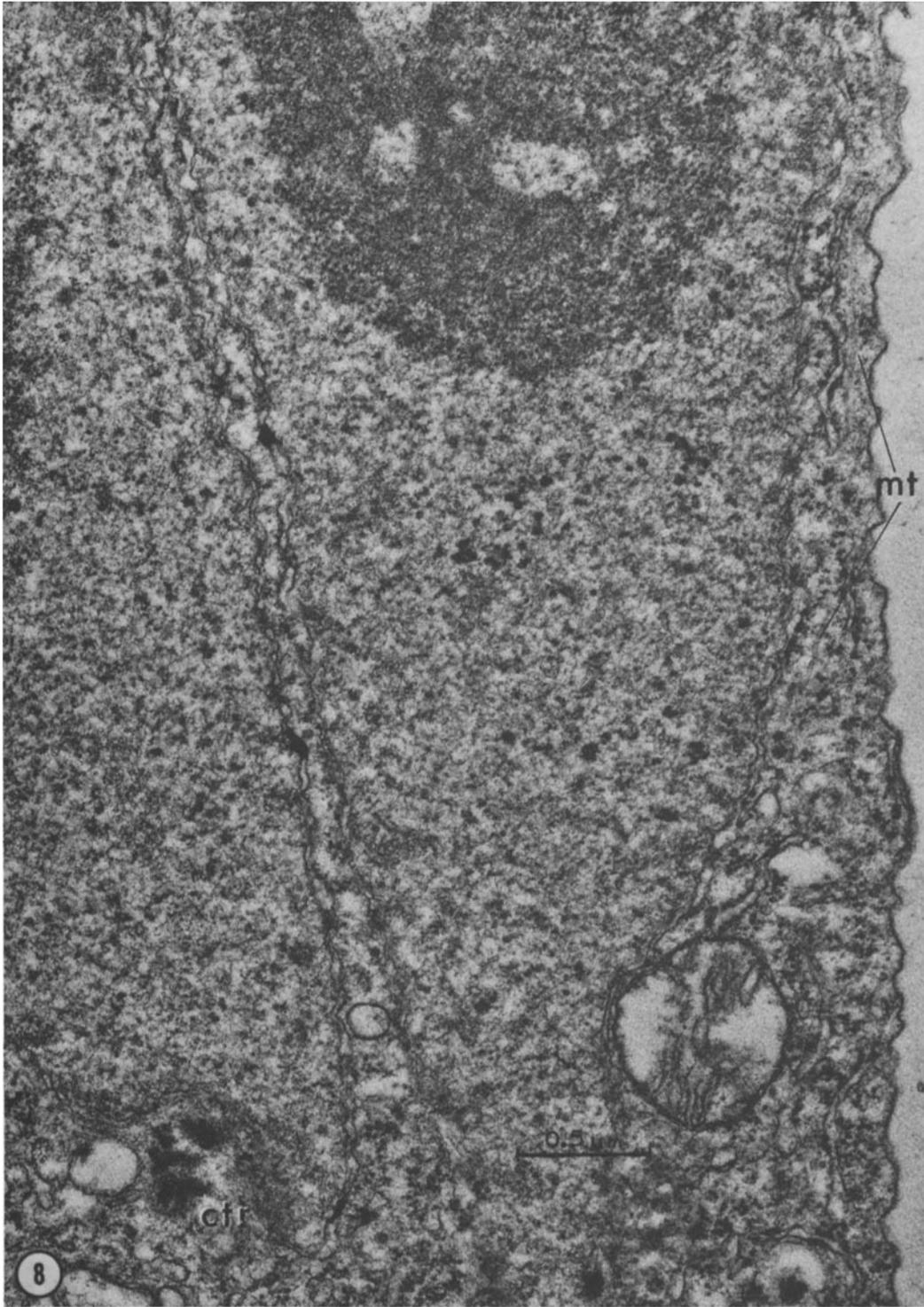


FIGURE 8 Longitudinal thin section of elongate aligned nuclei with centrioles (*ctr*) at their narrow tips. Longitudinally oriented microtubules (*mt*) are located near the plasmalemma.  $\times 40,000$ .

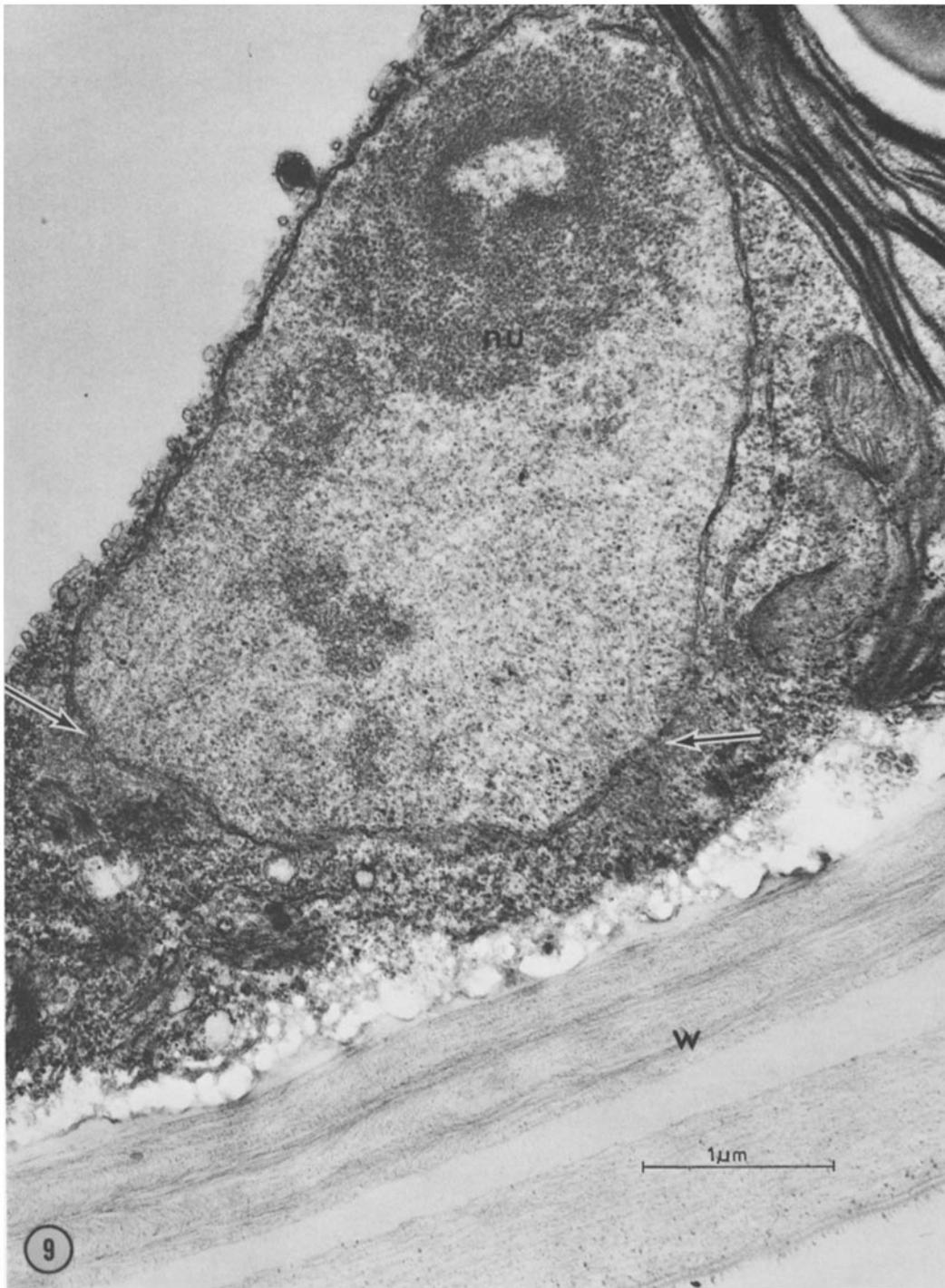


FIGURE 9 Late prophase nucleus with nucleolus (*nu*) and forming spindle poles (arrows). Note wall texture (*w*) and outer wall layer.  $\times 28,000$ .

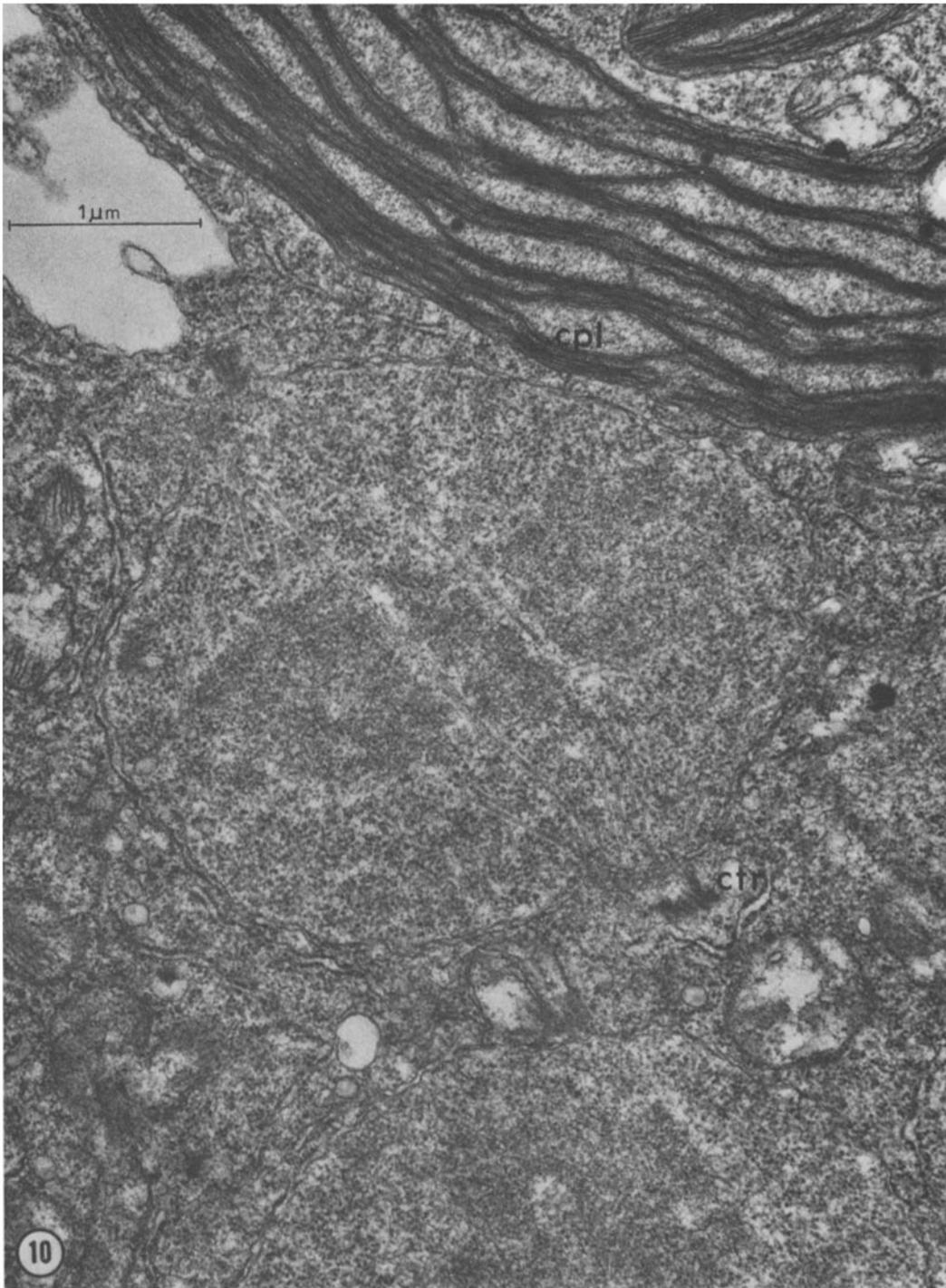


FIGURE 10 Metaphase nucleus with intact nuclear envelope with polar gaps and centrioles (*ctr*) outside the gaps. *cpl*, chloroplast.  $\times 28,000$ .

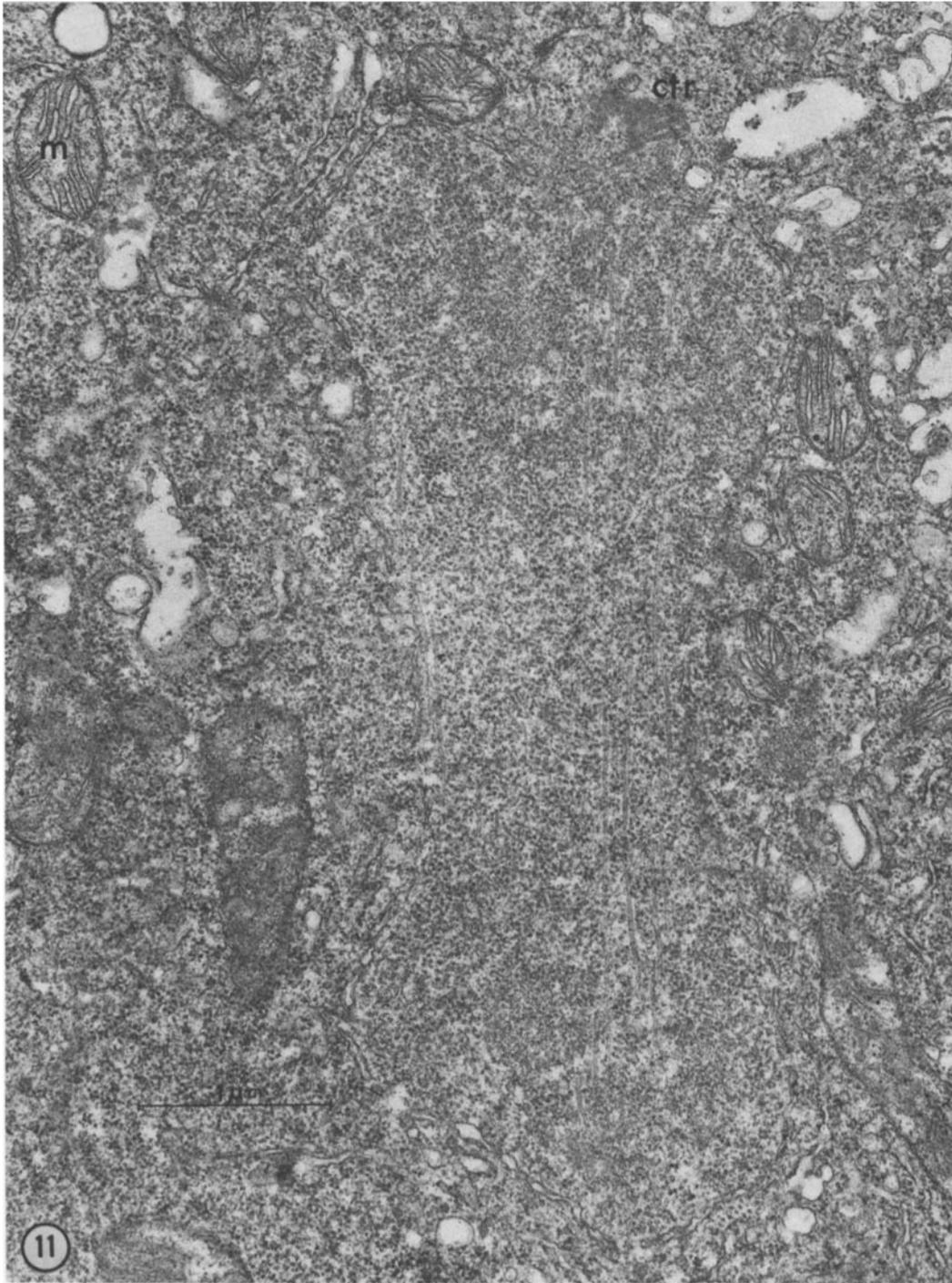


FIGURE 11 Anaphase nucleus with envelope perforate but mainly intact; note centrioles (*ctr*) at poles. *m*, mitochondrion.  $\times 28,000$ .

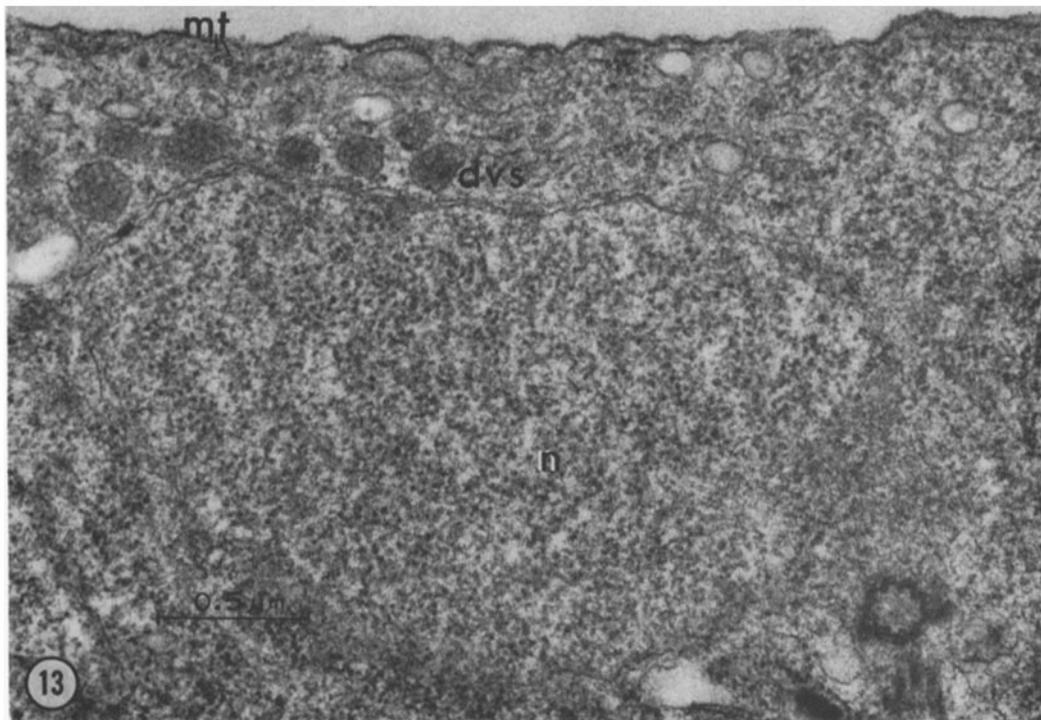
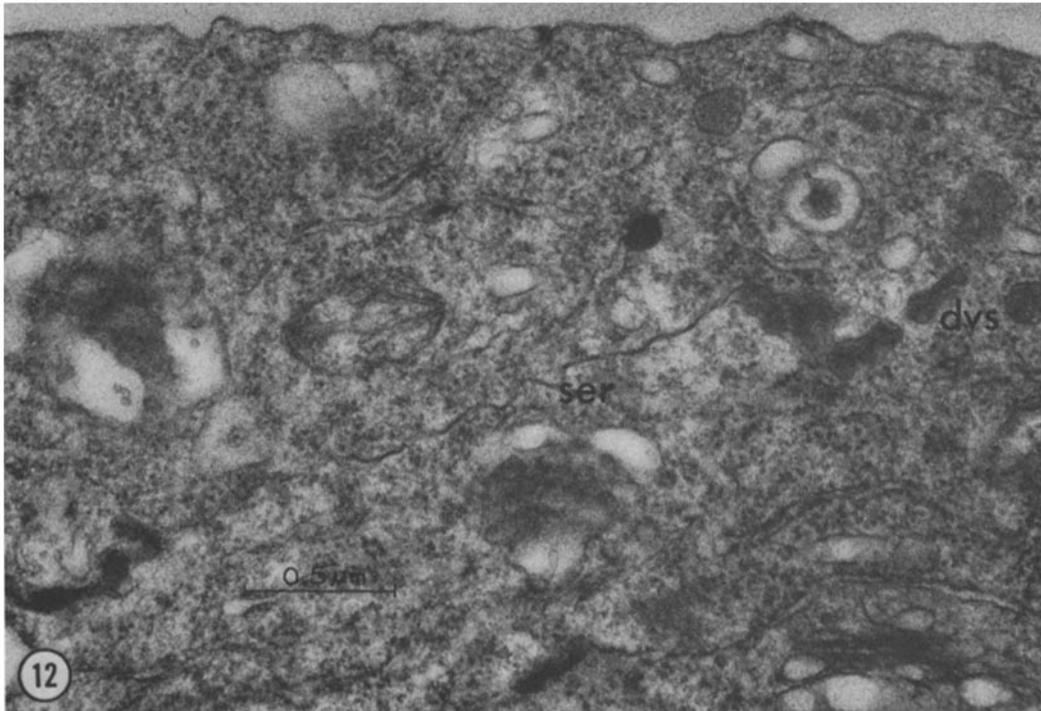


FIGURE 12 Formation of vesicles with dense contents (*dvs*) from smooth endoplasmic reticulum (*ser*).  $\times 40,000$ .

FIGURE 13 Longitudinal thin section showing microtubules (*mt*) at base of band when nuclei (*n*) are in division. Note vesicles with dense contents (*dvs*).  $\times 40,000$ .

asymmetrical broad-based, narrow-topped band was also encountered in this study, in the case of a particularly large band of nuclei in a tip cell. In this case, nuclei at the upper and lower edges of the band were in metaphase when nuclei in the center of the band were in late anaphase.

Although the nucleolus is still present at the time when chromatin is condensing (Fig. 9), it is not apparent at metaphase (Fig. 10) when the nucleoplasm contains a slightly greater density of granular material. After division the nucleolus is obvious again (Fig. 4), but we have not observed stages in its reformation.

**POSTMITOTIC MIGRATION AND CYTOKINESIS:** Within 0.5 h of the time that the premitotic, broad-based band begins to change in shape, most of the band contents are displaced apically away from the original band base location, and cytokinesis takes place near the original base location (Figs. 5 *j*, *k*; 6 *d*). The site at which the initiated septum appears is somewhat variable with relation to the base of the group of nuclei, since sometimes it is entirely below the group and sometimes within the basal part of the group.

In newly formed tip cells the bright band of recently divided nuclei remains evident for up to an hour, as it moves and disperses toward the tip of the cell (Fig. 6 *d*; Fig. 4, intercalary cell). The spreading and apical displacement of the band seems too rapid to be accounted for simply by the resumption of tip growth immediately after division, since it can shift 100  $\mu\text{m}$  in 30 min, and the overall rate of elongation of the tip cell is about 700  $\mu\text{m}$  in 24 h. Also, the distance from the tip of the cell to the top of the band decreases during this spreading, while the distance from the cell tip to the new septum remains the same.

Cytokinesis is accomplished by the infurrowing of an annular septum (Figs. 14–16). Its location may be determined as early as the asymmetric band stage before mitosis, when nuclei appear to be focused toward the base of the band. At that time, the group of vesicles with electron-dense contents is present in a small area of cytoplasm in the base of the band, near the plasmalemma (Figs. 7, 10). When nuclei in the band are at metaphase, this group of vesicles is in the same position, but in addition there is a group of two to three microtubules external to them (Fig. 13). Because these microtubules appear in precise or slightly oblique cross section when observed in longitudinal sections of cells, we believe that they are perpendicular to the longitudinal axis of the cells and are arranged around the cytoplasmic periph-

ery just internal to the plasmalemma. When the initiated septum is actually visible as a projection from the old cell wall, a larger group of 8–20 microtubules is located at the leading edge of the septum (Figs. 14, 15).

We have not observed other arrays of microtubules to be present in the cytoplasm beyond the advancing septum, in contrast with the reports of such microtubules in other green algae. We would expect that such microtubules would be visible to us since we can detect microtubules in the cytoplasm of interphase cells, in mitotic spindles, and in the small group closely associated with the growing septum.

During cytokinesis, the vesicles with electron-dense contents are located just internal to the leading edge of the septum (Figs. 13–15). The vesicles with electron-lucent contents are concentrated along the entire surface of the growing septum (Fig. 16). Numerous dictyosomes are also present in the region of the growing septum. The stainable fibrillar material present in the older cell wall is not visible either in the newest portions of the forming septum or in the dictyosomes or electron-lucent vesicles near it.

## DISCUSSION

### *Unusual Features of Mitosis and Cytokinesis in Acrosiphonia*

**SYNCHRONY:** The cytology of this unusual separation of nuclei into different dividing and nondividing roles in division has been discussed extensively by Jónsson (1962) and Kornmann (1965). Because the plants arise from uninucleate meiospores, or in some cases, from unfused gametes, they begin life as uninucleate cells. They are also capable of vegetative propagation from elongate rhizoids or from rhizoidal disks of small cells containing only a few nuclei per cell. For cells to become multinucleate, karyokinesis must occur a number of times in the absence of cytokinesis. In very young filaments which are undergoing rapid extension and expansion growth, a band of nuclei is formed, but *all* nuclei in the cell undergo division (Jónsson, 1962). In somewhat older filaments in which cells are still actively dividing vegetatively, the nuclei in the band divide while those in the ends of the cell do not (Jónsson, 1962; Kornmann, 1965; this paper). The basis for this transition is not known. However, it apparently is not permanent, since in the formation of gametes, cells are cleaved into uninucleate units, and there is no evidence to indicate that any nuclei are excluded from this process (Jónsson, 1962; Hudson, unpublished re-

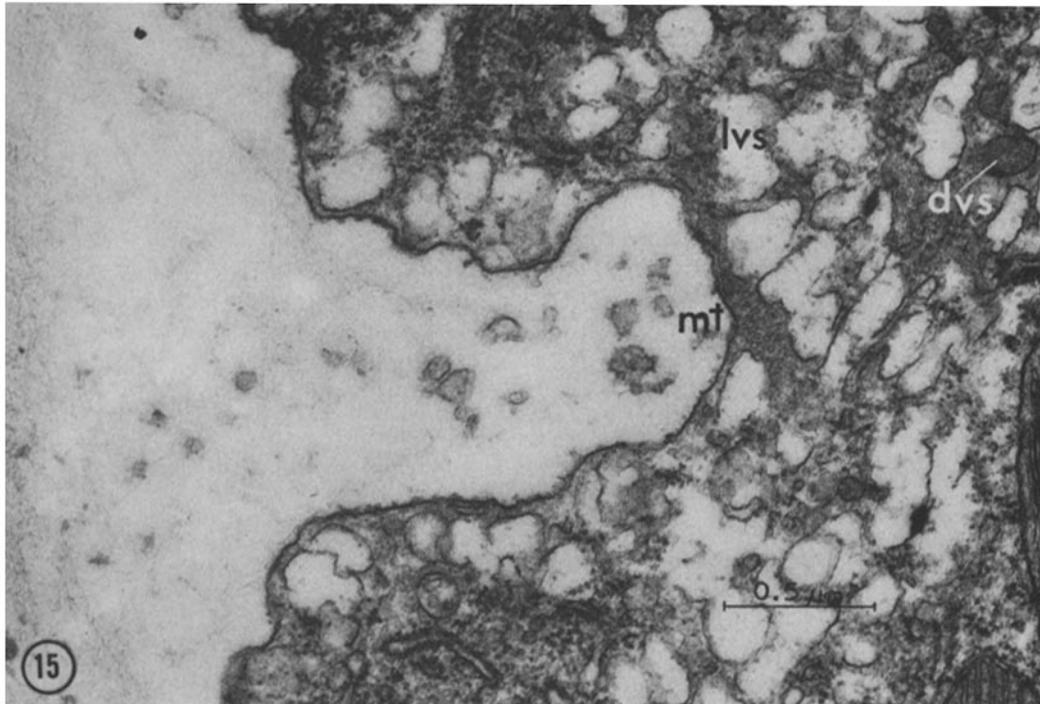
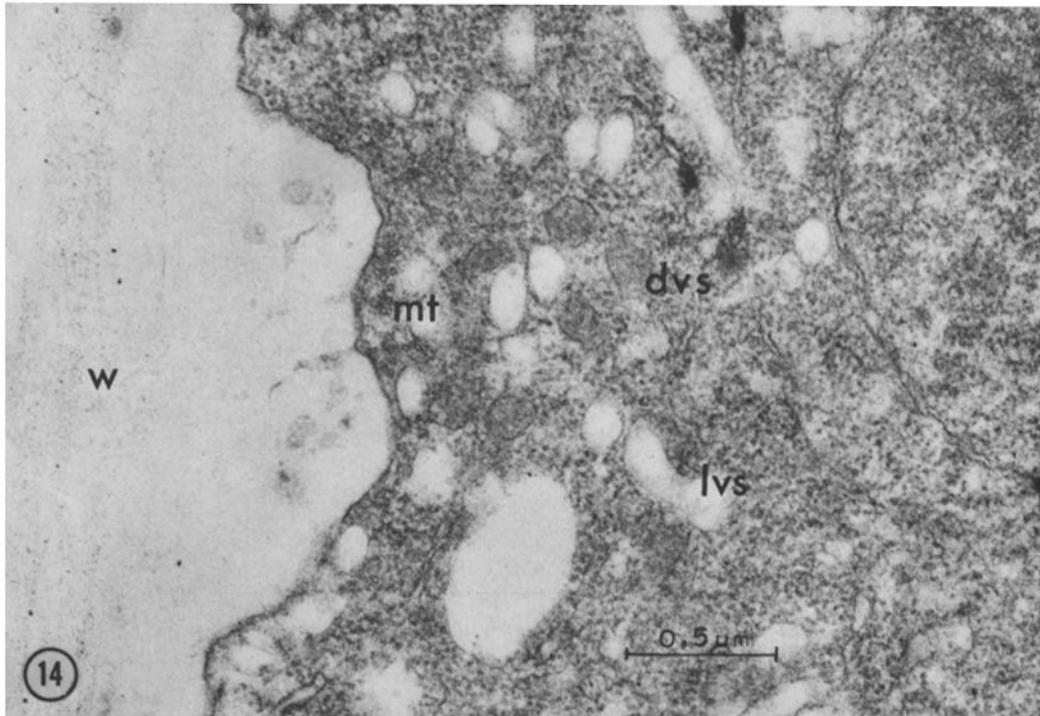


FIGURE 14 Longitudinal thin section showing septum initiation with associated microtubule group (*mt*) and vesicles with dense (*dvs*) and electron-lucent (*lvs*) contents. *w*, wall.  $\times 40,000$ .

FIGURE 15 Longitudinal thin section showing septum progression, continued association with microtubules (*mt*) and two types of vesicles (*dvs*, *lvs*).  $\times 40,000$ .

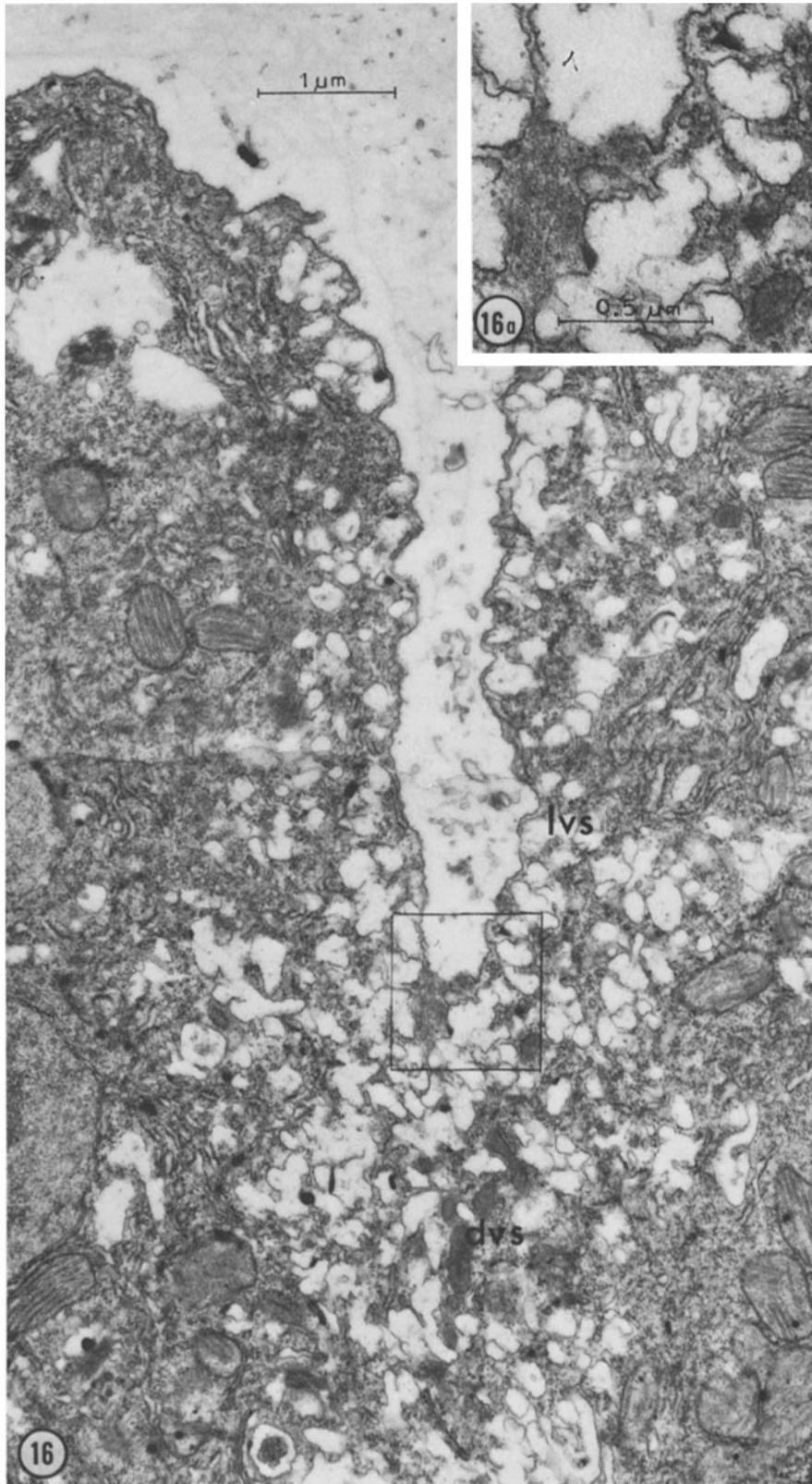


FIGURE 16 Longitudinal thin section montage showing septum progression, associations of electron-lucent vesicles (*lvs*) along entire edge of septum as well as in the region preceding the septum, where vesicles with electron-dense contents (*dvs*) are concentrated. Fig. 16 *a*, enlargement showing obliquely sectioned microtubules at septum edge. Fig. 16,  $\times 20,000$ ; Fig. 16 *a*,  $\times 44,000$ .

sults). The roles of the nuclei and the coordination of nuclear and cell division thus can vary during the life of the cells.

**CENTRIOLES:** The presence or absence of centrioles at different times in the same organism is not unique to *Acrosiphonia*; ephemeral centrioles are found in many plants which have flagellate cells at some time in their life histories. Centrioles commonly are found at the poles of the mitotic spindle even when there is no evidence that flagellate cells are about to be formed (Pickett-Heaps, 1969 a, 1971). However, in most such plants, centrioles are present continuously in interphase. In *Chara* (Pickett-Heaps, 1967), *Oedogonium* (Pickett-Heaps and Fowke, 1969), and *Bulbochaete* (Pickett-Heaps, 1973 b) there are no centrioles present at vegetative mitosis although male gametes with flagella are formed when sexual reproduction occurs. In the fungus *Labyrinthula* (Perkins and Amon, 1969; Porter, 1972) and in the green alga *Kirchneriella* (Pickett-Heaps, 1970), what seem to be very simple centrioles are produced just before mitosis and are not present in interphase. Thus the absence of centrioles in association with interphase nuclei in *Acrosiphonia* is unusual but not unprecedented.

**MOVEMENT OF NUCLEI:** Before division in *Acrosiphonia* the centrioles are located at the narrow end of a teardrop-shaped nucleus in the band, with the nucleolus in the broad opposite end. We have not been able to detect these nuclei in motion in living cells. However, the shape of the nucleus and the location of the centrioles is like that reported by Girbardt (1968) and by Wilson and Aist (1967) for moving nuclei in fungi, in which a kinetochore equivalent is present and centrioles are lacking. In the desmid *Closterium littorale* (Pickett-Heaps and Fowke, 1970), which also lacks centrioles, a similarly elongate nucleus is preceded by a microtubule center in relocation of the nucleus in a daughter cell after division.

Before mitosis in uninucleate cells it is not uncommon for the nucleus to take up a position different from that which it occupies at interphase. However, it is unusual for this repositioning to occur in multinucleate cells. In the giant amoeba *Pelomyxa carolinensis* (= *Chaos chaos*), Kudo (1947) reported that the entire amoeba contracts before mitosis and many nuclei divide in protrusions extending from the main body of the amoeba. Thus it is rather difficult to compare the positions of the nuclei at mitosis with the positions

in interphase because the whole organism has changed its shape. *Urospora*, another green alga quite similar to *Acrosiphonia*, provides the only other example of readily identifiable movement of nuclei to new positions in the cell before vegetative mitosis and cytokinesis in a multinucleate cell. However, in *Urospora*, (Kornman, 1966), all nuclei in a cell divide, not just the ones aligned in a band at the future site of cytokinesis.

### *Comparison with Other Multinucleate Organisms*

In a multinucleate cell, nuclear division may be synchronous, asynchronous, or may occur as a wave of division passing from one part of the cell to another (Agrell, 1964; Erickson, 1964; Grell, 1964). In studying the series of nuclear divisions in the formation of pollen in orchids, Heslop-Harrison (1966, 1968) found that cytoplasmic continuities between nuclei may be important in maintaining synchrony. In species in which septation occurs late in the series of mitoses, mitotic synchrony persists for more nuclear division cycles than in species in which septation begins after the first few cycles of mitosis. In *Drosophila* embryos, nuclear division is synchronous for the 12 mitotic cycles that precede the first cell division that leads to the formation of blastoderm (Fullilove and Jacobsen, 1971). However, some multinucleate algae have asynchronous division of nuclei within the same cell (Godward, 1966—Siphonocladales; Jónsson, 1962; Schussnig, 1931—*Cladophora*), so that although cytoplasmic continuity may promote or allow synchrony of nuclear division, it does not appear to impose synchrony.

In the multimicronucleate ciliate *Spirostomum*, it is reported that nuclear division is synchronous but that only 20–30% of the nuclei undergo mitosis at one time (Sesachar and Padmavathi, 1956). Whether the nuclei are assembled in groups before division and whether cytokinesis is closely related to mitosis, apparently have not been determined. However, according to Grell (1964), mitotic synchrony in multinucleate protozoans is rare, so that *Spirostomum* appears to be unusual among protozoans.

### *Comparison with Other Green Algae*

Mitosis and cytokinesis have been studied ultrastructurally in a number of green algae and comparison of the characteristics observed may be

useful in phylogenetic considerations (Pickett-Heaps, 1972 *b, c*; Pickett-Heaps and Marchant, 1972; Stewart et al., 1973).

Among the ultrastructural studies on mitosis and cytokinesis in green algae, only those on the vegetatively nonseptate *Bryopsis* (Burr and West, 1970) and on the coenobial *Hydrodictyon* (Marchant and Pickett-Heaps, 1970) can provide examples of these processes in multinucleate green algae. Both undergo septation regularly only during formation of motile uninucleate reproductive cells. Thus septation in those multinucleate-algae may be considered to be basically multiple cleavage of a multinucleate mass resulting in the formation of uninucleate units. This occurs in many organisms in early embryonic development or in formation of reproductive cells. Gametogenesis and zoosporogenesis in *Acrosiphonia* entail just such a cleavage pattern. In contrast, except for the present study on *Acrosiphonia*, members of the Cladophorales, which are composed of filaments of multinucleate cells which regularly undergo cytokinesis vegetatively, have not been studied ultrastructurally with regard to mitosis and cytokinesis.

**MITOSIS:** The closed mitotic spindle with polar perforations in *Acrosiphonia* is like that in many green algae, including *Chlamydomonas reinhardtii* (Johnson and Porter, 1968), *Ulva mutabilis* (Løvlie and Bråten, 1970), *Oedogonium* sp. (Pickett-Heaps and Fowke, 1969), *Bulbochaete hiloensis*, (Pickett-Heaps, 1973 *b*), *Chlorella pyrenoidosa* (Atkinson et al., 1971; Wilson et al., 1973), *Hydrodictyon reticulatum* (Marchant and Pickett-Heaps, 1970), and *Bryopsis hypnoides* (Burr and West, 1970). However, the perforations in the nuclear envelope at anaphase in *Acrosiphonia* also show some similarity to the partial breakdown of the nuclear envelope in *Stigeoclonium* and *Ulothrix* (Floyd et al., 1972 *a*) and *Microspora* (Pickett-Heaps, 1973 *a*). The complete breakdown of the nuclear envelope is reported in *Closterium littorale* (Pickett-Heaps and Fowke, 1970), *Cosmarium botrytis* (Pickett-Heaps, 1972 *a*), *Spirogyra* sp. (Fowke and Pickett-Heaps, 1969 *a*), *Chara* (Pickett-Heaps, 1967), *Klebsormidium flaccidum* (Floyd et al., 1972 *b*), and *K. subtile* (Pickett-Heaps, 1972 *c*).

The presence of centrioles at the poles of the mitotic nucleus does not appear to be directly related to the open or closed condition of the mitotic nucleus: *Ulothrix* (Floyd et al., 1972 *a*) and

*Klebsormidium* (Floyd et al., 1972 *b*; Pickett-Heaps, 1972 *c*) have both polar centrioles and dispersal of the nuclear envelope, while *Spirogyra* (Fowke and Pickett-Heaps, 1969), *Closterium* (Pickett-Heaps and Fowke, 1970), and *Chara* (Pickett-Heaps, 1967) have dispersal of the nuclear envelope but lack centrioles. Except for *Oedogonium* (Pickett-Heaps and Fowke, 1969) and *Bulbochaete* (Pickett-Heaps, 1973 *b*), species with an intact nuclear envelope have centrioles present just outside the gaps at the spindle poles. In *Stigeoclonium* (Floyd et al., 1972 *a*) the centrioles actually enter the nucleus at prophase. In *Chlorella* (Wilson et al., 1973) and *Chlamydomonas* (Johnson and Porter, 1968) the centrioles appear to be more directly related to initiation of the septum at cytokinesis than to mitosis.

**CYTOKINESIS:** Microtubules were not observed in the plane of septation in *Ulva* (Løvlie and Bråten, 1970), *Klebsormidium flaccidum* (Floyd et al., 1972 *b*), *K. subtile* (Pickett-Heaps, 1972 *c*), or in the present study of *A. spinescens*. Microtubules appear to be involved in septum formation in a number of green algae, where they are arranged in the plane of a developing cell plate, the group of microtubules being termed a "phycoplast" by Pickett-Heaps (1972 *b*). This group of algae includes *Oedogonium* (Pickett-Heaps and Fowke, 1969), *Bulbochaete* (Pickett-Heaps, 1973 *b*), *Chlorella pyrenoidosa* (Atkinson et al., 1971; Wilson et al., 1973), *Scenedesmus* (Nilshammer et al., 1972), *Ulothrix fimbriata* (Floyd et al., 1972 *a*), *U. belkae* (Stewart et al., 1973), *Stigeoclonium helveticum* (Floyd et al., 1972 *a*), *S. farctum*, *S. tenue*, *Chaetophora incrassata*, *Uronema confervicolum*, *Schizomeris leibleinii*, and *Draparnaldia plumosa* (Stewart et al., 1973). In *Microspora* (Pickett-Heaps, 1973 *a*) a phycoplast is associated with a combination of an annular septum and a cell plate. The presence of microtubules in the plane of septation in the *Drosophila* zygote (Fullilove and Jacobsen, 1971) indicates that this sort of involvement is not confined to green algae. By contrast, microtubules oriented perpendicular to the plane of septation, which along with vesicles fusing to form a cell plate constitute the phragmoplast found in higher plants, have been observed in *Chara* (Pickett-Heaps, 1967), which is not generally considered to be very closely related to the rest of the green algae. In *Spirogyra* (Fowke and Pickett-Heaps, 1969 *b*), septation begins by ingrowth of an annular septum and ends with a small

phragmoplast between the daughter nuclei. The phragmoplast in *Coleochaete* (McBride, 1970; Marchant and Pickett-Heaps, 1973) is initiated between the daughter nuclei during cytokinesis along the radius of the discoid thallus, but develops from a more lateral position during cytokinesis parallel to the circumference of the thallus.

Microtubules which appear to mark the location of future septation were first described in developing stomatal complexes in wheat, by Pickett-Heaps and Northcote (1966). Such microtubules have since been observed in some other higher plants (Burgess and Northcote, 1967—*Phleum*; Cronshaw and Esau, 1968—*Nicotiana*) and in some green algae (Johnson and Porter, 1968—*C. reinhardtii*; Pickett-Heaps, 1970—*Kirchneriella*; Atkinson et al., 1971—*Chlorella*). Perhaps the one or two microtubules next to the just-initiated septum in *C. littorale* (Pickett-Heaps and Fowke, 1970) are comparable. The function of these groups of microtubules remains unclear (Pickett-Heaps, 1969 *b*; Burgess and Northcote, 1967). The microtubules in wheat are present at preprophase and disappear at later stages of mitosis, which led Pickett-Heaps (1969 *b*) to suggest that they might represent the effect of mobilization of microtubules from positions in the cytoplasm for use in the mitotic spindle. However, this does not seem to be likely in *Acrosiphonia*, in which more microtubules are present at the leading edge of the growing septum after mitosis than are present at the septum initiation site when they are first noticeable, at metaphase. In *Acrosiphonia*, the microtubules apparently form a band just internal to the advancing annular septum, somewhat like the position of the group of microtubules internal to the furrow in *C. reinhardtii* (Johnson and Porter, 1968).

## SUMMARY AND CONCLUSIONS

*Acrosiphonia* is like most of the green algae thus far investigated ultrastructurally in that kinetochores were not observed and the nuclear envelope is intact at mitosis, with polar gaps that open onto a dense amorphous area containing centrioles. It is like *Ulva* and *Klebsormidium*, in that it lacks either the phycoplast or the phragmoplast type of septation observed in many other green algae. It is distinct among the green algae and other plants because before mitosis it forms a large band of nuclei accompanied by a characteristic array of endoplasmic reticulum, dictyosomes, and vesicles,

bounded by the large reticulate chloroplast, a characteristic shared only by its close relative, *Urospora*. It is not known whether the migrated nuclei of *Urospora* are associated with centrioles and microtubules while nonmigrated nuclei lack this association, as is the case in *Acrosiphonia*. However, nuclei in *Urospora* do not appear to be role-separated, since regardless of their position, all nuclei in a cell of *Urospora* undergo synchronous division. As far as we know, the only parallel to mitotic-nonmitotic role separation among nuclei occurs in a multimicronucleate ciliate, *Spirostomum* (Sesachar and Padmavathi, 1956), which unfortunately does not appear to have been studied further. This kind of relationship among nuclei may represent just one sort of approach to the general problem of propagating the cells of multinucleate, multicellular organisms. Perhaps in *Acrosiphonia* the gathering of nuclei into a band is a way of insuring that at least some nuclei are distributed into each daughter cell, and in the special case of tip cells, of distributing the larger number of nuclei into the cell which will increase most rapidly in volume. The formation of the band of nuclei along with the accompanying cytoplasmic components also in some way might define the location of cytokinesis.

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