ULTRASTRUCTURE AND DIFFERENTIATION OF *HYDRODICTYON RETICULATUM*

I. MITOSIS IN THE COENOBIUM

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Summary

Newly developed processing techniques have made possible an ultrastructural study of mitosis in the coenocytic green alga, *H. reticulatum*. Centrioles, apparently persistent in the coenobia, replicated and migrated to the poles of the prophase spindle as microtubules appeared between them. A perinuclear envelope of endoplasmic reticulum enclosed the nucleus, centrioles, and cytoplasmic microtubules around the nucleus at prophase; it remained virtually intact until telophase. The nuclear envelope was deeply invaginated at the poles during prometaphase before the formation of large polar fenestrae. Through these openings extranuclear microtubules invaded the nucleus. Separation of the chromosomes was accompanied by considerable elongation of the spindle within a nuclear envelope that remained intact except for the polar fenestrae. Nuclear division in this alga is compared with that in various other organisms.

I. INTRODUCTION

Very few accounts of the ultrastructure of coenocytic algal cells have been published, no doubt due to the formidable problems of avoiding the gross damage and collapse in these large, highly vacuolate cells that follows standard preparative methods for electron microscopy. Recently, considerable development of techniques has allowed examination for delicate filamentous algae such as *Spirogyra* (Fowke and Pickett-Heaps 1969*a*, 1969*b*) and *Oedogonium* (Pickett-Heaps and Fowke 1969, 1970*a*, 1970*b*). These techniques have now been further refined to yield adequate preservation of large coenocytic cells.

The very large vegetative cells of the water net, *Hydrodictyon reticulatum*, are coenocytic and cylindrical in shape with a very thin layer of cytoplasm lining the cell wall and enclosing the large central vacuole. Cells are linked together to form extensive nets (Fig. 1). To reproduce both sexually and asexually the cytoplasm of the coenobia of this alga cleaves to form a multitude of tiny uninucleate zooids. This cytoplasmic cleavage and the various fates of the zooids will be dealt with in subsequent papers. For an extensive discussion of the life cycle of *H. reticulatum* Pocock's (1960) excellent paper should be consulted.

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Mitosis in *H. reticulatum* has been described by a number of light microscopists, most recently by Proskauer (1952); some ultrastructural observations on the early stages of mitosis in *H. africanum* have been reported by Northcote (1968). This paper will concentrate on details of nuclear division beyond the limit of resolution of the light microscope, and the results will be related to studies on mitosis in other organisms.

II. Materials and Methods

(a) *Materials*

*H. reticulatum** was collected from Coppins Crossing on the Molonglo River, A.C.T. It was maintained in culture using Hill's medium (details given by Pickett-Heaps 1970). The alga was grown in Petri dishes at 20°C with a 15 hr/9 hr light/dark cycle, the lighting being provided from underneath by two 40W daylight fluorescent tubes. For fixation, cells were taken from cultures contaminated with various protists, as *H. reticulatum* appeared to grow more vigorously in these conditions than in axenic culture.

(b) *Methods*

(i) *Fixation.*—Cells were fixed in 1% glutaraldehyde in Hill's medium (pH 6·8) at room temperature for 30 min. They were transferred to 3% glutaraldehyde in the same medium for 2 hr. After five washings in Hill's medium over 30 min, they were post-fixed overnight at 0°C in 1% OsO_4, again in Hill's medium. They were then washed in cold distilled water and each cell cut into two or three pieces which were embedded in 1% agar.

(ii) *Dehydration and Embedding.*—Cells, in agar, were run to absolute methyl cellosolve by 5% increments added every 10 min at 0°C. After three changes of absolute methyl cellosolve over 4–8 hr, the cells were run to absolute ethanol by 10% increments, made every 10 min, and left in the cold overnight with at least three changes of ethanol. Cold propylene oxide was added dropwise to the material in ethanol over 6 hr until the concentration of propylene oxide was about 70%. The material was then transferred to 100% propylene oxide and left in the cold overnight, brought to room temperature, and washed twice with propylene oxide. A mixture of Araldite and propylene oxide (1:1 v/v) was added dropwise until there was enough resin to cover the cells when all the propylene oxide was evaporated off. The material was placed in fresh resin after 2 days of slow evaporation of the propylene oxide. This Araldite was polymerized *in vacuo* at 60°C for 36–48 hr.

(iii) *Sectioning and Staining.*—Sections were mounted on coated grids and stained with uranyl acetate and lead citrate (Venable and Coggeshall 1965) for examination in an Hitachi HU-IIE electron microscope. Thicker sections were mounted on glass slides. stained with toluidine blue (Pickett-Heaps and Fowke 1969), and examined with a Zeiss Universal microscope.

(iv) *Living Material.*—Living cells mounted in the culture medium were photographed using both phase-contrast and Nomarski differential interference-contrast optics.

III. Observations

(a) *Light Microscopy of Living Material*

The small nuclei (3–5 μm diameter) were often obscured by the overlying chloroplast(s), making detailed observation of mitosis *in vivo* of little value. Figures 2–7† show adjacent nuclei undergoing mitosis synchronously. The prominent

* See Acknowledgments, p. 1185.

† The following abbreviations are used on Figures 2–28: *c*, centriole complex; *ch*, chromosome; *g*, golgi body; *k*, kinetochore; *n*, nucleus; *nc*, nucleolus; *nm*, nuclear membrane; *p*, pyrenoid; *pf*, polar fenestra; *rn*, reforming nucleus; *t*, microtubules; *w*, cell wall.

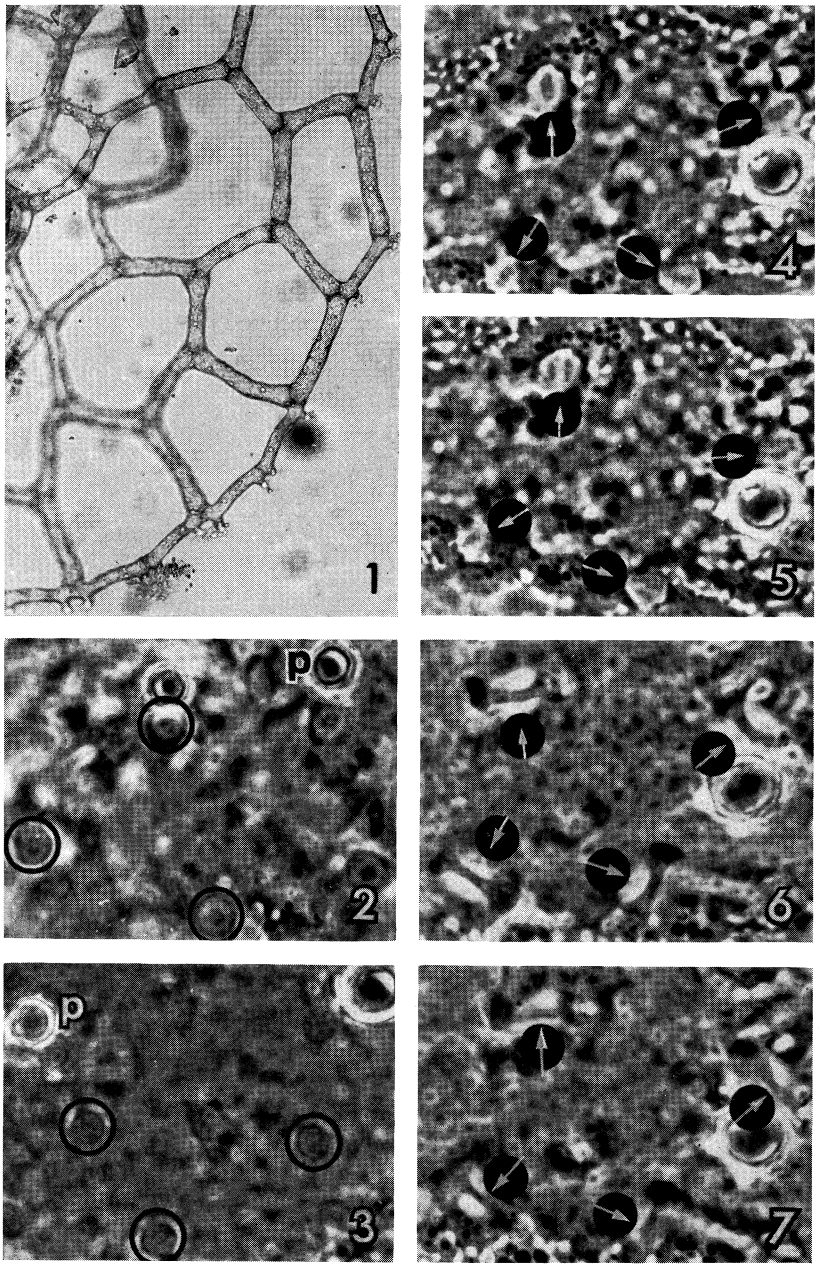
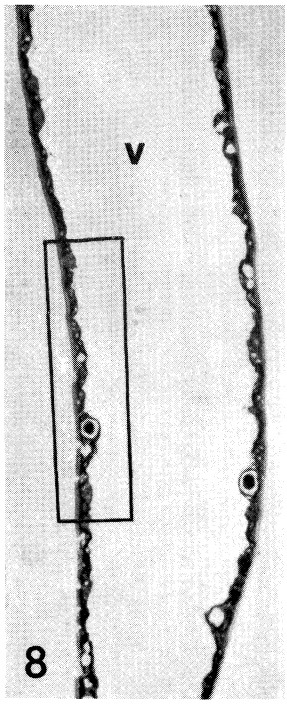


Fig. 1.—Part of a young net of *H. reticulatum*. ×110. [Figures 1–9 are light micrographs; Figures 10–28 are electron micrographs.]

Figs. 2–7. Living cells of *H. reticulatum* undergoing mitosis. In Figure 2 some interphase nuclei are ringed, and nucleoli may be observed. In Figure 3—prophase—the nucleoli have dispersed. Figures 4–7 show part of a series following mitosis in four nuclei within one cell. Arrows indicate the plane of the metaphase plate. *4*, metaphase; *5*, anaphase—early separation of chromatids; *6*, early telophase—spindle elongation may be noted; *7*, telophase. All phase-contrast. ×1400.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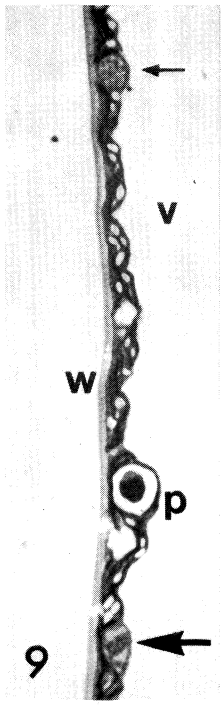
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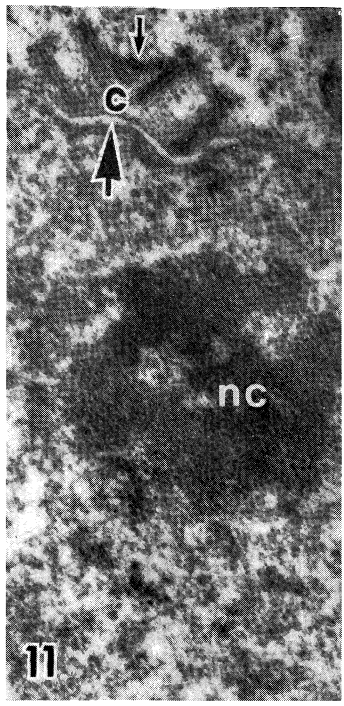
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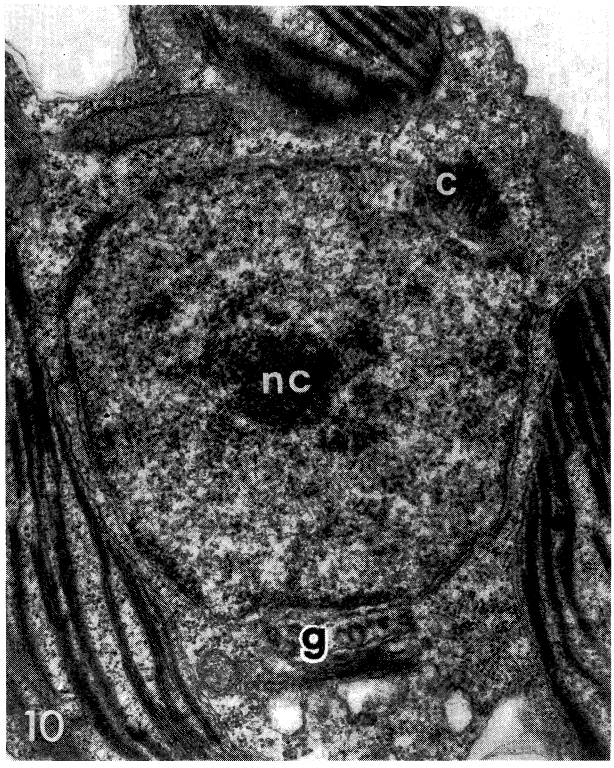
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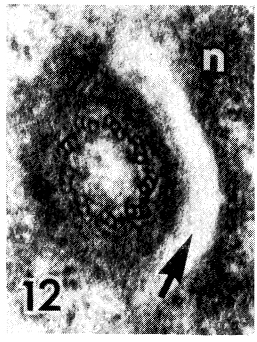
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nucleolus (Fig. 2) dispersed as the chromatin condensed at prophase (Fig. 3); a metaphase plate was clearly visible (Fig. 4) and separation of the chromosomes (Fig. 5) was accompanied by considerable elongation of the spindle (Figs. 6 and 7). The interphase to metaphase transition took about 20 min and metaphase to telophase only about 5 min. Mitotic waves, described by early workers (e.g. Timberlake 1902 and Proskauer 1952), were commonly seen in the coenobia.

(*b*) *Light Microscopy of Fixed Material*

The cells of *H. reticulatum* used were around 2 mm long and 0·2 mm in diameter, rather larger than those shown in Figure 1; unless they were cut after fixation and unless the subsequent dehydration and embedding in resin were very gradual, severe collapse was inevitable. The efficacy of our processing methods can be judged from Figures 8 and 9, which are light micrographs showing part of a longitudinally sectioned cell with its enormous central vacuole. The peripheral layer of cytoplasm was only very rarely detached from the cell wall; the tonoplast was sometimes damaged, ballooning into the vacuole.

(*c*) *Electron Microscopy*

Centrioles appeared to be persistent in the coenocytic stage of the life cycle but they have not been found in some of the uninucleate sexual stages (Marchant and Pickett-Heaps, unpublished data).

(i) *Interphase*

At interphase, the chromatin was usually diffuse (Fig. 10) but sometimes partially condensed near the nuclear envelope. The nucleolus apparently comprised two components (Fig. 11)—a dense matrix and loosely clustered granules about the size of ribosomes. Two centrioles, at right angles, lay adjacent to the nuclear envelope which was distended at this site (Figs. 10–13). A similar modification of the nuclear envelope has also been observed by Northcote (1968) in *H. africanum*. Microtubules were uncommon at interphase even near the cell wall where they might have been expected from observations on other algae and higher plants. Golgi bodies were frequently positioned with one face close to the nuclear envelope; coated vesicles often lay between the nuclear envelope and the face of the golgi body (Fig. 10).

Fig. 8.—Longitudinally sectioned cell of *H. reticulatum* showing the thin layer of cytoplasm enclosing the large central vacuole (*v*). The enclosed area is shown at higher magnification in Figure 9. ×560.

Fig. 9.—Peripheral cytoplasm of *H. reticulatum* containing two nuclei undergoing mitosis: prophase (small arrow), early anaphase (large arrow). Also note the large pyrenoid (*p*). ×1300.

Fig. 10.—Interphase nucleus with adjacent golgi body and centriole complex. ×26,000.

Fig. 11.—Part of an interphase nucleus showing the components of the nucleolus. The nuclear envelope is dilated near the centriole complex (large arrow). Note the bridge (small arrow) linking the centrioles. ×35,000.

Fig. 12.—Transverse section of a centriole complex showing the triplet structure surrounded by amorphous material. Note also the dilated nuclear envelope (arrow). ×65,000.

Fig. 13.—Centriolar replication at prophase; of the four centrioles, two are sectioned transversely and two tangentially. ×30,000.

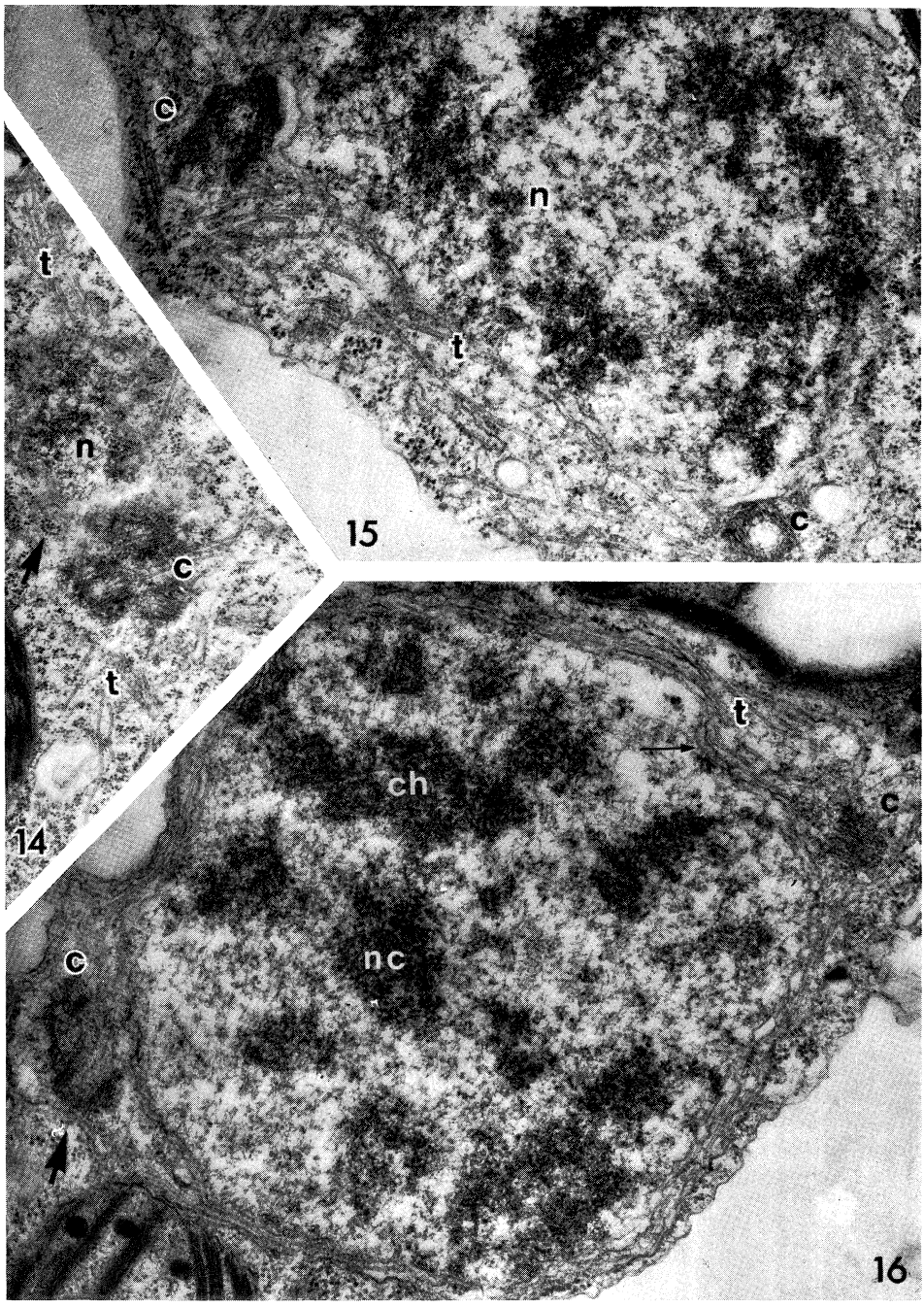

Fig. 14.—A section grazing the nucleus at prophase showing replicating centrioles, microtubules, and polysome spirals (arrow) on the nuclear envelope. ×20,000.

Fig. 15.—Separation of the centriole complexes to establish the poles of the spindle. ×30,000.

Fig. 16.—Nucleus at late prophase showing condensing chromatin, the nucleolus dispersing, and the centriole complexes at the poles. The perinuclear envelope (large arrow) encloses one centriole. Note the slight invagination of the nuclear envelope (small arrow). ×30,000.

(ii) *Preprophase*

Microtubules proliferated outside the nucleus near the centrioles. The nucleolus lost its dense matrix leaving a loose assembly of granules. The golgi bodies and nuclei separated at preprophase and remained so until late telophase.

(iii) *Prophase*

The centrioles, surrounded by amorphous material (Fig. 12), replicated (Figs. 13, 14), and the centriole complexes separated (Fig. 15) to establish the poles of the spindle, the nucleus becoming ensheathed with microtubules (Figs. 16, 17). Concurrently a layer of endoplasmic reticulum enveloped the nucleus and the surrounding layer of cytoplasm containing the centrioles and microtubules (Figs. 16, 17). Dispersion of the nucleolus rendered it almost indistinguishable from the condensing chromatin (Fig. 16).

(iv) *Prometaphase*

Ribosome-like particles were liberated into the nucleoplasm by the complete dispersion of the nucleolus (Fig. 19). Cytoplasmic microtubules began invading the nucleus through polar fenestrae (i.e. openings) forming in the nuclear envelope near the centrioles (Figs. 19, 20). Often the nuclear envelope was temporarily invaginated at the poles, presumably by these elongating microtubules (Figs. 16, 18, 19, 20).

(v) *Metaphase*

The paired chromosomes on the metaphase plate were traversed by continuous microtubules while chromosomal microtubules linked the kinetochores with the poles (Figs. 21–23). The polar fenestrae in the nuclear envelope enlarged but the perinuclear envelope of endoplasmic reticulum remained intact (Fig. 21). The centrioles usually lay in the polar fenestrae (Fig. 21) but Figure 22 shows an intercalation of the nuclear membrane between the centriole and the spindle. Elongation of the spindle started during metaphase (compare Figs. 16 and 21).

(vi) *Anaphase*

The separation of the chromosomes was accompanied by considerable elongation of the spindle (compare Figs. 4–7 with Figs. 22–25). Both nuclear and perinuclear envelopes remained essentially intact (apart from the polar fenestrae of the former) as they contracted between the separating chromosomes (Fig. 24).

(vii) *Telophase*

Mid-bodies were never found between the widely separated daughter nuclei. The perinuclear envelope dispersed and the restoration of intact nuclear envelopes preceded the disappearance of the continuous microtubules remaining between the daughter nuclei (Figs. 25, 26, 28). At late telophase, some of the centriole complexes appeared to move around the nuclear envelope until they were about 90° from the spindle axis (Fig. 27). Unlike in some other algae (*Oedogonium*, *Closterium*, *Ulva*, and other species) the post-mitotic nuclei in *H. reticulatum* did not come close together.

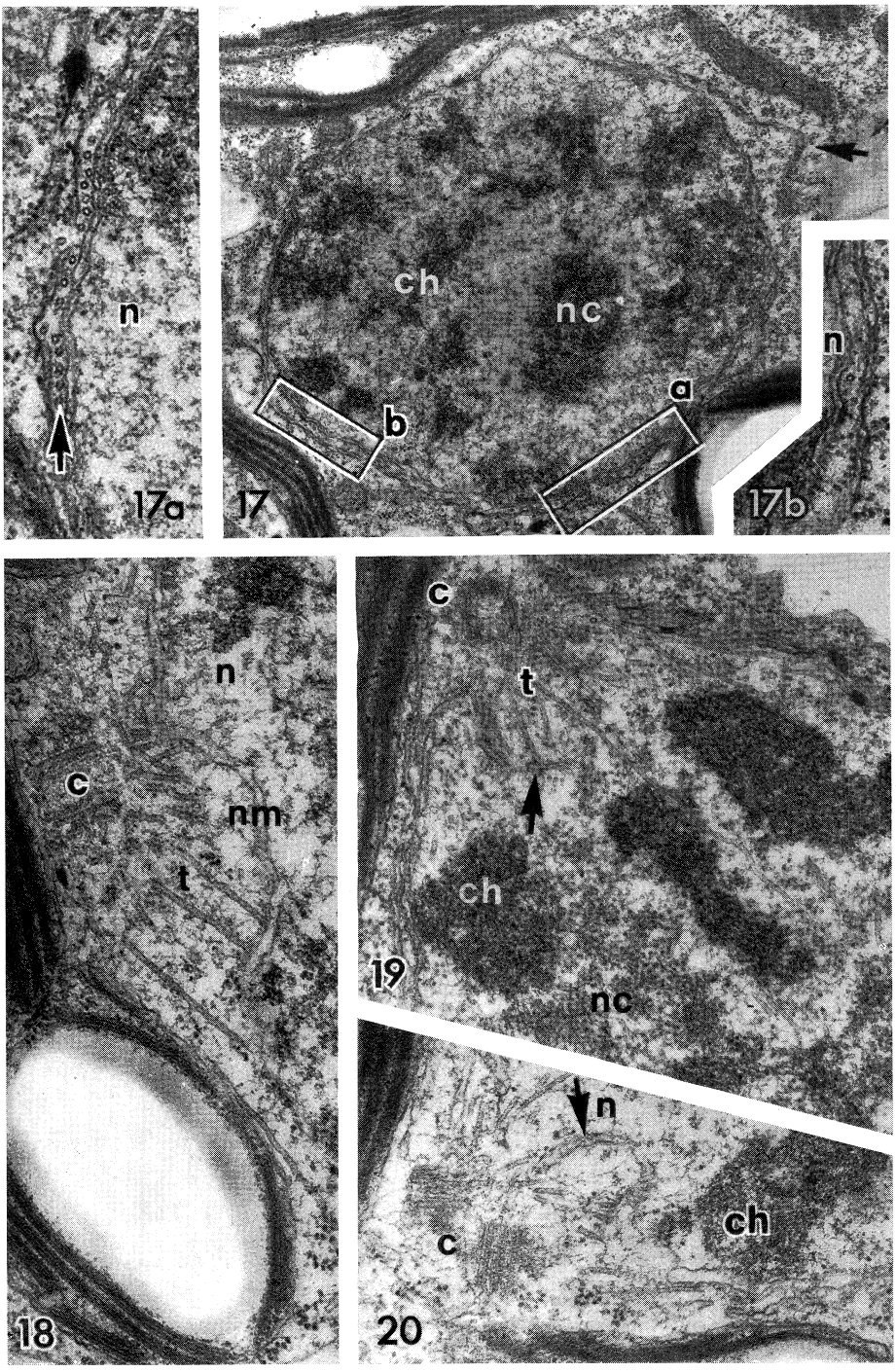

Fig. 17.—A nucleus at prophase almost completely surrounded by the perinuclear envelope (arrow). Fine detail of the enclosed areas is shown in Figures 17*a* and 17*b*. ×17,500.

Fig. 17*a*.—A band of transversely sectioned microtubules (arrow) lying between the nuclear and perinuclear envelope. ×45,000.

Fig. 17*b*.—Fine detail of the nuclear and perinuclear envelopes. ×45,000.

IV. Discussion

The dearth of published observations on the ultrastructure of highly vacuolate coenocytic cells, despite their importance in studies on ion transport, etc., probably reflects the difficulty of preserving these cells for electron microscopy. In our experience, ultrastructural information from cells damaged by conventional processing techniques can be misleading and render any discussion of the gross structure and spatial relationships between cytoplasmic components futile.

Unlike most of the algae studied in this laboratory, it was not essential to meticulously select young, actively growing cells for fixation which is usually necessary (Pickett-Heaps and Fowke 1969, 1970*a*) to avoid cytoplasmic denseness that obscures ultrastructural detail. Cells of some other stages of the life cycle of *H. reticulatum* do, however, exhibit this quality (Marchant and Pickett-Heaps, unpublished data).

In most higher plant and animal cells, the nuclear envelope fragments early in mitosis and reforms at telophase, while in many lower forms of both kingdoms the nuclear envelope remains more or less intact throughout mitosis. Both intact and open spindles are found among the algae. Those with open spindles include *Chara* (Pickett-Heaps 1967), *Prymnesium* (Manton 1964), and *Closterium* (Pickett-Heaps and Fowke 1970*c*); examples of those having closed spindles include species of *Oedogonium* (Pickett-Heaps and Fowke 1969), *Ulva* (Løvlie and Bråten 1970), *Chlamydomonas* (Johnson and Porter 1968), *Kirchneriella* (Pickett-Heaps 1970), and other species [see Pickett-Heaps (1969) for review]. During mitosis in *H. reticulatum*, the behaviour of the nuclear envelope resembles that of *Chlamydomonas* and *Kirchneriella* as it remains essentially intact except for large polar fenestrae.

We find most intriguing the formation of the perinuclear envelope at prophase and its disappearance at telophase, particularly as a similar structure has recently been discovered in *Kirchneriella* (Pickett-Heaps 1970), a related member of the Chlorococcales. This envelope cannot however be discerned from Northcote's (1968) micrographs of *H. africanum*. In *H. reticulation*, the perinuclear envelope apparently isolates the nucleus undergoing mitosis, the centrioles, and a thin layer of cytoplasm around the nucleus from the rest of the cell.

Northcote (1968, p. 183) says centrioles appear at the nuclear envelope of *H. africanum* at the beginning of prophase and he suggests that their occurrence might be significant in plant cells where no rigid cell wall is formed after mitosis. The basis of this latter hypothesis is not given and it appears refuted, for example, by work on spermatogenous cells undergoing mitosis in *Chara* (Pickett-Heaps 1968) and dividing vegetative cells of *Stigeoclonium* (Pickett-Heaps, unpublished data) which have centrioles and also cell plates forming rigid cell walls. Northcote (1968)

Fig. 18.—Prometaphase; polar invagination of the intact nuclear envelope. ×39,000.

Fig. 19.—The nuclear envelope (arrow) is deeply invaginated and fenestrated. Microtubules invading the nucleus. Note the dispersed nucleolus and the ribosome-like particles in the nucleoplasm. ×36,000.

Fig. 20.—Spindle pole with microtubules both inside and outside the nucleus. The nuclear envelope is invaginated (arrow). ×36,000.

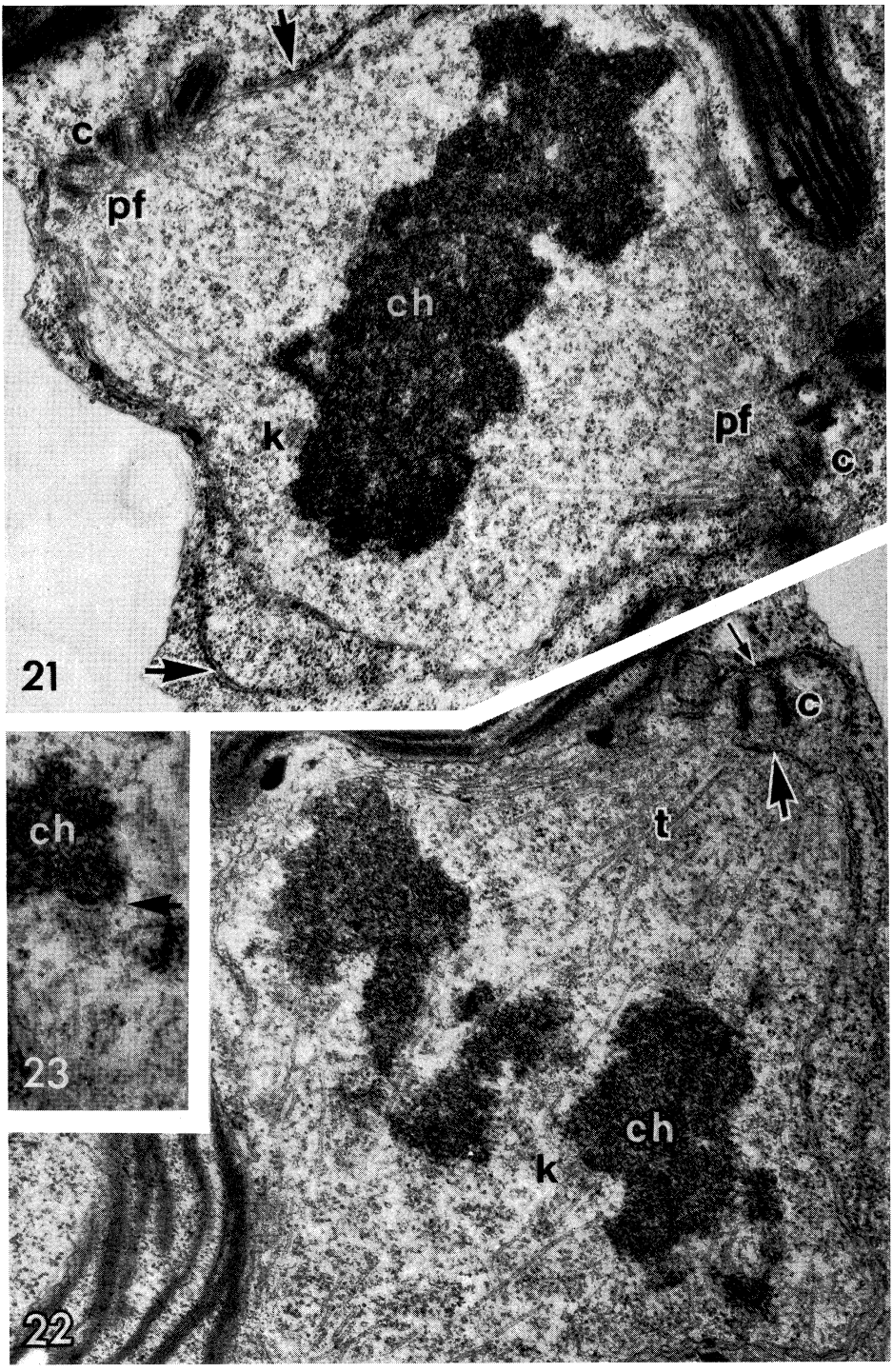

Fig. 21.—A nucleus at metaphase with centrioles lying in the polar fenestrae. The perinuclear envelope (arrows) is shown both closely pressed and separated from the nuclear envelope. ×26,000.

Fig. 22.—Centriole (*c*) between the perinuclear envelope (small arrow) and the nuclear membrane (large arrow). Note the distinct kinetochore (*k*). ×29,000.

Fig. 23.—A kinetochore at metaphase. ×39,000.

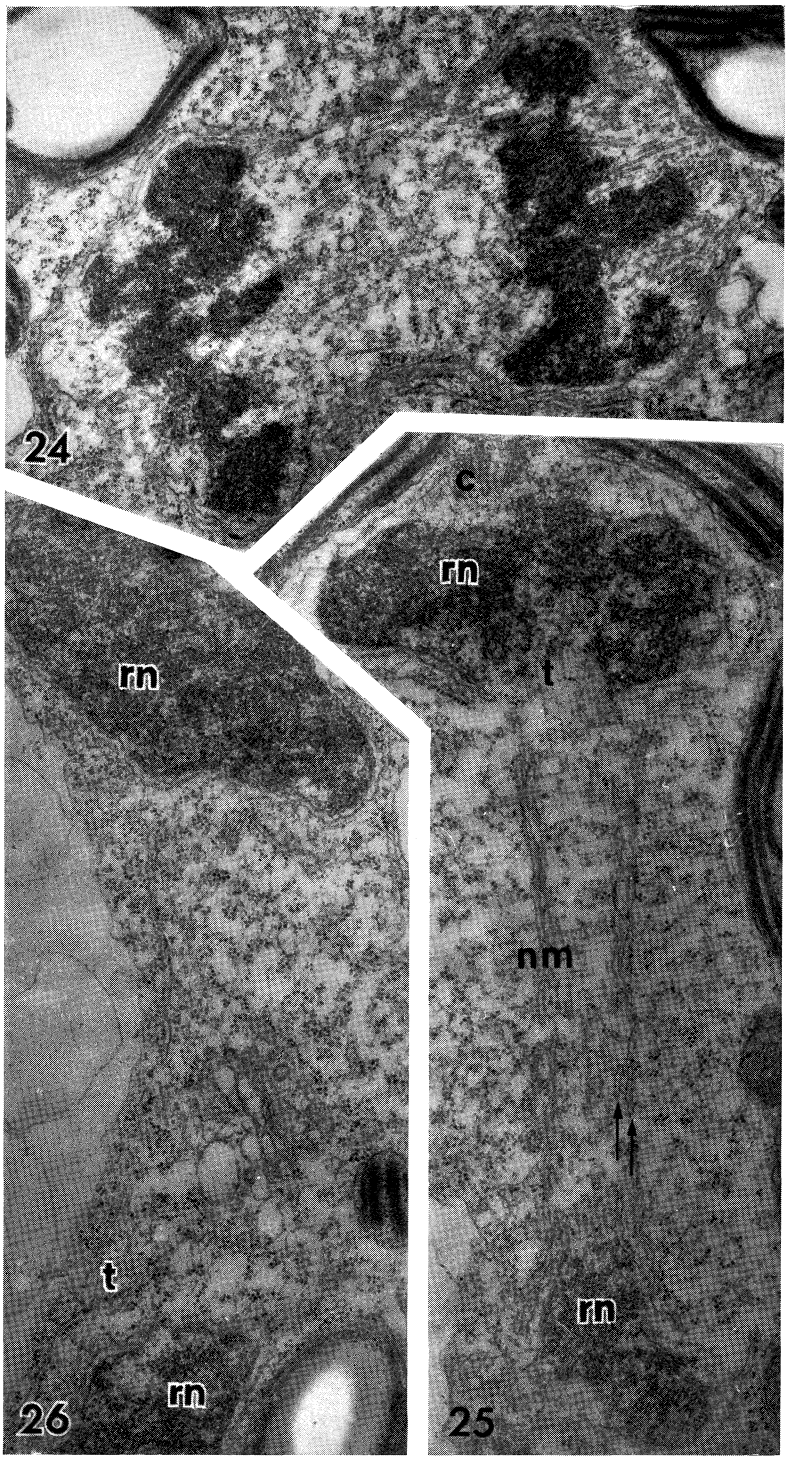

Fig. 24.—Anaphase; intact nuclear envelopes contracting between the separating chromosomes. ×20,000.

Fig. 25.—Telophase; both the nuclear and perinuclear envelopes (arrows) disintegrating. Note that the aggregated chromosomes and centriole (*c*) are still enclosed within the perinuclear envelope. Compare this figure with the light micrograph (Fig. 6) of telophase. ×25,000.

Fig. 26.—Nuclear membrane reforming around the daughter nuclei. Fragments of membrane and disorganized spindle microtubules remain. ×25,000.

similarly associates the presence of kinetochores with the absence of post-telophase wall formation; this also conflicts with the recent work on *Oedogonium* in which a 7-layered kinetochore and rigid transverse walls formed after cytokinesis are found (Pickett-Heaps and Fowke 1970*a*). In *H. reticulatum*, centrioles were apparently persistent in the coenobia which is not unexpected as the cytoplasm of the coenobia cleaves (Marchant and Pickett-Heaps, unpublished data) to form biflagellate zooids *not* at a specific stage of maturity but rather in response to changes in the external environment (Pocock 1960).

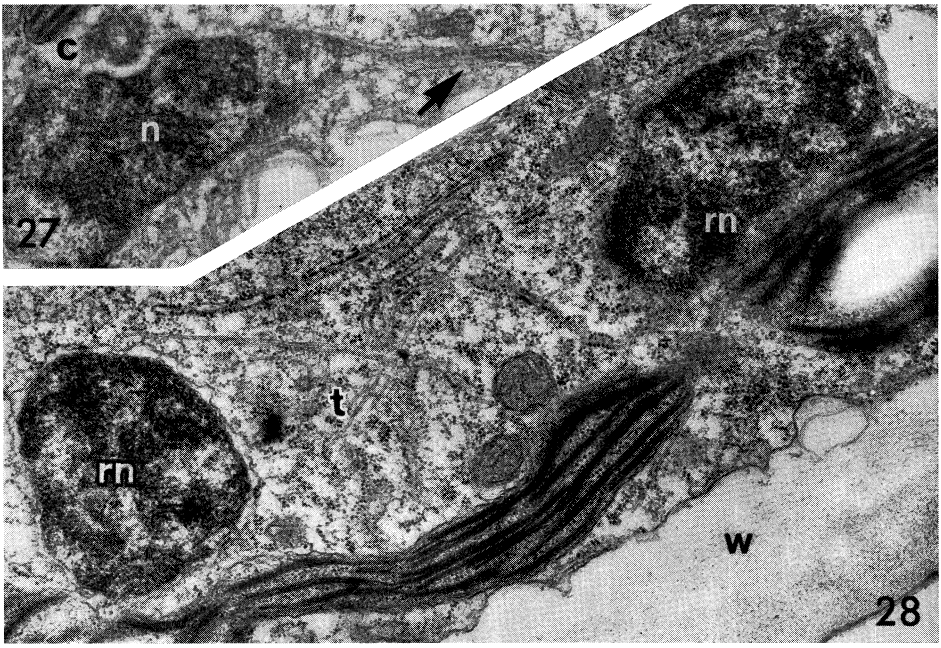

Fig. 27.—A telophase nucleus with centriole (*c*) at right angles to the axis of the spindle. Note the elongation of the nucleus (arrow). ×19,500.

Fig. 28.—Post-telophase daughter nuclei each enclosed in a single nuclear envelope. Other cytoplasmic components have invaded the space between the nuclei where some remnants of the spindle apparatus remain (cf. Fig. 26). ×21,000.

For reasons argued elsewhere (Pickett-Heaps 1969, 1970) we think it most unlikely that centrioles *per se* are involved in the actual formation of the spindle; in *H. reticulatum*, their function appears to be solely connected with some future production of flagella (see above). One rather striking piece of evidence for this non-involvement of the centriole in the spindle apparatus is shown in Figure 22 where nuclear membrane is found between the centriole and the spindle microtubules. This reminds one of various fungi which have extranuclear centrioles situated at the poles of closed spindles (Ichida and Fuller 1968; Lessie and Lovett 1968).

Figures 18, 19, and 20, showing cytoplasmic microtubules abutting the invaginated nuclear membrane, suggest that these invaginations may be caused

by elongation of the microtubules before fenestration of the nuclear membrane is complete. Similar observations on *Haemanthus* endosperm (Bajer and Molè-Bajer 1969), *Kirchneriella* (Pickett-Heaps 1970), and some animal cells (Threadgold 1967, p. 281) strongly suggest a movement of microtubules from the cytoplasm into the spindle during the early stages of mitosis.

A close, specifically orientated association between golgi bodies and interphase nuclei is known in a number of algae including species of *Ulva* (West and Pitman 1967), *Chorda* and *Giffordia* (Bouck 1965), *Kirchneriella* (Pickett-Heaps 1970), and other species. Such an association is evident in *H. reticulatum*, and, as in *Kirchneriella*, it is temporarily lost during mitosis. The activities of the golgi bodies will be discussed in detail when the differentiation of the coenobia to form zooids is described in a later paper.

V. Acknowledgments

We are most grateful to Dr. M. A. Pocock for confirming our identification of the *Hydrodictyon* species used and for other helpful advice. One of us (H.J.M.) gratefully acknowledges the receipt of a Commonwealth Post-Graduate Award.

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