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Fine Structure of *Cylindrocapsa* Zoospores

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With 40 Figures

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Summary

Quadriflagellate zoospores from an isolate of *Cylindrocapsa geminella* have been studied ultrastructurally. Each swimming zoospore is enclosed within a delicate, loose-fitting, outer investment which in grazing tangential section exhibits a pattern resembling wire gauze. The chloroplast is axial and possesses numerous, irregularly shaped lobes, each joined to the central pyrenoid region by a slender connection. A single pyrenoid per zoospore is typical, but as many as 3 per cell have been observed. The pyrenoids have a distinctive structure; the matrix is penetrated by branched cytoplasmic channels delimited by a double membrane system continuous with the chloroplast envelope. Formation of new pyrenoids by bipartition is common. Each zoospore has an eyespot consisting of a single layer of closely packed pigment chambers positioned directly beneath the chloroplast envelope near the cell surface. Contractile vacuoles are prominent near the flagellar bases and throughout their development they are associated with numerous coated vesicles; membrane continuity is often observed between the vesicles and the contractile vacuole. Subunits on the surface of the coated vesicles appear identical to similar subunits occurring on the outer surface of both expanding and fully expanded contractile vacuoles. Preliminary observations are given on the structure of the flagellar apparatus.

1. Introduction

Cylindrocapsa is one of 2 filamentous genera assigned to the *Cylindrocapsaceae*, a family included in the order *Ulotrichales* by many authorities (*e.g.*, FRITSCH 1945, SMITH 1950, RAMANATHAN 1964, BOURRELLY 1966) or in the separate order *Cylindrocapsales* by others (DESIKACHARY 1958, PRESCOTT 1962, ROUND 1971). Few morphological or taxonomic studies have been conducted on the family in recent years (IYENGAR 1957, BOURRELLY 1961, HOFFMAN and HOFMANN 1975) and the present report provides the first ultrastructural information on the group [1].

[1] In a recent paper, PICKETT-HEAPS and MCDONALD (1975) gave an ultrastructural account of mitosis and cell division in *Cylindrocapsa involuta*. However, as discussed later in the present paper, I do not believe the alga they studied is *Cylindrocapsa*.

Members of the *Cylindrocapsaceae* are oogamous and possess a combination of unusual structural features which has stimulated interest in the group's phylogenetic affinities with other green algae. The oogamous condition is not common in filamentous green algae, although it is characteristic of the *Oedogoniales, Sphaeroplea,* and the siphonous genus, *Dichotomosiphon.* Along with oogamy, members of the *Cylindrocapsaceae* demonstrate an unusual type of cell wall formation (for discussion see HOFFMAN and HOFMANN 1975) and appear to be further distinguished by possessing vegetative cells with a single axial chloroplast (IYENGAR 1939, 1957, BOURRELLY 1961, HOFFMAN and HOFMAN 1975). Occasional reports of a parietal chloroplast in *Cylindrocapsa* have been questioned by BOURRELLY (1961).

The present ultrastructural study is a continuation of investigations begun on zoospore formation in an isolate tentatively identified as *C. geminella* (HOFFMAN and HOFMANN 1975). It was initiated in the hope that the ultrastructural features of *Cylindrocapsa* zoospores and germlings would be useful in helping to resolve the phylogenetic affinities of the *Cylindrocapsaceae.*

2. Materials and Methods

The *Cylindrocapsa* isolate used in this study is clearly oogamous (Fig. 9). Although it forms quadriflagellate zoospores like *Cylindrocapsopsis,* its sexual characteristics are clearly those of *Cylindrocapsa.* This isolate has been tentatively identified as a strain of *C. geminella* Wolle which forms quadriflagellate rather than biflagellate zoospores. It has been discussed in an earlier paper (HOFFMAN and HOFMANN 1975) which also gives details on culture maintenance and zoospore induction.

Zoospores produced by induction were collected by pipette from the meniscus of the induction medium where they tended to concentrate. Before fixation, nonswarmers and debri were removed by centrifugation. Active zoospores were then allowed to concentrate again at the meniscus of the fluid in the centrifuge tube where they were collected by pipette and transferred to the fixative. Germlings were obtained by allowing time for the zoospores to settle prior to fixation.

Material for electron microscopy was fixed in 2 ways. One method involved fixation overnight at room temperature in 2% glutaraldehyde made up in soil-extract (*i.e.,* the supernatant from soil-water medium). After rinsing in soil-extract several times, the material was post-fixed for 6 hours in 2% OsO_4 at 0 C. Other material was fixed for 20 minutes in 2% OsO_4 made up in 0.1 M Sørensen's buffer (pH ca. 7.1). In both instances, the fixed material was dehydrated through a graded ethanol series to absolute ethanol which was then exchanged for propylene oxide. The dehydrated material was gradually infiltrated with a mixture of Epon 812/propylene oxide (1 : 3) and then polymerized in fresh pure Epon.

Sections were cut with a diamond knife on a Porter-Blum MT II ultramicrotome and mounted on grids with pure carbon films or carbon-stabilized, formvar films. Conventional methods were used to stain the sections with uranyl acetate followed by lead citrate (REYNOLDS 1963). Whole cells were prepared for electron microscopy by placing a drop of swimming zoospores on a coated grid, fixing them for 20–40 seconds in osmic acid vapor, drying the grids, washing the grids with distilled water to remove salts and other contaminants, and final drying followed by shadowcasting with gold/palladium in a vacuum evaporator. Sections and shadowcast whole-cell preparations were examined with a Hitachi HU-11A electron microscope.

3. Observations

3.1. General Features of Cellular Organization

Free swimming zoospores vary in shape from ovate (Fig. 10) to nearly spherical (Fig. 1) as seen in longitudinal section. In size, they range from 13–20 μm in length and 10–17 μm in width (HOFFMAN and HOFMANN 1975).

Near median, longitudinal sections of mature *Cylindrocapsa* zoospores (Figs. 1 and 10) show a consistent pattern in the arrangement of the major cell organelles. The pyrenoid (or occasionally several pyrenoids) is always near the center of the cell or slightly posterior. Immediately anterior to the pyrenoid is the nucleus (Figs. 1 and 10) within which one often observes a nucleolus (Fig. 1). Anterior to the nucleus are the contractile vacuoles (Figs. 1, 10, and 19) while the flagellar apparatus is found at the extreme anterior position (Figs. 1, 10, 19, and 32). The quadiflagellate nature of the zoospores can be readily determined in living cells (HOFFMAN and HOFMANN 1975) or with shadowcast preparations of whole cells studied with the electron microscope (Fig. 5).

Most of the zoospores examined possessed numerous, irregularly shaped, very dense globules distributed in an irregular fashion at the surface of the plasmalemma (Figs. 1, 6, 10, 19, and 32). These globules vary greatly in size (Fig. 1). Although they may occur anywhere on the plasmalemma, they are usually most common at the anterior end of the cell, particularly on the flagellar sheath (Figs. 1 and 32). They are also occasionally found on the inner surface of the contractile vacuole membrane (Figs. 6, 22, 26, and 29) and may even occur within the membranous elements of discharged contractile vacuoles. Their function is unknown.

The origin of these dense, surface globules is uncertain, but they may be derived from small vesicles occasionally observed within the cell which contain material of similar density. These dense vesicles should not be confused with the far more numerous and relatively larger vesicles of somewhat less density which are common in the anterior region of most zoospores (Figs. 1, 6, and 10). This latter type of vesicle is almost certainly Golgi derived and is similar in appearance and position to zoospore vesicles in other green algae which are thought to be associated with zoospore adherence during germination (*e.g.*, HOFFMAN and MANTON 1962, MANTON 1964, EVANS and CHRISTIE 1970).

Although the zoospore lacks a cell wall, it always possesses an unusual and very thin outer investment (Figs. 1–3, 6–8, 10, 15, 19, 20, 22, 27, and 38). This outer investment appears loose-fitting and a wide "space" frequently separates it from the plasmalemma, particularly at the posterior end of the cell. The investment completely surrounds the cell except for small discontinuities through which the flagella emerge (Figs. 1, 10, and 37). Structural details of the outer investment will be discussed at length later in the paper.

The shape of the axial chloroplast, with its central pyrenoid, is difficult to discern in most zoospores, especially when large grains of stromal starch are present (Figs. 1 and 10) which result in swollen regions in the chloroplast lobes. With single sections, this creates the impression that the zoospore contains many small chloroplasts. However, serial sections clearly show that the swollen segments are all interconnected, although these connections are sometimes very tenuous. A few thin connections are evident in Fig. 10.
Each zoospore contains a single eyespot within the chloroplast. This occurs near the plasmalemma in an equatorial position or slightly anterior (Fig. 1).
Numerous Golgi bodies are conspicuous within the zoospore. These are most predominant in a perinuclear position (Fig. 1) although they may also surround the pyrenoid as in Fig. 11. Mitochondria, although scattered throughout the zoospore, are usually most prevalent at the anterior end (Fig. 1). They are relatively elongate, sometimes branching, and exhibit numerous flattened cristae (Fig. 4) as is typical of many green algae. Frequently, one observes a dense amorphous region between a mitochondrion and an adjacent chloroplast lobe (Fig. 4, arrow).

3.2. Outer Investment

At low magnification, the outer investment appears in cross-sectional view as a delicate dense line (Figs. 1 and 10). At higher magnification (Figs. 2–3), it appears as a regular pattern of alternating dense and less dense regions which resembles a "dotted line" ca. 10–12 nm thick. The explanation for this pattern becomes apparent when one observes the investment in grazing tangential section (Figs. 7 and 8). A relatively large area of an investment is shown in such a section in Fig. 7, while a smaller region is shown at higher magnification in Fig. 8. The investment appears as a gauze-like network with

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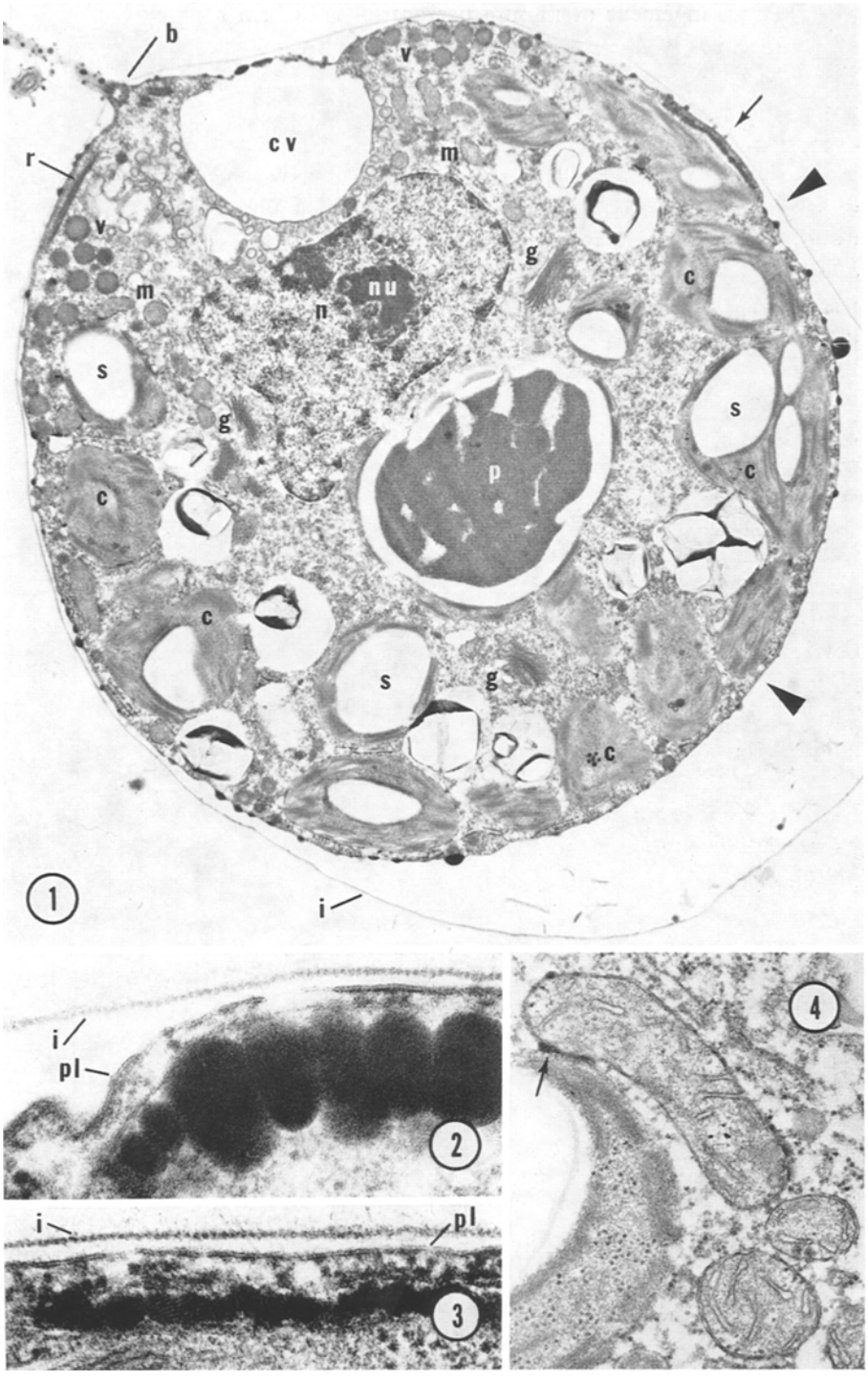
Abbreviations (apply to all figures):

b = basal body of flagellum, *c* = chloroplast, *cv* = contractile vacuole, *ce* = chloroplast envelope, *g* = Golgi body, *i* = thin outer investment, *p* = pyrenoid, *pl* = plasmalemma, *m* = mitochondrion, *n* = nucleus, *nu* = nucleolus, *r* = flagellar root; *s* = starch, *v* = dense vesicle characteristic of the anterior end of the zoospore. The cells in all illustrations were fixed with glutaraldehyde unless otherwise stated.

Figs. 1–4. Fig. 1. Median longitudinal section through a zoospore of *Cylindrocapsa* showing the relative positions of major cell organelles. Note particularly the nucleus, pyrenoid, contractile vacuole, outer investment, and eyespot (arrow). Three connected segments of a chloroplast lobe are shown between arrowheads. ×10,000. Fig. 2. Cross section through the thin outer investment illustrating its perforated nature which gives it the appearance of a "dotted line". Beneath it lies a part of the cell including pigment chambers of the eyespot. ×80,000. Fig. 3. Another cross section through the outer investment lying close to the cell surface. ×80,000. Fig. 4. Section through mitochondria showing flattened cristae. Note dense material between mitochondrion and adjacent chloroplast lobe (arrow). ×38,000



Figs. 1–4

a regular arrangement of minute perforations to form a pattern of two sets of parallel lines intersecting at right angles (Figs. 7–8). The parallel lines have a constant spacing with a periodicity of ca. 14–15.5 nm. The regular pattern of small perforations explains the "dotted line" appearance when examined in cross section.

Fig. 7 shows a relatively large area of investment with the regular gauze-like pattern. However, near the bottom and right-hand margins there appears to be some discontinuity in the pattern with a slight shifting in the orientation of different "patches" of parallel lines. Such discontinuities are apparently very common. A good example of many patch-like discontinuities within a small region is seen in Fig. 8.

3.3. The Chloroplast

Single sections through a zoospore (Figs. 1 and 10) present a somewhat confusing picture of chloroplast shape. In fact, the zoospore appears to contain many irregularly shaped chloroplasts when a single section is examined. Serial sections are required to determine that this is not the case. Although a complete study of all sections through a cell has not been obtained, there is sufficient evidence based on many series of sections to indicate that all chloroplast portions are interconnected within the zoospore to form a single axial chloroplast of complex shape.

The central portion of the axial chloroplast includes the region of the pyrenoid (or sometimes several pyrenoids). Thin connections radiate from this region and each expands and branches to form a complex peripheral lobe. Each lobe consists of a more or less ramified system of swollen segments joined by tenuous connections. Three connected segments of such an expanded peripheral lobe are shown in section on the right side of the zoospore in Fig. 1 (between the 2 arrowheads). Large grains of stromal starch frequently result in enormously swollen chloroplast segments (Figs. 1 and 10). Since the connections between swollen chloroplast segments are often very tenuous, it is seldom that a single section shows continuity between the pyrenoid and the peripheral portions. The swollen segment to the immediate left of the pyre-

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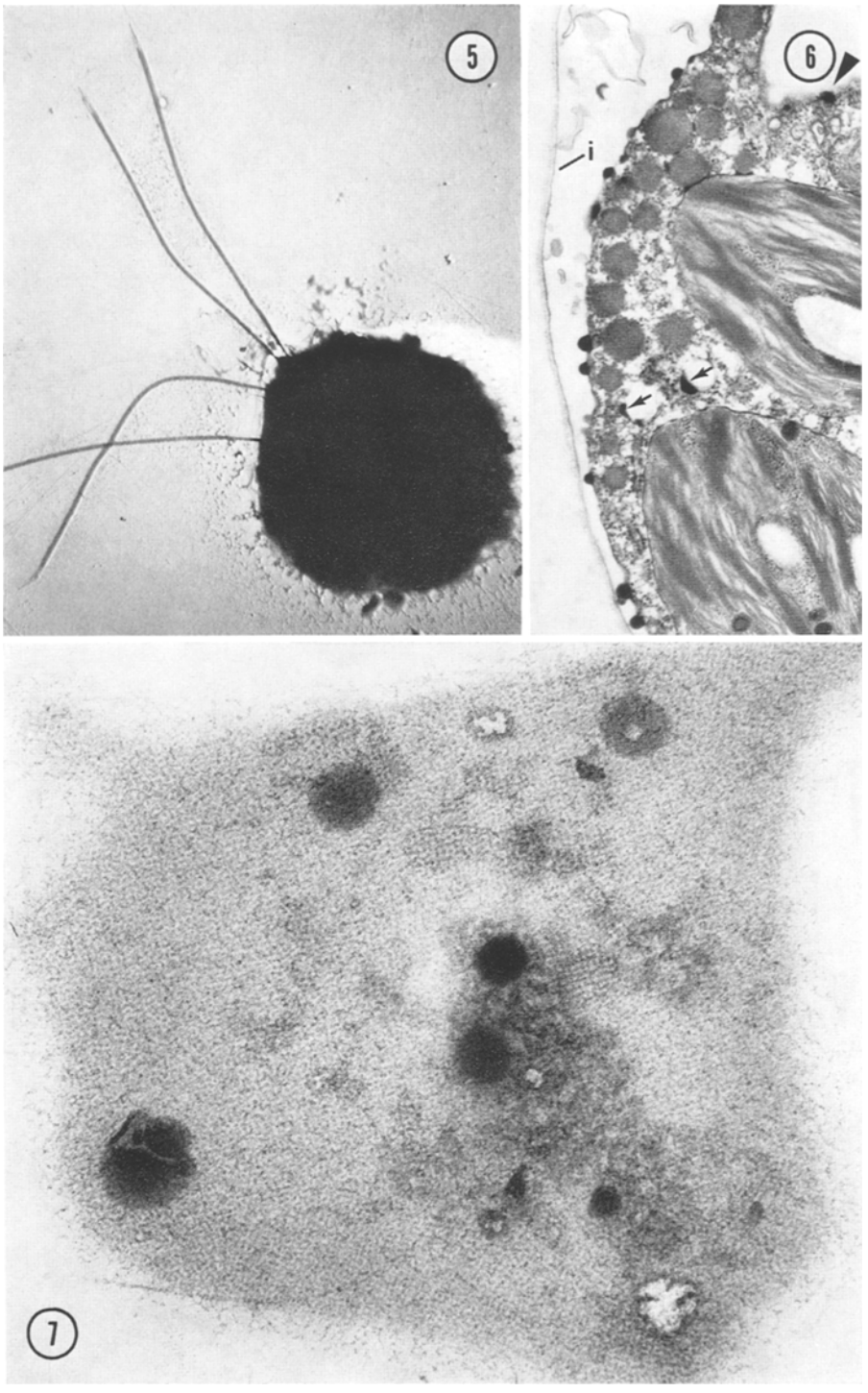
Figs. 5–7. Fig. 5. Shadowcast whole-cell preparation showing a zoospore with its 4 isokont flagella. The region of flagellar insertion is just beneath the cell at the upper left-hand margin. ×3,200. Fig. 6. Section through the anterior of a zoospore to contrast small dense surface globules with a group of larger, less dense vesicles within the cell. The latter may be associated with zoospore adhesion during germination. Note two vesicle-like, marginal pockets of an overlying contractile vacuole (as determined from adjacent sections) which contain material similar in density to that of the surface globules (arrows). Another dense globule is seen on inner surface of a contractile vacuole (arrowhead). ×25,000. Fig. 7. Grazing tangential section showing the gauze-like pattern at the surface of the outer investment. ×55,000



Figs. 5–7

noid in Fig. 10 (arrow) is shown in adjacent sections to be connected with the central pyrenoid region.

The nature of the chloroplast appeared virtually the same in all zoospores examined. However, the chloroplast lobes of young germlings are sometimes more readily demonstrated than those in zoospores. This is particularly true of those that possess relatively little stromal starch (Fig. 12). In such cells, the general shape of the axial chloroplast with its radiating lobes can be discerned in living cells with light microscopy (see Figs. 39–40 in HOFFMAN and HOFMANN 1975).

Fig. 12 shows a transverse section through a single-celled germling. Note the central pyrenoid region with the numerous radiating connections which expand into the branched peripheral lobes. Also evident are the cell wall (arrow) and the persistent outer investment. Relatively little stromal starch is present in this cell as compared to the zoospores shown in Figs. 1 and 10.

3.4. Pyrenoids

Typically, there is a single central pyrenoid in the chloroplast of each zoospore (Figs. 1 and 10). However, many zoospores possessed two pyrenoids (Fig. 11) and a few had three. Multiple pyrenoids arise within a zoospore through division of a single parental pyrenoid (Fig. 13); all stages in pyrenoid division are readily observed in a single fixation. Although multiple pyrenoids are common, most zoospores possessed only a single pyrenoid at the time of attachment.

Pyrenoid ultrastructure is very distinctive and represents a type found in a relatively few, but diverse, green algal genera (GIBBS 1962, MANTON and PARKE 1965, HOFFMAN 1968, RETALLACK and BUTLER 1970, SWALE and BELCHER 1971, MARKOWITZ and HOFFMAN 1974). *Sphaeroplea* also possesses pyrenoids of this type (unpublished observations). The dense matrix appears granular (Fig. 14) or, at times, finely fibrous. The matrix is penetrated by numerous, branched, cytoplasmic channels (Figs. 1, 10, and 12–14). At higher magnification, the channels are seen to be delimited by a double membrane system continuous with the chloroplast envelope (Fig. 14). A single layer of starch grains surrounds the matrix; the shape and size of the individual grains

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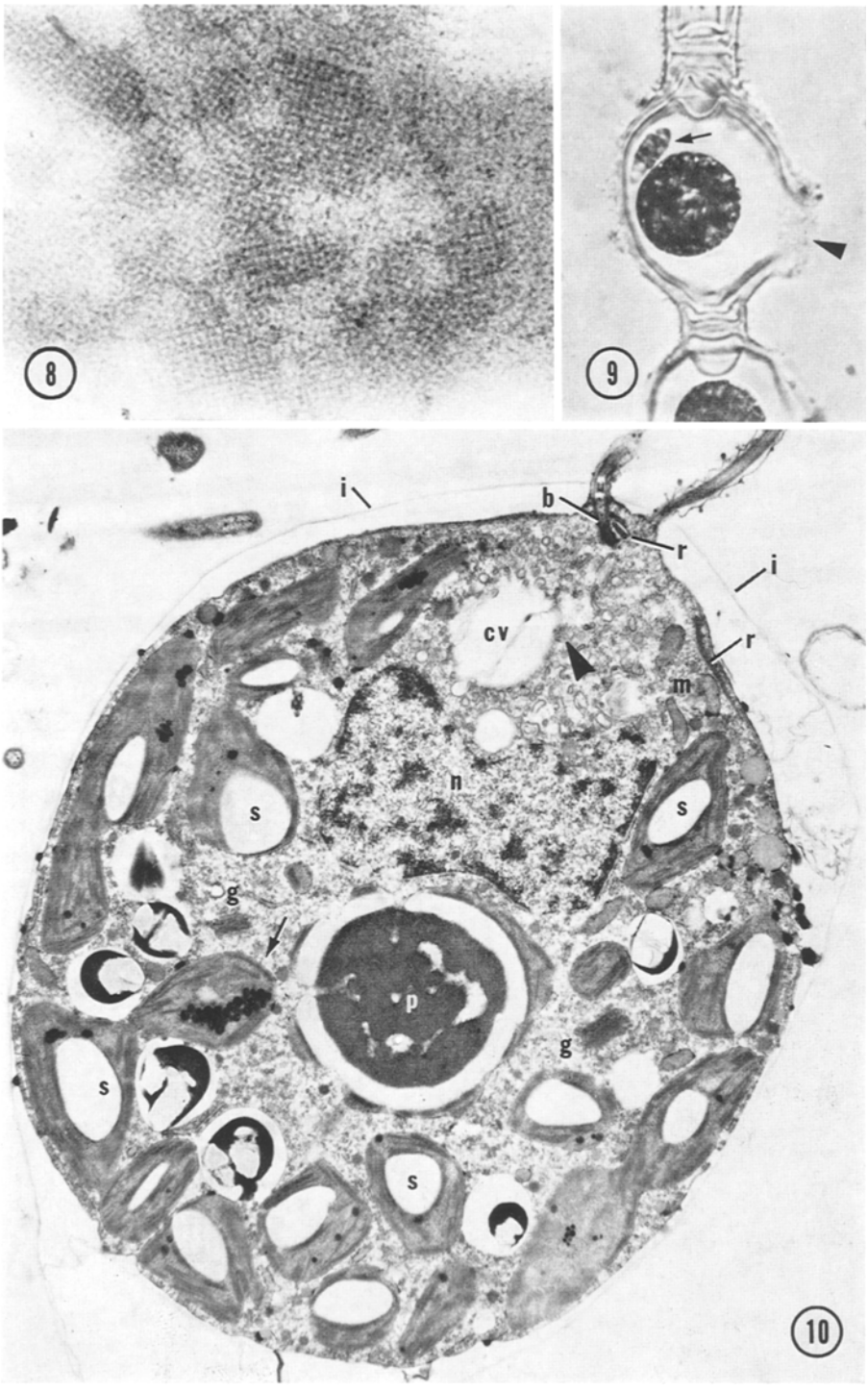
Figs. 8–10. Fig. 8. Small portion of the outer investment in grazing tangential section to show the gauze-like pattern (*cf.*, Figs. 2–3). Note discontinuities which result in distinct patches with different orientation in the sets of parallel lines. ×80,000. Fig. 9. Light micrograph of an oogonium which contained an egg and a swimming spermatozoid (arrow). Arrowhead indicates oogonial pore. ×600. Fig. 10. Median longitudinal section through a zoospore showing the relative positions of major cell organelles. A chloroplast lobe (arrow) is connected to the central pyrenoid region (as confirmed in adjacent sections) and an accessory vacuole appears to be fusing with a contractile vacuole (arrowhead). ×9,700



Figs. 8–10

vary considerably. The cytoplasmic channels which penetrate the pyrenoid matrix pass between adjacent starch grains. They appear devoid of any recognizable cytoplasmic constituents with the exception of ribosomes and an occasional membranous profile.

3.5. The Eyespot

Each zoospore possesses an eyespot positioned peripherally in one of the chloroplast lobes near the cell surface. It is typically found near the cell equator or slightly anterior (Fig. 1). As seen in longitudinal section (Figs. 2 and 15), the eyespot consists of a single layer of closely packed pigment chambers (*i.e.*, osmiophilic globules) positioned directly beneath the chloroplast envelope near the cell surface. A layer of cytoplasm separates the chloroplast envelope from the plasmalemma in this region (Fig. 15) although it is sometimes very thin (Fig. 2).

Immediately subtending the layer of pigment chambers is a conspicuous pocket of stroma which lacks thylakoids but includes many chloroplast ribosomes (Fig. 15). Occasionally, a few thylakoids intervene between the layer of pigment chambers and the stromal pocket as seen in Fig. 15. Tangential sections through eyespots (Figs. 17 and 18) also show the stromal pocket with its numerous ribosomes and occasional starch grains.

The close packing of the pigment chambers is best seen in tangential sections through eyespots (Figs. 16 and 17). In sectional profile the closely packed chambers appear six-sided (or sometimes five-sided) as is true of most algal eyespots.

Although eyespots typically consist of a single layer of pigment chambers, one was observed which appeared to have a poorly developed second layer (Figs. 17 and 18). Figs. 17 and 18 show different levels of section from a tangential series through a single eyespot. Fig. 17 shows a grazing section near the surface of the eyespot, while Fig. 18 passes through the base or margin of the eyespot. A small group of pigment chambers is shown in Fig. 18 (arrow) which apparently constitutes a partial second layer. Sections passing through the overlying levels showed the continuous primary layer of pigment chambers immediately above.

Tangential sections through the base of an eyespot (Fig. 18) show a ring-shaped assemblage of pigment chambers since each eyespot has a pronounced concavo-convex shape (*cf.*, Fig. 15). Serial sections in a tangential plane can

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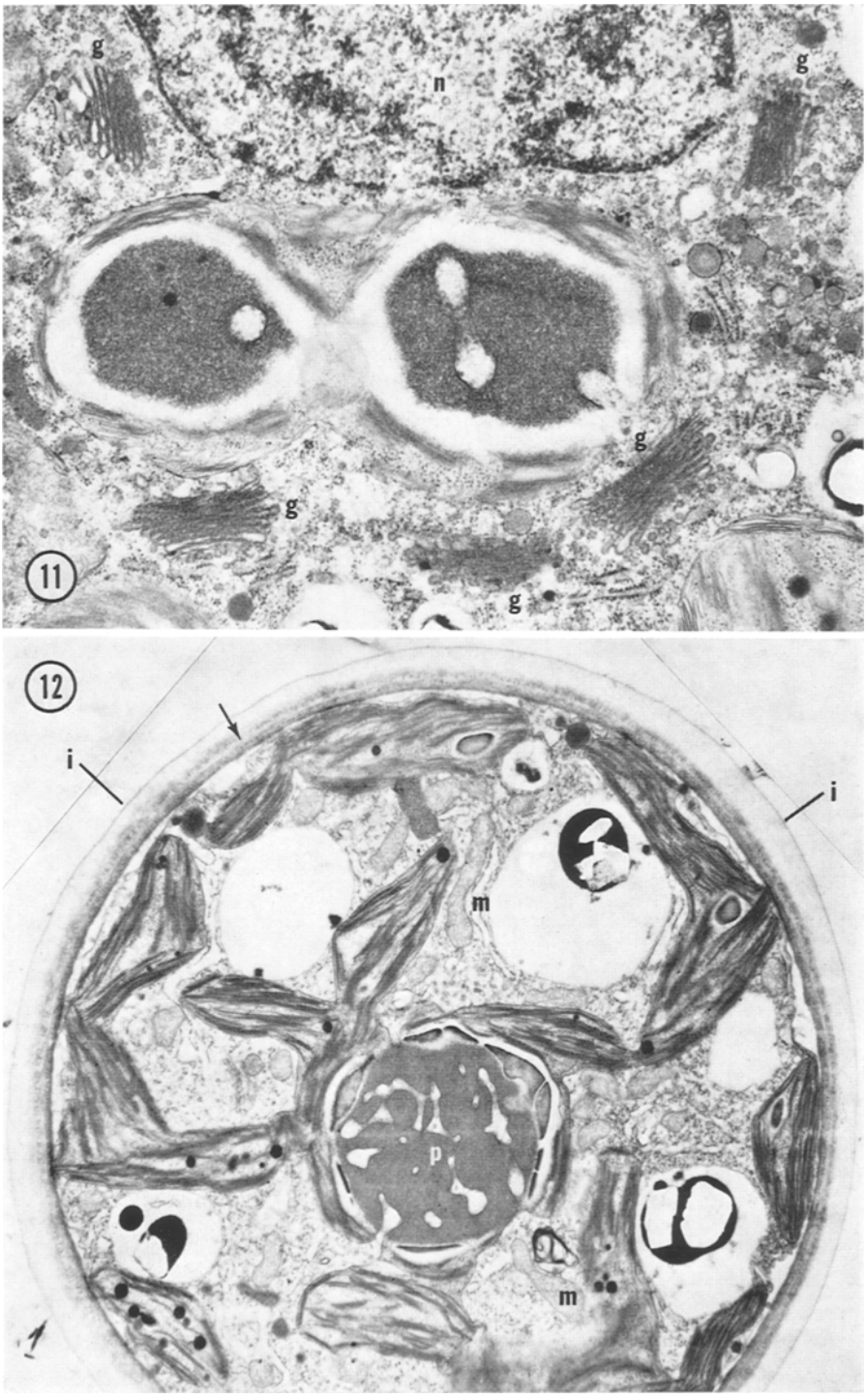
Figs. 11 and 12. Fig. 11. Section through a zoospore that contains 2 pyrenoids. Note also the nucleus, the numerous Golgi bodies, and elements of rough endoplasmic reticulum. ×20,000. Fig. 12. A transverse section through a young, single-celled germling to show the central pyrenoid region with its radiating chloroplast lobes. A thin cell wall is evident (arrow) as well as the persistent outer investment. ×9,600



Figs. 11 and 12

be used to determine the marginal configuration and size of eyespots. Most eyespots exhibited an ovoid profile and ranged in size at the margin from ca. 3.2–4.5 μm in length and 1.0–1.8 μm in width.

3.6. Contractile Vacuoles

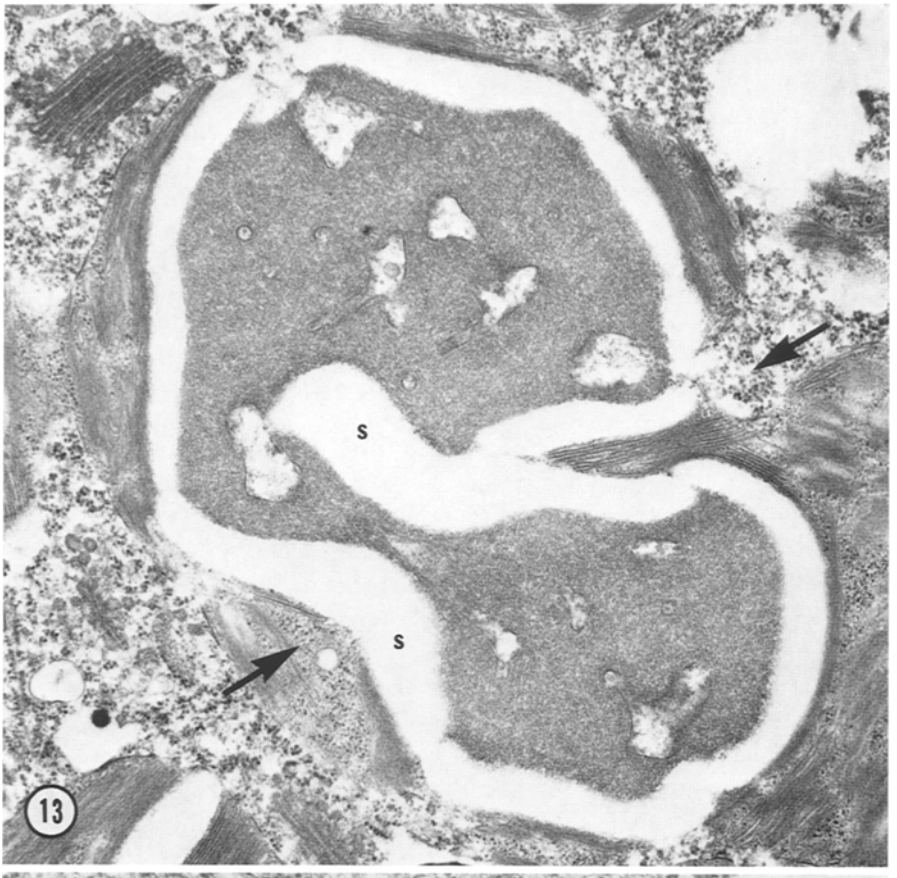
Contractile vacuoles are conspicuous features in the region between the nucleus and the flagellar bases (Figs. 1 and 19). Transverse sections through this region suggest that each zoospore possesses two contractile vacuoles. Portions of two expanded contractile vacuoles are shown in the longitudinally sectioned zoospore illustrated in Fig. 19; smaller, accessory vacuoles are evident between them. A contractile vacuole may appear in varying degrees of expansion, ranging from discharged (Figs. 20 and 21) to fully expanded (Fig. 27).

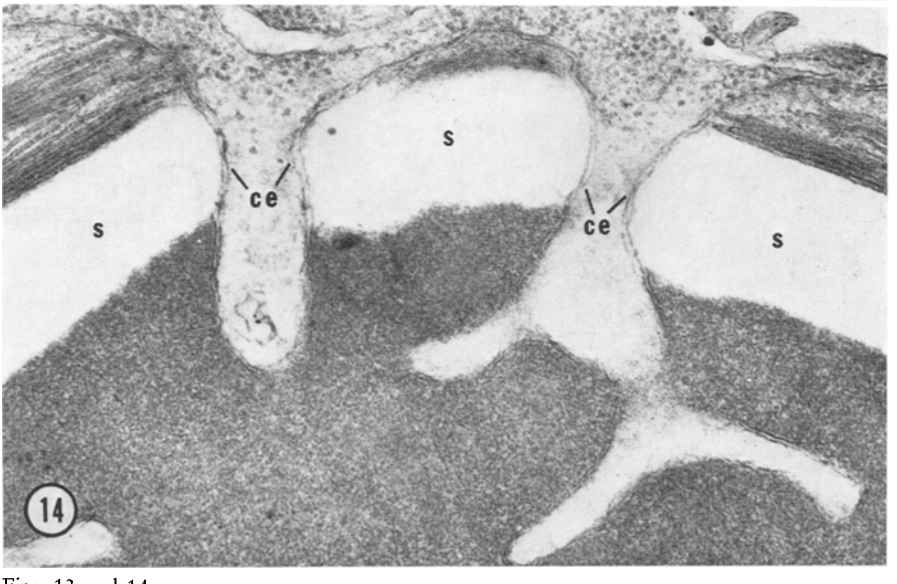
A discharged contractile vacuole appears as a complex and presumably interconnected system of convoluted membranes. In section, the membranes often give the appearance of large flattened vesicles. In association with these membranes one always finds small, spherical vesicles of a most distinctive nature. These are "coated" vesicles, so-called because they bear on their outer surface a coating of small subunits. Coated vesicles are shown in association with expanding contractile vacuoles in Figs. 22, 23, 25, and 26. The subunits are clearly seen, whether the vesicle is shown in cross section (Fig. 25) or in grazing tangential section (Fig. 26).

The appearance of contractile vacuoles varies somewhat with the fixation procedure. The discharged contractile vacuole of Fig. 20 is from a cell fixed with osmic acid, while that of Fig. 21 is from a cell fixed with glutaraldehyde. The membranes appear more dense in Fig. 20 thus making them more conspicuous. However, the coated vesicles in the osmic acid-fixed cell (Fig. 20) do not show the surface subunits as distinctly as in the glutaraldehyde-fixed cell (Fig. 21). In the former, the surface of each vesicle appears to bear minute, hair-like projections, while in the latter, the subunits are more obvious and look like those shown at higher magnification in Figs. 25 and 26.

During expansion of the contractile vacuole, membrane continuity is established between coated vesicles and the expanding vacuolar membrane (Figs. 22 and 23). The tripartite nature of both the vesicle and contractile vacuole membranes is evident (Fig. 23). Also, there appears to be at least occasional fusion between coated vesicles (Fig. 26, arrows).

Figs. 13 and 14. Fig. 13. Near median section through a pyrenoid which appears to be in a late stage of division. A thin connection joins the 2 major portions of the pyrenoid matrix at this level of section, but not in sections above or below this level. Putative plane of division is indicated by arrows. ×25,000. Fig. 14. Portion of a pyrenoid to show that the delimiting membranes of the cytoplasmic channels are continuous with the paired membranes of the chloroplast envelope. ×45,000





Figs. 13 and 14

Subunits on the coated vesicles appear spherical when the vesicles are seen in cross section (Fig. 25 and some in Fig. 26). However, in grazing tangential section, the closely packed subunits on the surface of the vesicle appear angular with 5 or 6 sides (Fig. 26).

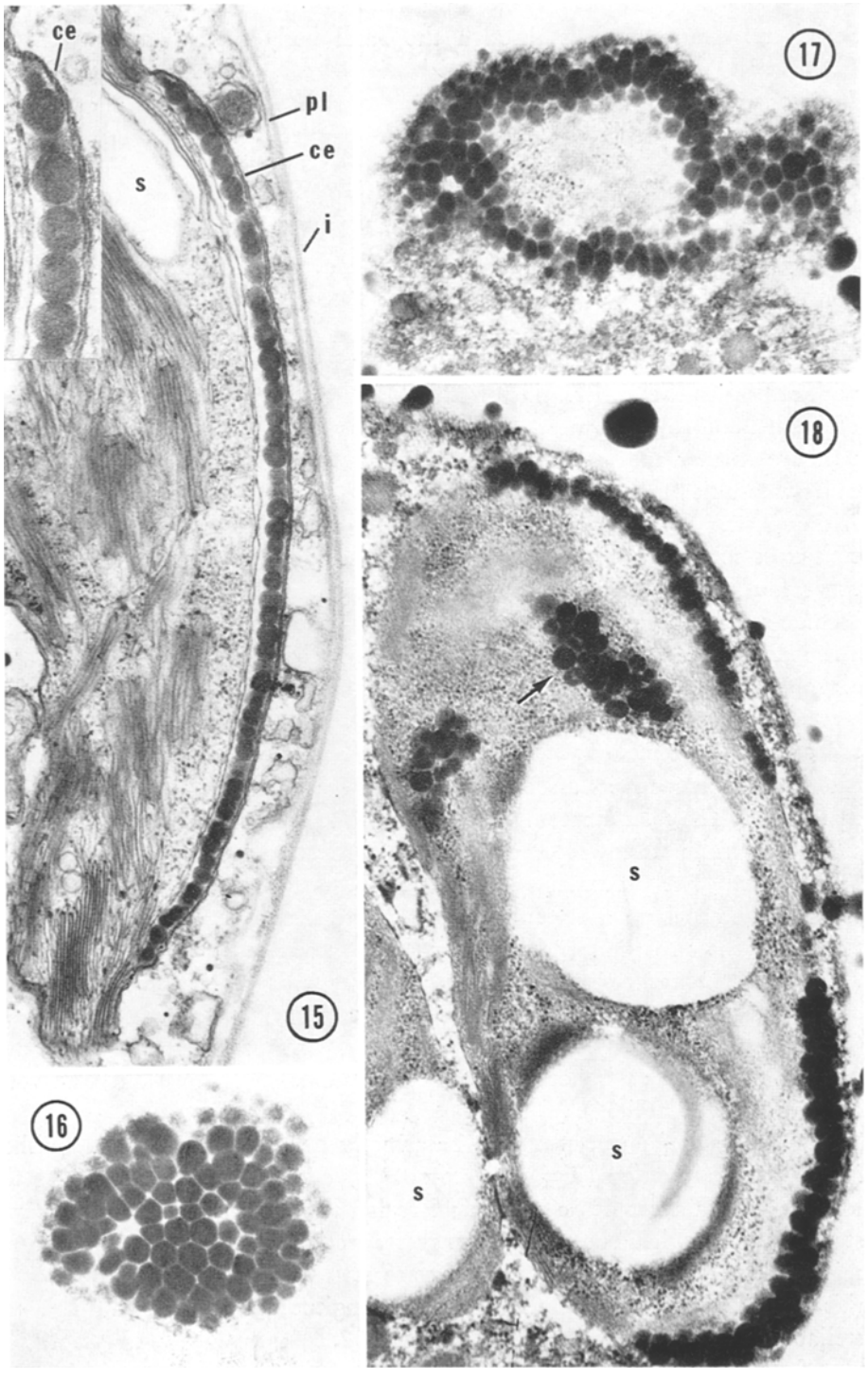
Furthermore, it was frequently possible to distinguish relatively large areas of the contractile vacuole membrane that bore subunits indistinguishable from those on coated vesicles. This was true of both expanding and fully expanded vacuoles (Figs. 27 and 28). Grazing tangential sections at the surface of expanded contractile vacuoles indicate that such subunits are sometimes associated with much of the membrane surface (Fig. 24). As on the surface of coated vesicles, these amassed subunits appear in tangential section as a network of five- and six-sided meshes.

3.7. Flagellar Apparatus

The flagellar apparatus occupies the anterior-most position in the zoospore (Figs. 19 and 32). It consists of four basal bodies with associated isokont flagella, four cruciately arranged flagellar roots which alternate with adjacent basal bodies, and a complex variety of fibrous components.

The four basal bodies of a flagellar apparatus are shown in Fig. 30. All of them appear in oblique transverse section with the exception of #3 which clearly shows the characteristic triplet pattern of the component fibers. One basal body (#2) has just been grazed by this section at its proximal-most end. The four basal bodies are oriented in a diamond-shaped pattern with members of one diagonally opposed pair (#1 and 3) very close together and positioned midway between the more widely separated members of a second diagonally opposed pair (#2 and 4). This pattern was consistent in every zoospore examined. Thus, it was possible to distinguish a bilateral symmetry in the flagellar apparatus with the proximal ends of one opposing pair of basal bodies very close together, while the proximal ends of the other opposing pair were more widely separated.

Figs. 15–18. Fig. 15. Longitudinal section through an eyespot showing a single layer of pigment chambers. Note subtending thylakoids (3 at upper margin and one extending all the way) which separate the pigment chambers from an underlying stromal pocket filled with ribosomes. ×30,000. Fig. 15 (inset). Portion of same eyespot at higher magnification. ×60,000. Fig. 16. Section tangential to the surface of an eyespot to show closely packed pigment chambers. ×30,000. Fig. 17. Another eyespot in tangential section (*cf.*, Fig. 18). ×30,000. Fig. 18. Same eyespot as seen in Fig. 17 but at a deeper level of section which passes through the base of the eyespot and shows the peripheral "ring" of pigment chambers (*cf.*, Fig. 15). Micrograph is oriented at ca. 90° rotation with respect to Fig. 17. A small group of pigment chambers (arrow) appears to form a partial second layer in this atypical eyespot. The central region is the stromal pocket with its ribosomes and two large starch grains. ×30,000



Figs. 15–18

Serial, transverse sections through the basal body region indicate that a fibrous band cross-connects the basal bodies of one diagonally opposed pair. However, the other pair is not similarly connected. The connected pair is always the one with the basal bodies closest together (*e.g.*, #1 and 3 in Fig. 30). This fibrous connective band is most readily demonstrated in sections which pass longitudinally through the basal bodies (Figs. 19 and 32). Its contact with the basal bodies occurs midway along their length, in a position which represents the basal-most part of the distal end. Similar connective bands in this position have been observed in cells of *Chlamydomonas* and have been called *distal fibers* by RINGO (1967).

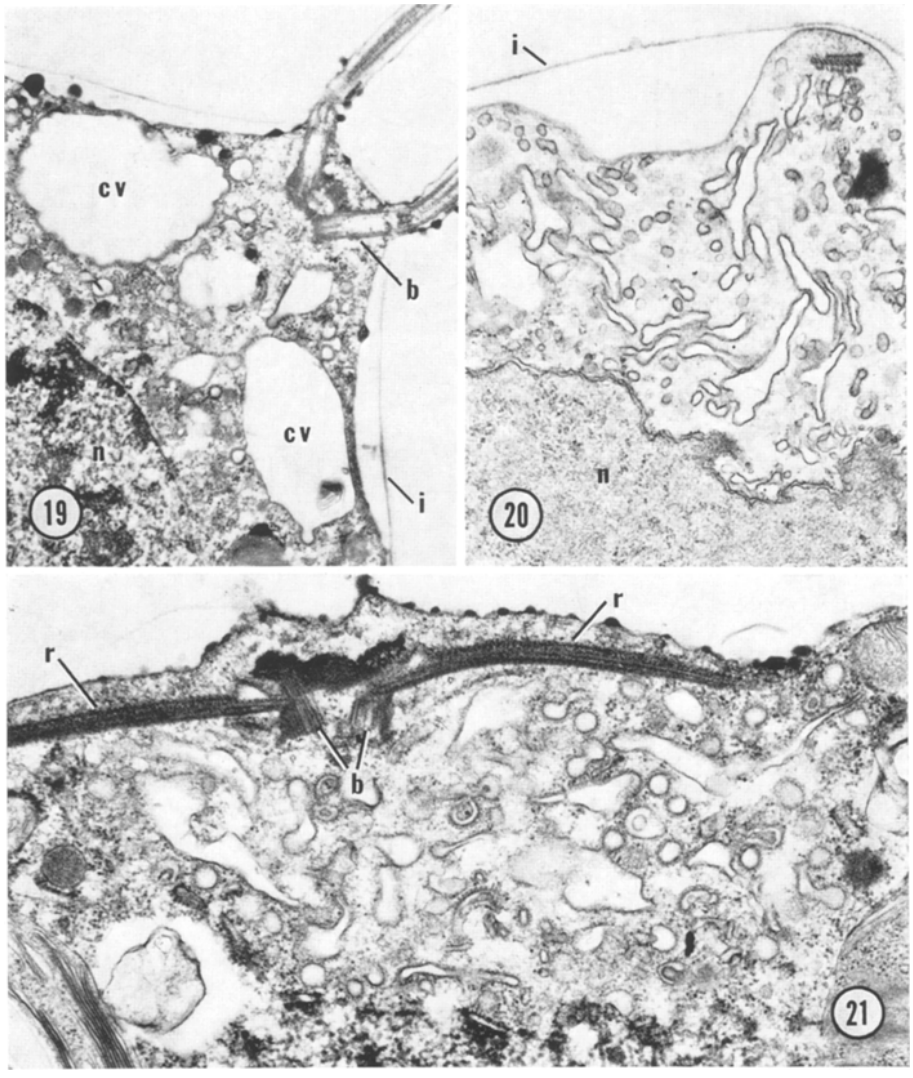
The basal bodies shown in Fig. 32 are positioned at an angle of ca. 45 degrees with respect to each other. The proximal ends of this diagonally opposed pair of basal bodies nearly meet. Basal bodies of the other diagonally opposed pair are further apart; one would lie above this plane of section while the other would lie beneath.

Although the *Cylindrocapsa* flagellar apparatus includes only one distal fiber that cross-connects a pair of diagonally opposed basal bodies, there are several other fibrous components that are associated with the basal bodies. These include a set of four striated fibrous bands which connects the basal bodies in a ring. Each fibrous band passes between and connects two adjacent basal bodies; all four together form a ring. One of these connective bands is shown in Fig. 38. Certain species of the quadriflagellate alga *Carteria* are also known to possess a similar set of connective bands between basal bodies (LEMBI 1975).

The basal body with its associated flagellum is shown in longitudinal section in Fig. 33. The microanatomy of the basal body, flagellum, and intervening transition region follows the expected pattern already determined for most green algae. The proximal and distal ends of the basal bodies are readily distinguished in both longitudinal and transverse section (Figs. 32–36). The lumen at the proximal end contains traces of an electron dense material (Fig. 33) and transverse sections through any portion of this region show the characteristic "hub and spoke" arrangement of the cartwheel pattern (Fig. 34). The distal portion of the basal body lacks the material responsible for the cartwheel pattern as is noted in both longitudinal (Fig. 33) and transverse sections (Figs. 35 and 36).

The transition region between the basal body and flagellum is distinguished in longitudinal section (Fig. 33, arrow 4) by a dense septum traversing the lower end of a short dense cylindrical region that comprises the well known stellate pattern when observed in transverse section (Fig. 37). The two central microtubules of the flagellar axoneme are seen to terminate just anterior to this transition region (Fig. 33). Other longitudinal sections through the stellate region are shown in Figs. 19, 27, and 32.

The four flagellar roots are arranged in a cruciform pattern and alternate between basal bodies as occurs in motile cells of most green algae. The roots



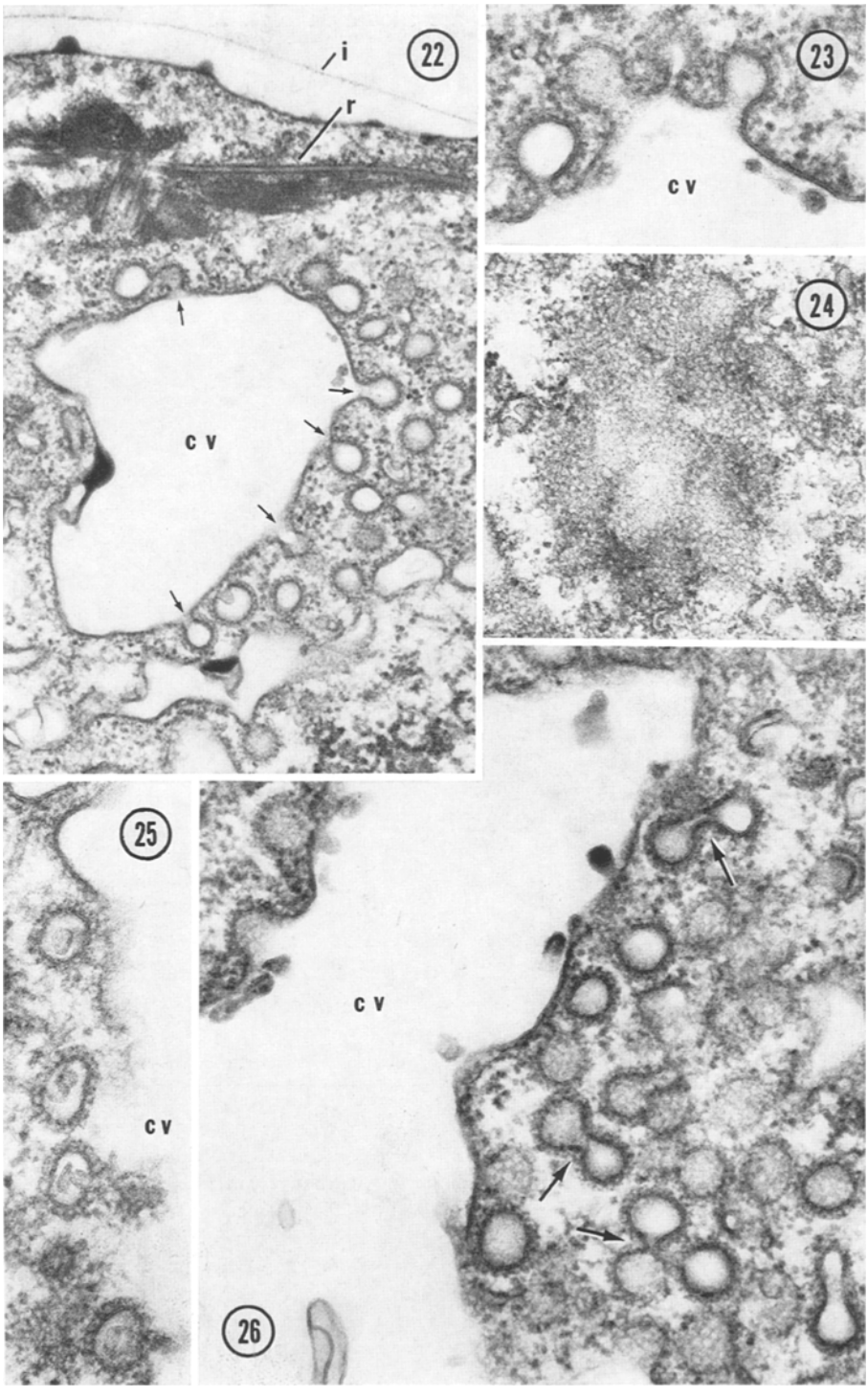
Figs. 19–21. Fig. 19. Median longitudinal section through the anterior end of a zoospore showing portions of two contractile vacuoles with smaller, accessory vacuoles between. Also note two flagella with their associated basal bodies. ×15,000. Fig. 20. Convoluted membrane system of a discharged contractile vacuole fixed with OsO_4 (*cf.*, Fig. 21). ×20,000. Fig. 21. Anterior end of a zoospore showing convoluted membranes of a discharged contractile vacuole fixed with glutaraldehyde. Note also portions of two basal bodies and associated flagellar roots. ×25,000

have their origin at the anterior end of the zoospore and each passes between two adjacent basal bodies and extends posteriorly for an undetermined distance just beneath the plasmalemma (Fig. 40).

At least two of the roots are compound in that each consists of both microtubular elements and striated fibers as illustrated in two different planes of longitudinal section (Figs. 21, 30, and 31). Fig. 30 shows the region of the flagellar apparatus in a grazing section tangential to the surface of the zoospore. This section passes obliquely through two layers of a single compound root—an outer layer appearing as a broad band of many microtubules, and an inner layer consisting of at least several striated fibers (arrows). In contrast, Fig. 31 is a longitudinal rather than a tangential section through the anterior end of a zoospore. The complete anterior end of a root is shown as it passes above parts of two basal bodies seen at the lower right. In this section, three distinct superimposed components of the root can be distinguished. The outermost component (labelled 1) occurs only at the anterior-most end of the root and appears as a thin striated fiber. The middle component (labelled 2), as seen here in "side view", is the same ribbon-like band of many microtubules which was observed in tangential section in Fig. 30. The innermost component (labelled 3) represents the same layer of striated fibers which was also observed in Fig. 30. The two opposed roots illustrated in Fig. 21 have been sectioned in much the same plane as in Fig. 31 and the three component parts can be distinguished at the anterior end of each (particularly the root on the right). All three components are present at the anterior end of each compound root, but as the root passes posteriorly beneath the cell surface, first the outer component terminates, and then the inner component. Only the middle, microtubular component extends the entire length of the root.

It is still not certain whether or not all four roots have the same complex structure as described above, but at least two opposed roots demonstrate this compound condition (Fig. 21). It is evident, however, that all four roots of a zoospore include a broad band of many microtubules.

Figs. 22–26. Fig. 22. Section through a contractile vacuole showing the closely associated coated vesicles. Note membrane continuity between some of the coated vesicles and the contractile vacuole (arrows). ×40,000. Fig. 23. Coated vesicles presumably fusing with a contractile vacuole. Note tripartite nature of vesicle membranes and their continuity with the contractile vacuole membrane. ×75,000. Fig. 24. A section which has grazed the surface of a contractile vacuole. The net-like appearance is due to the closely amassed subunits on the membrane surface which resemble those on coated vesicles (*cf.*, Figs. 26 and 28). ×50,000. Fig. 25. Cross sections through coated vesicles near the margin of a contractile vacuole. Note the circular profiles of the subunits on the membrane surface (*cf.*, Fig. 26). ×75,000. Fig. 26. Coated vesicles closely associated with a contractile vacuole. Many of the vesicles are seen in grazing tangential section and the amassed subunits on their surface form a net-like pattern. Some vesicles appear to be fusing with each other (arrows). ×60,000



Figs. 22–26

Transverse sections though roots are shown in Figs. 33 and 38–40. Cross sections through the anterior end of two roots from different zoospores are shown in Figs. 33 and 38. Both roots are adjacent to basal bodies and at this level of section both have a 5 + 1 arrangement of microtubules, with five in a straight row and a sixth lying beneath and surrounded by a dense amorphous material. In neither instance is there any evidence of an outer, striated component as described above. These roots may represent a second type within the cell. The dense material beneath the band of microtubules is sometimes resolved into distinct units (Fig. 33), but it is uncertain if these represent striated fibers as described above for the compound roots. A few roots were observed in transverse section at the anterior-most end; these sections exhibited a 3 + 1 arrangement of microtubules rather than the 5 + 1 pattern.

Serial sections, which pass transversely through the anterior end of individual roots, demonstrate conclusively that the number of microtubules varies as the root extends posteriorly. The same root is shown at different levels of transverse section in Figs. 38–40. The microtubules are arranged in a 5 + 1 pattern near the anterior end (Fig. 38), while a short distance away posteriorly, the same root shows a pattern of 8 + 1 as three additional microtubules have been added (Fig. 39). The new microtubules appear to be those at the right end of the row. The level of section in Fig. 40 is much further away posteriorly, and now 14 microtubules are seen all in a row directly beneath the plasmalemma. This represents the maximum number of microtubules observed in flagellar roots of *Cylindrocapsa*. The three different levels of section also show a difference in the amount of dense material underlying the microtubular band, with the most observed in Fig. 39 and the least in Fig. 40.

In a similar manner, each of the four roots of a zoospore has been shown to possess a band of microtubules varying in number from a few (at the anterior) to many as the root proceeds posteriorly. This is evident not only from serial sections cut transversely through roots, but also in longitudinal sections cut in the plane illustrated in Fig. 30. Only three microtubules can be counted at the extreme anterior end of the root in Fig. 30 (at the right between basal

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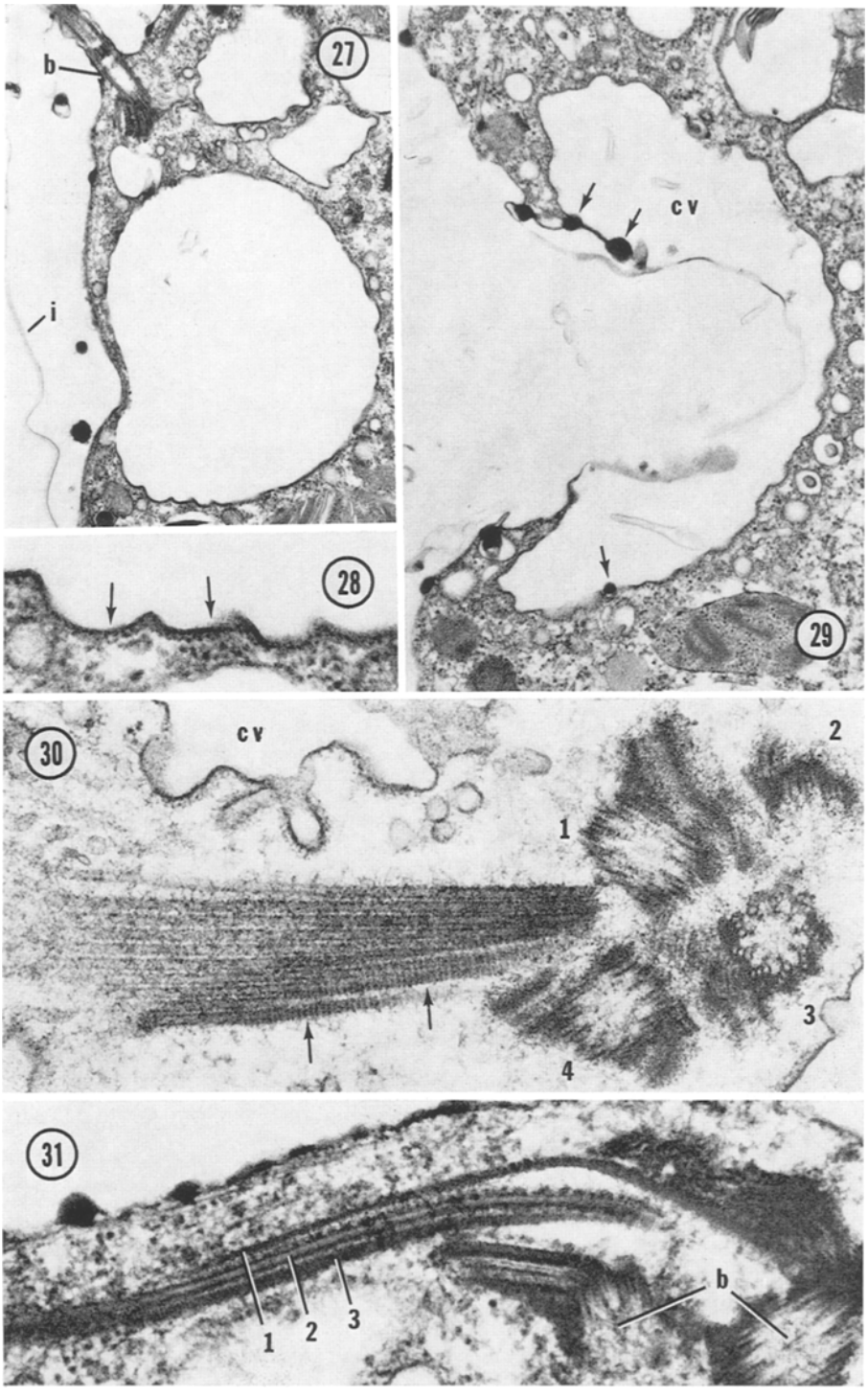
Figs. 27–31. Fig. 27. A fully expanded contractile vacuole prior to discharge. Smaller, accessory vacuoles lie immediately above. ×14,000. Fig. 28. Portion of same contractile vacuole (*cf.*, Fig. 27) but at higher magnification to show small subunits amassed on the membrane surface (between arrows). ×60,000. Fig. 29. Contractile vacuole at the time of discharge (*i.e.*, systole). Small dense globules appear on what was formerly the inner surface of the contractile vacuole membrane (arrows). ×25,000. Fig. 30. Section through the flagellar apparatus to show parts of the 4 basal bodies (labelled 1–4). One of the flagellar roots is seen in oblique tangential section and includes a band-like component consisting of many microtubules and an underlying component formed of at least several striated fibers (arrows). OsO_4 fixation. ×60,000. Fig. 31. Longitudinal section through the anterior end of a zoospore to show the 3 components of a compound flagellar root (*cf.*, Figs. 21 and 30). ×60,000



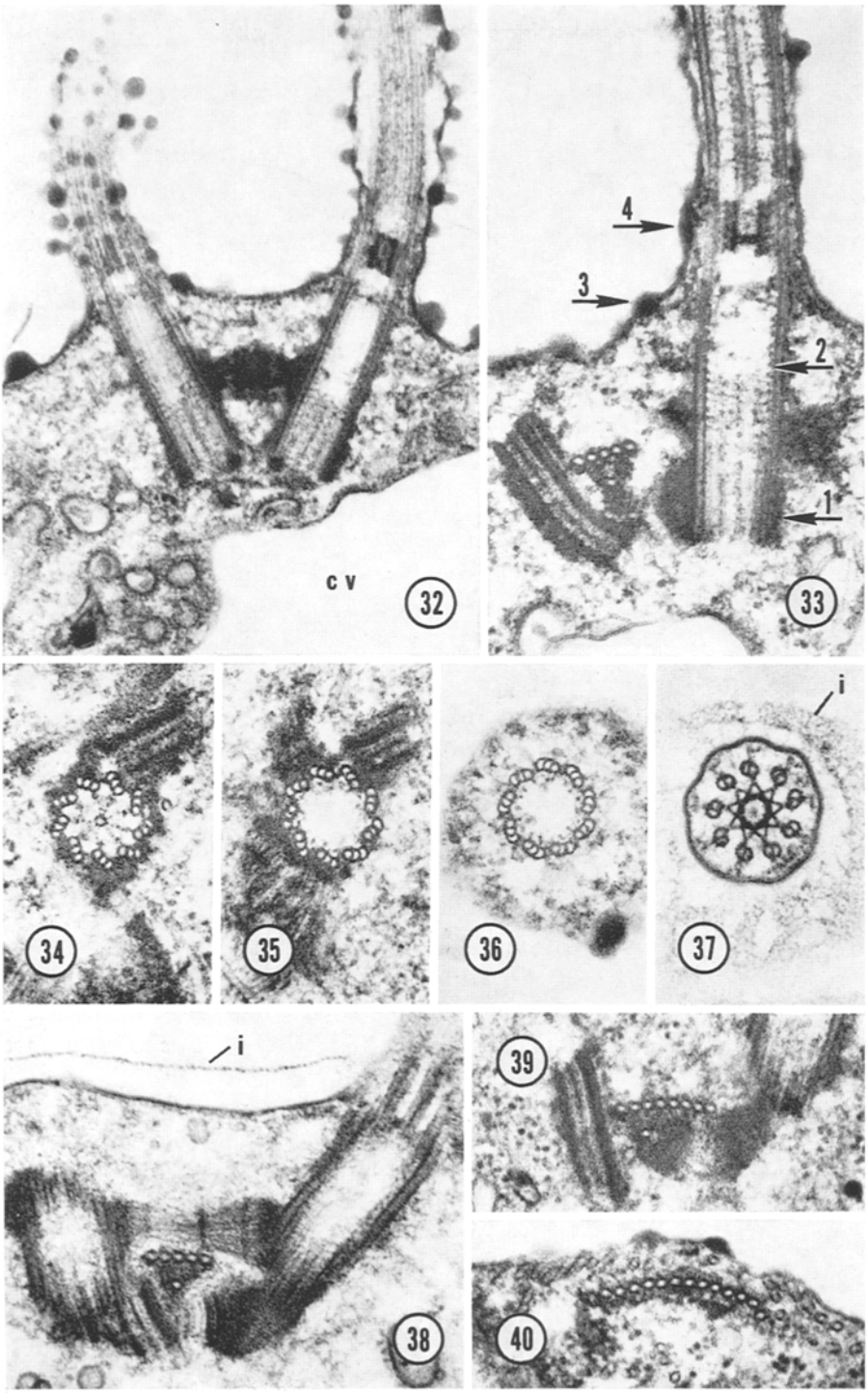
Figs. 27–31

bodies), while a short distance away, posteriorly, there are 9 or perhaps 10 microtubules. The new additions all appear to be initiated at one side of the band as noted earlier in the series of transverse sections (Figs. 38–40).

4. Discussion

Zoospores of *Cylindrocapsa geminella* were found to possess a number of unusual structural features including the thin, outer investment. No exact parallel to the investment is known in any other green alga. A superficial resemblence may be seen, however, in the thin, net-like layer which surrounds the vegetative cells of *Scenedesmus quadricauda* (BISALPUTRA and WEIER 1963, BISALPUTRA, WEIER, RISLEY, and ENGELBRECHT 1964). However, the net in *Scenedesmus* is somewhat thicker and has hexagonal (rather than square) meshes which are much larger than those of the *Cylindrocapsa* investment (ca. 80 vs 15 nm). The theca of certain prasinophytes (*e.g., Platymonas* and *Prasinocladus*) is also quite different from the *Cylindrocapsa* investment in being much thicker and nonperforated (MANTON and PARKE 1965, PARKE and MANTON 1965). Although the zoospore investment appears delicate in *Cylindrocapsa*, it persists as the zoospore becomes attached and develops into a single-celled germling. It is ruptured and eventually sloughed off as the one-celled germling divides to initiate the filament. The function and chemical nature of the zoospore investment in *Cylindrocapsa* remain to be determined.

Figs. 32–40. Fig. 32. Longitudinal section through two, diagonally opposed basal bodies which are connected by a fibrous band midway along their length. Basal body on the right shows the associated flagellum and transition region. ×40,000. Fig. 33. Longitudinal section through a basal body with its associated transition region (arrow 4) and flagellum. Dense material in the lumen distinguishes the proximal end of the basal body from the distal end. Note cross section through a flagellar root to the left of basal body. Numbered arrows refer to various planes of cross section illustrated in Figs. 34–37. ×60,000. Fig. 34. Cross section through the proximal end of basal body. Note the nine sets of triplet microtubules and the characteristic cartwheel pattern in the lumen. Corresponds to level of section #1 in Fig. 33. ×60,000. Fig. 35. Cross section through distal end of basal body showing absence of cartwheel pattern. Corresponds to level of section #2 in Fig. 33. ×60,000. Fig. 36. Another cross section through distal end of basal body, but corresponding to level of section #3 in Fig. 33. ×60,000. Fig. 37. Cross section through the stellate pattern of the transition region. Corresponds to level of section #4 in Fig. 33. ×60,000. Figs. 38–40. Different levels of cross section through the same flagellar root. Fig. 38. Section near the anterior end of the root showing a 5 + 1 arrangement of the component microtubules. The root lies between two basal bodies which are connected by a fibrous band. This band, although similar in appearance, is not equivalent to that shown in Fig. 32 (see text for explanation). ×60,000. Fig. 39. Cross section of same root but at a level of section slightly posterior to that shown in Fig. 38. Three microtubules have been added to give an 8 + 1 arrangement. ×60,000. Fig. 40. Cross section of same root at a more posterior level of section. Now there are 14 microtubules forming a single layered band (*cf.*, Fig. 30). ×60,000



Figs. 32–40

Ultrastructural study has confirmed that zoospores and germling cells of *C. geminella* possess axial chloroplasts as was documented earlier for vegetative cells of both *Cylindrocapsa* and *Cylindrocapsopsis* (BOURRELLY 1961, IYENGAR 1939, 1957, HOFFMAN and HOFMANN 1975). The isolate of *C. geminella* used in this study has a chloroplast of complex shape with numerous, ramified lobes radiating from the central pyrenoid region. Although the chloroplast may appear stellate in light micrographs (HOFFMAN and HOFMANN 1975), this is not so obvious in individual electron micrographs, particularly in the case of the zoospores.

Pyrenoids of this *Cylindrocapsa* isolate have the same distinct structural characteristics which are known to occur only in a few other green algae including: *Platymonas* (GIBBS 1962, MANTON and PARKE 1965); all three genera of the *Oedogoniales* (HOFFMAN 1968, RETALLACK and BUTLER 1970, MARKOWITZ and HOFFMAN 1974); the chlorococcalean alga, *Ankyra* (SWALE and BELCHER 1971); and the siphonous genus, *Sphaeroplea* (unpublished observations). Still other green algae will, no doubt, be found to possess the same distinctive type of pyrenoid. It is obvious that the similarity of pyrenoid ultrastructure among these diverse algae does not imply phylogenetic affinity. However, this type of pyrenoid may characterize all members of the *Cylindrocapsaceae* as it characterizes all members of the *Oedogoniales*.

The coated vesicles which are associated with contractile vacuoles were especially conspicuous in this investigation since they were abundant in each cell and demonstrated the surface subunits very clearly. The origin of coated vesicles is uncertain in *Cylindrocapsa*. Coated vesicles of identical appearance have been shown to be produced by Golgi bodies in some algae. This was demonstrated particularly well in *Ochromonas* by COLE and WYNNE (1973) who showed that the coated vesicles in this alga migrate from the Golgi bodies to the cell surface where they fuse with the plasmalemma. There was no suggestion in *Ochromonas* that they ever fused with the contractile vacuole as occurs in *Cylindrocapsa*. In the present study on *Cylindrocapsa*, there was no evidence that the coated vesicles were derived from Golgi bodies nor did there appear to be a close relationship between Golgi bodies and contractile vacuoles as has been reported in some algae [*e.g.*, *Vacuolaria*, SCHNEPF and KOCH (1966); *Euglena*, LEEDALE (1967); *Chaetosphaeridium* MOESTRUP (1974)]. The coated vesicles appeared only in the region of the contractile vacuoles and were never observed near the numerous Golgi bodies examined. The Golgi bodies produced vesicles, but these did not bear subunits on their surface. Although one can not discount the possibility that some of these Golgi-derived vesicles might later develop into coated vesicles, there was no suggestion that this occurred. As far as can be determined, coated vesicles in *Cylindrocapsa* zoospores arise in association with the convoluted membranes that persist following discharge of a contractile vacuole.

The association between coated vesicles and contractile vacuoles has been

reported in numerous algae (see DODGE 1973), although the complete significance of this relationship has never been resolved. It is generally accepted that contractile vacuoles have the primary function of osmoregulation. The prevailing opinion, as summarized by DODGE (1973), suggests that the contractile vacuole expands as associated vesicles (in this case coated vesicles) fuse with the contractile vacuole and thus discharge their contents into it. In turn, the membrane of the expanded contractile vacuole fuses with the plasmalemma and thereby discharges its contents outside the cell. However, there is little in the literature concerning the significance of the subunits on the coated vesicles or, in organisms such as *Cylindrocapsa*, of the subunits on the contractile vacuole membrane itself.

In their study of coated vesicles in *Ochromonas*, COLE and WYNNE (1973) discuss the possible role of the vesicle coating in membrane unfolding and fission—a suggestion proposed by ROTH and PORTER (1962) and further supported by the work of KANESEKI and KADOTA (1969) who investigated coated vesicles in mammalian brain cells. In a more recent paper, COLE and WYNNE (1974) have further amplified this concept in a study on endocytosis in *Ochromonas*. Since coated vesicles occur in many types of cells, including many lacking contractile vacuoles, the functional significance of the coating must be fundamental in nature and not unique to the function of contractile vacuoles. Thus, if one accepts the proposal of ROTH and PORTER (1962), the membrane coatings in *Cylindrocapsa* may be important in membrane fission which would be the prelude to subsequent fusion of coated vesicles with the contractile vacuole, and would also precede the fusion of the contractile vacuole membrane with the plasmalemma. However, one could speculate on other fundamental roles for the membrane coatings. For example, they may be associated instead with the selective retention of certain ions or molecules which might otherwise be transported across the membrane. A conclusive demonstration of the fundamental role played by these membrane coatings remains to be shown.

Although each *Cylindrocapsa* zoospore forms two primary contractile vacuoles, smaller accessory vacuoles may also be present nearby (Figs. 19 and 27). Accessory vacuoles have been described in *Euglena* (LEEDALE 1967), while in *Vacuolaria* many small vacuoles arise in the Golgi region and fuse together to form the contractile vacuole (SCHNEPF and KOCH 1966). Accessory vacuoles may be more common in green algae than presently recognized. Fig. 10 shows what may represent the fusion of an accessory vacuole with a contractile vacuole (arrowhead).

The flagellar root system in *Cylindrocapsa* zoospores is most unusual. The roots of at least one opposing pair are compound in nature with three superimposed components: an outer fibrous component occurring only at the anterior end; a middle component consisting of a band of many microtubular elements that extends the entire length of the root; and an inner component

consisting of several striated fibers that are also only present at the anterior end (Figs. 21, 30, and 31). All four roots include a band of many microtubules which, in one pair, number up to 14 in certain levels of section (Fig. 40). This is the greatest number of microtubules per root known for any green alga with a cruciate root system (MOESTRUP 1972, Table 1). The only alga that approaches this condition is *Microthamnion,* with up to 8 microtubules per root (illustrated but not discussed by WATSON and ARNOTT 1973, Fig. 9).

Flagellar roots in *Cylindrocapsa* are also unusual in that the number of microtubular elements in a root changes dramatically from a few to many (up to 14) as the root extends posteriorly from the region of the basal bodies (Figs. 38–40). In most other algae the microtubular elements of a root remain constant in number over the entire length (MOESTRUP 1972). *Microthamnion* (WATSON and ARNOTT 1973), like *Cylindrocapsa,* appears to be an exception. In both genera, the number of microtubules per root increases as the root extends posteriorly. In *Cylindrocapsa* this change in number occurs over a short distance near the anterior end. This is probably true also for *Microthamnion* in which a change from 5 to 8 can be noted in the published micrographs of WATSON and ARNOTT (1973).

Recently, PICKETT-HEAPS and MCDONALD (1975) have investigated the ultrastructure of an algal isolate reputed to be *Cylindrocapsa involuta* (strain LB 653 in the Culture Collection of Algae at Indiana University; STARR 1964). However, an ultrastructural comparison of this isolate with my isolate of *C. geminella* shows they are so different that it is inconceivable to consider them in the same genus. Strain LB 653 was originally isolated by E. A. GEORGE and is listed in the Cambridge culture collection as CCAP No. 314/1. On the basis of my own light microscopic examination of this isolate (obtained from the culture collections of both Indiana and Cambridge University) and correspondence with Mr. GEORGE and Dr. PICKETT-HEAPS, I have concluded that this alga is not *Cylindrocapsa.* Furthermore, oogamy, which is characteristic of *Cylindrocapsa,* has not been demonstrated for this isolate as far as I have been able to ascertain. The systematic identity of this alga, if it isn't *Cylindrocapsa,* remains to be determined.

The two isolates are compared ultrastructurally as follows. It has been clearly shown (PICKETT-HEAPS and MCDONALD 1975) that each vegetative cell of strain LB 653 possesses a single *parietal* chloroplast rather than an *axial* chloroplast as described in the present paper on *C. geminella.* Furthermore, the structure of the pyrenoid in the two isolates is totally different. The pyrenoid in strain LB 653 lacks cytoplasmic channels and the matrix is traversed instead by membranes continuous with thylakoids as reported by STEWART, MATTOX, and FLOYD (1973) and illustrated by PICKETT-HEAPS and MCDONALD (1975). A phycoplast is not associated with cytokinesis in strain LB 653 according to PICKETT-HEAPS and MCDONALD (1975). In marked contrast, a distinct phycoplast is associated with cytokinesis in my isolate of *C. geminella*

(unpublished data which will appear in another paper). Furthermore, there is no precocious formation of a cleavage furrow in *C. geminella* cytokinesis, as has been described for strain LB 653. Other distinctions can be detected by comparing the prominent sheath and the thick, lamellated cell walls in filaments of *C. geminella* (HOFFMAN and HOFMANN, 1975) with the published illustrations of strain LB 653 (PICKETT-HEAPS and MCDONALD 1975). The zoospores of strain LB 653 remain to be studied ultrastructurally. However, I predict that the flagellar apparatus will be very different from that of *C. geminella*.

The ultrastructural distinctions between strain LB 653 and my isolate of *C. geminella*, particularly those associated with cytokinesis, make it clear that the two organisms should not be included in the same genus. The absence to date of demonstrated oogamy in strain LB 653, its parietal chloroplasts, and the basic ultrastructural differences between it and *C. geminella*, are major reasons for questioning its identity as *Cylindrocapsa*.

In contrast to strain LB 653, my isolate of *C. geminella* demonstrates the combination of structural features generally associated with the genus *Cylindrocapsa* (RAMANATHAN 1964, BOURRELLY 1966, CHRISTENSEN 1966, ROUND 1971). Thus, it is unequivocally oogamous (Fig. 9); the filament possesses both a distinct sheath and cell walls which become thick and lamellate (IYENGAR 1939, RAMANATHAN 1964, HOFFMAN and HOFMANN 1975); and each cell possesses a single, axial chloroplast (IYENGAR 1939, 1957, BOURRELLY 1961, HOFFMAN and HOFMANN 1975).

It is clear that more information is needed on other isolates of the family *Cylindrocapsaceae* before the group can be ultrastructurally characterized with any degree of certainty. The systematic position of strain LB 653 must be resolved and, until that time, it should not be considered as representative of the *Cylindrocapsaceae* in drawing phylogenetic conclusions. Thus, the suggestion by PICKETT-HEAPS and MCDONALD (1975) to include the family *Cylindrocapsaceae* in the order *Ulvales* (*sensu* STEWART and MATTOX) should be reconsidered.

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