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OBSERVATIONS ON THE CELL STRUCTURE OF  
*OSCILLATORIA LIMOSA* AGARDH.\*

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The prevalent understanding of the cellular structure of the blue-green algae for many years has been that the cells consist of two regions, a chromatoplasm and a centroplasm (Fritsch, 1945). The photosynthetic pigments are associated with the chromatoplasm which is located at the periphery of the cytoplasmic portion of the cell. The nuclear processes are associated with the centroplasm which occupies the central region of the cell. Recent cytological techniques, such as the use of Feulgen reactions, azure stains, and far red light, demonstrate that the above concept is valid (Ris et al., 1961; Hopwood et al., 1960). The recent use of electron microscopy has revealed structural features within the cells which make it possible to recognize a more elaborate scheme of organization in the cells of this group of algae. Ris et al. (1961) examined several species of blue-green algae with the electron microscope, among them species of the Genus *Oscillatoria*. They proposed and discussed an organization in which the typical blue-green algal cell consists of three regions: a central nucleoplasm which is Feulgen positive

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and which appears to be fibrous on the ultra-structure level, a peripheral region of the cytoplasm which is divided into a series of lamellae and vesicles, and a cell boundary consisting of three layers, an outer membrane, an inner investment and a cytoplasmic membrane on the outer surface of the cytoplasm.

Investigations by Lefort (1960) and Hopwood et al. (1961) contributed to Ris' concept. All of these authors comment that many structures associated with the higher plant cells are absent in blue-green algae. That is, a genetic mechanism, mitochondria, golgi apparatus, and chloroplasts have not been observed. Two major problems are: first, to describe the internal organization in terms of structural features, and secondly, to associate these structural features with processes known to occur within the cells. The intent of this paper is to present some additional observations on the ultra-structure of *Oscillatoria*.

When electron micrographs are interpreted one must always remember that the patterns formed are the result of scattering of electrons because of dense areas within the cells. These electron-dense areas may be caused by deposition of substances during fixing, during subsequent staining, or by the chemistry inherent in the cell prior to any treatment. I am aware of these differences in the appearance of micrographs which are related to different methods of treatment of the cells and to differences in their physiological states. The resolution of these differences will involve all disciplines of cell study. The physiology associated with newly-discovered structures is as yet largely conjectural. It is with these limitations in mind that I discuss the cellular structure of *Oscillatoria*.

#### MATERIALS AND METHODS

*Oscillatoria limosa* Agardh., growing abundantly in tanks in the University greenhouse, was used in this experiment. In these tanks the alga forms a "bloom" during spring months, then gradually disappears during summer and re-appears again the next spring. Specimens were collected during the actively-growing phase and again during the time when the algal quantity was reducing, which was assumed to be a period of slow growth. No special culture conditions were maintained except to insure that the specimens were from well-lighted areas free from contaminants such as dirt or other plant material.

After collecting, specimens were fixed immediately for 20 min using a 1 percent solution of  $\text{OsO}_4$  buffered to pH 7.4 or a 2 percent solution of  $\text{KMnO}_4$ . The material was dehydrated in the usual alcohol series from 50 to 100 percent in four steps of 10 min each. Three changes of the 100 percent alcohol of 10 min each were followed by embedding in methacrylate, Epon, or Vestopal.

Methacrylate embedding proceeded from 100 percent alcohol to a 1:1 mixture of uncatalyzed methacrylate and absolute alcohol, then to 100 percent methacrylate catalyzed with Luperco. After 20 min in each of the latter two steps the specimens were placed in capsules filled with catalyzed methacrylate and polymerized with ultra-violet light. The methacrylate used was a mixture of 4 parts butyl methacrylate to 1 part methyl methacrylate.

For Epon embedding the alcohol series was followed by two changes of Propyl oxide, then into a 1:1 mixture of Propyl oxide and Epon and finally into Epon in capsules and polymerized at 60° C for 72 hr.

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#### ABBREVIATIONS

Inclusion bodies: a—chromosome-like bodies, b—oval granular bodies, c—large dark staining bodies (cyanophycin granules of some authors), d—oval, structured bodies, e—dark bordered small cavities, R—rod-shaped bodies occurring particularly in the lamellar region of the cytoplasm.

OM—outer membrane, I—inner investment, G—grana-like structures, N—nucleoplasm, S—sheath.

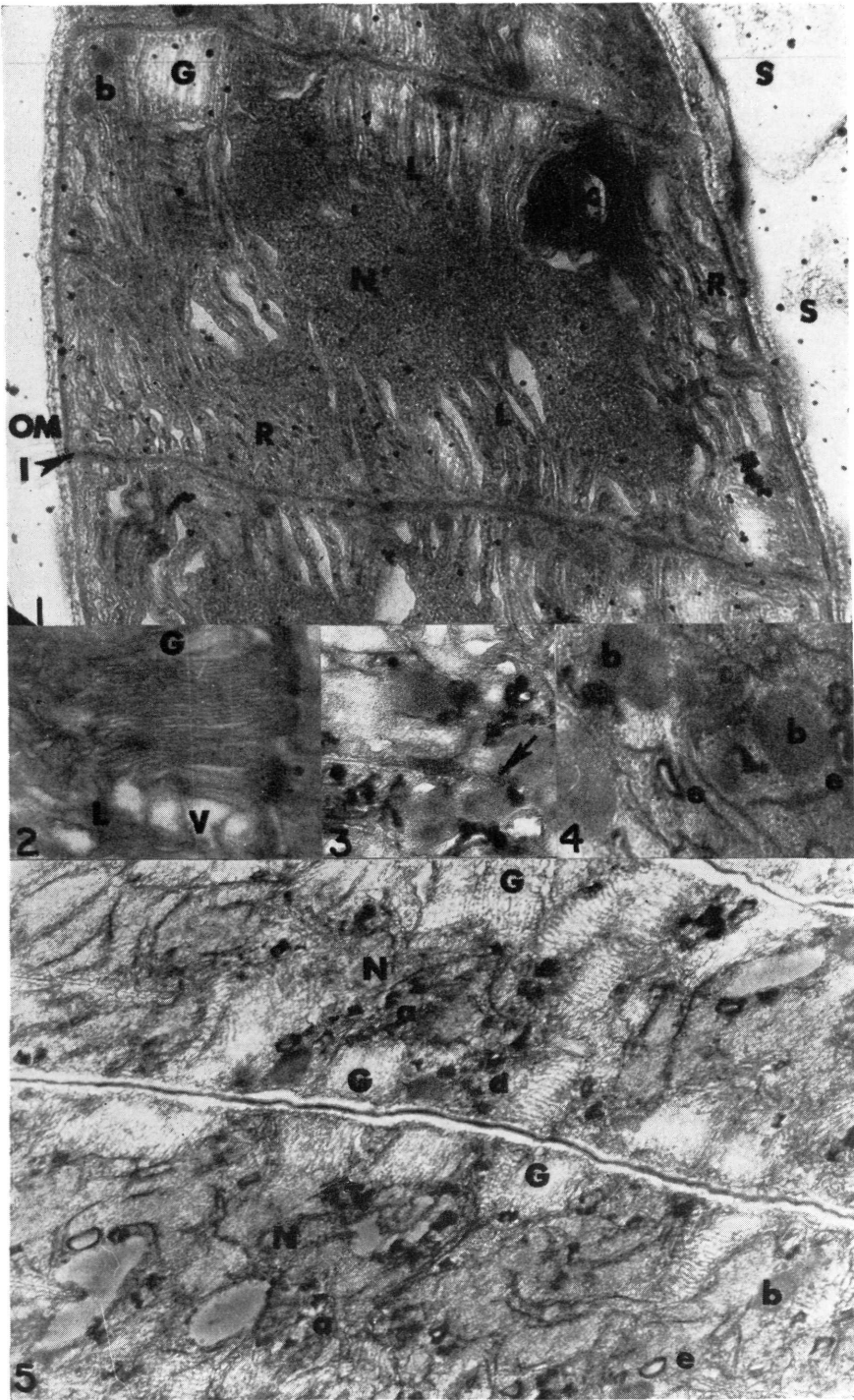


FIGURE 1. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in Epon (25,000 $\times$ ).  
 FIGURE 2. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in methacrylate. (60,000 $\times$ )  
 FIGURE 3. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in methacrylate. (60,000 $\times$ )  
 FIGURE 4. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in methacrylate. (60,000 $\times$ )  
 FIGURE 5. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in Vestopal (30,000 $\times$ )

For Vestopal embedding, the specimens were dehydrated in acetone and embedded according to the method described by Pease (1960).

Blocks were sectioned with glass knives on a Porter-Blum microtome, stained with either uranylacetate or lead hydroxide and observed with an RCA, EMU 3f electron microscope.

#### RESULTS AND DISCUSSION

*Oscillatoria limosa* Agardh., is a filamentous alga with trichomes ranging from 2 to 6  $\mu$  in diameter. Each cell is approximately 2  $\mu$  thick. Cells are arranged in the filament as a series of short discs, or very like a stack of "poker chips." The end wall of the apical cell of the filament is usually convex. During the past year specimens of *Oscillatoria limosa* Agardh. in different stages of growth were examined with the electron microscope. The results of various methods of fixation, dehydration, and embedding were compared.

The ultra-structure of *O. limosa* fits the general pattern proposed by Ris et al., (1961) for blue-green algae. The entire trichome is covered by a sheath which appears to be fibrous and which forms an image of greater contrast when stained with uranyl acetate (fig. 1-S). The outer cell walls appear to be multi-layered structures (fig. 1, 7-OM). When embedded in Epon the external layers of outer walls appear undulate and in contact with the heavily stained inner layer at regular intervals. There is evidence that the outer membrane described by Ris is composed of more than one layer (fig. 7-OM). All cell walls are undulate in appearance in many sections. The undulate appearance of the outer membrane may be an extrusion pattern related to the formation of the sheath, but it is my opinion that the undulate, wavy appearance of membranes is at least in part due to shrinkage of cells during processing. This opinion is strengthened by experiments conducted with swelling agents. When embedded in methacrylate the external cell walls appear laminate (fig. 7). The end walls of adjoined cells are definitely of a three-parted nature and appear to develop centripetally. Centripetal differentiation of the end walls begins with a thin extension of material which is continuous with the inner investment (fig. 1, 3, 6). This material becomes thicker as it ages and as deposition of other layers of the cell wall occurs upon it.

Immediately within the cell wall is a continuous layer of cytoplasm which also appears undulate and which is marked by a deeply staining outer boundary (fig. 1, 5, 6). The cytoplasm near the periphery of this outer layer is divided into a number of lamellae (fig. 1, 6, 7). These lamellae appear as wide bands of cytoplasm lying parallel to each other and generally are oriented so as to be at right angles to the end walls of the cells. Between each band of cytoplasm is a sac-like vesicle which probably corresponds to the pseudo-vacuoles or gas vacuoles visible under the light microscope (fig. 2, 6, 7, 8). Toward the center of the cells the cytoplasmic structure appears to be granular, stains darker than the cytoplasm in the lamellar region and is continuous, not dissected or lamellar (fig. 1, 5, 6, 8).

A distinct structural variation in the patterns of the lamellar regions is shown (fig. 1, 2, 5). These areas appear to have definite boundaries and are composed of a series of parallel lamellae much smaller than the usual lamellae in the periphery of the cell. The lamellae within each area are parallel, but the areas are oriented randomly with respect to the direction of the lamellae. These structures are in many respects similar in appearance to the grana in chloroplasts of higher plants (Weier, 1961) and extend further the thought that this is the area associated with the photosynthetic pigments.

All lamellae are preserved much better when  $\text{KMnO}_4$  is used as a fixative than when  $\text{OsO}_4$  is used. The  $\text{OsO}_4$  results in a better preservation of cytoplasmic components.

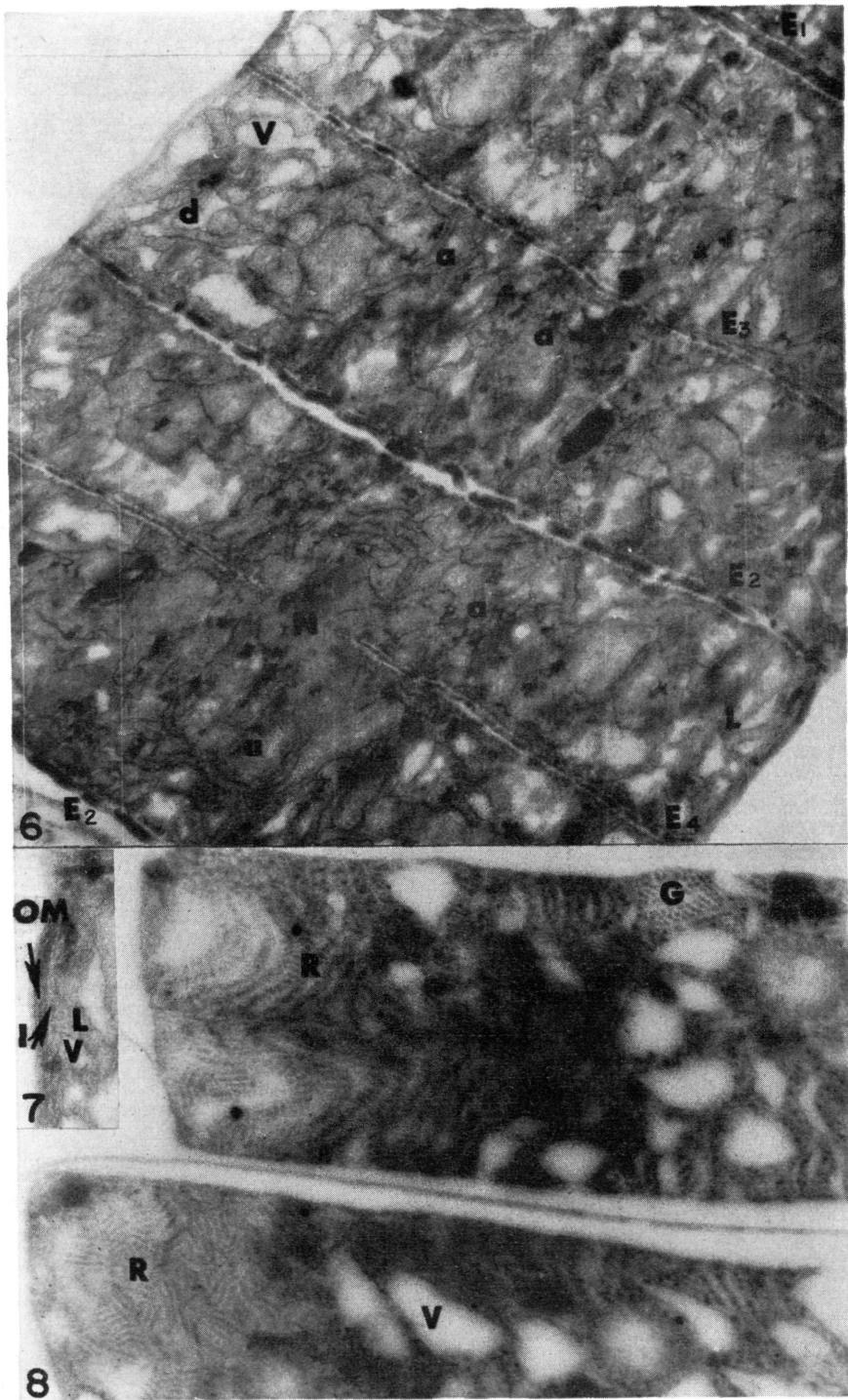


FIGURE 6. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in methacrylate ( $25,000\times$ ) E<sub>1</sub> to E<sub>4</sub>, oldest to youngest and walls.

FIGURE 7. *O. limosa* fixed in  $\text{KMnO}_4$ , embedded in methacrylate ( $60,000\times$ )

FIGURE 8. *O. limosa* fixed in  $\text{OsO}_4$ , embedded in methacrylate ( $30,000\times$ )

Figure 8 shows the appearance of the cells when fixed with  $\text{OsO}_4$ . The lamellae are not retained, but the cytoplasm appears as a series of short rods which are round in cross section, oval when obliquely cut, and short parallel rods when cut lengthwise. The appearance of definitely outlined areas (fig. 8) as a series of dots or parallel lines, where grana-like areas ordinarily appear (fig. 1, 2, 5-G) suggests that the grana-like areas are similar in composition to the cytoplasm in which the same rod-like bodies appear (fig. 1-R, 8-R). If these rod-shaped bodies in the cytoplasm and the grana-like areas are of the same material it would suggest that a reorientation of certain cytoplasmic constituents occurs perhaps in association with the formation of chlorophyll, and in photosynthetic activity. Structurally this appears to be a separation of a large mass of cytoplasm into more finely-divided lamellae than are usually found in the lamellar region.

Inclusions within the cytoplasm are varied. Some (fig. 4-e, 5-e) appear as small, irregularly shaped holes with a deeply staining border. These bodies appear primarily in the nucleoplasm. Round bodies of a granular texture appear in the lamellar regions when uranyl acetate stain is used (fig. 1-b, 4-b). The occasional appearance of oval-shaped, structured bodies is noted (fig. 6-d). However, these occur only occasionally and may represent fixation artifacts. There are also small, solid electron-dense bodies of an irregular shape that consistently appear in the central portion of rapidly-dividing cells (fig. 5-a, 6-a). These structures resemble the mitotic figures of chromosomes in their contorted outlines. There is no basis other than their appearance and distribution to suggest that these electron-dense bodies in the nucleoplasm are related to chromosomes. Investigations are now being conducted to determine if DNA is associated with these bodies.

Some of these inclusions have been observed and described by other authors. They have been called cyanophycin granules, mitochondrial equivalents, products of photosynthesis, volutin, and by other names. At present there is no basis for anything other than speculation as to their origin and physiology.

#### SUMMARY

*Oscillatoria limosa* Agardh. was fixed in  $\text{KMnO}_4$  or  $\text{OsO}_4$  and embedded in methacrylate, Epon, or Vestopal for electron microscopy. The results of these different methods of fixation and embedding are compared. The cells of this species of blue-green alga fit the general pattern of cell organization proposed by Ris et al. (1961) for blue-green alga. Grana-like areas, not previously described are demonstrated. Attention is directed to inclusion bodies which are at present the subject of further investigations.

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