Arch. Mikrobiol. 69, 79—91 (1969)

The Ultrastructure of the Marine Blue Green Alga, *Trichodesmium erythraeum*, with Special Reference to the Cell Wall, Gas Vacuoles, and Cylindrical Bodies

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Received August 15, 1969

Summary. The marine blue green alga, *Trichodesmium erythraeum*, was studied with electron microscopy in an attempt to elucidate the structural basis for its rapid lysis when removed from its marine environment. In this connection, it was found that a thining of the electron-dense layer of the longitudinal wall at the site adjacent to transverse wall attachment was responsible for lysis. The underlying biochemical basis for this change has not been elucidated because of the extreme difficulties of maintaining and growing the alga in culture under defined conditions. Several other features of considerable interest also were found. Especially interesting is the very regular array of gas vacuoles in the form of a hollow cylinder which shields most of the photosynthetic system. It was suggested that the gas vacuoles might possibly function optically, having adaptive value in protecting the free-floating alga from excessive radiation. In addition, a detailed structure of the cylindrical bodies was presented, and its structure with the photosynthetic lamellae was compared. On the basis of sectoring to form fragments of double lamellar units from the cylindrical body which are identical in structure to the photosynthetic lamellae, it has been postulated that the cylindrical body may be the site of synthesis for the photosynthetic system in *Trichodesmium erythraeum*.

The genus *Trichodesmium* (Skujaelia) was described in 1830 by Ehrenberg. This filamentous cyanophycean alga forms free-floating bundles of trichomes which lack sheathes and exhibit a similarity to *Oscillatoria* (Fritch, 1965).

Trichodesmium is unique in that it probably represents one extreme in the environmental range of the Cyanophyta. In particular, the habitat of *T. erythraeum* is planktonic, and this alga frequently has been observed floating on the surface in great masses in temperate oceanic areas, including the Gulf of Mexico (Curl, 1959). In the in-shore regions adjacent to Port Aransas, Texas, it is commonly present on the ocean surface in spring and fall blooms. Despite the report of its possible attached growth

in the benthos (Feldmann, 1932), repeated attempts to demonstrate epiphytic or attached growth in the Gulf and bay environments near Port Aransas have been unsuccessful.

Trichodesmium has not been grown in unialgal culture although one of us (CVB) has made repeated attempts to culture this organism. These accumulated attempts at culture over the past five years suggest, that either some unusual chemical requirements are not satisfied by diverse natural extracts, or that an external physical factor is critical to the maintenance of viability. For example, cellular integrity is preserved longer if the collected material is kept between 15—20° C. It is also significant that material collected from the sea surface invariably lyses within several minutes when any appreciable quantity of cells is present in the sample.

The study of the nutrition, growth, biochemistry, and life history of *Trichodesmium* must await its successful isolation and subsequent growth in pure culture. Nevertheless, it is of interest to study the ultrastructure of this enigmatic organism in order to better understand the structural basis of its rapid lysis and for comparison with the ultrastructure of other blue green algae. As far as we are aware, there have been no published electron microscopic studies of this organism.

Materials and Methods

Trichodesmium erythraeum was collected several miles offshore from the Port Aransas jetties and immediately processed for electron microscopy on shipboard. The material was embedded in 1.5% agar (in sea water) and the mass fixed in a freshly prepared 3% acrolein-3% glutaraldehyde solution, buffered in 0.2 M sodium cacodylate (1:1 v/v), pH 6.3. The material was stored in the buffered fixative for about 24 hr at 5° C while in transit to Austin, Texas. The agar blocks were post-fixed for 1 hr at room temperature (23° C) in 2% OsO_4 at pH 6.3 in 0.2 M sodium cacodylate (1:1 v/v), after which they were rinsed three times in distilled water. Following post-fixation, the material was placed in a 0.5% aqueous solution of uranyl acetate at 5° C for 12 hr. The blocks were rinsed in distilled water and embedded according to the procedures of Mollenhauer (1964). Sections were prepared with a Dehmer diamond knife on a Porter-Blum MT-2 microtome and post-stained on the grid with 0.5% uranyl acetate and Millonig's (1961) lead citrate. The material was examined with a Siemens Elmiskop I at 60 kv. For light microscopy, 1 μ sections of the fixed material were cut and observed with a Zeiss WL microscope equipped with phase contrast and bright field optics.

Observations

The fine structure of polyhedral bodies and structured granules (Figs. 5 and 6) is similar to other cyanophycean algae (Pankratz and Bowen, 1963). The following organelles are somewhat different from the general features exhibited by most Cyanophyta. No heterocysts were observed.

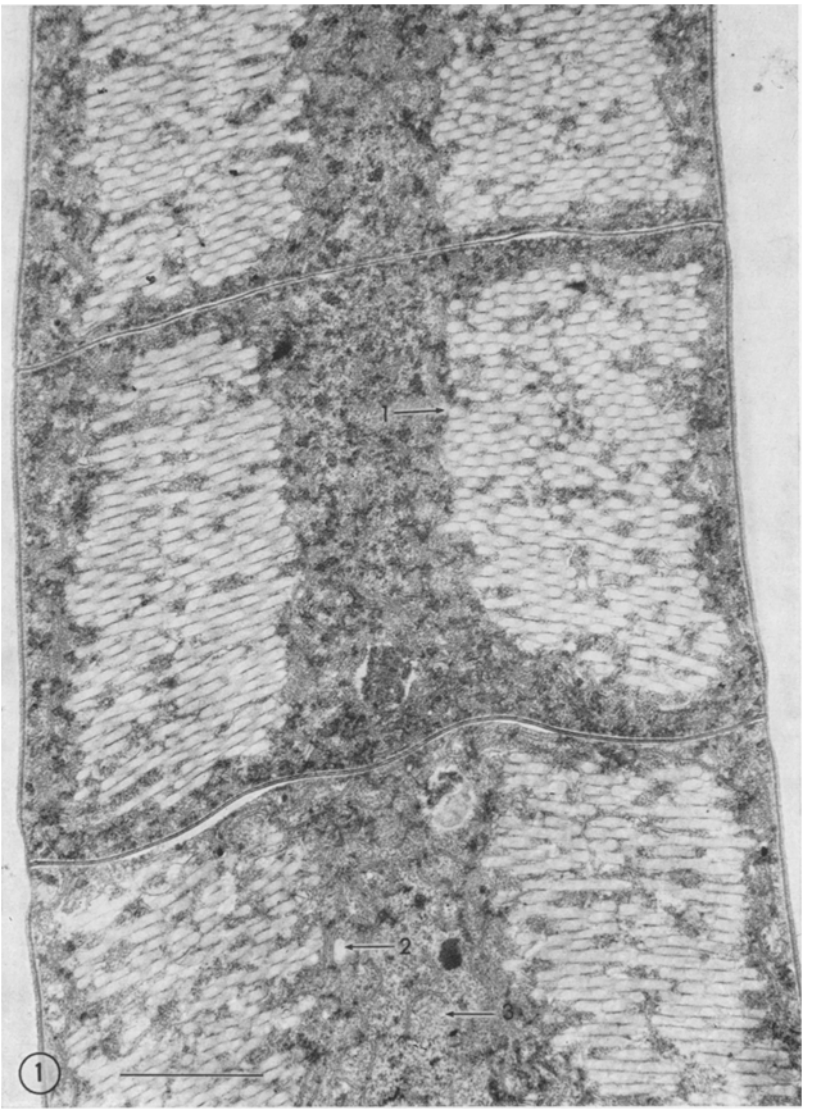

Fig. 1. Median longitudinal section through intact filament showing concentric arrangement of gas vacuoles (arrow *1*), photosynthetic lamellae (arrow *2*), and nucleoplasmic region (arrow *3*). ×17,800. Magnification scale = 1.0 μ except where noted otherwise

Gas Vacuoles

The gas vacuoles occupy as much as 60—70% of the cell volume (Fig. 1). Transverse sections through the cell show that the gas vacuoles are arranged radially to form a hollow cylinder. The longitudinal axis of the individual gas vacuole is always perpendicular to the longitudinal axis of the filament. The gas vacuoles appear as relatively electron-transparent fusiform structures with apiculate ends (Figs. 2, 3). Their average diameter is 780 Å with a range from 750 Å to 850 Å. The gas vacuole is reported to be a hollow structure whose membrane is freely permeable to the gas it contains (nitrogen, oxygen, argon) (Walsby, 1969). To the periphery of the gas vacuole membrane is an electron-transparent zone which is the same density as the internal region. Interspersed among the expanded gas vacuoles are appressed membranes whose total diameter is about equivalent to a unit membrane (Bowen and Jenson, 1965). These membranes also are surrounded by an electron transparent zone (Fig. 3, arrow 1). Because the total membrane diameter of the appressed structures is exactly twice the diameter of an expanded gas vacuole membrane, these structures are interpreted as collapsed gas vacuoles.

Arrays of gas vacuoles stack radially two layers deep to form the hollow cylinder (Figs. 1, 2). Tangential sections through the hollow cylinder reveal the presence of fissures. In non-lysed cells, the intact hollow cylinder of gas vacuoles is positioned equi-distant from the transverse and longitudinal cell walls (Fig. 1). In cells which have lysed, the gas vacuoles frequently collapse (Fig. 8).

Photosynthetic Lamellae

The photosynthetic lamellae are scattered throughout the cell; however, most are located in the central core region of the cell (Fig. 1, arrow 2, Fig. 9, arrow 1). Frequently, they penetrate the inner area of the hollow cylinder of gas vacuoles and extend to the cell surface in the non-vacuolate zone adjacent to the transverse walls. The structure of the individual photosynthetic lamella is a double unit membrane (75 Å diameter for each) separated by an electron-transparent space of 100 Å. They are not usually stacked together. Associated with the photosynthetic lamellae are amorphous electron-dense structures which we possibly equate with the biliproteins (phycoerythrin and phycocyanin) (Fig. 9, arrow 2). These electron-dense masses appear to be closely associated with the cylindrical bodies and will be discussed below.

Nucleoplasm

The nucleoplasmic region is not so well defined as in other cyanophycean algae. It occupies the central core and is delimited by the

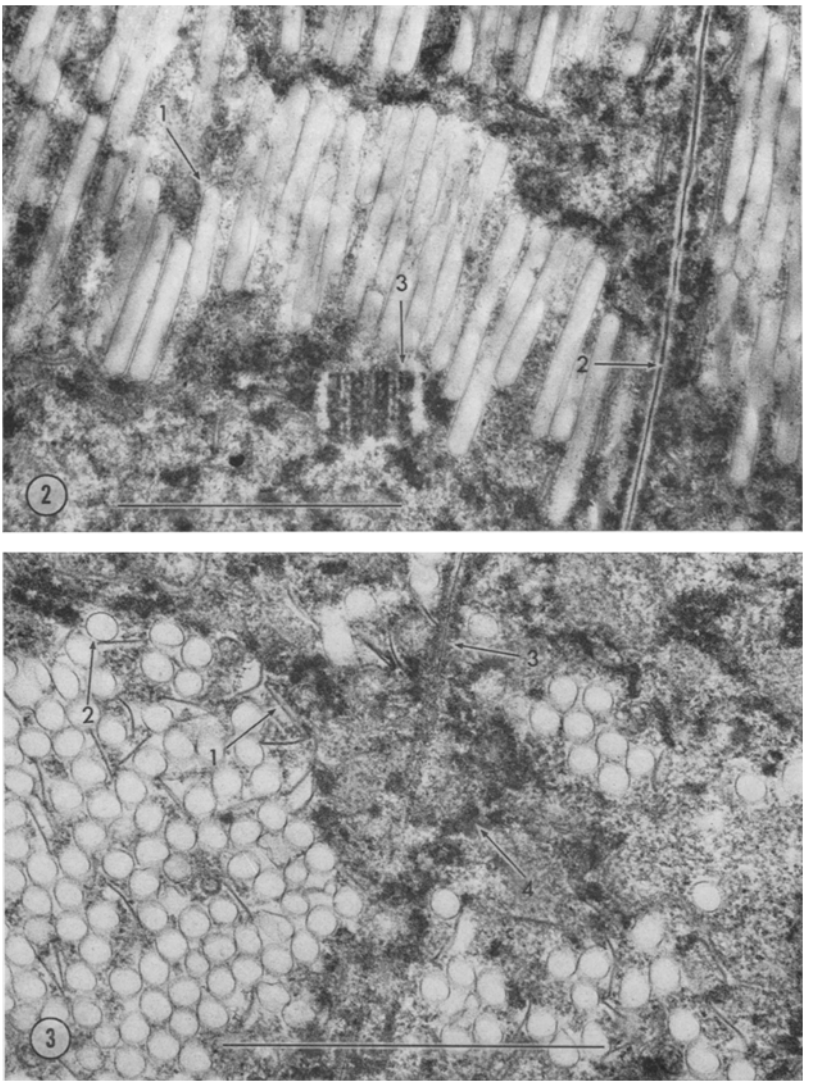

Fig. 2. Portion of intact cell which is sectioned through the longitudinal axes of the gas vacuoles. Arrow *1* illustrates the angular terminal portion of fully inflated gas vacuoles. Arrow *2* dipicts the transverse wall of the filament. Arrow *3* illustrates a cylindrical body in longitudinal section. ×36,600

Fig. 3. Section through gas vacuole region as in Fig. 2 but at right angles to the longitudinal axis of the gas vacuoles. Some of the vacuoles are collapsed and form a typical "unit membrane" (arrow *1*). The membranes of the fully expanded vacuoles have a diameter less than the unit membrane (arrow *2*). The transverse wall (arrow *3*) is incompletely formed. Note the photosynthetic lamellae and the association of an electron dense substance, probably the phycobilin pigments, with the photosynthetic lamellar system (arrow *4*). ×48,000

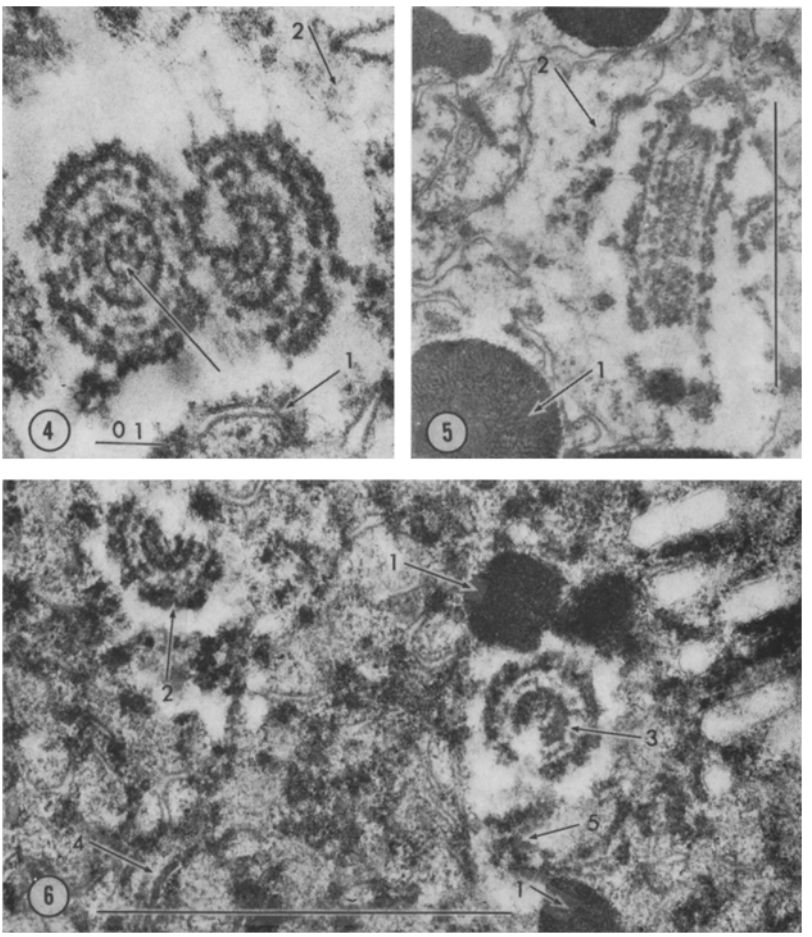

Fig. 4—6. Cylindrical bodies

Fig. 4. A transsection through two cylindrical bodies showing the alternate electron-transparent and electron-dense concentric regions. Note the photosynthetic lamellae (arrow *1*) and the fibrillar material of the nucleoplasm, presumably DNA (arrow *2*). The cylindrical body at the right is partially sectored. Central core at arrow *3*. ×77,600

Fig. 5. Longitudinal section through cylindrical body. Note possible polyphosphate granule (arrow *1*) and coated photosynthetic lamellae (arrow *2*) in proximity to the cylindrical body. ×35,900

Fig. 6. Cross section through nucleoplasmic region of cell showing polyhedral bodies (arrow *1*) and two cylindrical bodies (arrow *2*, *3*). Again note the association of electron dense material on the surface of the photosynthetic lamellae (arrow *4*). The cylindrical body in arrow *3* apparently has just produced electron dense substance from the sector which is aparently being incorporated or associated with the photosynthetic lamellae at Arrow *5*. ×53,500

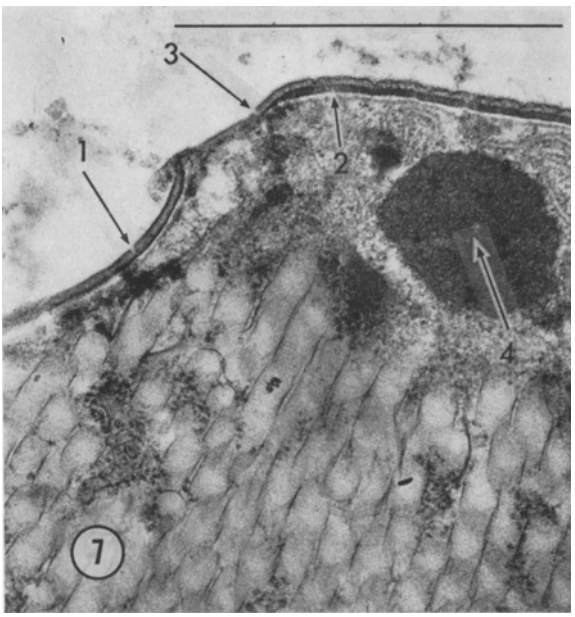

Fig. 7. Transverse wall (arrow *1*) and longitudinal wall (arrow *2*) at the time of lysis. A structural basis for lysis is the diminishing electron dense zone of the longitudinal wall (inner layer) as it approaches the transverse wall (arrow *3*). Arrow *4* illustrates the polyhedral body. ×50,000

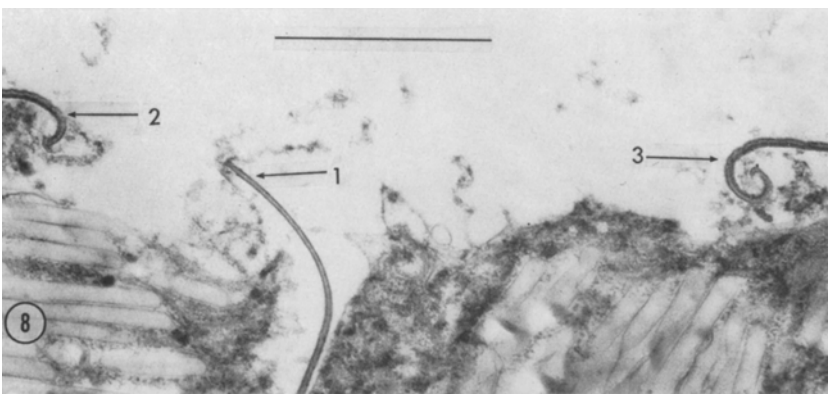

Fig. 8. Longitudinal section through a recently lysed filament showing homogeneous transverse wall (arrow *1*) and stratified circinate longitudinal walls (arrows, *2*, *3*). ×28,300

hollow cylinder of gas vacuoles (Fig. 1). The photosynthetic lamellae frequently traverse the nucleoplasm (Fig. 9, arrow 5).

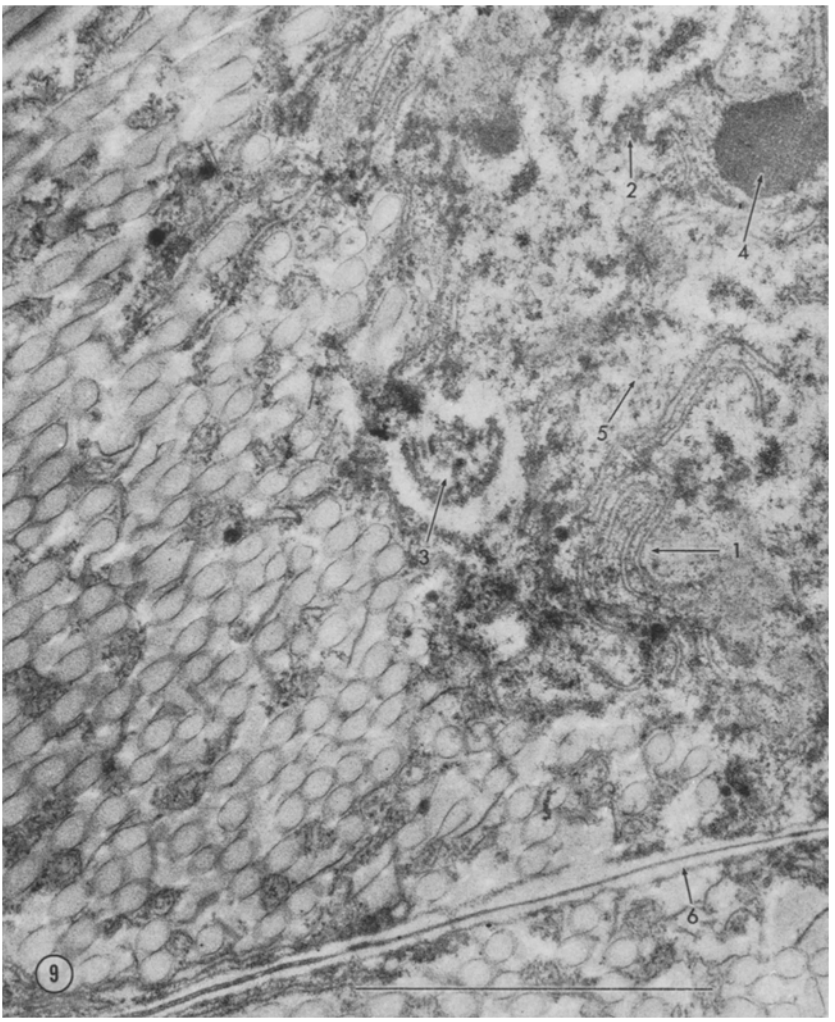

Fig. 9. Median longitudinal section through intact cell showing the photosynthetic lamellae (arrow *1*) and the associated phycobiolin pigments (arrow *2*), a cylindrical body (arrow *3*), a polyhedral body (arrow *4*), the fibrillar material of the nucleoplasm (arrow *5*), and a transverse cell wall at arrow *6*. ×46,600

Cylindrical Bodies

Unusual structural features of *Trichodesmium* include 1—3 cylindrical bodies in the core region of each cell. Their general appearance is similar to structures described by Pankratz and Bowen (1963) in *Symploca muscorum*. The diameter of the cylindrical bodies is rather constant

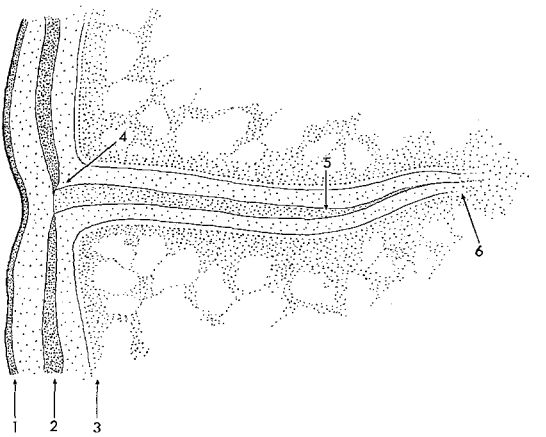

Fig. 10. Schematic diagram of the cell wall of *Trichodesmium erythraeum*. *1* outer membrane; *2* electron-dense layer of the longitudinal wall (inner membrane) which tapers at the juncture of this wall with the transverse wall at *4*; *3* plasmalemma; *5* homogeneous electron dense layer of the transverse wall; *6* plasmalemma at the site of developing transverse wall

(0.2—0.3 μ) while the length of such structures is more variable (Figs. 2, 5). Transverse sections of cylindrical bodies reveal a consistent pattern of two concentric, double lamellar units that alternate with two electron-transparent spaces (Fig. 4). The central core of the cylindrical body is a rod 200 Å in diameter (Fig. 4, arrow 3). Transverse sections of the cylindrical body show frequent sectoring which results in a dissociation of part of the double lamellar units from the parent structure (Fig. 6, arrow 5). These individual dissociated fragments have the same structure as the photosynthetic lamellae. Polyhedral bodies are also found in close association with the cylindrical bodies (Figs. 6, 9). A schematic diagram of our interpretation of the cylindrical body is shown in Fig. 11.

Cell Wall

An electron transparent region of 100 Å is found to the periphery of the plasmalemma (Fig. 7). This interspace is common to the longitudinal and transverse walls. The next outermost layer is the inner layer (Fig. 7, arrow 2). This is an electron-dense layer of 200 Å which is not present in the transverse wall. In the longitudinal wall, the inner layer tapers at each junction where the transverse and longitudinal walls meet (Fig. 7, arrow 3). This weakened zone probably represents the structural basis for the rapid lysis. Lysis always occurs at this

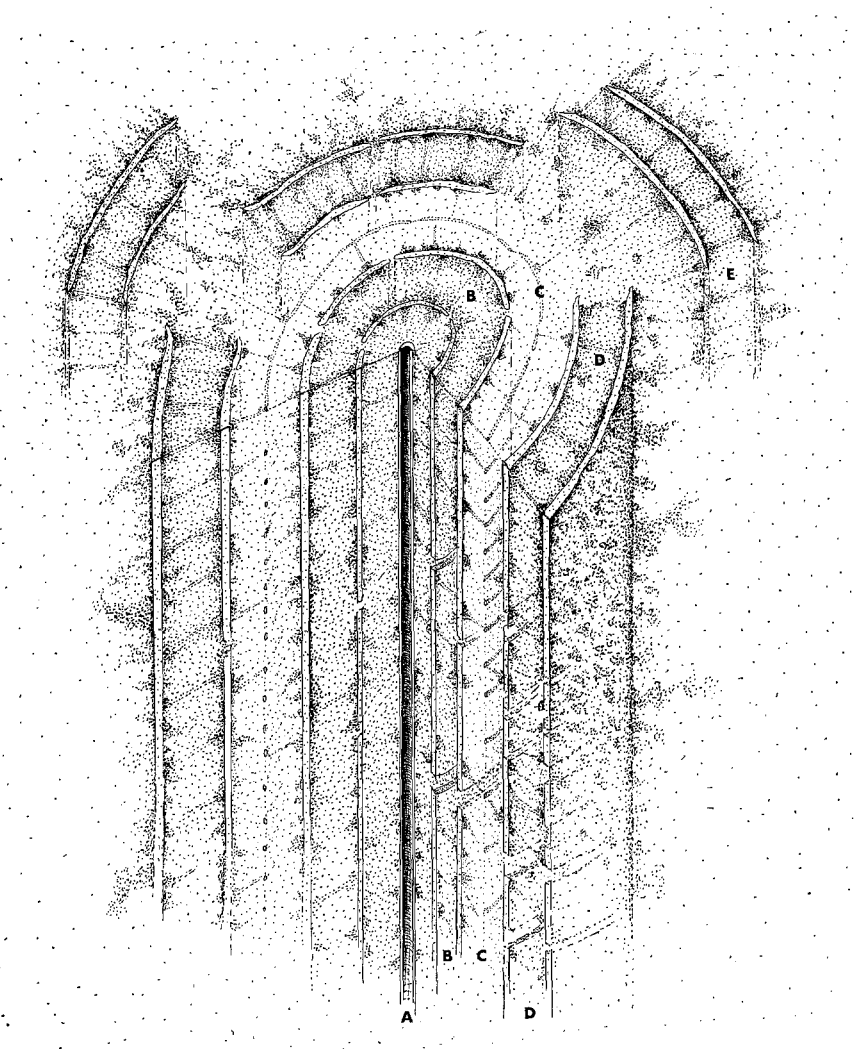

Fig. 11. Schematic representation of the cylindrical body in *Trichodesmium erythraeum*. *A* hollow core or rod; *B* and *D* the two concentric double lamellar membranes with associated electron dense amorphous material separated by an electron-transparent space; *C*. *E* a portion of the outer double lamellar unit which has separated from the cylindrical body leaving a sector

juncture resulting in a separation of the longitudinal wall from the transverse wall (Fig. 8). Structures equivalent to rings of pores (Pankratz and Bowen, 1963) have not been observed in this region. The longitudinal

wall is apparently under tension since it invariably curls inwardly, whereas the transverse wall remains largely in its original configuration (Fig. 8).

The outermost dense layer of 70 Å is the outer membrane of the longitudinal wall and is separated from the inner layer by an electron-transparent space of 100 Å.

The transverse wall is not stratified like the longitudinal wall and has an electron density intermediate between that of the outer and inner wall layers (Fig. 7, arrow 1). Fig. 10 schematizes the structure of the longitudinal and transverse walls.

Discussion

The ultrastructure of *Trichodesmium erythraeum* presents several interesting points not heretofore observed in cyanophycean algae. The first is the particular localization of the initiation of lysis at the juncture of the longitudinal and transverse cell walls. The structural basis for ensuing lysis as presented here suggests some orderly, directed event and not a simple random dispersion of the cell wall in several areas at once. If this tapering electron-dense layer (Fig. 7, arrow 3) is composed of mucopolymer, as suggested by Allen (1968) for *Anacystis nidulans* and *Gloeocapsa alpicola*, then it could be postulated for *Trichodesmium* that a localized structural alteration may occur for this product at the time of its deposition, or that a lysing enzyme specific for this region of the cell wall is under some morphogenetic control. That the longitudinal wall is structurally different from the transverse wall is clearly seen in the electron micrographs and is further indicated by the curling only of the longitudinal wall upon lysis (Fig. 8).

The correlation between the structural basis of lysis and environmental conditions which provoke it cannot be substantiated at present; however, the modification of the outer wall may be a response to the ionic environment. Bacterial outer membranes are known to have clearly defined structural and chemical responses to the ionic environment (Brown, 1964).

We believe that the gas vacuoles having such an orderly arrangement and occupying such a large volume of the cell in *Trichodesmium* are not solely a flotation device. We suggest, in addition, that they may protect the central core of the cell from the deleterious light intensities and/or wavelengths of full sunlight (Rupert, 1964) which *Trichodesmium* cannot avoid receiving in the floating condition. There are at least two optical effects that we would attribute to the gas vacuole system. The first is an interference effect perpendicular to the long axis of the vacuole. For the average diameter of the gas vacuole of 780 Å, zero order reflection

will occur around 320 mμ. The second effect, internal reflection, would operate along the long axis of the gas vacuole and would diverge light away from the central area of the cell, thus confining the main transmission of light to the gas vacuole region.

In spite of the reddish appearance of regions containing gas vacuoles (Smith and Peat, 1967), this observation was not uniform among the algae examined, and these authors pointed out that the peculiar optical properties of a non-refractile reddish appearance could possibly be due to the photosynthetic lamellae which contain the photosynthetic pigments. The cells of *T. erythraeum* exhibit a red color, but this may be due to the presence of an abundance of c-phycoerythrin. Because of the predictable localization of the hollow cylinder of gas vacuoles, it is more likely that the observed reddish color in this region is related to an optical effect produced by the gas vacuoles.

The structure of the gas vacuoles in *T. erythraeum* is similar to that found in other cyanophycean algae (Bowen and Jenson, 1965; Jost and Matile, 1966; Smith and Peat, 1967; Walsby and Eichelberger, 1968; Walsby, 1969) and in bacteria (Larson, Omang, and Steensland, 1967; Stoeckenius and Kunau, 1968), despite *Trichodesmium*'s somewhat different structural appearance and biochemical properties (Parker, van Baalen, and Maurer, 1967). We agree with Smith and Peat (1967) that one cannot actually classify the membrane which delimits the gas vacuoles as a "half-unit membrane" and furthermore support the position of Stoeckenius and Kunau (1968) that they are not typical membranes in the sense that they lack lipids.

No detailed structural analysis of the cylindrical bodies has been reported in the literature although reports of their presence have been mentioned passingly (Pankratz and Bowen, 1963). The level of resolution of these structures achieved in this communication permits us to suggest that the double lamellar structures of the cylindrical bodies are very similar, if not identical, to the photosynthetic lamellae. Thus, it would not be unreasonable to assume that the cylindrical bodies in *T. erythraeum* may represent sites of synthesis of the photosynthetic system. The abundant sectoring of the cylindrical bodies and adjacent fragments of double lamellae suggests this as a means of continued synthesis, release, and transport of photosynthetic fragments until their final incorporation into the anastomosing network. If the phycobilin pigments are associated with the photosynthetic lamellae (Gantt, Edwards, and Conti, 1968), then it is possible that the cylindrical bodies may be the sites of phycobilin biosynthesis. The correctness of this view must await a careful electron microscopic and biochemical investigation of pigmentation changes as influenced by known and defined growth parameters.

The authors wish to thank Mr. A. E. Walsby of Westfield College, London, for the helpful discussion, comments, and suggestions, and Mr. John Batterton for collecting the material. Work was supported in part by research grant WP-01057-07 to RMB and NSF GB 4721X and GB 8461 to CVB.

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