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A Cytomorphological and Cytochemical Study of Cyanophyta I. An Electron Microscope Study of *Oscillatoria princeps*.

by

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The structure of the cyanophyta cell has long been a subject of controversy (cf. Poljansky and Petruschewsky, 1929; Geitler, 1932). During the last decade, however, Bringman (1950) and Herbst (1953, 1954) have regarded the globular bodies found in the cells as equivalent to the nuclei of higher organisms.

In the cells of *Oscillatoria princeps*, *O. limosa*, *Scytonema crispum* and others, however, one of us (N. S.) has recognized many thin threads which run sinuously throughout the protoplast and has regarded them as the chromonemata or the chromosomes. It is intended in this study to see the structure of the cells in *Oscillatoria princeps* in more complete details with an electron microscope.

The trichomes of the plant were fixed 1 % osmium tetraoxide dissolved in phosphate buffer at pH 7.4. Then the material was dehydrated and embeded in a mixture of methyl and n-buthyl methacrylate, fellowed by the cutting with a glass knife.

Result

Before entering into the main subject of this study, the result of the observation with the optical microscope is summarized as follow:

Many sinuous threads distribute not only in the central (centroplasm) but also in the peripheral part (chromatoplasm) of the protoplast when the cells are fixed with Flemming's, Benda's and other fixatives, followed by the staining with haematoxylin (Fig. 1). These threads are also stained with acetocarmine, more or less deeply, but are not colored by the Feulgen nucleal staining.[1] The diameter of the threads is about 300 mμ or more in the fixed material. No chromatophore is visible in the cells, and chlorophyll and other pigments disperse diffusely in the peripheral part of the protoplast.

Observation by an electron microscope. The chromonemata or the chromosomes stated above are easily distinguished from other organellae in the protoplast

1) According to the result of the biochemical study carried out by Shinke and Ishida, however, the protoplast contains a fairly large amount of DNA.

by their size and shape. Fig. 2 represents a part of the protoplast in which many elongated profiles of the chromonemata are shown. These chromonemata are about 200 mμ or more in diameter and are found throughout the protoplast. That is, the chromonemata do not construct a definite nucleus in this plant. Each chromonema is composed of three elements; they are, a thin limiting membrane, a chromonema matrix which appears structureless or homogeneous, and many fine threads.

These fine threads run, in most cases, just about perpendicular to the longer axis of the chromonemata and parallel to one another at more or less regular intervals (Fig. 2). Such arrangement as stated above, could be interpreted as representing the oblique or longitudinal sections of a bundle of fine threads which make up a short-pitched helix. Fig. 3 represents a portion of Fig. 2 at higher magnification. In this microgram, two of the fine threads appear to run closely and they interlace each other, composing a helix as indicated by an arrow. The diameter of each fine thread is estimated to be about 10–20 mμ.

The chromonema marked *c'* in Fig. 2, however, has a large diameter and contains numerous fine threads which do not show regular orientation. This may be regarded as a chromonema, existing before the longitudinal splitting. Fig. 4 represents the details of the irregular arrangement of the fine threads at a higher magnification than Fig. 2.

A small area of the peripheral part of the protoplast is shown in Fig. 5. In this microgram, there are many lamellae (*l*) which are found in the peripheral part of the protoplast and run parallel to each other. The lamellar structure is also recognized in the peripheral part of the protoplast in *O. limosa* (*l* in Fig. 6). Considering the fact that the peripheral part of the protoplast is not stained with neutral red, these lamellae are not regarded as the endoplasmic reticulum. It is not improbable to assume that the lamellae are organellae equivalent to these found in the chloroplast of *Vaucheria* shown in Fig. 7, because assimilation products (*a*) are found in the lamellae in *Oscillatoria* and *Vaucheria*.

Several granules (*g*), the diameter of which is 100–200 mμ are visible both in the peripheral and central parts of the protoplast (Fig. 2). It was not determined in this study whether these granules are mitochondria or assimilation products, but they are electron dense and appear as relatively homogeneous granules in which no internal detail can been seen.

No organellae which had "*cristae mitochondriales*" were recognized in the present observation.

Many dividing cells were observed in this plant, but both the diameter and the length of the chromonemata or chromosomes appeared not to be different to those in the resting cells.

It must be noted that the cell walls of this plant have numerous small perforations with a diameter of 60–100 mμ. Fig. 8 represents a longitudinal section of a trichome, in which several perforations (*p*) are seen in the cell

wall. In the surface view, these perforations appear to form themselves more or less regularly (Fig. 9).

Our tentative conclusion obtained from the present study is as follows:

In the cells of *Oscillatoria princeps*, the chromonemata or the chromosomes are dispersed throughout the protoplast and do not constitute a definite nucleus. The lamellae which constitute a chromatophore in green algae, do not construct the chromatophores in this plant. In respect to the cellular organization, therefore, the cells of *Oscillatoria princeps* is assumed to be more primitive than those of other organisms.

The pesent authors wish to expess their cordial thanks to Professor N. Higashi of Kyoto University and Professor M. Shigenaga of Nara Women's University who kindly permitted us the use of their electron microscopes.

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Explanation of Plate I

All figures except Fig. 1 which is a microphotograph taken by an optical microscope, are electron micrograms.

Fig. 1. *Oscillatoria princeps.* A transversal section of a trichome fixed with Flemming's solution and stained with Heidenhain's haematoxylin. Many thin threads run sinuously throughout the protoplast. × 1,500.

Fig. 2. *O. princeps.* Sections of three cells. The profile marked *c* represents a part of a chromonema or a chromosome. Lamellae (*l*) are deformed in this figure. × 20,000.

Fig. 3. *O. princeps.* A part of Fig. 2 at higher magnification. Profiles of two chromonemata which have many fine threads are shown. Two thin threads interlace each other as indicated by an arrow. × 45,000.

Explanation of Plate II

Fig. 4. *O. princeps.* A profile of a thick chromonema in which fine threads run somewhat irregularly. The general appearance of this profile closely resembles that of the nuclei of higher organisms. × 45,000.

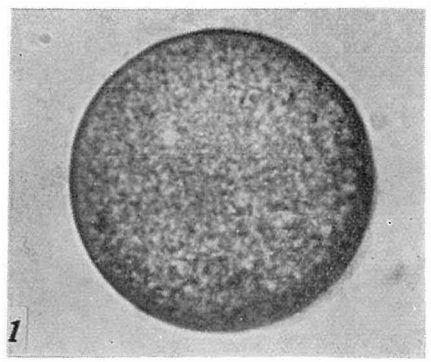
Fig. 5. *O. princeps.* A part of the peripheral region of a cell in oblique section. Several perforations are visible in the cell wall (*w*). Many lamellae (*l*) run parallel to the surface of the trichome. × 20,000.

Fig. 6. *O. limosa.* A part of an oblique section of a trichome. Many lamellae (*l*) with assimilation products (*a*) are seen. Chromonemata are deformed in this microgram. × 20,000.

Fig. 7. *Vaucheria sp.* A chromatophore with lamellar structure. Assimilation products (*a*) are seen in the lamellae. × 20,000.

Fig. 8. *O. princeps.* A part of a longitudinal section of a trichome. Several perforations (*p*) are visible in the cell wall. × 20,000.

Fig. 9. *O. princeps.* A surface view of a piece of a cell wall showing many perforftions. × 20,000.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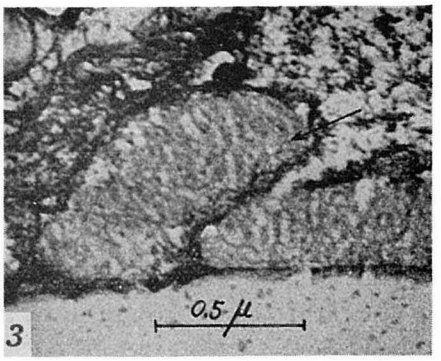
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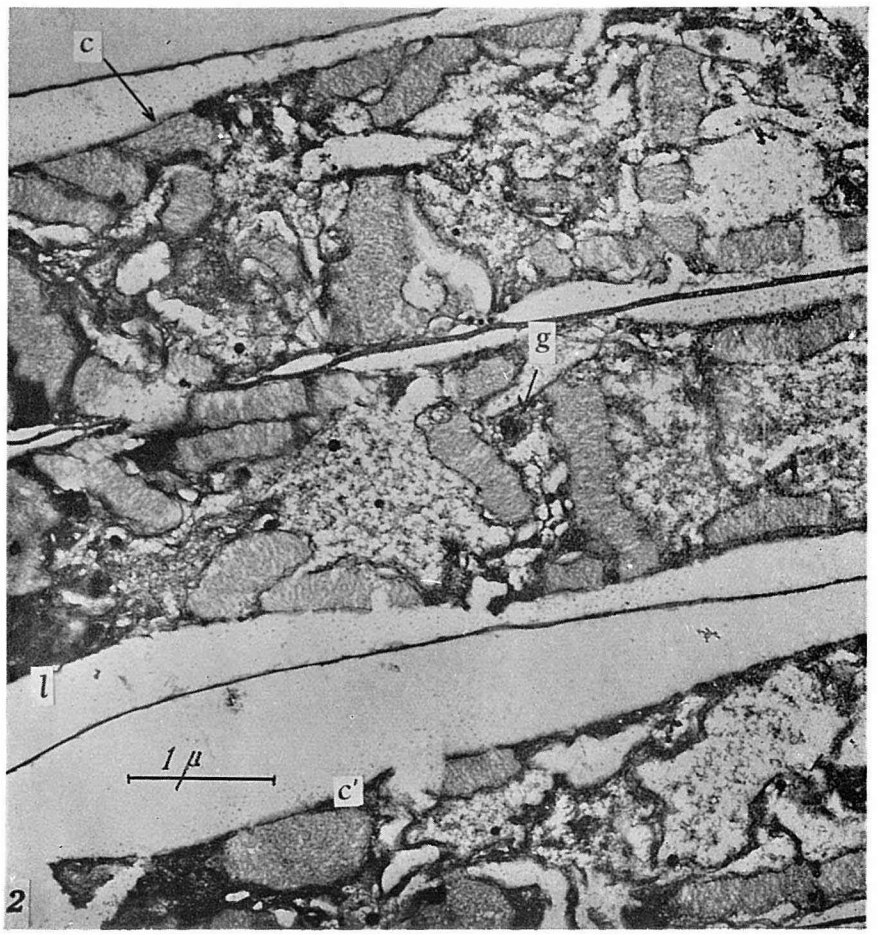
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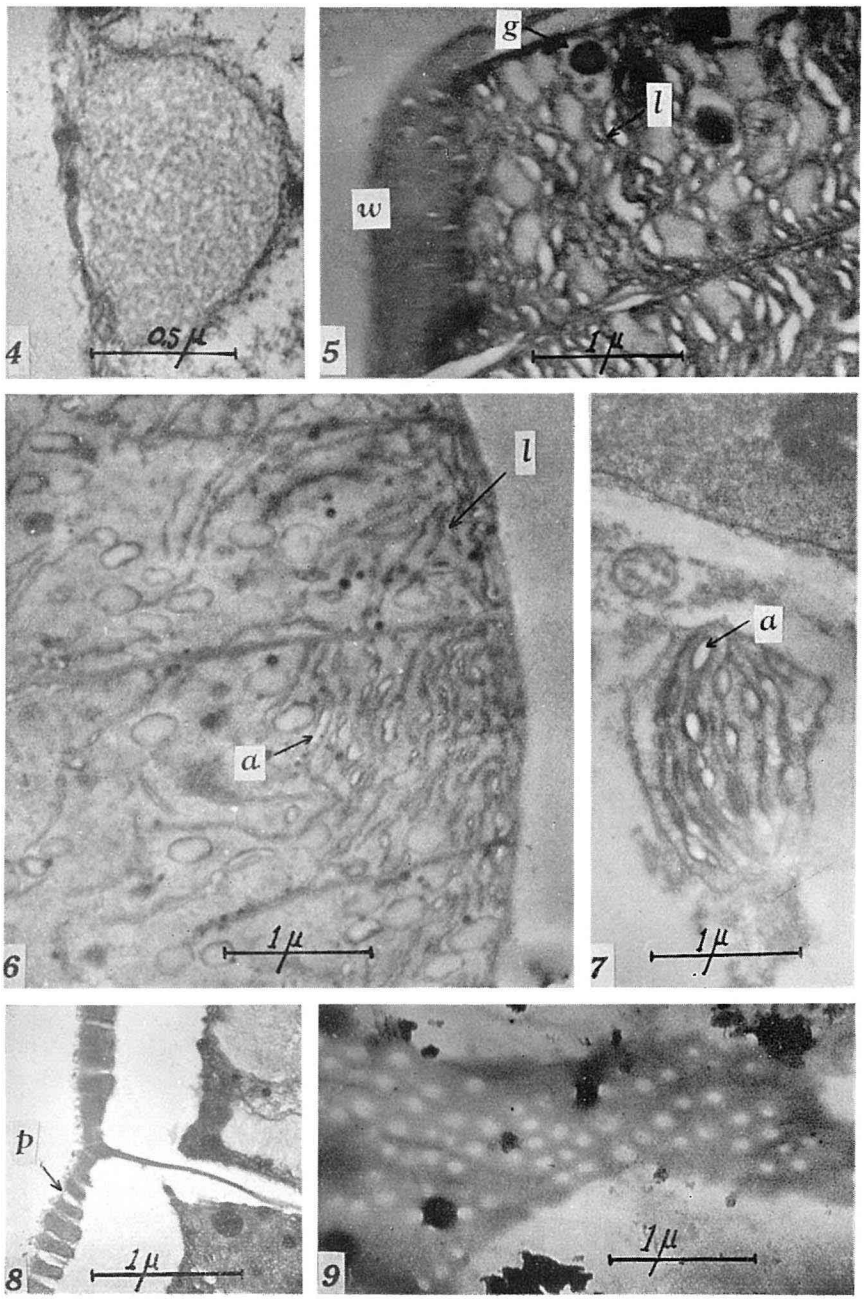
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