THE ULTRASTRUCTURE OF *PORPHYRIDIUM CRUENTUM*

E. GANTT and S. F. CONTI

From the Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire

ABSTRACT

An electron microscopic examination of *Porphyridium cruentum* revealed the presence of mitochondria which had been reported absent in this aerobic organism. The chloroplast in this red alga was found to contain small granules (about 320 A) regularly arranged along the parallel chloroplast lamellae. The chloroplast granules differ in size and staining intensity from the ribosomes located in the cytoplasm. Two tubular elements are described. One type (450 to 550 A) is associated with the Golgi bodies. Another type (350 A), in the cell periphery, is believed to connect the endoplasmic reticulum and the cell membrane. Daughter nuclei were found to be positioned at opposite ends of the cell prior to commencement of cell division. Cytokinesis is accomplished by an annular median constriction causing the gradual separation of the chloroplast, pyrenoid, and other cell organelles, resulting in two equal daughter cells. No appreciable differences were observed between cells grown in high light (400 ft-c) and low light (40 ft-c). Structural differences between young and old cells were compared.

As one of the few unicellular forms of the Rhodophyta, *Porphyridium cruentum* has long been of interest. Studies on its morphology and cytology (6, 10, 20) have shown it to be spherical in shape containing an eccentric nucleus and a large red chloroplast. The simplicity of its morphology and cell division, plus a lack of sexual reproduction, have led to its uncertain taxonomic position in the subclass Bangiodeae (18). Its simple unicellular nature and the ease with which it can be cultured (8) have made it useful as an experimental tool particularly in elucidating the role of pigments involved in photosynthesis (2, 3).

In their study of the ultrastructure of *P. cruentum*, Brody and Vatter (4) reported that mitochondria were absent. The possible lack of mitochondria in an aerobic plant cell would be rather significant, especially in a cell such as *P. cruentum* which has Golgi bodies and membranes delimiting the nucleus and chloroplast (4). In view of the fact that *P. cruentum* has these eucaryotic characteristics and because of the presence of mitochondria in other red algae (1), we felt that a re-investigation of the ultrastructure of this alga was warranted.

In the course of this investigation we have made observations on the general ultrastructure, especially in regard to the photochemical apparatus and cell division of *P. cruentum*.

MATERIALS AND METHODS

An axenic culture of *P. cruentum* Naegeli was obtained from Dr. M. B. Allen (Laboratory of Comparative Biology, Kaiser Research Institute, California). Cultures were grown in 300 ml of an artificial sea water medium (8) in 500-ml Erlenmeyer flasks. An inoculum (20 ml) from a 6- to 8-day-old culture was used. Cultures were illuminated by a bank of "cool white" fluorescent lights (incident light intensity of 400 ft-c) and maintained at 21 ± 1°C in a New Brunswick Incubator-Shaker. Aeration of cultures was accomplished by passing a continuous stream of 5 per cent CO_2 in 95 per cent air through the medium. Neutral density filters were used for growing cultures at

low-light intensity (40 ft-c). Synchronization of cell division was attempted by following a light regime (400 ft-c) of 120 hours of light, 24 or 48 hours of dark, followed by light periods of 5, 10, and 20 hours.

Cells were harvested by centrifugation (4,000 *g*) after incubation for 6 to 8 days. A variety of fixatives was examined, *i.e.* aqueous $KMnO_4$, acrolein and OsO_4, and glutaraldehyde with and without OsO_4 postfixation. The most satisfactory fixation procedure, based on apparent preservation of fine structure, proved to be similar to one described by Sabatini *et al.* (16). The cells were fixed with 4 per cent glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) for 2 hours, thoroughly washed several times with 0.1 M phosphate buffer, and postfixed for 2 hours with 1 per cent OsO_4 in 0.1 M phosphate buffer. After fixation, the cells were again rinsed in buffer prior to being suspended in 1 or 2 drops of melted 2 per cent agar. The hardened agar blocks, containing the cells, were put through a graded ethanol dehydration series and embedded in Epon 812 essentially as described by Luft (11). Sections were cut with a diamond knife on an LKB ultrotome, stained with 1 per cent uranyl acetate and basic lead hydroxide (13), and examined in a Philips-200 electron microscope.

OBSERVATIONS

The general appearance of *P. cruentum*, grown under an incident light intensity of 400 ft-c, is illustrated in Fig. 1. A chloroplast with a centrally located pyrenoid is the dominant structure in the cell. In the cytoplasmic region between the chloroplast and the cell membrane are contained all the structures normally associated with a eucaryotic cell. Surrounding the cell is a thin diffuse sheath, possessing a fibrillar appearance; the fiber-like components of the inner part of the sheath appear to be connected to the cell membrane (about 50 A) (Fig. 2). The thickness of the sheath varies with the age of a cell, being thicker in older cells.

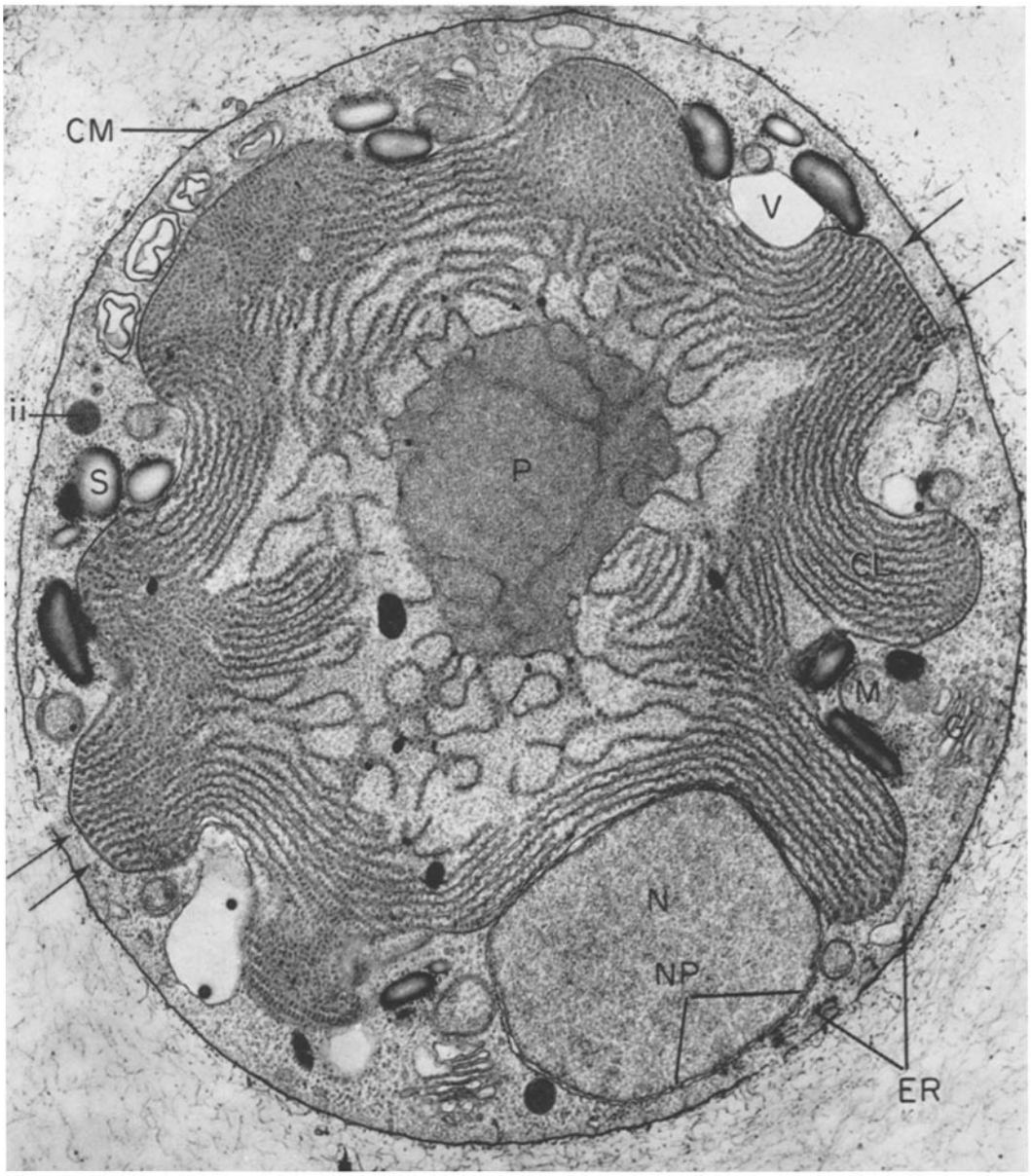
Mitochondria are indeed present in *P. cruentum*. Each mitochondrion typically consists of a chamber enclosed by a double membrane, with tubular cristae arising from the inner membrane (Fig. 3). Cristae appear as irregularly spaced circular or tubular profiles. In shape, the mitochondria are long and slender, and not infrequently branched (Fig. 4). Because of their slender shape and tortuous positioning between starch granules, chloroplast extensions, and vacuoles, they are usually observed in cross-section. One mitochondrion is frequently seen as several neighboring cross-sections. Only in regions through the periphery of a cell (Fig. 4) can one get a true picture of the mitochondria.

The nucleus is eccentrically located in cells which do not appear to be undergoing cell division. It is generally spherical to ellipsoidal in shape and bounded by a double membrane (about 200 A). Nuclear pores regularly interrupt the nuclear membrane (Figs. 1 and 4). Fig. 4 shows the nuclear pores to be circular with a darkly staining rim. Some portions of the outer layer of the nuclear membrane are covered by ribosomes. The nucleolus, which is more opaque than the granular nucleoplasm, is located in the region of the nucleus immediately adjacent to the chloroplast (Fig. 7).

The endoplasmic reticulum is neither extensive nor elaborate. It consists of a double membrane system, appearing in the form of tubules or vesicles, which follow the contour of the cell membrane (Figs. 1, 2, and 5). Connections between the endoplasmic reticulum and the nuclear membrane occur. Areas, such as those indicated by arrows in Fig. 2, strongly suggest that there are also connections between the endoplasmic reticulum and the cell membrane. Ribosomes are attached along the tubules and vesicles of the endoplasmic reticulum (Fig. 5). However, the majority of ribosomes are free of any membrane attachment, and are most easily observable in the cortical region of the cytoplasm (Fig. 4).

Golgi body cisternae are arranged parallel to one another as well as parallel to the cell membrane (Figs. 1 and 3). Surrounding the stacked Golgi cisternae are tubules which are circular to elliptical in profile (Fig. 3), with diameters ranging from 450 to 550 A. These tubules have a smaller average diameter than the inflated Golgi cisternae, and are arranged at right angles to the cisternae. Their greater concentration and arrangement around the Golgi bodies suggest that they are components of, or derived from, the Golgi bodies.

Small tubular elements (Fig. 4), with a diameter of about 350 A, are seen in the cortical region of the cell. They are most regularly observed as circular profiles or as short rods, and are believed to be extensions from the endoplasmic reticulum to the cell membrane (Fig. 2). Lightly stained strands, apparently composed of tubules with a diameter of 200 A, are occasionally observed in the peripheral cytoplasm and in nuclei of dividing cells. Although they fall within the size range of microtubules (9), their identification remains uncertain due to their indefinite appearance.



FIGURES 1 TO 9 Cells grown for 6 to 8 days in the light (400 ft-c), whereas cells shown in Figs. 10 to 12 were exposed to 5 days of light (400 ft-c), 1 day of dark, then 10 hours of light.

FIGURE 1. This micrograph is a cross-section of a cell grown under high light (400 ft-c). It is surrounded by a cell membrane (*CM*) and a diffuse sheath. The eccentrically located nucleus (*N*) is seen to possess a nuclear envelope with pores (*NP*) (See Fig. 7 for nucleolus). Occupying the central portion of the cell is a large chloroplast consisting of parallel-arranged lamellae (*CL*) and an opaque pyrenoid (*P*). Chloroplast lamellae are randomly arranged in and around the pyrenoid. That the chloroplast lamellae, with associated granules, are not fused to the chloroplast membrane is seen in the chloroplast extensions indicated by arrows. In the granular cytoplasm, between the chloroplast and the cell membrane, are the Golgi bodies (*G*), a sparse endoplasmic reticulum (*ER*) with occasional connections to the cell membrane, vacuoles (*V*), starch grains (*S*), mitochondria in cross-section (*M*), and some darkly stained unidentified bodies (*ii*). The endoplasmic reticulum and the Golgi bodies are oriented parallel to the cell membrane. × 20,000.

Unlike that of most other algae, the chloroplast of *P. cruentum* does not contain starch between the photosynthetic lamellae nor around the pyrenoid. Starch grains of various sizes and shapes, lacking a bounding membrane, are randomly distributed throughout the cytoplasm. They vary greatly in staining intensity when treated with lead hydroxide (Figs. 1, 10, and 11). Cytoplasmic vacuoles, located among cytoplasmic organelles and starch grains, are clearly distinguishable from starch grains by having a distinct unit membrane and generally being larger in size (Figs. 1 and 7).

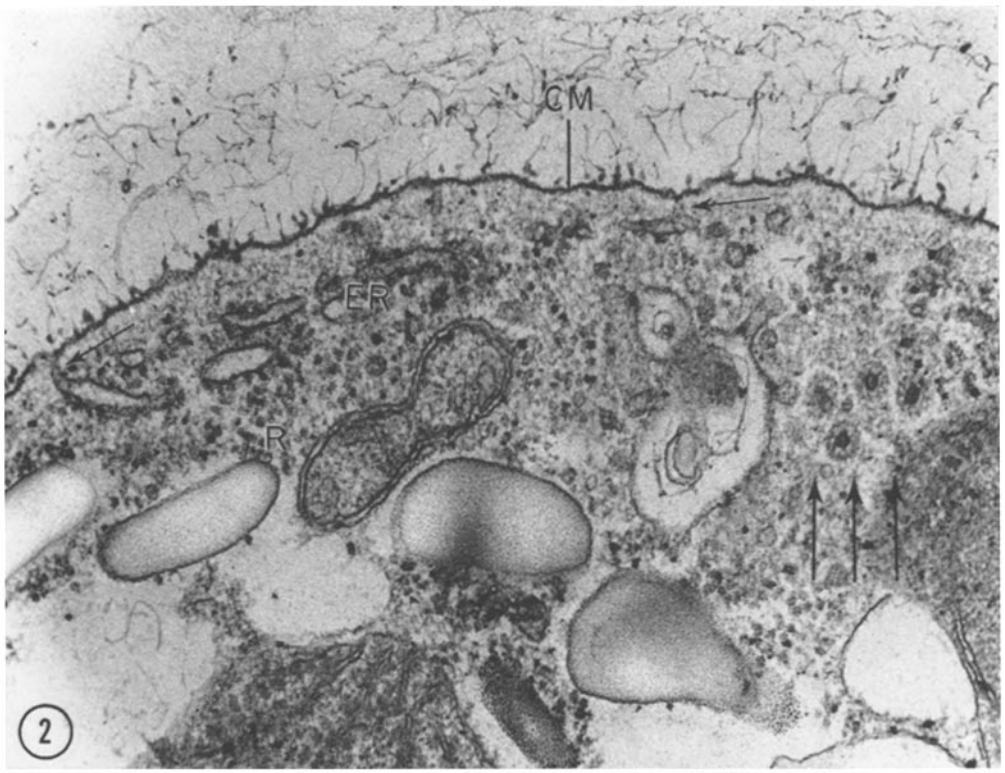
Two other types of structures, consistently observed in the cytoplasm, bear description. One of these components appears as a round, opaque body surrounded by a double membrane (Fig. 1). In size and shape it is comparable to a small mitochondrion seen in cross-section. The second type is smaller, but structurally more complex. It consists of a densely stained core enclosed by a more lightly stained, radially segmented halo (Fig. 2). The entire body measures about 900 A in diameter. Careful examination of its appearance in a variety of cells sectioned in different planes led to the assumption that it is rod shaped.

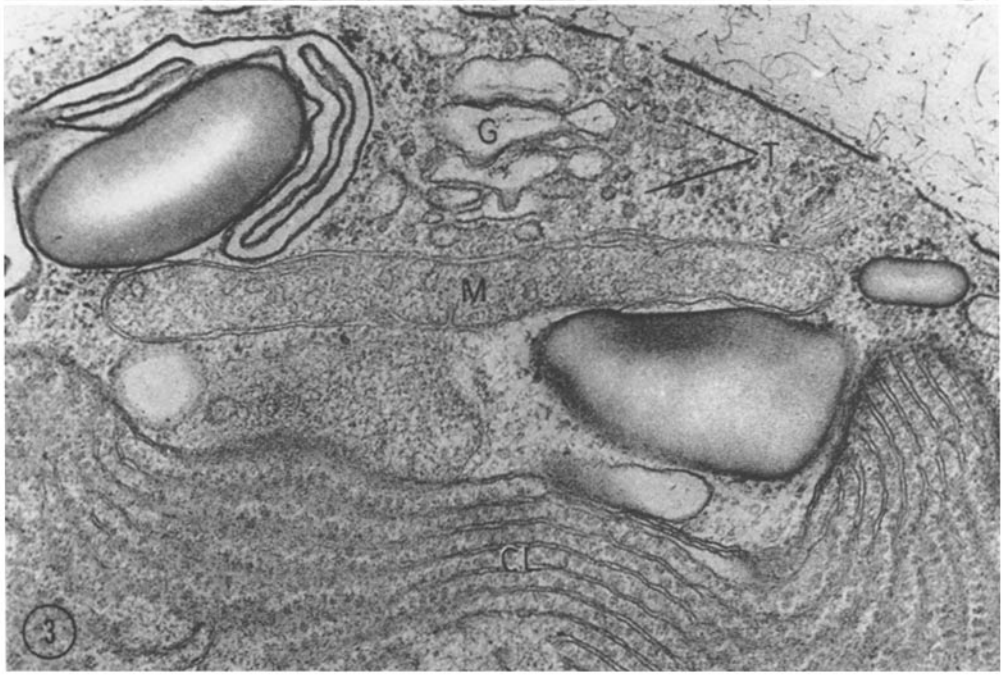
Mature cells contain a single, well developed chloroplast which occupies a major portion of the cell (Figs. 1, 10, and 11). Protruding in various directions from the main body of the chloroplast are chloroplast extensions. These extensions are integral parts of the chloroplast body exemplified by the direct continuation of the photosynthetic lamellae from the main chloroplast body into the extensions. A double limiting-membrane (about 200 A) encloses the chloroplast. The photosynthetic lamellae are double-membraned and generally arranged parallel to one another, with a spacing of about 500 A (or more) between the membrane pairs. Each lamella (as defined by Brody and Vatter (4)) is about 200 A wide and in section appears as two membranes separated by a space (Figs. 1 and 6). The number of parallel lamellae is greatest in the peripheral area of the chloroplast, especially in the extensions. Around the pyrenoid the parallel arrangement of the lamellae is decreased. Lamellae extend into and through the pyrenoid. Fusion and rebranching of adjacent lamellae, sometimes forming a honeycomb pattern (Fig. 6), is regularly found. Occasionally, chloroplast lamellae assume a helical or spiral pattern in the chloroplast extensions. Though fusion occurs between adjacent lamellae, no direct connections between the membranes of the lamellae and the limiting membrane of the chloroplast have been found. The lamellae terminate in close proximity to the chloroplast membrane (Fig. 1), but do not seem to fuse with it.

A characteristic feature of the chloroplast of *P. cruentum* is the association of small granules (about 320 A) with the chloroplast lamellae. These granules are located only on the "outer" side of each lamella (Fig. 8), *i.e.* the side facing away from its own membrane pair. As seen in Fig. 9, the granules are not present on the chloroplast membrane. A highly regular 2-dimensional spacing of the granules is evidenced in grazing sections over several lamellae (Fig. 7). It has not, as yet, been determined whether the granules are only associated with the lamellae, or are an integral part of

FIGURE 2 This micrograph shows a section of the periphery of a cell. Fibrillar sheath extensions radiate from the cell membrane (*CM*). Light arrows indicate connections between the endoplasmic reticulum (*ER*) and the cell membrane. Some ribosomes (*R*) are attached to the endoplasmic reticulum while some are not associated with a membrane. A group of unidentified bodies is indicated by bold arrows. Each body, with an approximate diameter of 900 A, has a darkly stained core surrounded by a lightly stained segmented halo. × 45,000.

FIGURE 3 A longitudinal section of a mitochondrion (*M*) is shown in this micrograph. Tubular cristae are connected with the inner membrane of the double membrane surrounding the mitochondrion. Above the mitochondrion is a stack of distended Golgi cisternae. Small tubular elements (*T*) (450 to 550 A) are clustered around the Golgi body (*G*). A portion of the chloroplast shows granules associated with the chloroplast lamellae (*CL*). × 38,000.


CM
ER
R
2


G
T
M
C.L.
3

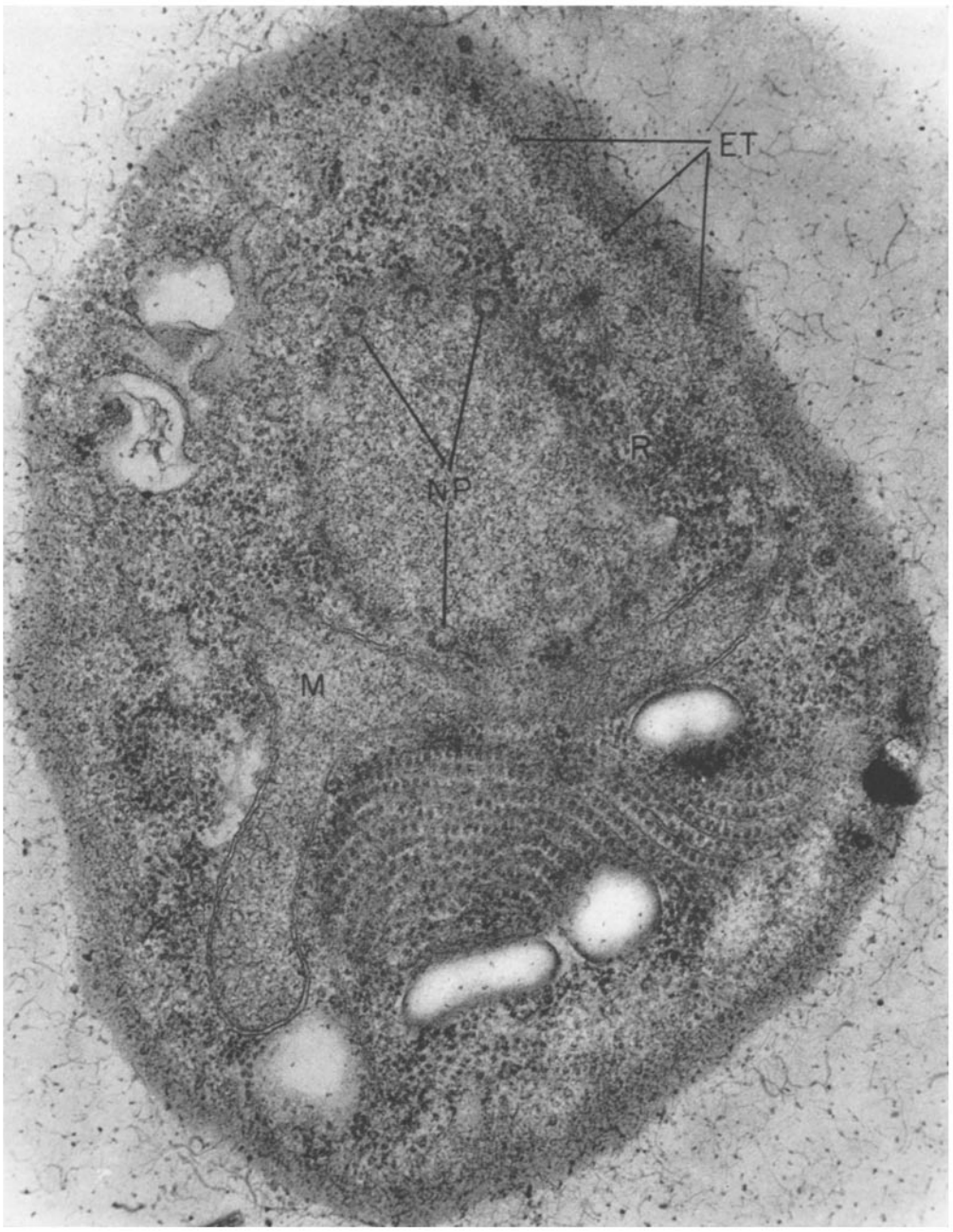


FIGURE 4 The section represented here is from the cortical portion of a cell. Only in a section of this type can one see the concentration of unattached ribosomes (*R*) in the cytoplasm, and the numerous cross-sections of small tubules (*ET*) which serve as connections between the vesicles of the endoplasmic reticulum and the cell membrane. Below the branching mitochondrion (*M*) is a portion of a chloroplast extension showing the chloroplast granules. Above the mitochondrion is part of a nucleus with round, closely spaced nuclear pores (*NP*) possessing a dark rim. × 40,000.

the lamellar structure. However, they are always found in contact with the lamellae, and are never free in the interlamellar matrix.

The granules are most clearly seen in cells fixed in glutaraldehyde, or glutaraldehyde followed by postfixation in 1 per cent OsO_4. Fixation with $KMnO_4$ caused destruction of the granules. Preliminary studies indicate that the appearance and structure of the granules is not affected by treatment of cells with ribonuclease.

In an attempt to synchronize cell division, cultures were exposed to a light-dark-light regime. Examination of these cultures revealed that many of the cells were undergoing division. Although complete synchronization of cell division was not obtained by the varied light regime, the number of dividing cells was increased, thus increasing the chances of observing the ultrastructural changes accompanying cell division.

Commencement of cell division is illustrated in Figs. 10 and 11. Prior to cytokinesis nuclear division occurs, and the daughter nuclei are positioned at opposite ends of the cell and separated from one another by the large chloroplast (Fig. 11). Cytokinesis occurs by an annular centripetal constriction of the median region of the cell. As the cleavage furrow progresses, an ever narrowing cell isthmus results (Fig. 12). An apparent localized stretching of the central pyrenoid and chloroplast (Fig. 10), especially of the chloroplast lamellae (Fig. 12), occurs in the cell isthmus. The constriction results in the division of the chloroplast, pyrenoid, and cytoplasmic content into two equal daughter cells. It is of interest to note that the cell membrane does not invaginate independently, as in cell division of some algae, but that it merely follows with the cleavage furrow. Furthermore, sheath production appears to continue actively in the cleavage region, since numerous sheath "fibrils" extend radially from the cell membrane (Fig. 12).

Manifestations of nuclear division are not yet clear and only some preliminary observations can be included. Cells from partially synchronized, actively dividing cultures are characterized by a greater irregularity of nuclear shape and nuclear location. The nuclei, instead of having their usual compact spherical shape, have one or two large blunt nuclear projections. Accompanying the irregular muclear shape is a change in nucleolar position. Instead of a single nucleolus lying adjacent to the chloroplast, sometimes two nucleoli can be seen, each one located at the tip of a nuclear projection. Some nuclei of dividing cells contain groups of small tubules (200 A). Whether the intranuclear tubules are part of a spindle is not yet known.

Older cells (3 weeks) are distinguishable by possessing several structures which are either rarely found or less abundant in younger cells (6 to 8 days). The sheath is much thicker and more compact than that of young cells, and the fibrillar sheath elements assume a concentric arrangement. Laminated, whorl-like bodies, located in the cytoplasm or chloroplast, range in complexity from a simple type as seen in Figs. 7 and 11, to large net-like elaborations. Large osmiophilic bodies are observable within the cytoplasm, while smaller ones are localized between the chloroplast lamellae (Fig. 8). Cells also occasionally contain lamellar structures (Fig. 11) resembling proplastids.

No unusual ultrastructural differences were observed between cells grown under high light (400 ft-c) and low light (40 ft-c). There are no apparent changes in appearance or concentration of chloroplast granules, and the variability in the number of lamellae is about the same under both light conditions. As expected, the starch grains are more numerous in cells grown under high-light intensity.

DISCUSSION

This study shows the existence of mitochondria in the unicellular red alga *P. cruentum*. The significance of the presence of mitochondria in this cell, having all other characteristics of eucaryotic cells, needs hardly be emphasized. Although the structure of the mitochondria of this cell is typical, they can be easily missed, since they are rather thin and are located between starch grains and other cell components. The fact that Brody and Vatter (4) did not observe mitochondria in *P. cruentum* may have been due to OsO_4 fixation, which appeared to cause a slight cell shrinkage, and because their cells were either full of starch or highly vacuolated. Under these conditions mitochondria, which usually are seen in cross-sections, are hard to identify.

During the preparation of this paper a similar study (19) appeared, also showing the presence of mitochondria in *P. cruentum*.

Glutaraldehyde with OsO_4 postfixation proved to be an excellent fixative. It not only allowed the observation of mitochondria, for they were also

observable with $KMnO_4$, and acrolein fixation, but it gave the best over-all cell preservation. Basically, this study can be regarded as an extension of the electron microscope work on *P. cruentum* begun by Brody and Vatter (4). As such, the present work has essentially confirmed their findings; however, it has added further information specifically on the mitochondria, "microtubules," endoplasmic reticulum, nucleus, cell division, and the chloroplast structures.

Several small discrepancies exist between our findings and those of Brody and Vatter (4). We did not find starch grains surrounded by a membrane as they did. There is also some difference between our measurements of the cell membrane (about 50 A), chloroplast limiting-membrane (about 200 A), and chloroplast lamella (about 200 A) and their measurements of 100, 100, and 150 A, respectively. These variations in the thickness of membranes are probably due to the different fixation procedures.

Golgi vesicles are involved in primary cell wall formation (17, 21) in higher plants. In this alga, which has a sheath instead of a rigid wall, there is no indication of Golgi vesicle involvement in sheath formation. The attachment of the sheath "fibrils" to the cell membrane (Fig. 2) leads us to assume that the cell membrane is active in sheath production. Bouck (1) similarly suspected that the cell membrane of *Lomentaria baileyana*, another red alga, released material to the cell wall.

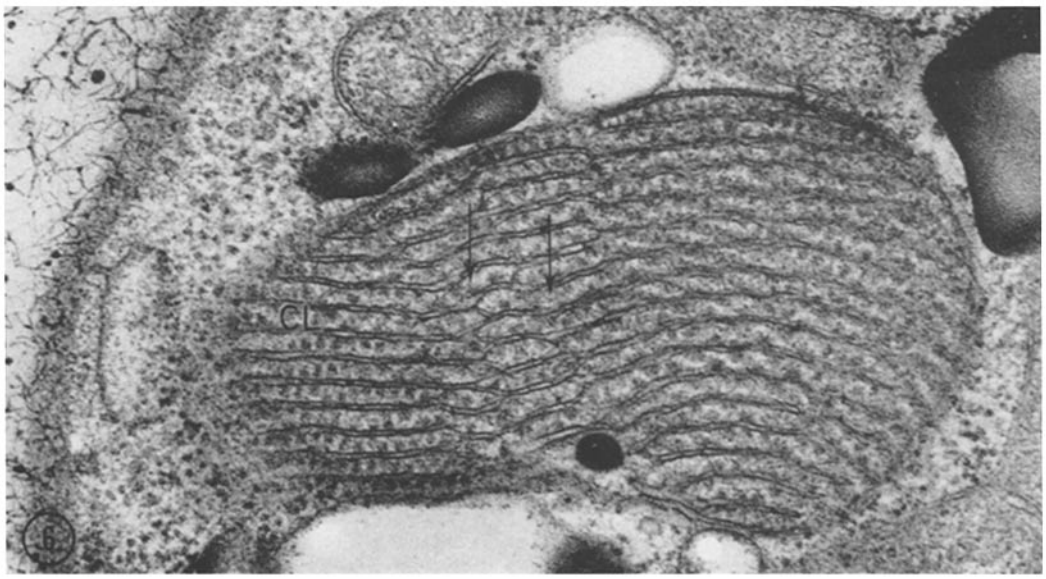
The gross manifestations of cell division in *P. cruentum* are more reminiscent of the division of some animal rather than plant cells. This pinching division, made possible by the lack of a rigid wall, differs in that no spindle fibers are present here. In some other algae (12), cell division immediately follows nuclear division, while spindle fibers from the nuclear division are still present. This leads us to the question of how nuclear division occurs in *P. cruentum*. Geitler (5, 6) also observed that nuclear division is completed before cell division begins. He states that nuclear division is typical (5) and implies that spindle formation is involved (6). Whether the changes in nuclear shape and the presence of intranuclear fibrils in cells of rapidly dividing cultures are typical of nuclear division remains to be resolved.

In appearance the chloroplast lamellae of this alga (Figs. 1 and 6) are in conformity with the generalization that chloroplast lamellae of red algae consist primarily of single layered discs (1, 4, 7, 14). To our knowledge, the small granules (320 A) regularly arrayed on the chloroplast membranes (Figs. 6 and 7) have not been reported. Although the exact nature of these lamellar granules must await their isolation, several possibilities may be entertained. These structures might correspond to ribosomes, quantasomes, sites of accessory pigments, or even fixation artifacts. The possibility that these granules are artifacts is quite improbable, since they are observed only on one side of each membrane, and are not present on the limiting chloroplast membrane (Fig. 9). Their uniform arrangement, distribution, and size (320 A), as well as their apparent resistance to ribonuclease digestion, make them questionable as ribosomes. They are considerably larger than spinach quantasomes (15) (155 *vs.* 320 A), but they have a two-dimensional crystalline array and location similar to that described for quantasomes. The difference in size might possibly be accounted for by the different preparative procedures. Since it is clear that the accessory pigments are restricted to the chloroplast and must be within or on the chloroplast lamellae (4), these granules may be

FIGURE 5 This micrograph illustrates the attachment of densely stained ribosomes to vesicles of the endoplasmic reticulum (*ER*). Note that the granules in the centrally located portion of the chloroplast (*C*) are larger in size and less electron-opaque than the ribosomes. *G*, Golgi body; *S*, starch. × 43,000.

FIGURE 6 Interconnections between adjacent chloroplast lamellae (*CL*) can be seen in this section of a chloroplast extension (arrows). Such interconnections are common and most readily seen in the chloroplast extensions. The regularly arranged chloroplast granules are distinct. × 50,000.


G
ER
C
ER
S
5


CL
6

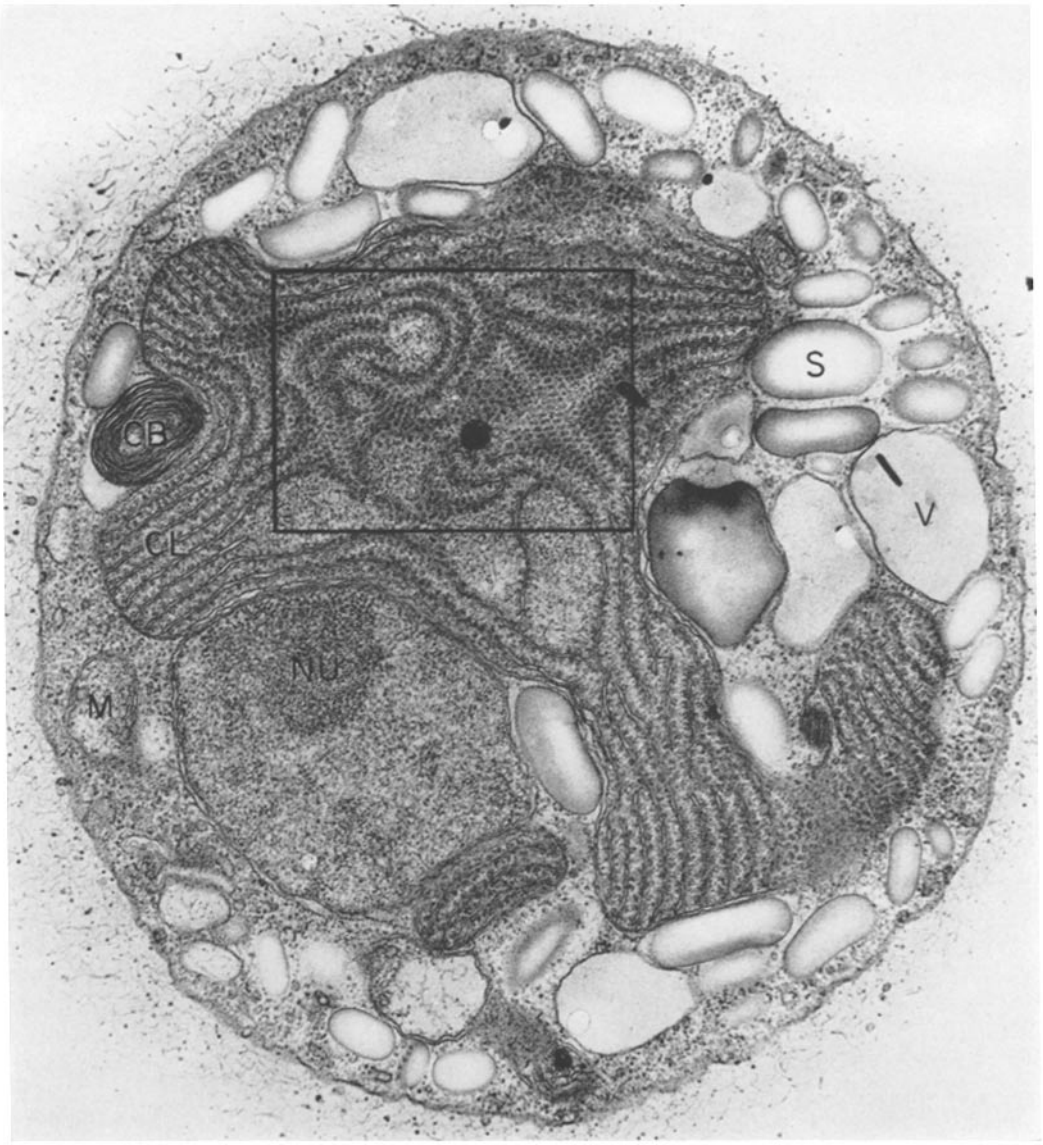


FIGURE 7 This micrograph represents a non-central section of a cell. The nucleolus (*NU*) is shown in its typical position facing the chloroplast. Chloroplast granules (320 A) show an orderly arrangement along the chloroplast lamellae (*CL*). A regular 2-dimensional spacing of the granules is evident in a grazing section over several lamellae (box). A simple concentric body (*CB*) is seen on the left. The cytoplasmic area is filled with starch (*S*), vacuoles (*V*), and mitochondria (*M*). × 27,000.

sites of accessory pigments. Attempts are now being made to determine the nature of these granules.

This study was supported in part by Public Health Service Training Grant 5 TL- GM-961-03 and Research Career Program Award 1 K3- GM-8716-01 (S. F. Conti) from the National Institutes of General Medical Sciences and by research grants from the National Science Foundation (GB-2387) and the Public Health Service (GM-08565).

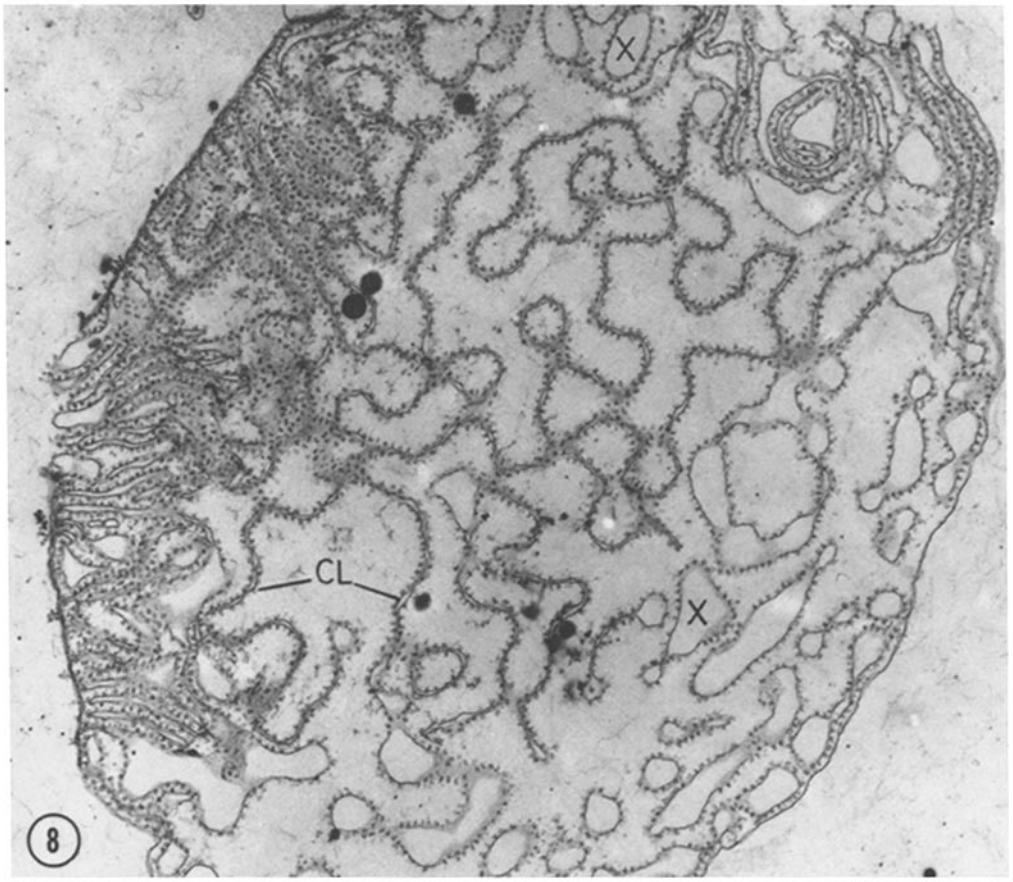
Received for publication, November 24, 1964.

REFERENCES

1. BOUCK, B. J., Chromatophore development, pits, and other fine structure in the red alga, *Lomentaria baileyana*, *J. Cell Biol.*, 1962, **12,** 553.
2. BRODY, M., and BRODY, S. S., Light reactions in photosynthesis, *in* Physiology and Biochemistry of Algae, (R. A. Lewin, editor), New York, Academic Press, Inc., 1962, 3.
3. BRODY, M., and EMERSON, R., The effect of wave length and intensity of light on the proportion of pigments in *Porphyridium cruentum*, *Am. J. Bot.*, 1959, **46,** 433.
4. BRODY, M., and VATTER, A. E., Observations on cellular structures of *Porphyridium cruentum*, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 289.
5. GEITLER, L., Über einige wenig bekannte Süsswasser Organismen mit roten oder blaugrünen Chromatophoren. Zugleich ein Beitrag zur Kenntnis pflanzlicher Chromatophoren, Revue Algologique, 1924, **1,** 357.
6. GEITLER, L., Furchungsteilung, simultane Mehrfachteilung, Lokomotion, Plasmoptyse und Okalogie der Bangiacee *Porphyridium cruentum*, *Flora* (Jena), 1944, **137,** 300.
7. GIBBS, S. P., The ultrastructure of the chloroplasts of algae, *J. Ultrastruct. Research*, 1962, **7,** 418.
8. JONES, R. F., SPEER, H. L., and KURY, W., Studies on the growth of the red alga *Porphyridium cruentum*, *Physiol. Plant.*, 1963, **16,** 636.
9. LEDBETTER, M. C., and PORTER, K. R., A "microtubule" in plant cell fine structure, *J. Cell Biol.*, 1963, **19,** 239.
10. LEWIS, I. F., and ZIRKLE, C., Cytology and systemic position of *Porphyridium cruentum* Naegeli, *Am. J. Bot.*, 1920, **7,** 333.
11. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
12. MANTON, I., Observations with the electron microscope on the division cycle in the flagellate *Prymnesium parvum* Carter, *J. Roy. Micr. Soc.*, 1964, **83,** 317.
13. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 736.
14. MITRAKOS, K., Feinbau und Teilung bei Plastiden einiger Florideen-Arten, *Protoplasma*, 1960, **52,** 611.
15. PARK, R. B., and BIGGINS, J., Quantasome: size and composition, *Science*, 1964, **144,** 1009.
16. SABATINI, D. D., BENSCH, K. G., and BARRNETT, R. J., Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation, *J. Cell Biol.*, 1963, **17,** 19.
17. SIEVERS, A., Über die Feinstruktur des Plasmas wachsender Wurzelhaare, *Z. Naturforsch.*, 1963, **18,** 830.
18. SMITH, G. M., The Fresh-Water Algae of the United States, New York, McGraw-Hill Book Company, Inc., 1950, 612.
19. SPEER, H. L., DOUGHERTY, W., and JONES, R. F., Studies on the fine structure of the red alga *Porphyridium cruentum*, *J. Ultrastruct. Research*, 1964, **11,** 84.
20. VISCHER, W., Zur Morphologie, Physiologie und Systematik der Blutalge *Porphyridium cruentum*, *Verhandl. Naturforsch. Ges., Basel*, 1935, **46,** 66.
21. WHALEY, W. G., KEPHART, J., and MOLLENHAUER, H. H., The dynamics of cytoplasmic membranes during development, *in*, Cellular Membranes in Development, (M. Locke, editor), 22nd Symposium of the Growth Society, New York, Academic Press, Inc., 1964, 135.

FIGURE 8 This micrograph represents a section of a chloroplast whose lamellae are distorted by swelling. Although the parallel, and close, orientation of the lamellae (*CL*) is absent, the granules are still present along the "outer" side of each membrane. Swelling also caused the separation of membrane pairs, resulting in vesicles (*X*) with a smooth "inner" surface and rough "outer" surface. The dark round structures between the loose lamellae are osmiophilic bodies. × 19,000.

FIGURE 9 Granules are absent from both layers of the chloroplast limiting-membrane (*CLM*). They are present on the chloroplast lamellae immediately below. × 40,000.


X
CL
X
8

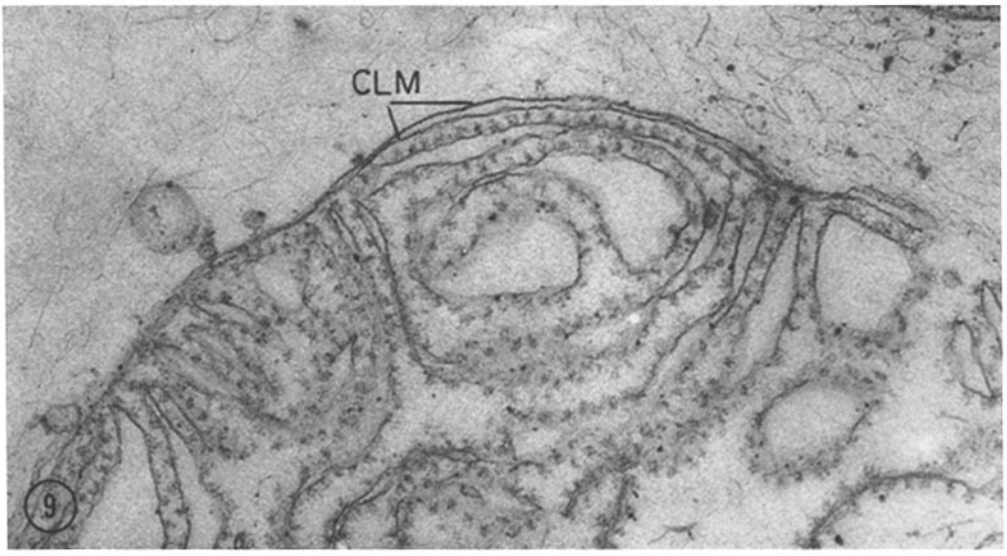

CLM
9

FIGURE 10 Represented is a section of a dividing cell. The elongation of the chloroplast and its pyrenoid (*P*) is due to the median annular constriction by which cell division proceeds. Only one of the daughter nuclei (*N*) is seen. Note that there is no independent invagination of the cell membrane and no unusual thickening in the cleavage furrow (arrows). The cell organelles and inclusions, such as mitochondria (*M*), osmiophilic bodies (*OB*), and numerous starch grains (*S*), are evenly distributed throughout the cytoplasm. × 25,000.

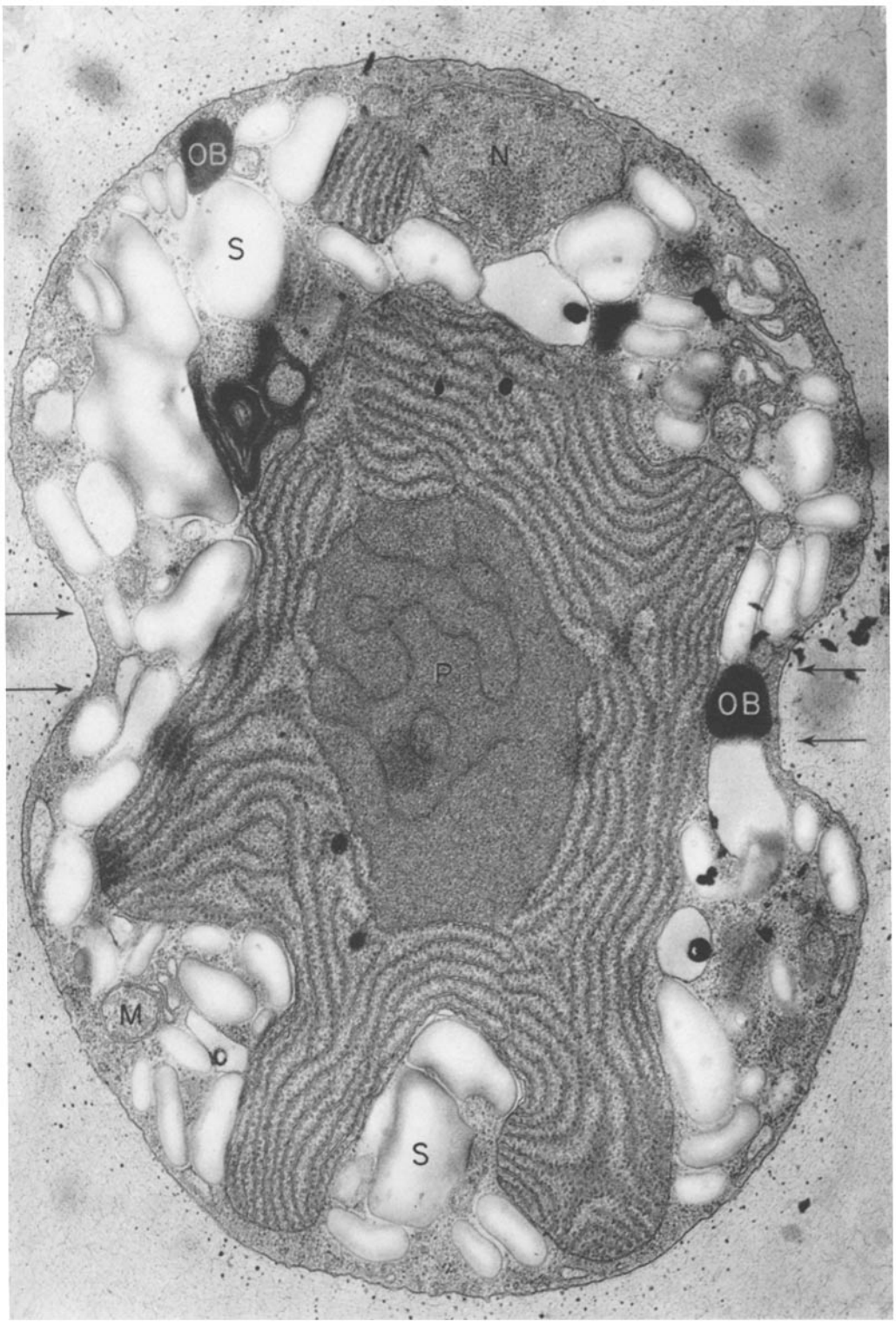
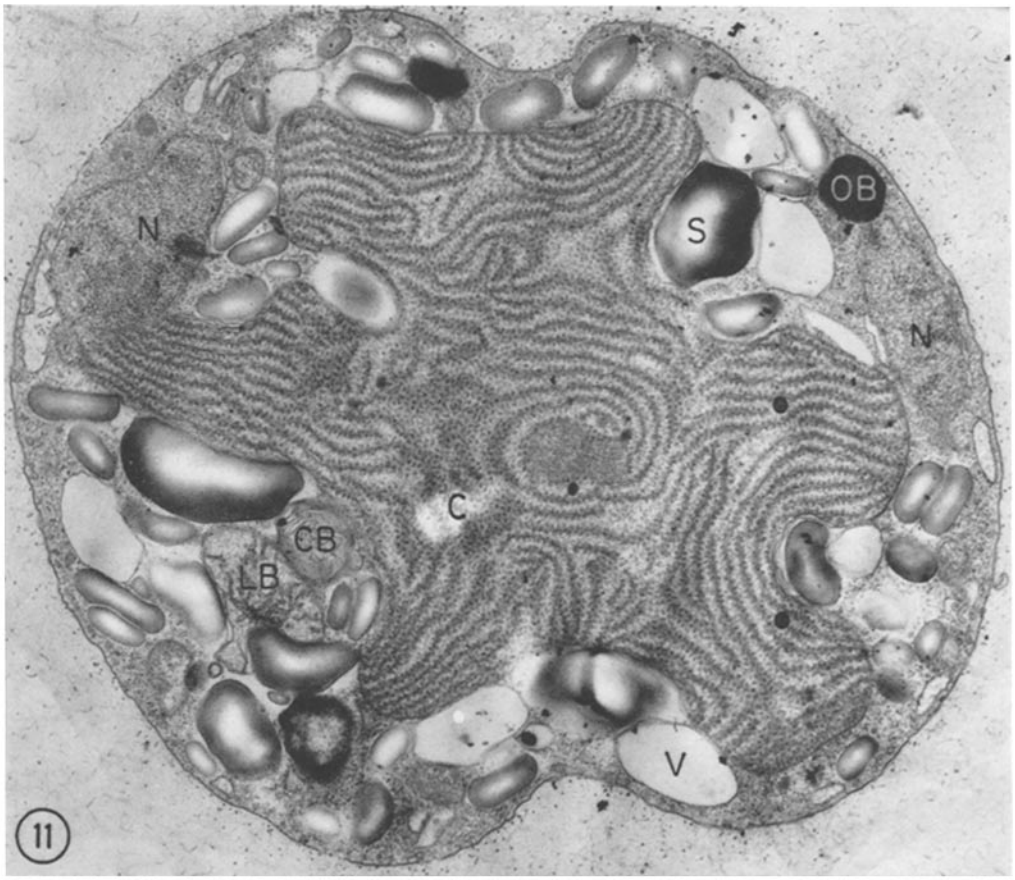
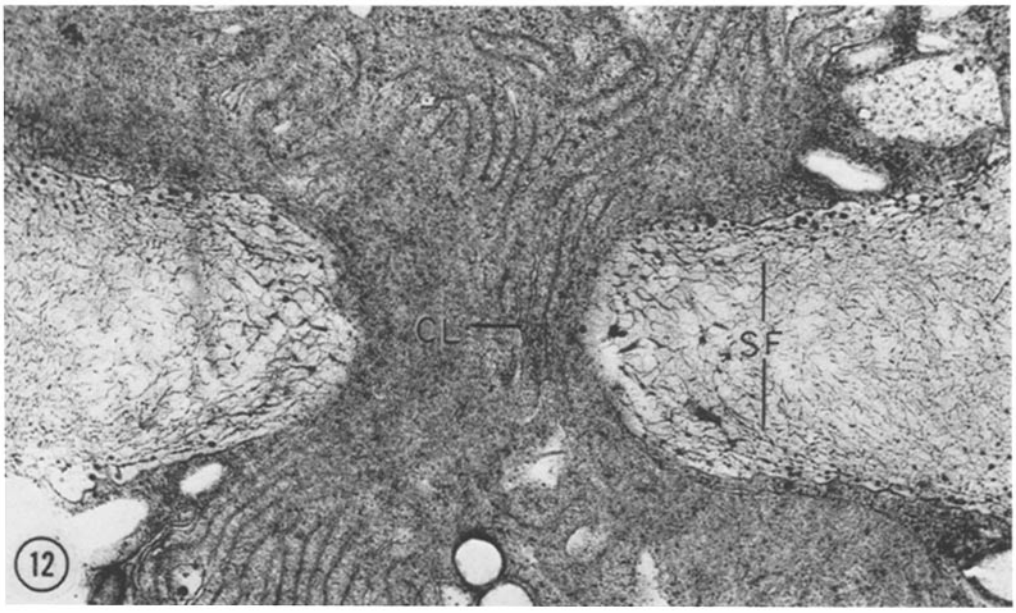

OB
N
S
P
OB
M
S

FIGURE 11 Micrograph of another dividing cell. The daughter nuclei are located at opposite ends of the cell. Each nucleus (*N*), surrounded by a membrane, lies appressed to the cell membrane. Commencement of cell division is indicated by the cleavage furrow. Among the vacuoles (*V*), starch grains (*S*), mitochondria, and osmiophilic bodies (*OB*) are two adjacent bodies, one with concentric lamellae (*CB*) and one lamellar body (*LB*) reminiscent of a proplastid. The latter two structures are more abundant and elaborate in old cells. × 18,000.

FIGURE 12 A longitudinal section of the cell isthmus, which resulted from the median annular constriction in cell division, is shown in this micrograph. Note the chloroplast lamellae in the isthmus. The cell membrane is indistinct on both sides of the isthmus since the cut was tangential to the cell membrane. The density and somewhat radial arrangement of the sheath "fibrils" (*SF*) suggest that sheath material is produced during the division process. × 32,000.



11



12