

ELECTRON MICROSCOPE STUDIES ON BLUE-GREEN ALGAE

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ABSTRACT

Several species of blue-green algae were studied in thin sections with the electron microscope. Our electron micrographs confirm the view that the cell of blue-green algae is different and simpler in organization than the typical plant or animal cell. On the other hand, the general pattern of ultrastructure is the same as that found in bacteria and *Streptomyces*. The cell boundary is formed by a double membrane which consists of two typical unit membranes. Situated in between these membranes is the dense inner investment or wall which continues uninterrupted into the cross-walls. The cells always contain photosynthetic lamellae, nucleoplasm with DNA, small granules resembling ribosomes, and often also a number of larger granules of various sorts. The photosynthetic membranes either form the boundary of vesicles or flattened sacs, or, when the lumen of the vesicles disappears and the vesicular surfaces of the membranes zip together, they appear as lamellae made of two closely applied unit membranes. These vesicles or lamellae are disposed irregularly through the cell or arranged in parallel stacks of two or more. A thin layer of cytoplasm always separates the lamellae. The nucleoplasm is composed of masses of fine fibrils about 25 A thick and is either dispersed through the cell or concentrated in polymorphous reticular structures near the center of the cell. The improved resolution of the electron microscope makes it obvious that the terms "chromatoplasm" and "centroplasm" commonly used in the description of blue-green algae are really misleading. There are not different kinds of cytoplasm, but the cell consists of various structural (and functional) units like the ones mentioned above, which are arranged in the cell in a number of ways characteristic for each species or for different physiological or developmental states.

INTRODUCTION

The development of new cytological techniques, especially phase microscopy, cytochemical methods, and electron microscopy of thin sections has stimulated renewed investigations into the long controversial organization of blue-green algae. The most significant result of recent studies is the realization that the structure of blue-green algae, like that of bacteria, is fundamentally different from the organization of typical cells found in higher plants and animals (9). This difference in structural organization raises interesting questions in regard to structure-function relationships. What is

the localization of enzyme systems for cellular respiration in the absence of mitochondria? What structures carry on photosynthesis where no chloroplasts are present and how do these structures compare with chloroplasts? There are no chromosomes nor mitotic figures: What is the structure of the genetic system? The understanding of the simpler organization of these cells will perhaps also throw some light on the evolutionary history of the more complex cell of higher forms.

Most students of blue-green algae distinguish between the chromatoplasm, the centroplasm, and

various granular inclusions (9, 11). The chromatoplasm is said to contain the photosynthetic pigments and published electron micrographs show it to have a lamellar structure (2, 14, 17, 19, 20). In the centropoplasm both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) have been demonstrated. The Feulgen reaction indicates that the DNA occurs in highly variable patterns from a finely granular distribution to irregularly lobed or network-like bodies (4). Published electron micrographs have failed to show any regular structures in these areas.

In this electron microscope study of several species of blue-green algae we want to present some further information on the ultrastructure of these cells, especially on the nature of the photosynthetic lamellae and the structure of the "nuclear equivalents" or "chromatin bodies."

MATERIALS AND METHODS

In view of the conflicting opinions on the structure of blue-green algae even in some of the recent work it is difficult to avoid the impression that at least part of the material examined was not in normal condition and that different investigators have dealt with the same algae in various stages of development. We have, therefore, fixed only material in well defined physiological condition and in active phase of growth. In fixing for electron microscopy we have taken advantage of recent experiences with fixation of bacteria (24).

The algae¹ investigated were: *Anacystis nidularis* (Myer's strain) *Diplocystis aeruginosa* (*Microcystis aeruginosa*), *Synechococcus cedrorum* (van Niel's strain) identified by Drouet as *Gloeocapsa alpicola*, *Oscillatoria princeps*, *Lyngbya Birgei*, *Nostoc muscorum*, *Calothrix parietina*, *Gloeotrichia echinulata* isolated by Gerloff *et al.*, *Anabaena cylindrica*, *Mastigocladus laminosus*, *Porphyrosiphon Notarisii*, *Nostoc ellipsosporum* and *Fischerella muscicola* isolated by Singh (unpublished) from Indian soils. These algae were maintained in unialgal and bacteria-free cultures. They were grown in Erlenmeyer flasks in Gorahm's medium at $23 \pm 1^\circ\text{C}$. and were illuminated from above by daylight fluorescent lamps at an intensity of 180 foot-candles. Some of the cultures were aerated by bubbling compressed air through the medium. Actively growing cultures in logarithmic phase were photographed in light above $600 \mu\mu$

¹ We wish to thank Dr. G. P. Fitzgerald of the University of Wisconsin for his aid in the culture of the algae.

which gives excellent contrast for chlorophyll-containing structures.

For the Feulgen reaction the algae were centrifuged and fixed in ethanol-acetic acid 3:1 for 10 minutes, hydrolyzed in *N* HCl at 60°C ., and stained with the Schiff's reagent for 45 minutes. They were then squashed on a slide. The coverslip was removed after freezing and the preparation mounted in piccolyte. Some algae were also stained with purified methyl green and pyronine before and after treatment with ribonuclease (1).

For electron microscopy the cells were fixed in buffered OsO_4 according to the method of Ryter and Kellenberger (24). After fixation they were centrifuged and, if necessary, embedded in 2 per cent agar, treated for 2 hours in 0.5 per cent uranyl acetate, dehydrated in ethanol, and embedded in araldite (NYSEM). Samples from several species were also treated with versene instead of uranyl acetate (24). Thin sections were cut on a Porter-Blum microtome and mounted on carbon films or on naked 400-mesh grids. Some sections were stained for 2 hours in saturated aqueous uranyl acetate or on lead hydroxide (28) for 15 to 30 minutes. Micrographs were taken with a Siemens Elmiskop IIb on Gevaert plates (Scientia 19D50) developed in D-19.

RESULTS

With the increased resolution provided by the electron microscope it becomes apparent that the generally accepted differentiation of the blue-green algal cell into chromatoplasm and centropoplasm is entirely artificial and misleading. There are no sharp boundaries dividing the cell into special regions or types of "plasm." Instead we find a number of cell components with characteristic ultrastructure. These are distributed through the cell in patterns which differ from one species to another, or in developmental stages within a species. We distinguish the following components: (1) cell envelopes; (2) cytoplasmic lamellar systems; (3) nucleoplasm; (4) ribosomes; (5) various granular inclusions. A typical arrangement of these elements is shown in Fig. 1.

1. Cell Envelopes

The cell boundary in blue-green algae consists of a double membrane with dense wall material of varying thickness between the two components. Outside of this membrane complex is the characteristic fibrous sheath (Fig. 1, S).

(a) *Inner or Plasma Membrane:* On the surface of

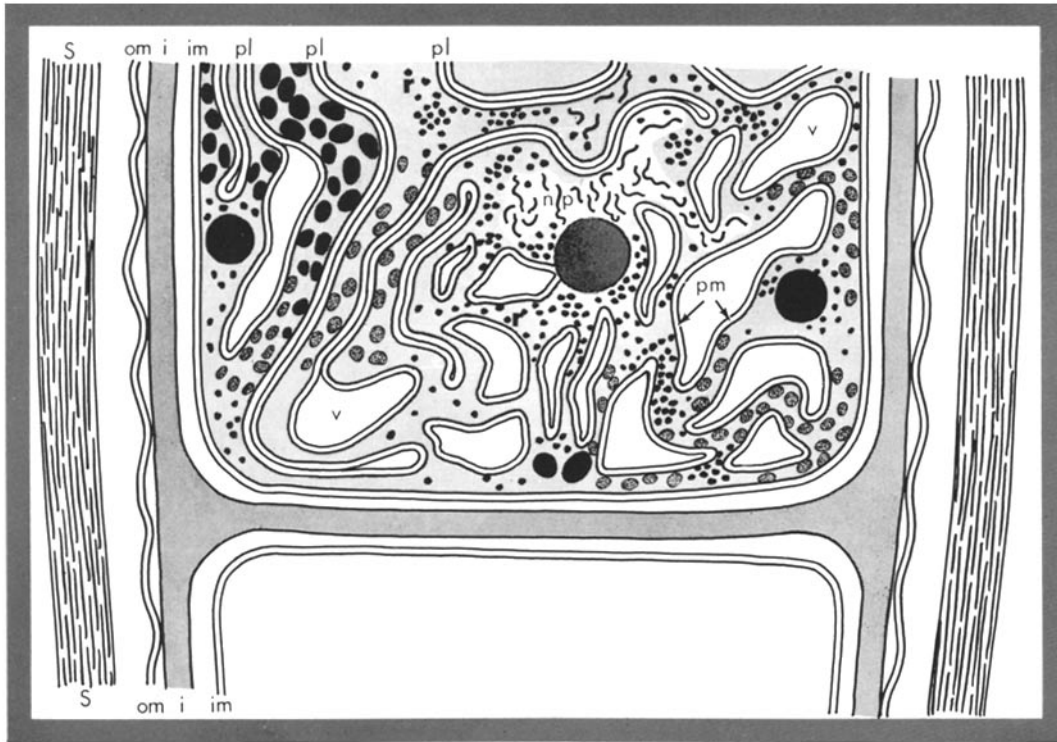


FIGURE 1

Diagram of a cell of *Calothrix* showing the typical structures found in blue-green algae. *np*: nucleoplasm with 25 Å fibrils. *v*: vesicles bounded by photosynthetic membranes (*pm*). Collapse of these vesicles leads to formation of photosynthetic lamellae (*pl*). The cytoplasm contains granules about 150 Å thick which appear to be ribosomes (*r*) and various other inclusions of unknown nature. The cell boundary is formed by an inner (*im*) and outer membrane (*om*) with the dense homogeneous inner investment (*i*) in between. Surrounding the cell is the fibrous sheath (*S*).

the cytoplasm one finds a typical plasma membrane about 70 Å thick and consisting of two dark lines separated by a light area of constant thickness (Figs. 2, 5, 14, *im*). It corresponds thus to the unit membrane found in many other biological membrane systems (22).

(b) *Dense layer (inner investment)*. Just outside of the plasma membrane is a layer of dense homogeneous material. It varies in thickness from about 100 Å (*Anacystis*) to about 200 Å (*Calothrix*, *Nostoc*, *Gloeotrichia*, *Anabaena*, *Gloeocapsa*, *Fischerella*) and 2000 Å or more in *Oscillatoria*. In this case it is perforated by pores that vary from 300 to 600 Å in diameter. This dense material continues directly into the septa or cross-walls, where it lies between the plasma membranes of the adjacent cells (Figs.

2, 5). Cell division begins with a thickening of this material which then extends into the cell like a diaphragm with decreasing aperture, pushing the inner membranes ahead of it until it fuses in the center.

In *Porphyrosiphon*, during formation of hormogonia, the cross-walls split down the middle and show a peculiar grid-like pattern at the point of separation (Fig. 8).

(c) *Outer membrane*. On the outside of the dense layer there is another thin layer with the structure of a unit membrane, *i.e.*, about 70 Å thick and showing two dense lines in profile (*om* Figs. 1, 2, 5, 9, 12, 14). It generally has a rather wrinkled or wavy appearance as if bound to the dense layer at irregular intervals and bulging out in between.

(d) *Sheath*: In contact with the outer membrane or separated from it by a space is the sheath characteristic of blue-green algae. In electron micrographs it appears as a mass of oriented interwoven fibrils and may be subdivided into several layers of different consistency (*s*, Figs. 2, 16).

2. Cytoplasmic Lamellar Systems

The most striking components of blue-green algae are the complex lamellar systems which make up a large part of the cytoplasm in most species. To understand better the nature of these lamellae we shall first consider the situation in *Calothrix* (Figs. 1, 2, 14, 15). The multitude of outlines is rather confusing at first, but becomes clearer when we realize that the cytoplasmic mass is characterized by the presence of dense particles of about 100 to 150 Å diameter (which we take to be ribosomes) and somewhat larger and less dense aggregates of irregular outline. With this in mind the peripheral areas of the cell now are seen to be occupied by a network of randomly undulating layers of cytoplasm about 350 Å thick (Fig. 1; Fig. 2, *c*). They are bounded on each side by a unit membrane about 70 Å thick (Fig. 15). Between these layers of cytoplasm are areas of very low density which will be called intralamellar vesicles (*v*). The unit membranes thus have a cytoplasmic side and a vesicular side. In certain places these vesicles are

obliterated so that the membranes touch with their vesicular sides. In this way packets of lamellae are formed in which we find alternating cytoplasmic layers (about 350 Å) containing cytoplasmic granules and double layers of unit membranes (lamellae) about 140 Å thick and homogeneous in density. These relationships are illustrated in Fig. 1. Fig. 2 shows the cytoplasmic layers (*c*) separated by vesicles (*v*) and Fig. 18 several photosynthetic lamellae (*pl*) in *Calothrix*. The vesicles vary greatly in size. In some cells they are completely collapsed and the cell is then traversed by irregularly winding lamellae (*Oscillatoria*, *Porphyrosiphon* Figs. 5, 8) or by lamellae arranged parallel to the cell surface and alternating with cytoplasmic layers (*Anacystis*, *Gloeocapsa*, Figs. 9, 10). In other cells the vesicles are more or less swollen at least in some parts of the cell (*Calothrix*, Fig. 2).

The pattern of membrane distribution described for *Calothrix* is also found in *Nostoc*, *Mastigocladus*, *Gloeotrichia*, *Anabaena* and *Fischerella*. In *Synechococcus*, *Anacystis*, and *Gloeocapsa* the membranes are more regularly arranged, generally parallel to the cell surface and filling the peripheral part of the cell. Usually there are cytoplasmic layers from 160 to 350 Å thick alternating with lamellae 140 Å thick (Figs. 12, 13).

The mass of granular and non-granular cytoplasm is traversed and dissected by the membrane system. Usually this is in the form of an irregular

FIGURE 2

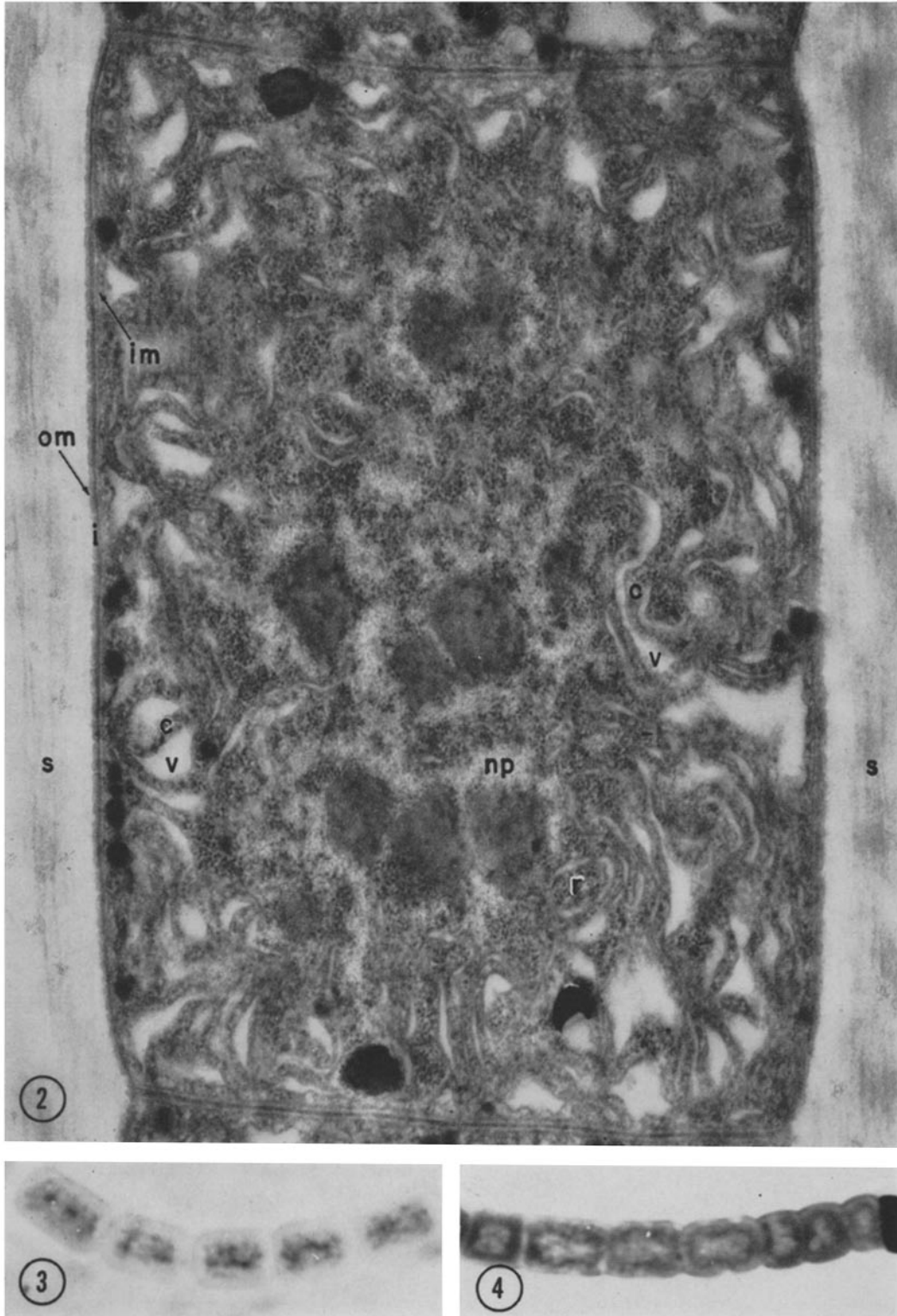
Electron micrograph of a cell of *Calothrix parietina* (compare with Fig. 1). The cell is surrounded by a fibrous sheath (*s*). The cell boundary is formed by three layers: the wrinkled outer membrane (*om*), the dense inner investment (*i*), and the plasma membrane (*im*). The photosynthetic membranes form vesicles of irregular shape (*v*) which separate thin layers of cytoplasm (*c*). When these vesicles collapse so that the membranes touch, the typical photosynthetic lamellae are formed. The nucleoplasm (*np*, corresponding to the Feulgen positive areas in Fig. 3), is distributed as a polymorphous network throughout the more central part of the cell. It has very low density except for fibrils of about 25 Å thickness. Dense particles about 150 Å in diameter which are most likely ribosomes (*r*) are present in groups, generally around the nucleoplasm, but also in more peripheral cytoplasmic portions. In the central area one finds also large granules whose nature is not known. $\times 45,000$.

FIGURE 3

Photomicrograph of *Calothrix parietina* stained with the Feulgen reaction. $\times 2000$.

FIGURE 4

Living cell of *Calothrix parietina* photographed in red light to show the distribution of photosynthetic pigments. $\times 2000$.



network of lamellae about 140 Å thick (Figs. 5, 8, 17). Short pieces of lamellae may be completely surrounded by granular cytoplasm at least in the plane of the section. In some cells one finds stacks of membranes in which the lamellae are separated from each other by layers of cytoplasm as thin as 160 Å (Figs. 12, 13). In a few preparations of *Oscillatoria* the lamellar membranes have been separated in spots giving rise to intralamellar vesicles. The membrane system now has the appearance of a network of undulating flattened sacs. It is not known what causes this swelling of the intralamellar space.

In *Oscillatoria* one finds another membrane differentiation which is clearly distinct from the lamellae. It is always associated with the plasma membrane along the cross-walls and forms a more or less spherical mass of tightly packed undulating membranes (*sg*, Fig. 5). These bodies correspond to the "structured granules" of Drews and Niklowitz (8) which according to these authors stain with Janus green *in vivo* and reduce tetrazolium salts. Further work, however, is necessary before it can be stated that these structures are indeed "mitochondrial equivalents."

How are these lamellar systems formed? At present one can say very little about this. Some pictures of *Oscillatoria* indicate that at least in part they arise like membrane systems in other forms through fusion of vesicles (15).

3. Nucleoplasm

In blue-green algae the distribution of DNA is very similar to that in bacteria (23) as shown by the Feulgen reaction (Figs. 3, 6). There are no chromosomes, nor interphase nuclei with a nuclear membrane, but the DNA occurs in structures of most variable shapes, either distributed in small droplets or in a polymorphous reticular structure. Usually the bulk of it is concentrated in the central part of the cell. Our present study demonstrates that the nuclear equivalents of blue-green algae resemble those of bacteria not only in the light microscope, but also in their ultrastructure. As first shown by Ryter and Kellenberger (24) a characteristic property of bacterial nucleoplasm is its lability towards fixation. Depending on the postfixation treatment it may have an irregular coarse structure or a fine fibrillar organization with fibrils as thin as 25 Å predominating. The even distribution of such minute fibrils is obtained by treating the cells after fixation with uranyl acetate. Versene treatment instead of uranyl acetate leads to a very coarse structure of the nucleoplasm. Figs. 1, 2, 9, 10, 16 to 18 illustrate the appearance of the nucleoplasm in various blue-green algae after treatment with uranyl acetate. The nucleoplasm is a polymorphous structure of low density containing fibrils of about 25 Å thickness. These fibrils tend to agglutinate into irregular complexes if fixed in Palade's buffered OsO₄ (Fig. 19) and especially with post-

FIGURE 5

Electron micrograph of two cells from a filament of *Oscillatoria princeps*. The filament is limited on the outside by the outer membrane (*om*), the thick inner investment with pores, and the plasma membrane (*im*). The inner investment extends into the cross-walls, lined on each side by the plasma membranes of the adjacent cells.

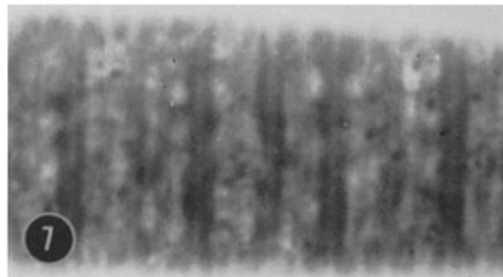
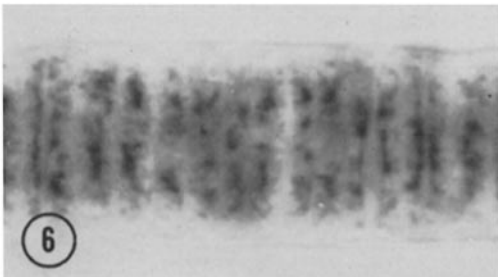
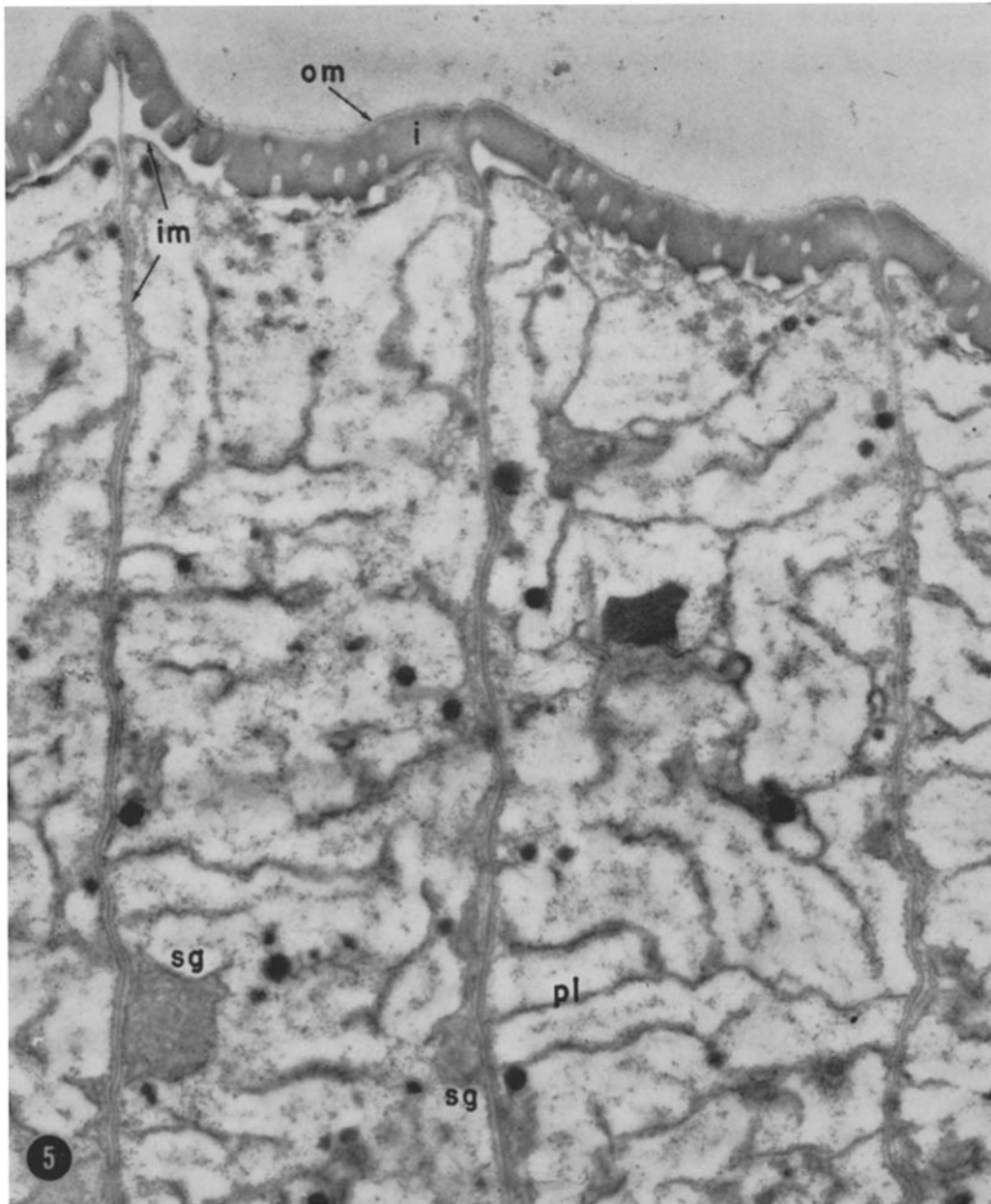
Along the cross-walls there are several "structural granules" (*sg*). The cytoplasm is transected by the system of photosynthetic lamellae (*pl*). × 25,000.

FIGURE 6

Photomicrograph of *Oscillatoria princeps* stained with the Feulgen reaction. × 2000.

FIGURE 7

Living cells of *Oscillatoria princeps* photographed in red light to show the distribution of photosynthetic pigments. × 2000.



fixation treatment in versene (Fig. 20). Most of this material is usually situated near the center of the cell, but portions of it may be found even in peripheral cytoplasmic layers (Figs. 2, 16).

4. Ribosomes

In all cells the most ubiquitous cytoplasmic components are dense granules about 100 to 150 A in diameter which stand out with sharp contrast especially after staining with uranyl acetate. They have the same size and appearance as ribosomes in bacteria and in cells of higher organisms. They occur in higher concentrations in those regions which are high in RNA content as shown by staining with pyronine before and after ribonuclease. We, therefore, assume for the present that these granules represent ribosomes. The final evidence of course will have to come from chemical and enzymatic analysis of granules isolated from cell homogenates.

In the cells of most species the ribosomes are concentrated in the central part of the cell in association with the nucleoplasm but they are also found in cytoplasmic layers everywhere in the cell and often also between photosynthetic lamellae (*r*, Figs. 2, 9, 10, 16, 17, 18, 20).

5. Other Granular Inclusions

The cytoplasm of most species of blue-green algae contains several kinds of granules of various sizes and appearance in electron micrographs. Most of them have been described before (*cf.* 5, 7, 19, 20, 27). We should like to call attention to granules about 250 A in diameter which have a special affinity to lead hydroxide and are found especially between the photosynthetic lamellae in many species (Fig. 10). They may represent a product of photosynthesis.

Until a systematic study of these inclusions is

made using modern methods of cell fractionation very little can be said about their nature. But the recognition and description of these structures in intact cells prepares the way for a systematic biochemical and functional analysis.

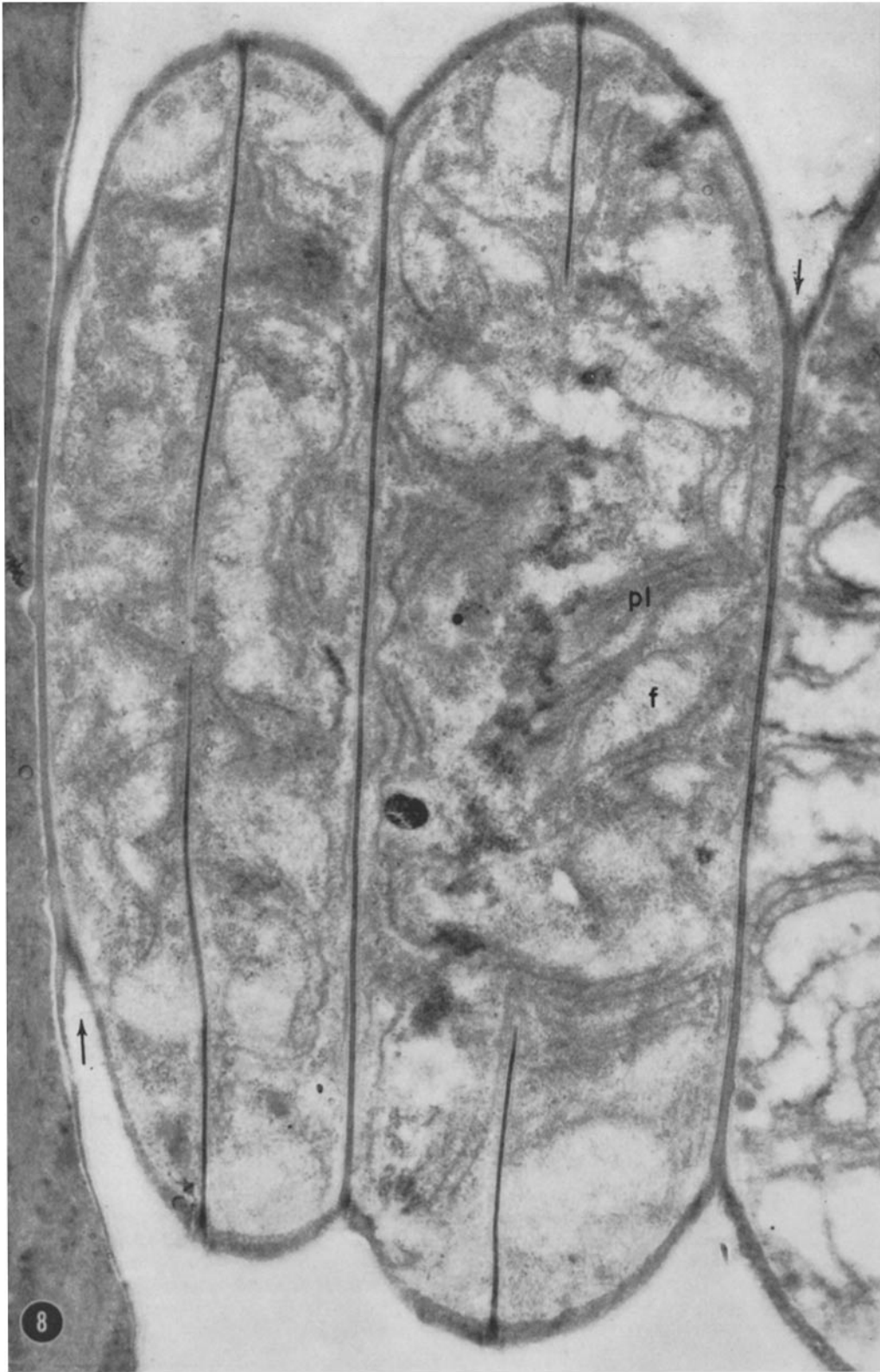
DISCUSSION

Recent cytological studies on blue-green algae, either with the light microscope or electron microscope, have established incontrovertibly that the cellular organization of these organisms is simpler and quite different from that of higher forms (*cf.* 9, 11). It is comparable, on the other hand, to the organization of bacteria and of *Streptomyces* (12). Attempts to find cell components analogous to those characteristic of higher cells can only confuse the issue. The real problem is to describe the ultrastructure of these primitive cells and analyze the structure-function relationships as found here in comparison to those established for animal and plant cells.

It might be useful at this point to compare briefly the cell structure of blue-green algae with cells of other groups. The cell surface of blue-greens as seen from our electron micrographs consists usually of a double layer of unit membranes within which there is a dense material of variable thickness forming the wall or inner investment. The cell divides through extension inwards of this material analogous to a closing diaphragm until a continuous septum is formed. In *Oscillatoria brevis* the inner investment may be missing and the cell border is formed by a double membrane about 200 A thick (17). Electron micrographs of cell walls in bacteria and *Streptomyces* suggest a very similar organization. In some bacteria the inner investment is absent and the two unit membranes are separated by a less dense material (electron micrographs of *Rhodospirillum*, 13, and of *E. coli*, 16). As in blue-greens the outer membrane

FIGURE 8

Electron micrograph of a hormogonium separating from a filament of *Porphyrosiphon Notarisii*. New cross-walls are seen extending into both cells, randomly separating the cell content into two portions. The inner investment shows a grid-like structure at the points where adjacent cells separate from each other (arrows). Photosynthetic lamellae (*pl*) crisscross the cells. A filamentous material of unknown composition (*f*) fills the space between the lamellae. $\times 30,000$.



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appears often wrinkled. *B. cereus*, on the other hand, possesses a dense wall and cell division proceeds in the same manner as in blue-greens (5, 6). Similar wall structures appear in the electron micrographs of *Streptomyces* by Glauert and Hopwood (12).

A comparison of the photosynthetic structures of blue-green algae and chloroplasts of plants is particularly interesting. Photomicrographs of living cells, using light which is absorbed specifically by their photosynthetic pigments, show that these pigments are not evenly distributed but form a pattern characteristic for each species (Figs. 4, 7, 11). On the basis of such observations most students of blue-greens have distinguished between the photosynthetic "chromatoplasm" and the "centroplasm." Under certain conditions even the light microscope reveals a lamellar organization of this chromatoplasm (10) and electron micrographs have provided further information on these lamellae (2, 14, 17, 19, 20). Membranous fragments obtained from homogenized blue-green algae were shown to contain all the chlorophyll (3). We can accept, then, that in blue-greens the chlorophyll is bound to membranes just as in chloroplasts (for recent reviews of chloroplast structure see 14, 25). How do these lamellae compare in the two systems? To begin with it should be emphasized that blue-green algae do not have either chloroplasts or grana. They contain lamellae, which in their origin and structure are very similar to the lamellae of chloroplasts. Perhaps the entire cell could be compared to an intact chloroplast. The morphological unit of the photosynthetic structures in both cases is a membrane about 70 Å thick. It is present as the lining of vesicles of various sizes which seem to be able to fuse into larger complexes or break up into smaller ones like other membrane complexes of cells (*cf.* 15, 18). These vesicles are usually collapsed so that the membranes touch with their vesicular

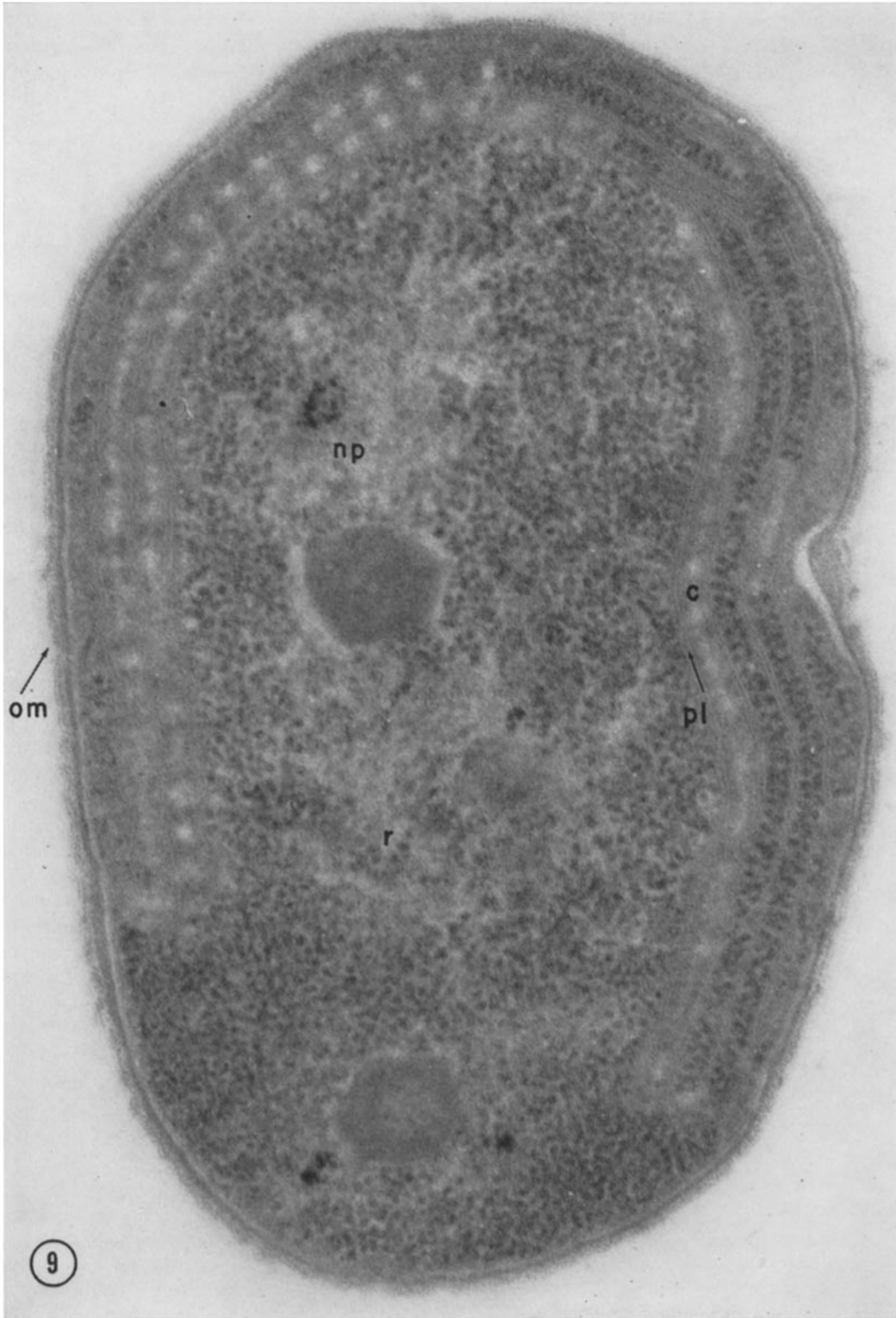
surfaces. In this fashion double-membraned lamellae are formed, about 140 Å thick. In blue-greens these lamellae traverse the cytoplasm either in a very irregular way or they may form complex lamellar systems not unlike those of chloroplasts, though no organization resembling the grana of higher plants is found. The lamellae are usually separated by cytoplasm which often forms layers of regular thickness between the lamellae or vesicles. In tightly packed lamellar systems the cytoplasmic layers may be as thin as 160 Å. The concentration of lamellae and the way they are arranged varies greatly from one species to another and in different stages of development. Some cells are tightly packed with lamellae, others have few vesicles or lamellae here and there through the cytoplasm, resembling certain chloroplast mutants (29). It would be interesting to find out whether light is necessary for the maintenance of lamellae in blue-greens as it is in the chloroplast.

The DNA-containing structures of blue-green algae have the same ultrastructure as the chromatin bodies or nuclear equivalents in bacteria. In view of their highly variable morphology and the absence of a limiting membrane the term "nucleoplasm" (24) is most descriptive. Just as in bacteria it has a low density and appears uniformly light in living cells observed with the phase microscope. After fixation with Kellenberger's procedure the nucleoplasm is composed of fine fibrils about 25 Å thick. These fibrils have the tendency to fuse into irregular complexes. The nucleoplasm of blue-greens, like that of bacteria, thus differs markedly in its ultrastructure from the organization of chromosomes and true nuclei (*cf.* 21).

The membrane bounded "chromonemata" containing fibrils 100 to 200 Å thick described by Shinke and Ueda in *Oscillatoria* (26) have nothing to do with nuclear structures but correspond to the sausage-shaped cytoplasmic areas which contain a material that in some cells looks fibrous

FIGURE 9

Electron micrograph of *Anacystis nidulans*. The central part of the cell is filled with dispersed nucleoplasm (*np*) and ribosome-like granules (*r*) about 150 Å in diameter; the nature of the two large granules is not known. On the outer part are several concentric layers of photosynthetic lamellae (*pl*). The outer membrane (*om*) is about 70 Å thick and shows the two dense lines characteristic of osmium-fixed unit membranes. $\times 115,000$.



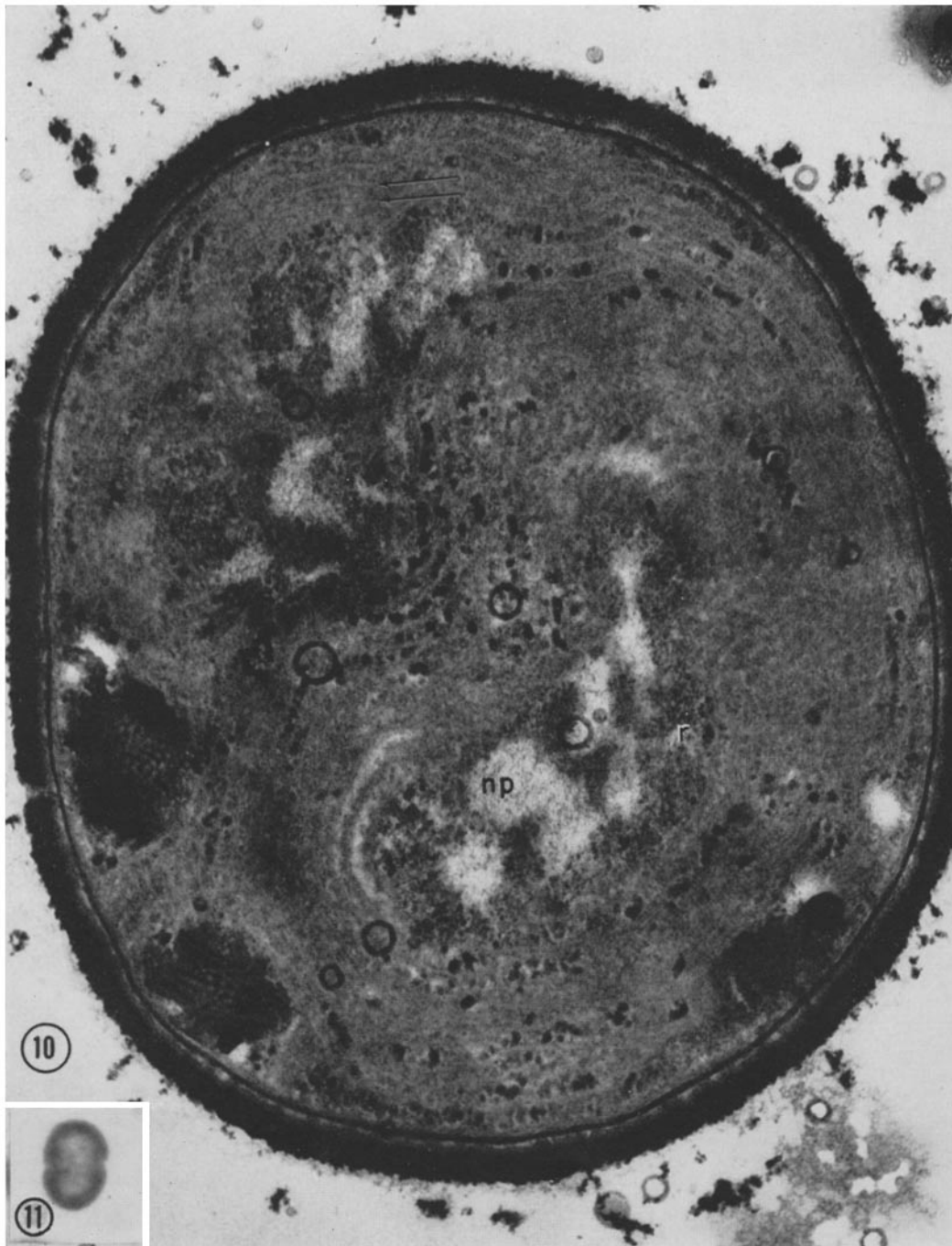


FIGURE 10

Electron micrograph of *Gloeocapsa alpicola* stained for 15 minutes in lead hydroxide; two areas of nucleoplasm (*np*) and associated ribosome-like granules (*r*) are embedded in tightly packed layers of photosynthetic lamellae (arrows). Heavily stained granules 250 to 300 Å in diameter are seen between some of the lamellae; they may represent a product of photosynthesis. $\times 60,000$.

FIGURE 11

Living cell of *Gloeocapsa alpicola* photographed in red light to show the distribution of photosynthetic pigments. $\times 2000$.

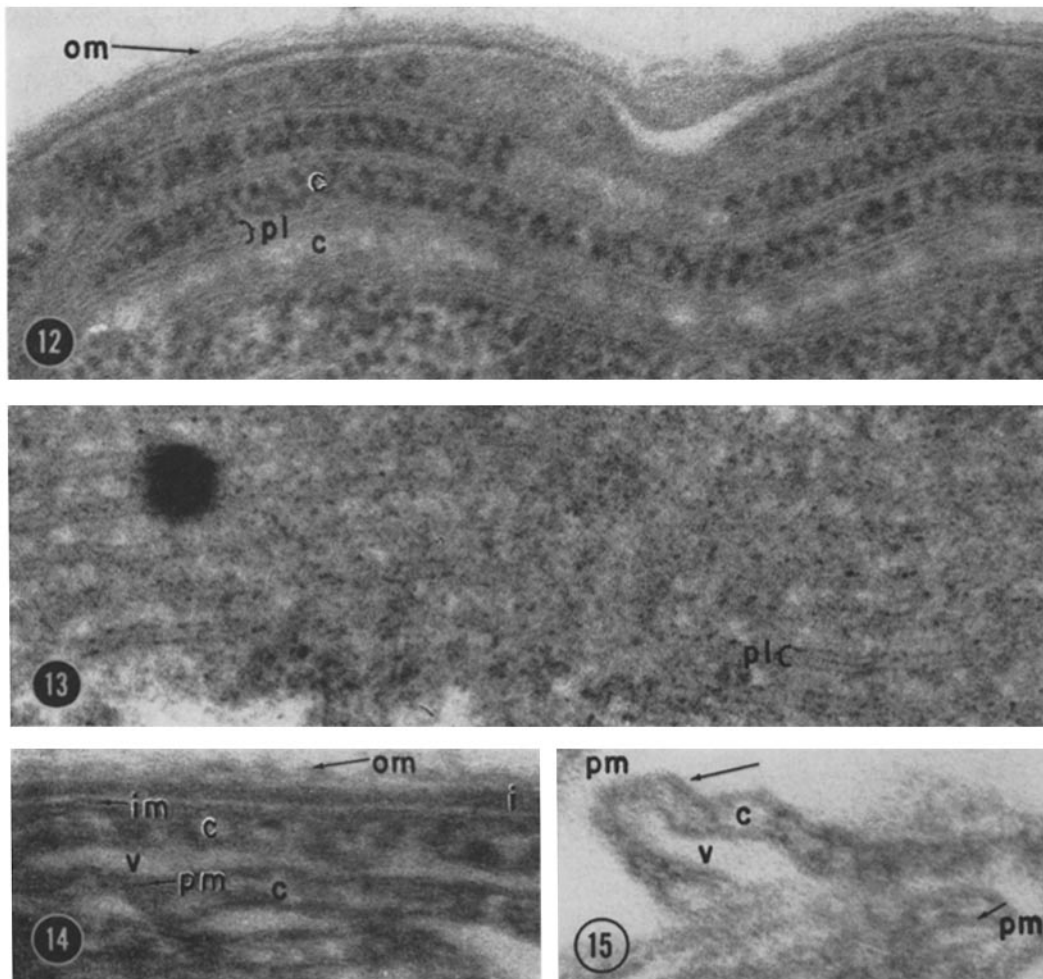


FIGURE 12

Electron micrograph of *Anacystis nidulans* showing several parallel photosynthetic lamellae (*pl*) separated by layers of cytoplasm (*c*) containing dense granules which are about 150 Å in diameter and appear to be ribosomes. The photosynthetic lamellae consist of two unit membranes closely appressed and 140 Å thick. (compare with Fig. 1). Stained with uranyl acetate. $\times 140,000$.

FIGURE 13

Gloeocapsa alpicola. Electron micrograph of section through a packet of parallel photosynthetic lamellae (*pl*) which are separated by layers of cytoplasm about 150 Å thick; stained with lead hydroxide. $\times 140,000$.

FIGURES 14 and 15

Electron micrographs of *Calothrix parietina* showing the unit membrane structure of the outer membrane (*om*), the plasma membrane (*im*) and the photosynthetic membranes (*pm*) between the cytoplasmic layers (*c*) and the vesicles (*v*). Compare with Fig. 1. $\times 140,000$.

(Fig. 8, f). The apparent membranes around these structures are in reality parts of the photosynthetic lamellae of these cells.

The cytoplasm of blue-green algae is generally rich in granules from 100 to 150 Å in diameter which have all the characteristics of ribosomes. They are, of course, less abundant in regions occupied by photosynthetic lamellae, but are also found in the cytoplasmic layers between the lamellae. This distribution which parallels that of the nucleoplasm is responsible for the concept of "centroplasm," a special region of the cell usually centrally located and reacting positive with tests

for DNA and RNA. The analysis with the electron microscope demonstrates, however, that ribosomes and nucleoplasm are found all through the cell, depending of course on the distribution and concentration of photosynthetic lamellae. The term "centroplasm" is, therefore, meaningless and should be dropped just as the term "chromatoplasma" should be replaced with "photosynthetic lamellae."

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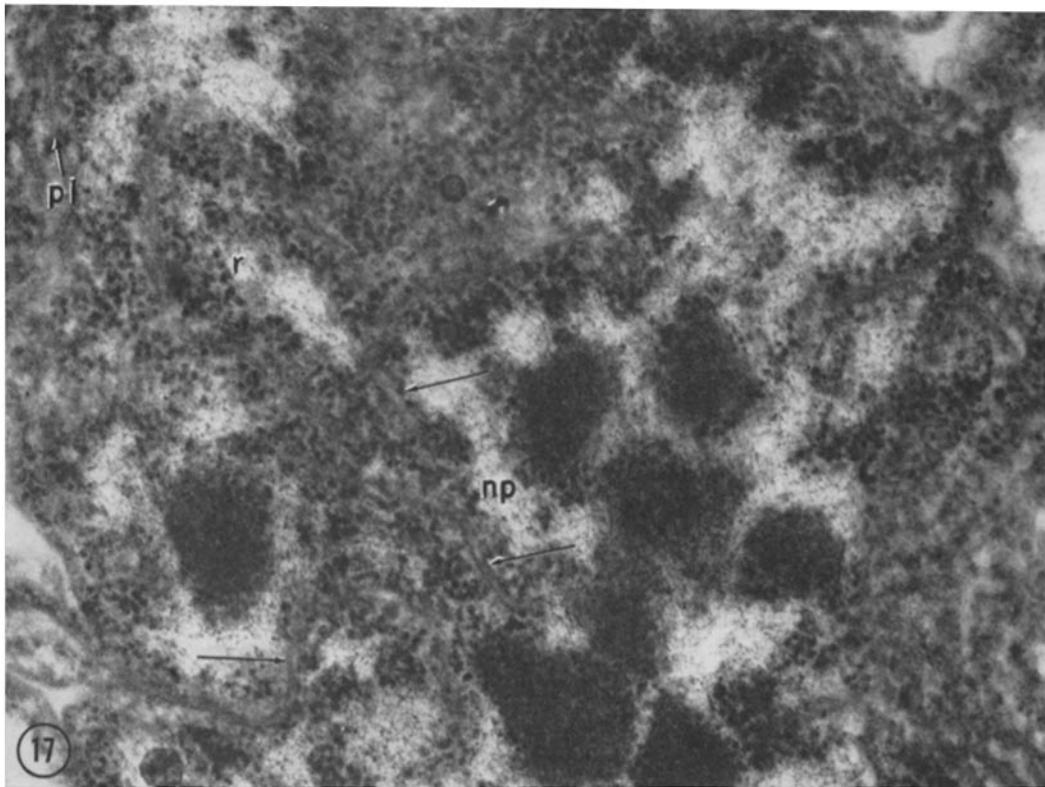
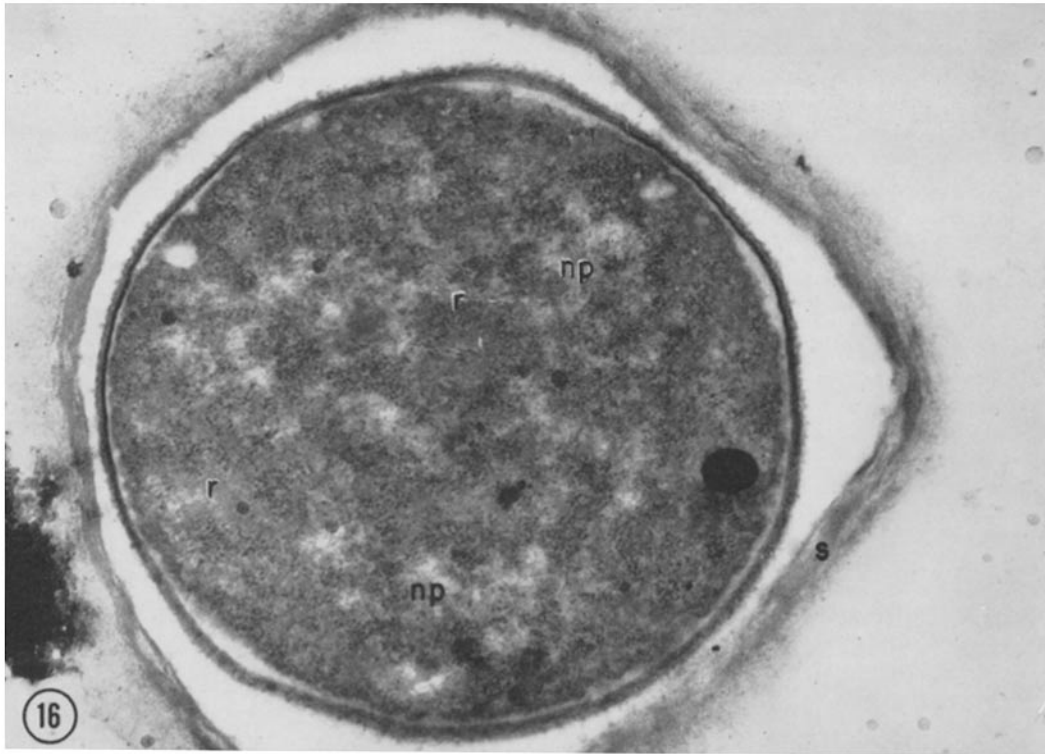
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FIGURE 16

Electron micrograph of spore of *Fischerella muscicola*. The nucleoplasm is distributed through the cell. A few photosynthetic lamellae are scattered through the cytoplasm. The fibrous sheath surrounds the cell. $\times 30,000$.

FIGURE 17

Electron micrograph of central area from a cell of *Calothrix parietina*. Nucleoplasm (*np*) and photosynthetic lamellae (*pl*, arrows) are intermixed; ribosome-like granules (*r*) are scattered through the area. There is no sharp separation into centroplasm and chromatoplasma.



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FIGURES 18, 19, and 20

Electron micrographs of nucleoplasm (*np*) illustrating the effect of fixation procedure on the appearance of the dense material in the nucleoplasm.

FIGURE 18

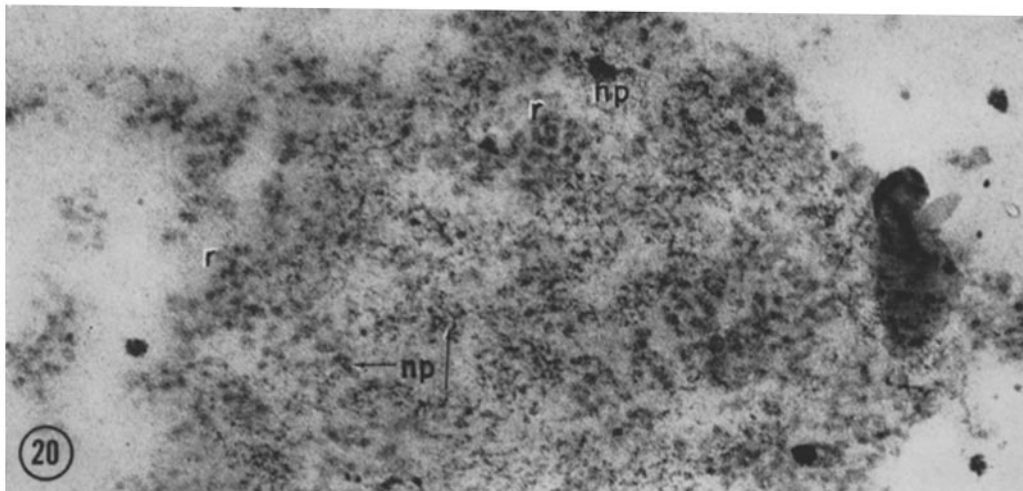
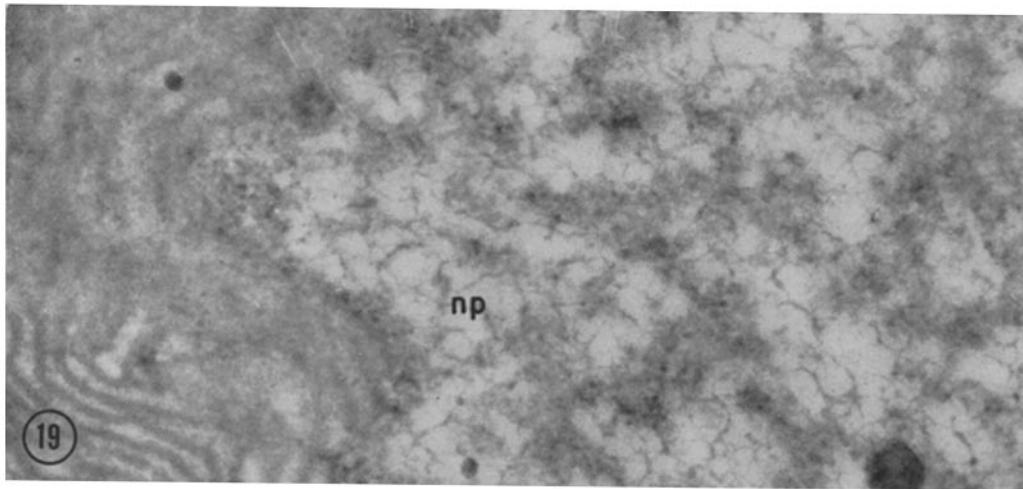
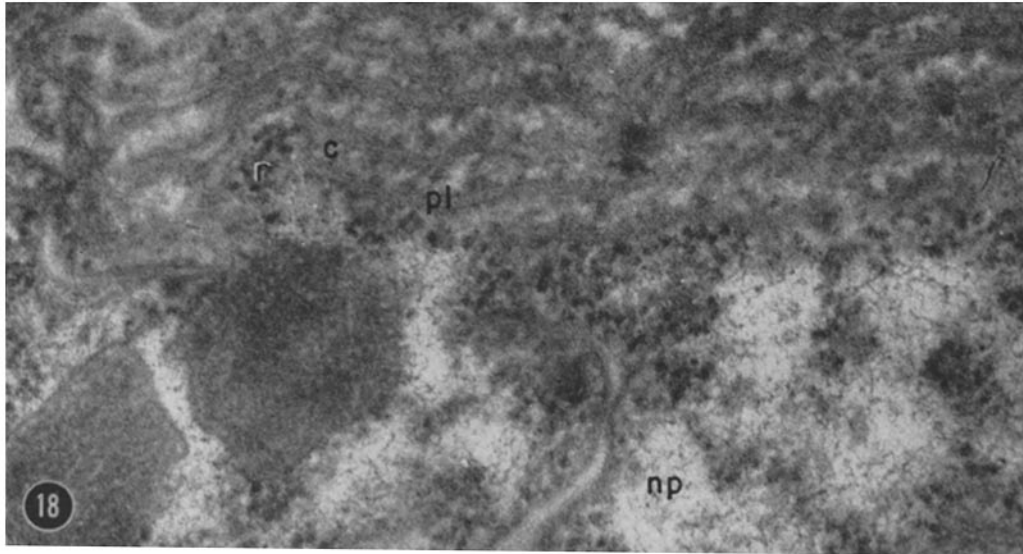
Calothrix parietina, fixation according to Kellenberger (24) posttreatment with uranyl acetate. The nucleoplasm is filled with fine fibrils, the thinnest about 25 Å thick. These fibrils probably correspond to the DNA molecules present in the nucleoplasm. $\times 100,000$.

FIGURE 19

Nostoc muscorum, fixation with Palade's buffered OsO_4 . The dense content of the nucleoplasm forms anastomosing complexes of varying thickness. $\times 75,000$.

FIGURE 20

Calothrix parietina, fixation according to Kellenberger (24) posttreatment with versene, section stained with uranyl acetate. The dense material of nucleoplasm (DNA?) is clumped into irregular complexes (arrows). Scattered in between the areas of nucleoplasm (*np*) are dense ribosome-like granules (*r*). The reaction of the nucleoplasm to fixation and posttreatment with uranyl acetate and versene is similar to that described for bacterial nucleoplasm (24). $\times 100,000$.



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