STRUCTURE AND FUNCTION OF THE ALGAL PYRENOID. I. ULTRASTRUCTURE AND CYTOCHEMISTRY DURING ZOOSPOROGENESIS OF *TETRACYSTIS EXCENTRICA*

R. Malcolm Brown, Jr. and Howard J. Arnott

Department of Botany and Cell Research Institute, University of Texas, Austin, Texas 78712

Reprinted from JOURNAL OF PHYCOLOGY
Vol. 6, No. 1, (1970)
pp. 14–22
Made in United States of America

STRUCTURE AND FUNCTION OF THE ALGAL PYRENOID. I. ULTRASTRUCTURE AND CYTOCHEMISTRY DURING ZOOSPOROGENESIS OF *TETRACYSTIS EXCENTRICA*[1]

R. Malcolm Brown, Jr.[2] *and Howard J. Arnott*

Department of Botany and Cell Research Institute, University of Texas, Austin, Texas 78712

SUMMARY

The fine structure of the pyrenoid in the mature vegetative cell of Tetracystis excentrica *Brown and Bold is described. During zoosporogenesis, the pyrenoid undergoes regression, and the ultrastructure of this process is described in detail. The ground substance undergoes dissolution, and reticulate fibrillar structures appear as well as intruding chloroplast thylakoids. Pyrenoid-associated starch plates diminish, and quantities of starch not associated with the pyrenoid are produced. New pyrenoids appear late in the division cycle after all other major organelles associated with the motile cell have been formed. Zoospore pyrenoids develop in thylakoid-free spaces of the chloroplast which are similar to the DNA-containing regions. The new pyrenoid ground substance, which is loosely fibrillar, arises in close proximity to starch grains which may be formed in the stroma. Then the zoospore pyrenoid produces 2 hemispherical starch plates identical to those in the mature vegetative cell. Zoospore pyrenoids lack the 2 convoluted thylakoids between the starch plates and the ground substance characteristic of those in the mature vegetative cell. Instead, the thylakoids are identical to those of the chloroplast at first, and then develop into a convoluted state in the vegetative cell. Cytochemical tests for DNA, RNA, and protein were made for the cytoplasm, nucleus, nucleolus, and pyrenoid. Conclusive evidence is presented for the presence of RNA in the cytoplasm and nucleolus, DNA in the nucleus, and protein in the pyrenoid. The tests did not conclusively demonstrate the presence or absence of DNA and RNA in the pyrenoid; however, they suggested that small amounts of both DNA and RNA may be present.*

INTRODUCTION

The pyrenoid, first described by Schmitz in 1882 (***25***), has since been the subject of many investigations. Schmitz defined the pyrenoid as a highly refractive body in the chloroplast. We know that the pyrenoid is found in some genera of all divisions of algae except the Cyanophyta (see ***8***). Most investigations have revealed that this body contains protein and that it is the site of starch accumulation (***6,12***); however, little is known about other constituents it may contain (***7,10,11,13,15,18,26***).

Czurda (***12***) reported that there are 2 ways in which pyrenoids may multiply: fragmentation and *de novo*. According to Czurda, *de novo* pyrenoid formation occurs only in those algae that have "endogenous cell formation" resulting in autospores, zoospores, or gametes. Little is known of the fate and subsequent reappearance of the pyrenoid during zoospore formation (***17***). It was the object of this investigation to study the fine structure of the pyrenoid during zoosporogenesis in order to elucidate its regression and reappearance. In addition, the pyrenoid was studied cytochemically.

MATERIALS AND METHODS

Axenic cultures of *Tetracystis excentrica* Brown and Bold were routinely maintained on slants of 3N BBM medium (***9***) solidified to 1.6% with Difco Bacto Agar. Standard conditions of culture included a 12–12 hr diurnal light-dark cycle utilizing a light intensity of 250 ft-c and a temperature of 22 C.

In preparation for zoospore induction, cultures were grown for about 10–12 days under standard conditions until large populations of vegetative cells had been produced. Cultures were made to divide synchronously to produce ca. 100% zoospores merely by transfer from a solidified medium (the agar slant) to liquid 3N BBM. This transfer need only be temporary because no inhibition in zoospore production was observed when the cell mass was overlaid with 1.6% agar medium only 3 min after transfer to liquid. Light intensities above 500 ft-c inhibit zoospore production, and darkness has no effect on it. Anaerobic conditions also inhibit zoospore production.

For electron microscopy, aliquots of agar-embedded material were fixed for 1 hr at room temperature (ca. 22 C) in 3% acrolein–3% glutaraldehyde buffered to pH 6.5 in 0.1 M cacodylate. Following fixation, the material was rinsed 3 times in 0.1 M cacodylate buffer, pH 6.5, and then postfixed for 1 hr, at 22 C, in 2% OsO_4 buffered in 0.1 M cacodylate, pH 6.5. Following osmication, the algae were rinsed in 3–4 changes of deionized water and placed in an aqueous solution of 0.5% uranyl acetate for 12–24 hr at 5 C. The cells were rinsed in deionized water, dehydrated in ethanol, and embedded according to the procedures of Mollenhauer (***22***). The blocks were sectioned on Porter-Blum MT-1 and MT-2 microtomes, and the sections poststained with uranyl acetate and lead citrate (***21***) for 5 min each, respectively. The algae were examined with an RCA EMU 3F microscope at 100 kv and a Siemens Elmiskop I at 60 kv.

Light microscopic examinations were made with a Zeiss WL microscope equipped with bright field, dark field, fluorescent, and phase optics.

For cytochemical studies, whole cells were scraped from the agar surface of actively growing cultures and cell monolayers spread on the surface of microscope slides. Care was taken to prevent the preparations from drying or else the cells would become impermeable to the subsequent reagents.

[1] *Received April 19, 1969; revised August 5, 1969.*

[2] Present address: Department of Botany, The University of North Carolina, Chapel Hill, North Carolina 27514.

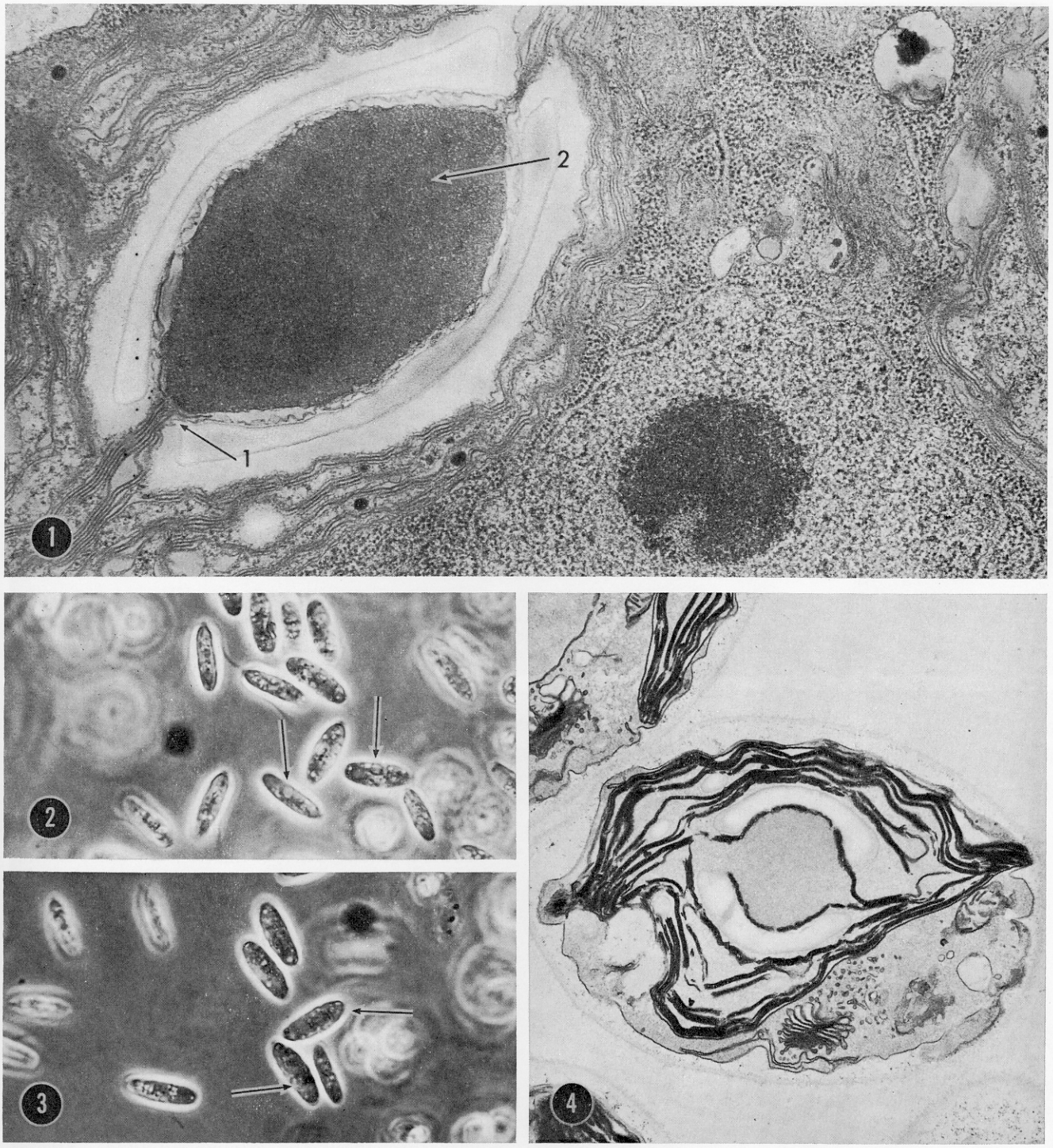
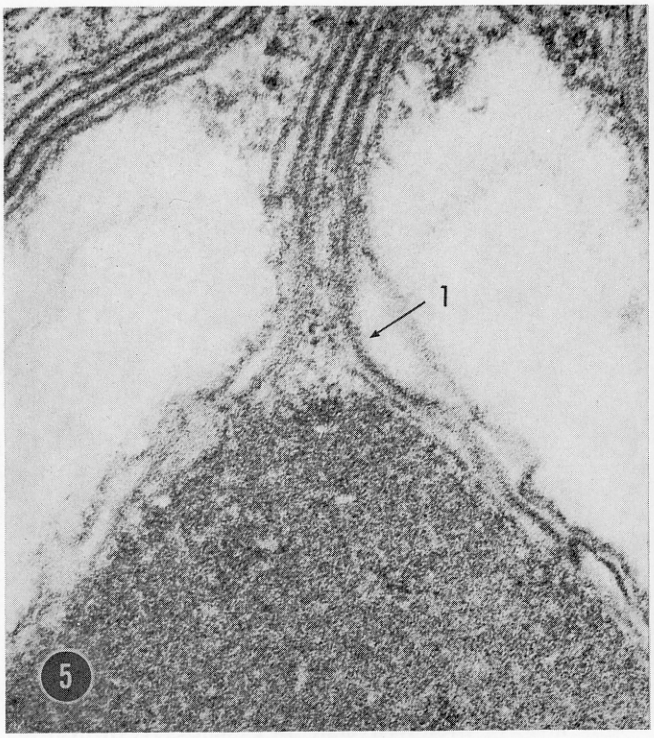


FIG. 1–13. Cells of the green alga, *Tetracystis excentrica*. FIG. 1, 5–13 fixed by the acrolein/glutaraldehyde–osmium tetraoxide technique. FIG. 1. Median section through a portion of a mature vegetative cell of *T. excentrica*. Note the ellipsoidal, electron-dense ground substance of the pyrenoid which is surrounded by 2 hemispherical starch plates. Note the 2 convoluted thylakoids between the starch plate and ground substance and the connection with stroma chloroplast thylakoids at arrow 1. × 30,800. FIG. 2. Living cultures, photographed with phase-contrast optics, showing flagellated zoospores just released from zoosporangia. Note the equatorial pyrenoid (*arrows*). × 840. FIG. 3. Young ellipsoidal vegetative cells, recent products of quiescent zoospores (*arrow 1*). Phase contrast. × 840. FIG. 4. Young vegetative cell fixed with 2% $KMNO_4$, 5 C for 1 hr. × 14,000.



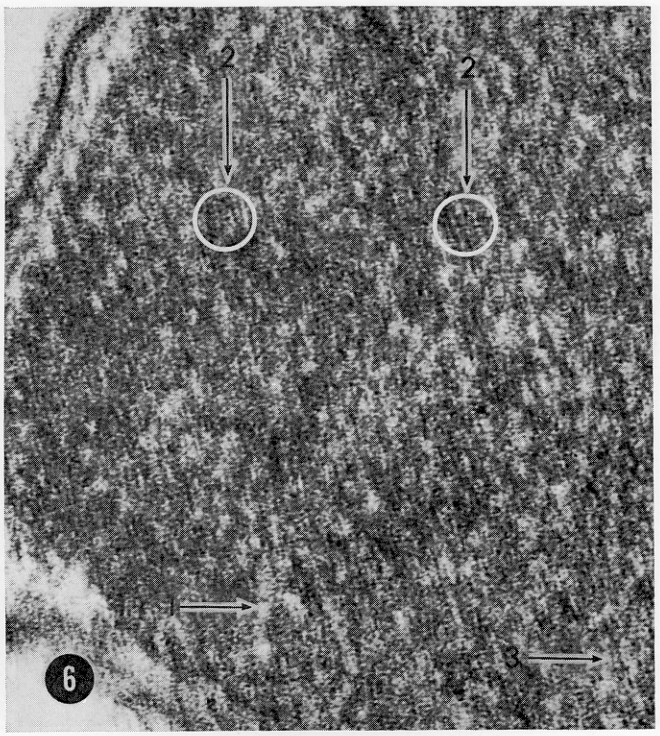


FIG. 5. Pyrenoid of mature vegetative cell at juncture of chloroplast and pyrenoid thylakoid (*arrow 1*). Note coarsely granular ground substance and convoluted pyrenoid thylakoids. × 125,800. FIG. 6. Details of the ground substance of pyrenoid of mature vegetative cell. Note the electron-transparent regions (*arrow 1*), the helical fibrillar structures (circles at *arrow 2*), and loose helical fibrils (*arrow 3*). × 176,400.

The acridine-orange methods used were those of von Bertalanffy *et al.* (*2*). The source of acridine orange was Chroma Gesellschaft. For the DNAase test, specimens which had been fixed in ether alcohol were incubated in 0.02% deoxyribonuclease (Sigma) in 0.003 M/$MgSO_4$, pH 6.5 adjusted with 0.5 M phosphate buffer. The slides were then washed in distilled water after appropriate incubation at the proper temperature (Table 1) and processed with the acridine orange technique. The ribonuclease tests consisted of incubating ether alcohol- or Carnoy-fixed specimens in 0.02% ribonuclease (Sigma) in distilled water, pH 6.5 adjusted with 0.05 M phosphate buffer at specified times and temperatures (Table 1).

Total nucleic acid extraction was accomplished by incubating the cells in 5% trichloroacetic acid (TCA) at 90 C for 30 min, and with 1N HCl at 60 C for 30 min.

In addition to enzyme extractions, RNA was extracted with 10% perchloric acid at 20 C for 5 hr and at 60 C for 45 min.

For protein extraction, 0.2 mg pepsin (Calbiochem) per ml of 0.02N HCl was employed.

The Mercuric Bromophenol Blue Method (*20*) was used for protein detection. Azure B at pH 4 (*14*) was used for nucleic acid detection. For both of these methods, the material was fixed for 2–4 hr in Carnoy's Fixative (*19*).

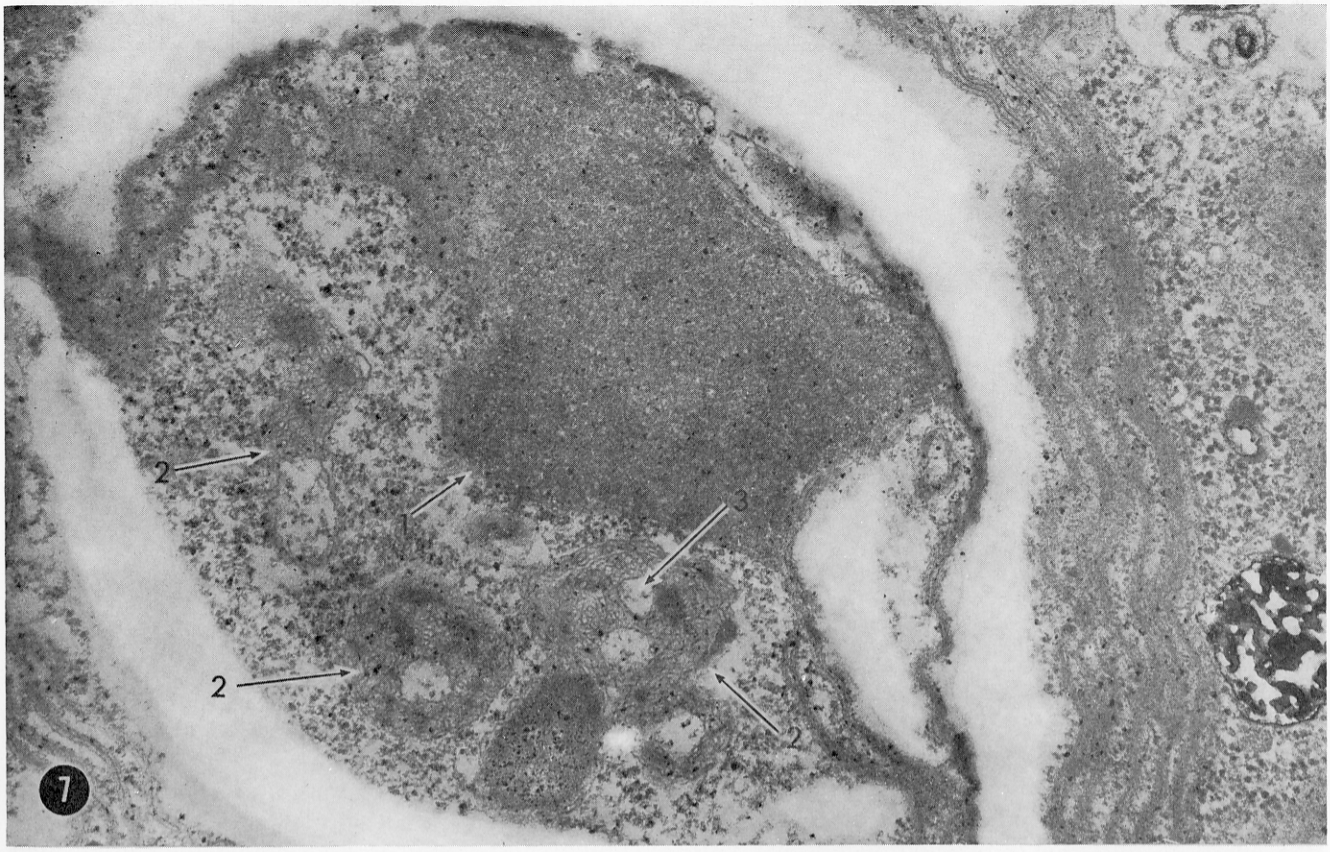
OBSERVATIONS AND RESULTS

Pyrenoid fine structure in the nondividing cell. The specific epithet of *T. excentrica* is based on one of its most obvious morphological features, a pyrenoid which is located excentrically in the mature vegetative cell. Furthermore, the pyrenoid is always ensheathed by 2 hemispherical starch plates (Fig. 1). Chloroplast thylakoids do not penetrate the pyrenoid ground substance. They occur as single, convoluted discs on the surface of the ground substance adjacent to the starch plate. Thylakoids in the chloroplast stroma are stacked in 4–6 discs and are not convoluted (Fig. 5). The pyrenoid-associated thylakoids always join the stroma thylakoids at the juncture of the 2 hemispherical starch plates (Fig. 1, 4).

Acrolein-glutaraldehyde fixation as well as permanganate reveals 2 structural entities in the starch plate (Fig. 1, 4). An inner, electron-dense core is surrounded by a more electron-transparent sheath which always lies in close proximity to the ground substance, usually by contact with the convoluted pyrenoid-associated thylakoids.

The elliptical pyrenoid ground substance is colorless in living preparations. It is composed of an electron-dense matrix in which are embedded tightly packed fibrils which seem to be composed of a helix (Fig. 6, *arrow 2*). Clusters of electron-dense bodies appear throughout the ground substance (Fig. 1, *arrow 2*). Interspersed throughout the latter are small electron-transparent regions of irregular shape and size (Fig. 6, *arrow 1*). It should be noted at this point that permanganate-fixed material (Fig. 4) reveals only a homogeneous ground substance.

Pyrenoid fine structural changes during zoospore formation. Mitosis occurs within 1–2 hr after zoospore induction; mature zoospores with stigmata, flagella, and new pyrenoids are produced within 8–10



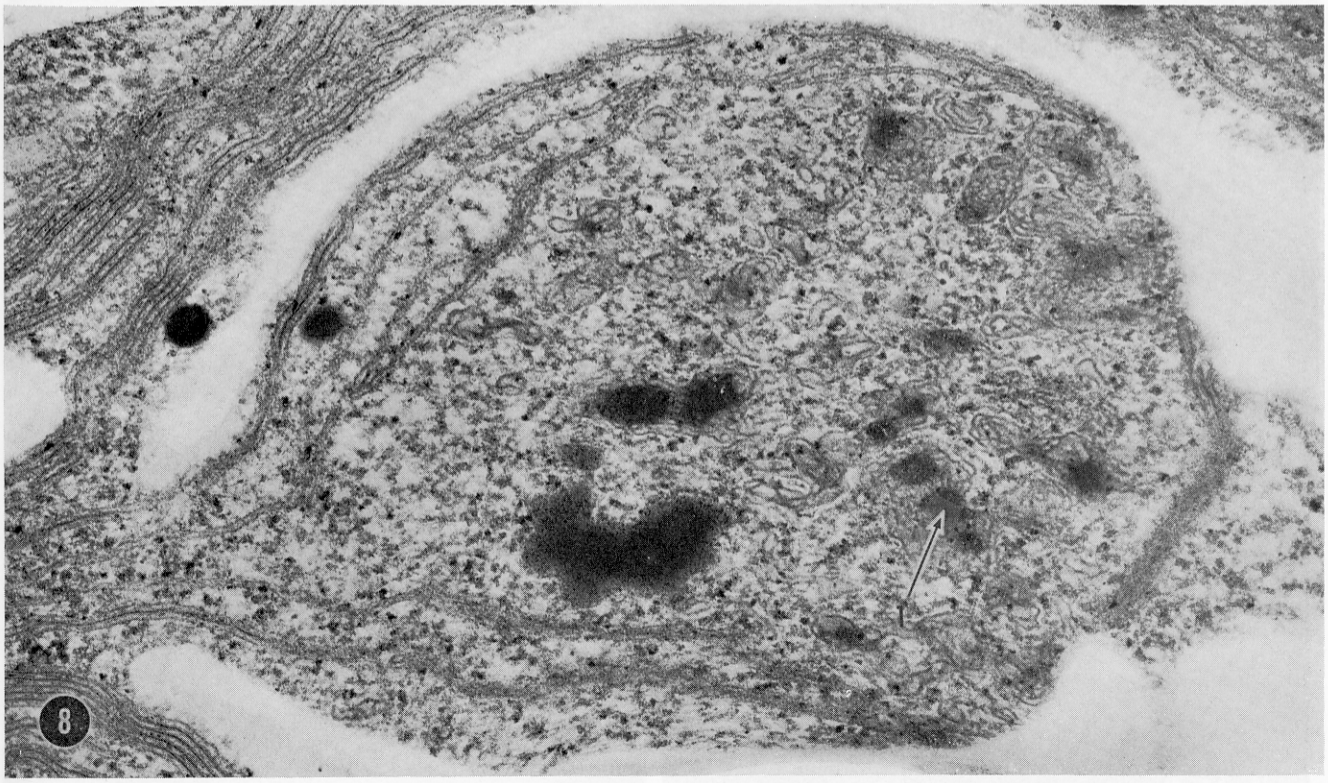


FIG. 7, 8. Stages of pyrenoid regression. FIG. 7. Early stage of pyrenoid regression. Note the dissolution of the electron-dense ground substance (*arrow 1*), the compact reticulate fibrillar structures (*arrow 2*). Note also the diminishing starch plates and compare with Fig. 1. × 50,600. FIG. 8. Later stage of pyrenoid regression at which the dissolution of the ground substance is now complete. Note the intruding chloroplast thylakoids and the unraveling reticulate structures, each with an osmiophilic core (*arrow 1*). × 63,000.

TABLE 1. *Cytochemical tests of* Tetracystis excentrica.

Treatment	Reaction		
	Nucleus	Pyrenoid	Cytoplasm
AO Control	Green	Dark green	Light greenish brown
AO DNAase extracted[a]	Nonfluor	Green	Same
AO RNAase extracted[b]	Green	Yellow-green to nonfluor	Same
AO 1N HCl extracted[c]	Red	Green	Yellow-green
AO Pepsin extracted[d]	Green	Nonfluor	Reddish brown
AO Pepsin extracted, followed by DNAase[e]	Nonfluor	Nonfluor	Light green
AO 5% TCA extracted[f]	Nonfluor	Green	Yellow-green
AO Perchloric acid extracted[g]	Green	Dark green	Yellow-green
Mercuric Bromphenol Blue[h]	Negative	Strongly positive	Weakly positive
Azure B[i]	Light green	White to very light green	Clear with dense blue spots

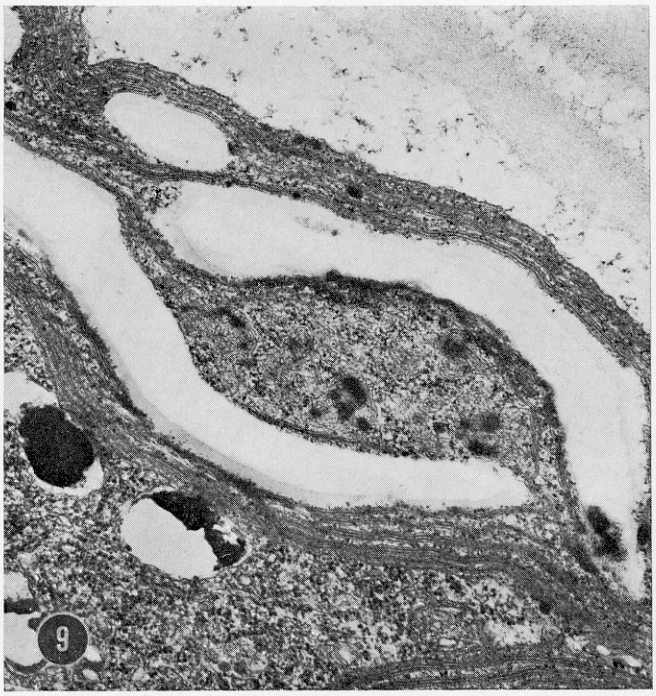
[a] DNAase, 20 min at 37 C; [b] RNAase, 3 hr at 37 C; [c] 1N HCl, 30 min at 60 C; [d] pepsin, 1 hr at 22 C; [e] pepsin, 3 hr at 22 C followed by DNAase for 30 min at 37 C; [f] TCA, 30 min at 90 C; [g] perchloric acid, 45 min at 60 C; [h] mercuric bromphenol blue, for proteins, 15 min at 22 C; [i] Azure B, for nucleic acids, pH 4.0, 15 min at 22 C.

hr. One of the first indications of incipient cell division is the initiation of pyrenoid regression. This is frequently observed at the light microscopic level as the 2 starch plates begin to diminish in size. The ultrastructure of the initiation of pyrenoid regression is shown by a progressive reduction of the outer layer of the starch plate (Fig. 1, 7, 8).

Within 3–4 hr after induction, the original pyrenoid ground substance becomes clear and empty as observed with the light microscope. In this case, the only marker for the location of the pyrenoid is the diminishing remains of the 2 original encompassing starch plates which have now diminished in size. These move away from each other along the longitudinal axis of the pyrenoid.

Ultrastructurally, the changes accompanying the regression of the pyrenoid are quite striking. The ground substance undergoes dissolution (Fig. 7, *arrow 1*). Several fibrillar structures, previously undetected, then appear (Fig. 7, *arrow 2*). These fibrils form a network cluster which is oriented over the surface of a membrane-free "core" (Fig. 7, *arrow 3*). The area formerly occupied by the ground substance now becomes coarsely granular and has some resemblance to the interthylakoidal regions of the chloroplast. Accompanying this striking change of the ground substance, the convoluted thylakoid discs disappear and are replaced by intruding thylakoids identical in structure to those of the chloroplast (Fig. 8). At this stage the original ground substance has undergone complete dissolution, and the tightly clustered fibrillar structures begin to unravel. An osmiophilic "core" is associated with the fibrillar structures (Fig. 8, *arrow 1*).

A later stage is shown in Fig. 9 where the starch



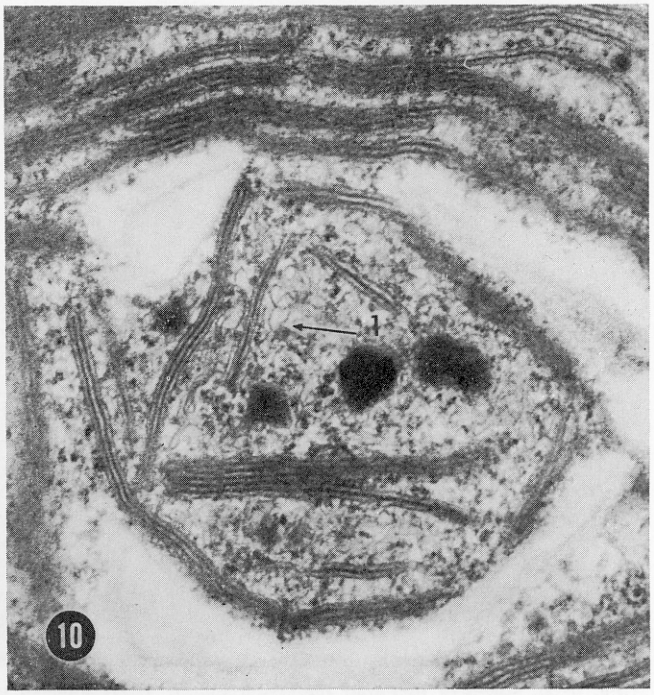


FIG. 9, 10. Final stages of pyrenoid regression. FIG. 9. Remains of pyrenoid of parental cell in one of the zoospore protoplasts. Note the displacement of the starch plates. × 27,500. FIG. 10. Illustrating abundant chloroplast thylakoid intrusion and fully unraveled fibrillar structures (*arrow 1*). × 120,000.

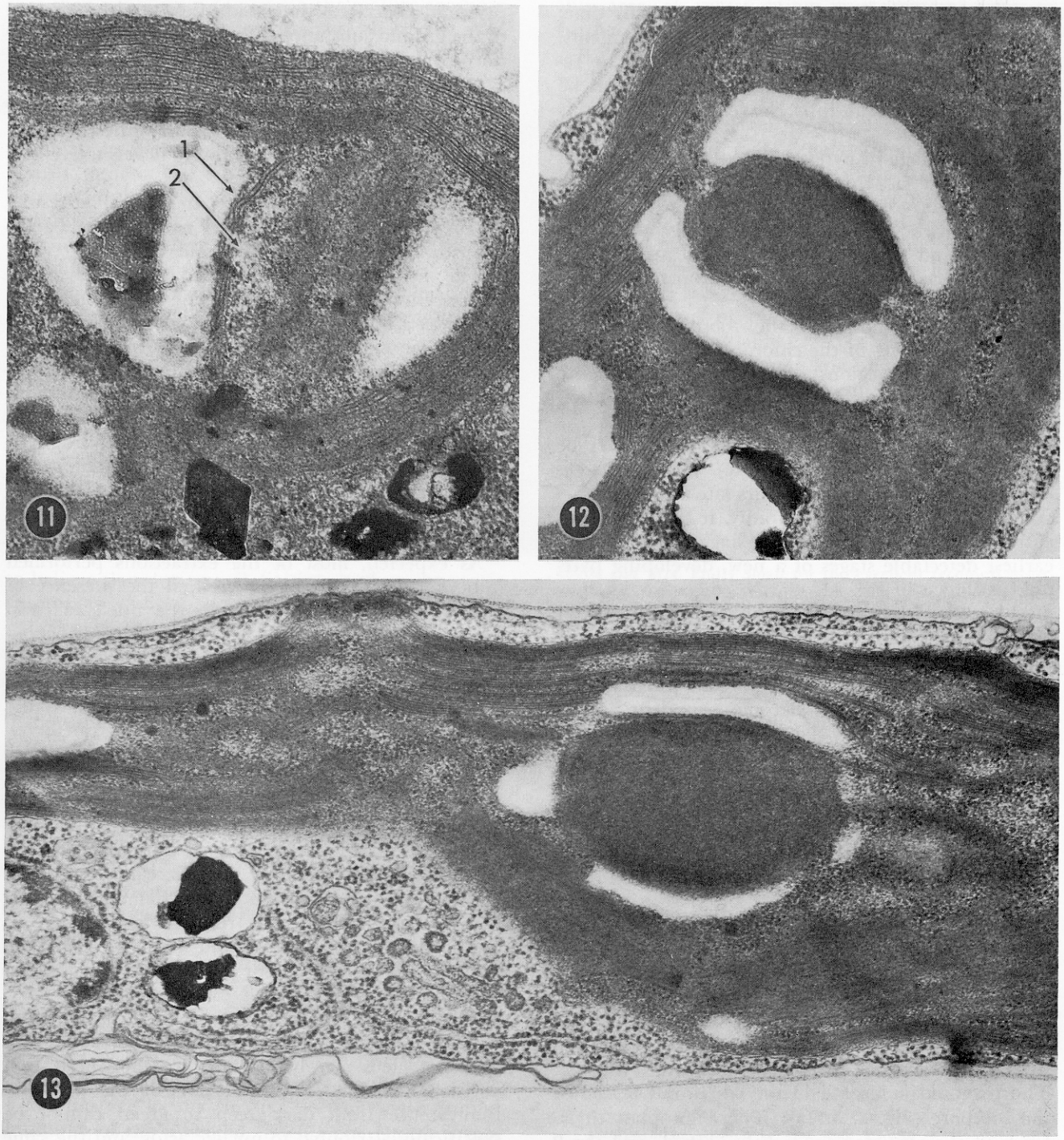


FIG. 11–13. Showing the formation of the new pyrenoid in the zoospore. FIG. 11. Earliest detectable stage of pyrenoid formation in the zoospore in which a loosely packed fibrillar ground substance has formed in a thylakoid-free area of the chloroplast. The starch to the left of the ground substance may not be pyrenoidal in origin. Note the chloroplast thylakoids (*arrow 1*) which first form and delimit the area to become the ground substance. Also note the 70 A fibrils in the electron-transparent region (*arrow 2*). × 42,900. FIG. 12. Completely formed pyrenoid in mature zoospore. Note and compare the hemispherical starch plates with Fig. 1. Note also that the 2 thylakoids between the starch plate and ground substance are not yet convoluted (see also Fig. 13). × 44,000. FIG. 13. Median longitudinal section through a zoospore showing the equatorial position of the pyrenoid. Posterior nucleus to the left. Note the compact chloroplast thylakoids as well as the nonconvoluted pyrenoid-associated thylakoid. Stroma starch at left. × 42,900.

plates are beginning to be displaced. By this time, the cell has divided into 4–16 protoplasmic segments, depending on the size of the parent cell. The remains of the pyrenoid-associated starch still can be detected in one of the fully cleaved segments at this stage.

The final stage in pyrenoid regression occurs with the intrusion of chloroplast thylakoids into the former matrix area of the pyrenoid (Fig. 10). Here, the fibrillar bodies have become fully unraveled (*arrow 1*), and the matrix has the same structure as the interthylakoid regions of the chloroplast. Finally, the 2 starch plates dissolve.

The new pyrenoids of the zoospores form in lamellar-free spaces of the chloroplast, similar to the DNA-containing regions (***4,5,16,23***). The zoospore pyrenoids have been observed only in the late division stages after all other organelles associated with the zoospore have formed (an exception to this may be the stigma which also develops late). Because daughter-cell pyrenoid formation occurs late in the division cycle, and apparently very rapidly, it has been difficult to obtain many different stages. One of the earliest detectable stages of a new, developing pyrenoid is shown in Fig. 11. Earlier stages would show only lamellar-free areas of the chloroplast, devoid of any structural similarity to the pyrenoid shown in Fig. 11.

The new pyrenoids form in close proximity to stroma starch which has become abundant at this stage. In the very early stages of pyrenoid formation in the zoospore shown in Fig. 11, it is not certain whether the starch is stroma-formed or pyrenoid-produced. At a slightly later stage (Fig. 12), the starch is clearly pyrenoid-associated, just like that in the mature vegetative cell.

Pyrenoid ground substance formation occurs in the lamellar-free areas of the chloroplast as a rather loosely arranged fibrillar system (Fig. 11). The chloroplast thylakoid intrusion (Fig. 11, *arrow 1*) occurs before the electron-dense, fibrillar ground substance has been formed, and at this stage, it is identical to thylakoid structure of the chloroplast. Note also the clear area immediately adjacent to this intruding thylakoid which has 70 A fibrils (Fig. 11, *arrow 2*). The pyrenoid is located in the equatorial region of the zoospore (Fig. 2, 3, 13). The chloroplast thylakoids of the zoospore are tightly packed, and the 2 pyrenoid-associated thylakoids between the surface of the ground substance and the starch plates remain unconvoluted in the actively motile zoospore (Fig. 12, 13). The pyrenoid of the zoospore is structurally identical to that of the mature vegetative cell by the time the zoospore has become quiescent and begins to enlarge into an ellipsoidal vegetative cell (Fig. 3, *arrow* 1; Fig. 4).

TABLE 2. *Summary of cytochemical tests on* Tetracystis excentrica.[a]

	RNA	DNA	Protein
Nucleus	−	+	−
Nucleolus	+	−	−
Pyrenoid	+?	+?	+
Cytoplasm	+	−	+

[a] Mitochondrial nucleic acids and proteins were not specifically detected in *T. excentrica* by our methods.

Cytochemical studies of the pyrenoid. The results of the cytochemical tests performed with *T. excentrica* are summarized in Table 1.

The acridine orange revealed a typical green fluorescence for the nucleus. The cytoplasm, however, was a light, greenish-brown which is indicative of low concentrations of RNA (***2***). In some cases, this was masked by a failure of the fixative to remove all of the chlorophyll (which has a red autofluorescence). If the preparation becomes the least bit dry after smearing a monolayer of cells, the chlorophylls will not be effectively removed. The pyrenoid exhibited a dark emerald-green fluorescence and was clearly distinguishable from the fluorescent chloroplast.

As expected, most of the extractions performed indicated and conclusively verified the presence of DNA in the nucleus, RNA in the nucleolus and cytoplasm, and protein in the pyrenoid. Mitochondrial nucleic acids were not detected by these methods.

The presence or absence of nucleic acid in the pyrenoid was not conclusively demonstrated, although the tests do seem to indicate the possibility of the presence of DNA and RNA in this region. One of the problems obviously is the considerable masking of these compounds by the great abundance of protein. The RNAase test gave a positive reaction for RNA in the pyrenoid, but one cannot rule out possible impurities in the RNAase, namely proteases. Likewise, the DNAase test resulted in a change in the fluorescence of the pyrenoid which could be due to impurities of RNAase or proteases in the preparation.

Nucleic acid extraction with 1N HCl seems to have been incomplete because the red fluorescence of the nucleus merely indicates a depolymerization of the DNA (***2***). The alteration of the pyrenoid fluorescence from dark-green to green cannot be specifically attributed to nucleic acids, and the same holds for the cytoplasmic change. Five percent trichloroacetic acid appears to have been more effective in removing DNA than 1N HCl, but it did not seem to have any additional effect on the pyrenoid and cytoplasm.

A summary of the cytochemical tests is presented in Table 2.

DISCUSSION

While it is well documented that pyrenoids undergo regression during zoospore formation and

that they can appear *de novo* in the mature zoospore (*6,12*), very little has been known about the fine structure of this process (*17*). The evidence presented here indicates that during zoospore formation an ordered regression of the pyrenoid takes place, which consists of dissolution of the ground substance and diminishing capacity for starch synthesis. A previously undescribed structure, the compact fibrillar network, appears in the pyrenoid matrix with the dissolution of the ground substance. What is the function of the fibrillar network? Although it is not the purpose of this report to attempt to answer this question, it is worthwhile to examine possible alternative functions in light of the structural evidence at hand.

If pyrenoids arise *de novo*, as would appear to be the case from light microscopy, is there a structure not detectable at the light microscopic level which is responsible for the transmission of a pyrenoid from the parent to offspring? That is, is there a pyrenoid progenitor? It is possible that the compact fibrillar structures are the progenitors of the pyrenoid in the zoospore. If the pyrenoid should contain DNA, as we have implied, then it would not be unreasonable to believe that during cell division (and consequently chloroplast division) the genetic information is distributed to the daughter chloroplasts, perhaps via the fibrillar network of the pyrenoid (*1*; see also *13*). Obviously, there is a considerable structural difference between this fibrillar network and the areas demonstrated to contain DNA in other plastids (*4, 5,16,23*).

An alternative hypothesis for the function of the fibrillar network is that it may represent nothing more than an assimilation phase (perhaps similar to the prolamellar bodies) of the new chloroplast thylakoids which invaginate into the dissolved region (Fig. 10). Another possibility is that the 2 previously appressed thylakoids are released as the ground substance dissolves.

Pyrenoid regression during zoosporogenesis gives us a clue at least to one of its suspected functions, namely, that of starch synthesis (see *24*). Concomitant with the dissolution of the ground substance of the pyrenoid a shrinkage of the hemispherical starch plates occurs. Perhaps the enzymes involved in starch formation are located in the electron-dense ground substance of the pyrenoid. Such a contention is supported by vegetative cell division where the pyrenoid does not undergo dissolution, and the starch plates do not diminish. Bisalputra & Weier (*3*) have shown in *Scenedesmus* that all of the chloroplast starch was originally produced from the surface of the pyrenoid. In *T. excentrica* starch appears in the stroma after pyrenoid regression, thus suggesting that not all starch synthesis is pyrenoid related during zoosporogenesis.

As to the presence of nucleic acid in the pyrenoid, this area of investigation must remain open to further study because the fluorescence or cytochemical tests did not conclusively settle whether nucleic acids are present in the pyrenoid. Other investigators have suggested that nucleic acids (RNA and DNA) may be present in the pyrenoid (*9,13,26*), but our data, as well as those of others, are open to question with respect to the purity of nucleases. The use of highly purified nucleases at the light and electron microscopic levels may validate the presence or absence of nucleic acids in the pyrenoid. In addition, the incorporation of labeled nucleic acid precursors (H^3 uridine and H^3 thymidine, for example) into specific regions of the pyrenoid ground substance would provide valuable evidence for the presence of nucleic acids in this region and might indicate which structural component represents the nucleic acid. Incorporation of labeled amino acid precursors might give evidence as to the structural nature of the protein component.

ACKNOWLEDGMENT

The authors wish to thank Drs. H. C. Bold and W. G. Whaley for reading the manuscript, and Mrs. Virginia Stork Carne and Mrs. Emily Sloan for the excellent technical assistance given with grace and understanding. Supported in part by a grant from the Department of the Interior, WP 01057-07, to R. M. Brown and H. C. Bold and from The University Research Institute, The University of Texas, Austin.

REFERENCES

1. Arnott, H. J. & Brown, R. M., Jr. 1966. Ultrastructural modifications in the pyrenoid during zoosporogenesis in *Tetracystis excentrica*. *J. Phycol.* 2(Suppl.):5.
2. von Bertalanffy, L. 1963. Acridine orange fluorescence in cell physiology, cytochemistry and medicine. *Protoplasma* **57**:51–83.
3. Bisalputra, T. & Weier, T. E. 1964. The pyrenoid of *Scenedesmus quadricauda*. *Am. J. Bot.* **51**:881–92.
4. Bisalputra, T. & Bisalputra, A. A. 1967. The occurrence of DNA fibrils in chloroplasts of *Laurencia spectabilis*. *J. Ultrastructure Res.* **17**:14–22.
5. ——— 1967. Chloroplast and mitochondrial DNA in a brown alga *Egregia menziesii*. *J. Cell Biol.* **33**:511–20.
6. Bold, H. C. 1951. Cytology of algae. *In* Smith, G. M. [ed.], *Manual of Phycology*, Chronica Botanica Co., Waltham, Mass., 203.
7. Bose, S. R. 1941. Function of pyrenoids in algae. *Nature* **148**:440.
8. Brown, R. M., Jr. 1967. The pyrenoid: Its structure, distribution, and function. *J. Phycol.* 3(suppl.):5–7.
9. ——— & Bold, H. C. 1964. Phycological studies. V. Comparative studies of the alga genera *Tetracystis* and *Chlorococcum*. *Univ. Texas Publ. No. 6417*.
10. Brown, R. M., Jr. & Wilson, R. 1968. Electron microscopy of the lichen *Physcia aipolia* (Ehrh.) NYL. *J. Phycol.* **4**:230–40.
11. Cook, P. 1967. A cytochemical study of acid phosphatase and nuclease activity in the plastid of *Closterium acerosum*. *J. Phycol.* **3**:24–30.
12. Czurda, V. 1928. Morphologie und physiologie des algenstarkekornes. *Bot. Centralb. Beih.* **45**:97–270.
13. Esser, K. 1967. Elektronenmikroskopischer Nachweis von DNS in den Pyrenoiden von *Strepthotheca thamesis*. *Z. Naturforsch.* **22b**:993.
14. Flax, M. H. & Himes, M. H. 1952. Microspectrophoto-

metric analysis of metachromatic staining of nucleic acids. *Physiol. Zool.* **25**:297–311.

15. GIBBS, S. P. 1962. The ultrastructure of the pyrenoids of green algae. *J. Ultrastructure Res.* **7**:262–72.
16. ——— 1967. Synthesis of chloroplast RNA at the site of chloroplast DNA. *Biochem. Biophys. Res. Commun.* **28**:653–7.
17. HOFFMAN, L. R. 1968. Observations on the fine structure of *Oedogonium*. V. Evidence for the *de novo* formation of pyrenoids in zoospores of *Oe. cardiacum*. *J. Phycol.* **4**:212–8.
18. HOLDSWORTH, R. H. 1968. The presence of a crystalline matrix in pyrenoids of the diatom, *Achnanthes brevipes*. *J. Cell Biol.* **37**:831–7.
19. JENSON, W. A. 1962. *Botanical Histochemistry; Principles and Practice*, W. H. Freeman, San Francisco, Calif.
20. MAZIA, D., BREWER, P. A., & ALFERT, M. 1953. The cytochemical staining and measurement of protein with mercuric-bromphenol blue. *Biol. Bull.* **104**:57–67.
21. MILLONIG, G. 1961. A modified procedure for lead staining of thin sections. *J. Biophys. Biochem. Cytol.* **11**:736–9.
22. MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* **39**:111–4.
23. RIS, H. & PLAUT, W. 1962. Ultrastructure of DNA-containing areas in the chloroplasts of *Chlamydomonas*. *J. Cell Biol.* **13**:383–91.
24. SALEMA, R. & BADENHUIZEN, N. P. 1969. Nucleic acids in plastids and starch formation. *Acta Bot. Neerl.* **18**:203–15.
25. SCHMITZ, F. 1882. *Die Chromatophoren der Algen*, Verlag von Max Cohen und Sohn, Bonn.
26. SIMON, M. F. 1954. Recherches sur les pyrenoids des Phaeophycees. *Revue Cytol. Biol.* **15**:73–105.