Cytology of Blue-green Algae

II. Unusual inclusions in the cytoplasm¹

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Structures of more or less regular occurrence in all blue-green algae include: a complex envelope usually consisting of a sheath, outer membrane, inner investment, and plasma membrane; the photosynthetic thylakoids; structured granules; β granules (lipoidal); α granules (polyglucosides); ribosomes; polyhedral bodies and nucleoplasm. A few structures of less frequent occurrence in blue-greens have been described. These include mesosome-like elaborations of the plasma membrane in *Anacystis nidulans* (Echlin 1964), the non-membranous cylindrical bodies in *Symploca muscorum* (Pankratz and Bowen 1963), the lamellar bodies in *Nostoc pruniforme* (Jensen and Bowen 1961), prolamellar-like bodies in *Anabaena* sp. (Lang and Rae 1967), the gas vesicles which form the complex gas vacuoles of many species (Bowen and Jensen 1965, Jost 1965), and polyphosphate bodies (Jensen 1968).

In the first paper of this series, Pankratz and Bowen (1963) described the fine structure of one blue-green alga, *Symploca muscorum*, in some detail. Since that time, we have studied the fine structure of some 60 different collections of blue-green algae including at least 30 species, mostly in the Nostocales. In addition to the structures found in all blue-green algal cells, we have found several different inclusions, most of which were observed in only one or, at the most, a few different collections. At the risk of oversimplification, it is convenient to place these elements into three categories—i.e., structures of primarily membranous, filamentous, or crystalline organization. This paper describes these unusual inclusions.

Materials and methods

Collections subjected to the most careful study are in the following list. Where the specific name appears in quotation marks, we have made no effort to verify the identification provided by the collector. I.S.U. numbers are indicated for material we currently have in culture.

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<i>Designation</i>	<i>Source</i>
<i>Anabaena spiroides</i>	Des Moines River, Iowa
<i>Anabaena</i> sp.	Lake Laverne, Ames, Iowa
<i>Aphanizomenon flos-aquae</i>	Lake Ahquabi, Indianola, Iowa
" <i>Calothrix crustacea</i> "	Kaiser Research Foundation M-13.1.1.
" <i>Calothrix parietina</i> "	Kaiser Research Foundation M-13.1.1.
<i>Cylindrospermum</i> sp.	Ledges State Park, Boone, Iowa
<i>Gloeotrichia pisum</i>	Little Wall Lake, Jewell, Iowa
<i>Gloeotrichia pisum</i>	Clear Lake, Clear Lake, Iowa
<i>Gloeotrichia echinulata</i>	Lake Okoboji, Milford, Iowa
" <i>Lyngbya</i> sp."	Indiana Culture Collection 487
" <i>Lyngbya</i> sp."	Indiana Culture Collection 488
" <i>Lyngbya</i> sp."	Indiana Culture Collection 621
" <i>Lyngbya</i> sp."	Indiana Culture Collection 622
" <i>Microcystis</i> sp."	Lake East Okoboji, Milford, Iowa
" <i>Nostoc calcicola</i> "	Indiana Culture Collection B382
<i>Nostoc carneum</i>	Lake Okoboji, Milford, Iowa
<i>Nostoc coeruleum</i>	Council Bluffs, Iowa
" <i>Nostoc commune</i> "	Indiana Culture Collection 584
" <i>Nostoc ellipsosporum</i> "	Indiana Culture Collection B383
<i>Nostoc linckia</i>	Silver Lake, Milford, Iowa
<i>Nostoc muscorum</i>	Indiana Culture Collection 486 (ISU J10)
<i>Nostoc muscorum</i>	Big Wall Lake, Dows, Iowa (ISU J15)
<i>Nostoc muscorum</i>	Franklyn Ott, University of Texas No. 079
<i>Nostoc pruniforme</i>	Lake Okoboji, Milford, Iowa (ISU J11)
<i>Nostoc pruniforme</i>	Indiana Culture Collection LB756
<i>Nostoc punctiforme</i>	Indiana Culture Collection 384
" <i>Oscillatoria borneti</i> "	Indiana Culture Collection LB143
" <i>Oscillatoria chalybea</i> "	Indiana Culture Collection 386
" <i>Oscillatoria formosa</i> "	Indiana Culture Collection LB390
<i>Oscillatoria princeps</i>	Lake Ahquabi, Indianola, Iowa
" <i>Oscillatoria tenuis</i> "	Indiana Culture Collection 428
" <i>Phormidium faveolarum</i> "	Indiana Culture Collection 216
" <i>Phormidium luridum</i> "	Indiana Culture Collection 426
" <i>Phormidium</i> sp."	Indiana Culture Collection 485
" <i>Plectonema boryanum</i> "	Kaiser Research Foundation M-9.2.1.
" <i>Spirulina major</i> "	Indiana Culture Collection LB552
" <i>Spirulina</i> sp."	Indiana Culture Collection LB770
" <i>Symploca muscorum</i> "	Indiana Culture Collection B617

An effort was made to culture each of our own collections. This was not always successful, since many blue-green algal species, particularly the planktonic forms, have special requirements. Consequently, samples of each of our collections were always fixed in the field.

Culture media used were Kratz and Meyers "C" (Kratz and Myers 1955), Chu 10 with soil extract with and without 2% agar (Chu 1942), soil extract agar (Starr 1960), soil water medium (Starr 1960), and Lazaroff madeia I and II both with and without 2% agar (Lazaroff and Vishniac 1962). Cultures were maintained at 21°C under 50–100 foot candles of illumination. A 12-hour alternating light and dark period was used.

Routine fixation was carried out at room temperature for 3 hours in 1% osmium tetroxide buffered at pH 6.1 containing calcium ions and amino acids (Pankratz and Bowen 1963). Unbuffered 4 and 5% potassium permanganate at room temperature for 5 minutes (Luft 1956, Mollenhauer 1959) was also used. Some samples were fixed in 3% glutaraldehyde followed by 1% osmium tetroxide (Sabatini, Bensch, and Barnett 1963). After dehydration, specimens were embedded in Epon 812.

Just before polymerization, a small amount of a sample in Epon was placed on a slide and squashed out under a cover slip. Incubation at 60°C for 24 hours produced high quality permanent slides for future reference. These were particularly useful for identification where we were unable to culture that collection.

Sections were cut on an LKB microtome with a diamond knife and, except for permanganate-fixed material, were stained with either lead or uranyl salts (Karnovsky 1961, Millonig 1961, Stempak and Ward 1964, Watson 1958 a, b). The study of adjacent, unstained, thick (1 μ) sections with a phase contrast microscope was useful where samples were not unialgal. Thin sections were studied with an RCA EMU-3F electron microscope.

Observations

Membranous stacks: Unusual "stacks" of membranous elements were observed in occasional cells of the *Cylindrospermum* sp. collection (Fig. 1). These stacks ranged from 0.3 to 0.7 μ in length and each was composed of about 20 parallel membranes. In no case were component membranes observed appressed to each other to form myelin-like arrays, but always they were separated by interlamellar spaces about 6 m μ in width. Though less electron-dense than the thylakoidal membranes, the stack membranes resembled the latter, being about 8.5 m μ thick and having the tripartite profile of unit membranes. Unlike the thylakoidal membranes which are always part of continuous closed systems, the stack membranes apparently have edges. We visualize each membrane as a simple sheet terminating at the margin of the stack. These inclusions were not observed in permanganate-fixed cells of *Cylindrospermum* sp. from the same collection. However, the low frequency of their occurrence may account for this, since we have only observed four such inclusions in the hundreds of cells of this collection studied.

Membranous spheres: Unique spherical membrane system were observed

in all vegetative cells and heterocysts of *Nostoc linckia* (Figs. 2, 3) with up to 30 such structures per cell. From 0.2 to 0.6 μ in diameter, these

Figs. 1-3. 1, *Cylindrospermum* sp. Profile of a "membranous stack" indicated by arrow. The cell wall (w) is composed of the outer membrane (om) and the inner investment (iv) distal to the plasma membrane (pm). Note the photosynthetic thylakoids (t) polyhedral bodies (pb) and ribosomes (r). $\times 100,000$. 2, *Nostoc linckia*. Profile of a "membranous sphere" in median section. Arrows point to 2 edges and a bifurcation of the membranous components. Several polyglucoside inclusions (α granules) are evident inside the sphere (a) as well as several lipoidal inclusions (β granules) outside (b). $\times 145,000$. 3, *Nostoc linckia*. A profile of a membranous sphere in median section at the upper left and several spheres in tangential section (arrows). $\times 100,000$.

"spheres" always exhibited circular profiles and appeared to consist of 2 or 3 membranous sheets wrapped around a common center. The membranes were each about $9\text{ m}\mu$ thick and although at first glance they had the tripartite appearance of unit membranes, upon closer examination, profiles of these

Fig. 4. Heterocysts of *Gloeotrichia pisum* (Little Wall Lake). Profiles of transverse sectioned membranous scrolls. One scroll (large arrow) at upper right is sectioned obliquely. Note inner and outer edges of scroll membranes (small arrows). $\times 100,000$.

membranes revealed subunits which resulted in an alveolar or chain-like appearance. Profiles of these membranes showed bifurcations and had ends (Fig. 2). No membranes extended into the 0.1 to 0.3 μ diameter central regions of spheres where ribosomes, α and β granules were found. Often spheres appeared to intersect one another in which case only one of the spheres was complete. The interruption of other spheres by a complete sphere (Fig. 3) suggests their sequential formation, the first formed sphere being complete. We have never observed any continuity between the membranes of adjacent spheres.

Scrolls: Unique "scroll-like" membrane systems were observed in heterocysts of *Gloeotrichia pisum* (Little Wall Lake) (Fig. 4). These were from 0.4 to 0.9 μ in diameter and ranged up to 2 μ in length. In cross section, these "scrolls" were seen to be composed of a single, clearly tripartite, unbranched membrane 11.5 m μ thick arranged in a spiral with 5 to 13 turns. About 150 m μ apart, these membranes also did not extend into the 0.3 μ central zone where α and β granules, ribosomes and even occasional thylakoidal elements occurred. When a scroll was sectioned longitudinally or obliquely, it was apparent that the general shape was cylindrical. Striking in appearance, the individual membranes were quite different from membranes previously described because of the prominent 5.5 m μ -wide central electron-transparent zone. The adjacent electron-dense layers of the membrane were asymmetrical, with the layers oriented to the outside and inside of the inclusion being 3.5 to 2.5 m μ in width respectively. At the membrane "edges" at the ends of the cylinder and at the inner and outer ends of the "scroll", the membranes simply terminated with no apparent continuity or connection between the two electron-dense zones of a single membrane. Ribosome-like particles 16 m μ in diameter were regularly arranged between the membrane and appeared to be in close contact with the innermost electron-dense zone of the membrane. A number of these scrolls were present in each heterocyst, up to 8 having been observed in a single section of one heterocyst.

Intrathylakoidal spheroids and rosettes: In about 1 out of every 100 cells of *Nostoc muscorum* (ISU J15) examined, the intrathylakoidal spaces contained a cluster of 10 or more small spheroids (Fig. 6). Each spheroid was about 30 m μ in diameter with an outer 3 m μ dense layer. On close study, the dense layer appeared granular, suggesting that it was composed of particulate subunits.

Rosette-like inclusions 85 to 90 m μ in diameter were observed in intrathylakoidal spaces of all cells of *Gloeotrichia pisum* (Little Wall Lake) collected in September 1963 and October 1964 (Fig. 5). Careful study of cells of this same species collected in the same area in August 1964, did not reveal these inclusions. Appearing to be aggregates of spheroids similar to those described above in *Nostoc muscorum* (ISU J15), profiles of these inclusions suggest a central 30 m μ spherical element surrounded by a number

of similar elements. The relative electron-dense appearance of the central element compared to the surrounding elements might be simply due to the fact that superimposed on it are the profiles of spheroids above and below it in the section.

In the intrathylakoidal spaces of *Gloeotrichia echinulata* clusters of 25 m μ

Figs. 5-7. 5, *Gloeotrichia pisum* (Little Wall Lake). Spheroids in intrathylakoidal spaces are in rosette-like aggregates. $\times 70,000$. 6, *Nostoc muscorum* (ISU J15). A mass of 30 m μ intrathylakoidal spheroids (arrow). Several structured granules (sg) are also present. $\times 70,000$. 7, *Gloeotrichia echinulata*. Clusters of 25 m μ "intrathylakoidal spheroids" (arrows). Note the expanded intrathylakoidal spaces (it) characteristic of this species. A structured granule (sg) is at the left. $\times 70,000$.

spheroids were occasionally observed (Fig. 7). These appeared to exhibit even more substructure than either the previously-described spheroids of *Nostoc muscorum* (ISU J15) or *Gloeotrichia pisum* (Little Wall Lake) and at high magnification appeared polygonal in profile.

Intrathylakoidal granules: Membrane-limited, structureless inclusions, with electron densities ranging from low to moderate, were observed in about

Figs. 8-9. 8, *Nostoc pruniforme* (ISU J11). Intrathylakoidal granules (ig) in vicinity of polyhedral bodies (pb). $\times 50,000$. 9, *Microcystis* sp. Intrathylakoidal granules (ig) showing bounding unit membrane. Note continuity with a thylakoid (arrow). $\times 100,000$.

one-third of the collections studied. Profiles of these bodies were circular, ranged from 200 to 400 $m\mu$ in diameter, and were bounded by typical unit membranes (Figs. 8, 9). Occasionally, the surrounding membranes were seen to be continuous with the thylakoidal membranes (Fig. 9).

Figs. 10-15. 10, *Cylindrospermum* sp. Longitudinal section of an aggregate of 4-5 $m\mu$ filaments which passes through a gap in the transverse wall (w). $\times 70,000$. 11, *Cylindrospermum* sp. Cross section of an aggregate of 4-5 $m\mu$ filaments. $\times 110,000$. 12, *Gloeotrichia pisum* (Clear Lake). Compound bundle of 8 $m\mu$ filaments in longitudinal section. $\times 70,000$. 13, *Gloeotrichia pisum* (Clear Lake). Compound bundle of 8 $m\mu$ filaments in cross section. Note associated 14 $m\mu$ filaments (arrow) in cross section. $\times 70,000$. 14 and 15, enlargements of portions of Fig. 13 showing cross sections of 14 $m\mu$ and 8 $m\mu$ filaments respectively. $\times 230,000$.

Fine filamentous inclusions: Filament from 5 to 7 m μ in diameter were found in interthylakoidal cytoplasm of several species. In each case, they were observed in aggregates but with variation in details of their structure and organization.

In about 1 out of 20 sections of cells of *Nostoc muscorum* (ISU J15), profiles of areas packed with 7 m μ diameter filaments were observed. The packets were about as long as the filaments, 0.3 μ , and ranged up to 0.4 μ in width. These inclusions seemed to be more frequent in cells from older cultures.

Less regularly-shaped aggregates of 4.5 m μ filaments were observed in cells of *Cylindrospermum* sp. (Figs. 10, 11). Maximum length of the individual filaments was difficult to determine, but profiles of aggregates as long as 2 μ and as wide as 0.4 μ were observed, although most were smaller. Often these filaments were quite parallel and were regularly spaced about 10 m μ on centers (Fig. 11). In one instance a bundle of these filaments was seen to extend between two adjacent cells through a gap 0.2 μ wide in the transverse cell wall (Fig. 10).

Very unique compound bundles of 8 m μ diameter filaments were observed in about one of every ten sections of cells of *Gloeotrichia pisum* (Clear Lake) (Figs. 12-16). These were roughly cylindrical, ranged up to 3 μ in length and 1 μ in diameter, and consisted of several disc-shaped aggregates, each 150 to 250 m μ thick. Individual filaments were parallel, oriented at right angles to the planes of the discs, and were roughly arranged in rows (Figs. 12, 16). Each 8.4 m μ filament appeared in cross section to be composed of a circular dense outer layer 2.8 m μ wide, and a less dense 2.8 m μ diameter central zone containing a dense central strand 1.5 m μ in diameter (Figs. 13, 15). Masses of even more regularly spaced filaments, larger and of different appearance, were frequently observed in association with these 8 m μ diameter filaments (Figs. 13, 14, 16). About 14 m μ in diameter, these filaments appeared to be hexagonal in cross-section. Relatively electron-dense throughout, they lacked the conspicuous central zone of the smaller filaments but appeared to have a particulate substructure. These filaments were parallel and arranged in rows. In cross section, they were seen in hexagonal arrays with a minimum center-to-center spacing of 15.5 m μ and were apparently interconnected by radiating processes of "arms" (Fig. 14).

Plate-filament arrays: In *Nostoc pruniiforme* (ICC LB756), an unusual arrangement of filaments in association with a plate-like membranous structure was regularly observed (Figs. 17-19). The plates were always located close to the crosswall and usually parallel to it. Roughly circular, about 1.2 μ in diameter and 19 m μ thick, these plates in cross section appeared to consist of 3 layers, 2 outer electron-dense layers about 7 m μ thick and an inner, more electron-transparent layer about 5 m μ thick (Figs. 17, 19). Filaments about 14 m μ in diameter and from 240 to 340 m μ in length projected

out from the plate at right angles. In cross section, these filaments were spaced $7\text{ m}\mu$ apart in a repeating hexagonal pattern with an occasional fila-

Figs. 16-17. 16, *Gloeotrichia pisum* (Clear Lake). Compound bundle of $8\text{ m}\mu$ filaments in longitudinal section. Note associated $14\text{ m}\mu$ filaments in longitudinal section (arrow). $\times 70,000$. 17, *Nostoc pruniforme* (ICC LB756). Longitudinal section of filament. Plate-filament arrays (arrows) in both cells. $\times 70,000$.

ment missing (Fig. 18). Each filamentous element appeared to have an electron transparent inner core about $7\text{ m}\mu$ in diameter surrounded by an

Figs. 18-21. 18, *Nostoc pruniiforme* (ICC LB756). Cross section of filament near cell wall (arrow). Filaments of plate-filament array cut transversely. $\times 70,000$. 19, *Nostoc pruniiforme* (ICC LB756). Longitudinal section of potassium permanganate-fixed cells. Note poor preservation of filamentous component (arrow) of plate-filament arrays. The plate (p) is preserved. $\times 70,000$. 20, *Nostoc pruniiforme* (ISU J11). Convoluted inclusion. $\times 100,000$. 21, *Nostoc muscorum* (ISU J15). Inclusion like that shown in Fig. 20, but with straight elements. $\times 100,000$.

electron-dense layer $3.5\text{ m}\mu$ thick. With potassium permanganate fixation, only the plate was preserved (Fig. 19). These "plate and filament arrays" were observed in almost every cell of this collection when the trichome was sectioned in a median longitudinal plane.

Convolut ed inclusions: Occasionally sections *Nostoc muscorum* (ISU J15) and *Nostoc pruniforme* (ISU J11) showed roughly circular profiles of tubular-like systems up to 0.4μ diameter (Figs. 20, 21). These appeared to consist of electron-transparent, elongate elements $7\text{ m}\mu$ in diameter separated from one another by common $7\text{ m}\mu$ electron-dense walls. An alternative interpretation is that they represent closely appressed, rod-shaped inclusions each about $14\text{ m}\mu$ in diameter and with a $7\text{ m}\mu$ electron-transparent core. The individual elements were arranged in 3-dimensional whorled and convoluted patterns. Gunning (1965) has described similar structures in the chloroplasts of *Avena sativa*. He calls them "stromacenters" and believes they are aggregates of fibers $8.4\text{ m}\mu$ in diameter and of undermined length. Similar arrays, but with straight parallel elements, were occasionally seen in *Nostoc muscorum* (ISU J15) (Fig. 21). These may be homologous with the stromacenter-like elements in that same species or they may represent a different kind of paracrystalline element.

Lamellar bodies: The paracrystalline arrays called "lamellar bodies" by Jensen and Bowen (1961) were again found in the central part of cells of *Nostoc pruniforme* (ISU J11) in 1964 and 1965. This was the same material studied earlier and had been maintained in culture at Iowa State University for over 4 years. Similar structures were found in *Nostoc carneum*, but not in any other collection including *Nostoc pruniforme* (ICC LB756). These were observed in 1 of every 10 profiles of cells that were sectioned medially.

The lamellar bodies in *Nostoc pruniforme* (ISU J11) were from 0.1 to 1μ long, about $130\text{ m}\mu$ wide, and were not membrane-limited. The electron-dense striations, at right angles to the longitudinal axis, were $4\text{ m}\mu$ wide and spaced 15 to $30\text{ m}\mu$ apart (Figs. 22, 23). In some material finer lines parallel to the long axis were observed between the striations. These lines were about $3\text{ m}\mu$ wide and 5 to $6\text{ m}\mu$ apart. These crystals quite regularly had their ends in contact with polyhedral bodies.

The lamellar bodies in *Nostoc carneum* were very similar to those in *Nostoc pruniforme* (ISU J11) except for the fact that the $4\text{ m}\mu$ striations at right angles to the axis were only 10 to $15\text{ m}\mu$ apart. The fine longitudinal lines were $2\text{ m}\mu$ wide and were about $3\text{ m}\mu$ apart (Figs. 24, 25).

The spacing of the elements in all crystalline elements studied varied with the angle of the plane of sectioning to the axis of the crystals. However, the differences in spacing observed in the lamellar bodies of these two species represent a great many observations and appear to be valid and significant.

Areas with some similarities to lamellar bodies, but with poorly defined

boundaries and less regular in organization were occasionally observed in the central part of cells of *Nostoc pruniiforme* (ISU J11) (Fig. 26). In favorable sections, these appeared as straight or slightly curved striations 2 to 3 m μ

Figs. 22-26. 22, *Nostoc pruniiforme* (ISU J11). Longitudinal section of a lamellar body (arrow) associated with two polyhedral bodies (pb). $\times 70,000$. 23, enlargement of a portion of Fig. 22. $\times 220,000$. 24, *Nostoc carneum*. With lamellar body. $\times 70,000$. 25, enlargement of a portion of Fig. 24. $\times 220,000$. 26, *Nostoc pruniiforme* (ISU J11). Paracrystalline zone in central region of cell. $\times 70,000$.

wide, spaced about 20 m μ apart. In favorable sections, as many as three sets of parallel, straight or slightly curved, electron-dense striations 3 m μ wide and with centers 20 m μ apart were observed; typically two sets intersecting at 90° and the third set at 45° to this pair (e.g., 0°-180°, 90°-270°,

and 45° – 225°). About 1μ on a side, profiles of these inclusions are roughly square.

Intrathylakoidal crystals: Membrane-limited clusters of electron-dense, needle-like crystals were found in the peripheral cytoplasm of *Nostoc muscorum* (ISU J10), *Nostoc muscorum* (ISU J17), and *Nostoc ellipsosporum* (Fig. 27). Individual tapered crystals measured from 5 to $15\text{ m}\mu$ wide at their widest point and up to $40\text{ m}\mu$ in length. These inclusions appeared to be regular components of cells of these three species since the majority of sections showed several profiles per cell (Fig. 27). Unlike the structureless intrathylakoidal granules which tend to be centrally located, these crystalline inclusions were located peripherally.

Peripheral crystalloids: Nonmembrane-limited crystalline structures were observed in the peripheral cytoplasm of several species. These generally appeared in sections as striated structures with one or more sets of parallel densities, each density about $4\text{ m}\mu$ in width, with a periodicity from 6 to $9\text{ m}\mu$. In any one profile, the number of intersecting sets of striations observed, their periodicity and the general appearance of the inclusion varied not only with the species, but also with the angle of intersection of the plane of sectioning with the planes of crystalline organization of the inclusion.

The most precisely ordered crystals observed were seen in about 1 out of 20 cells of *Gloeotrichia pisum* (Little Wall Lake) (Figs. 28–30). In favorably sectioned material, these appeared as square or rectangular profiles with 0.5 to 0.7μ sides. Dense parallel striations $4\text{ m}\mu$ wide appeared to be spaced about $8\text{ m}\mu$ on centers and in many sections there was a suggestion of a similar set of striations at right angles to these. Occasionally profiles were observed (Figs. 29, 30) that suggested the basic organization of these inclusions might be a cuboidal lattice of electron-dense particulates, each about $4\text{ m}\mu$ in diameter at the corners of $8\text{ m}\mu$ cubes. It is apparent that such organization would rarely be visible in sections $50\text{ m}\mu$ thick. Instead, linear profiles would be expected, and in many cases even these would be obscured. In fact, at survey levels of magnification, ordered structure within these crystals was difficult to observe. Since these inclusions are about the same size, shape, and relative electron-density as profiles of the polyhedral bodies found in all blue-green algae we have studied, it seems probable that these crystals are of more frequent occurrence in cells of *Gloeotrichia pisum* (Little Wall Lake) than reported here.

In *Nostoc linckia* peripheral crystals, roughly square in profile, averaged from 700 to $800\text{ m}\mu$ in their longest dimension, although some were as large as 1 by 2μ . They were seen in about 1 out of every 10 sections of cells examined, which suggested that they were present in most cells. In profile, these crystals exhibited electron-dense parallel striations $4\text{ m}\mu$ wide which were spaced $6\text{ m}\mu$ on centers. In some cases, they appeared compound and seemed to consist of several smaller crystals, each with its parallel striations

at a different angle (Fig. 31).

Crystals observed in sections of *Nostoc coeruleum* varied in size from about 400 by 400 m μ to the largest observed, 700 by 800 m μ . They were observed in about 1 out of every 10 sections of cells. Although somewhat variable in appearance, these always exhibited striations 4 m μ wide spaced about 9 m μ on centers similar to those observed in *Gloeotrichia pisum* (Fig.

Figs. 27-31. 27, *Nostoc ellipsosporum*. Membrane-limited clusters of crystalline inclusions. $\times 70,000$. 28 and 29, *Gloeotrichia pisum* (Little Wall Lake). Crystalline inclusion. $\times 70,000$. 30, enlargement of a portion of Fig. 29. $\times 200,000$. 31, *Nostoc linckia*. Compound peripheral crystals. $\times 70,000$.

28). In some of these crystals, there was only one set of parallel striations; in others two intersecting sets of parallel striations could be seen such as was observed in some of the crystals in *Gloeotrichia pisum* (Figs. 29, 30).

Cylindrical bodies: The "cylindrical bodies", as originally described in the peripheral cytoplasm of *Symploca muscorum* by Pankratz and Bowen (1963), appeared as two concentric cylindrical densities up to 1μ in length and with a total diameter from 100 to $140\text{ m}\mu$. The central region was usually electron transparent but occasionally contained a dense core. We have verified the presence of these bodies in *Symploca muscorum* (ICC B617) and have found similar structures in *Lyngbya* sp. (ICC 621), *Lyngbya* sp. (ICC 622), *Oscillatoria tenuis* (ICC 428) and *Oscillatoria borneti* (ICC LB143). Despite a careful search, they were not found in the other collections of Oscillatoriaceae studied nor in any of the collections of Nostocaceae. These structures were present after osmium tetroxide, potassium permanganate, and aldehyde fixations. The several elements of the cylindrical body were diffuse and never sharply defined, and we were not able to detect any membranous, filamentous, or crystalline substructure.

Discussion

The great variety of inclusions found in the cells of a relatively few blue-green algal species studied to date is of considerable interest. Each of these inclusions was unique to one or a few species; in most cases it was found only in a single collection and was not present in other collections that key out to the same "species". Despite the fact that the blue-green algae are considered a most ancient group of organisms and that most of the evolution into the present taxa took place eons ago, it is difficult to believe that all of these complicated inclusions are merely cytoplasmic differentiations. It seems reasonable to assume that some, if not most, of the inclusions described are evidence of infections by intracellular symbionts of one sort of another.

Several viruses have been described as infecting blue-green algal cells (Safferman and Morris 1963, Schneider, Diener and Safferman 1964, Safferman and Morris 1964). One which was isolated from sewage was tested against 78 different algae of which 11 proved susceptible including species of *Lyngbya*, *Plectonema* and *Phormidium*. This phycovirus resembled a bacteriophage in that it contained deoxyribonucleic acid, had a polyhedral head about $66\text{ m}\mu$ in diameter and a short tail about $16\text{ m}\mu$ long. There is, however, no evidence that this virus forms ordered structures during its intracellular development except for the appearance of bacteriophage-like heads shortly before lysis (Smith *et al.* 1966).

Ueda (1966) found "virus-like, crystalline granules" in cells of *Oscillatoria princeps*. These appeared as loosely packed rods, $19\text{ m}\mu$ in diameter, and 0.3μ in length, each rod exhibiting a granular substructure of hexagonally-

packed, 3 m μ "subunit particles". These rods bore some resemblance to the 14 m μ filaments of the "plate-filament" arrays of *Nostoc pruniiforme* as well as the larger of the two associated filaments observed in *Gloeotrichia pismus* (Clear Lake).

Sections of cells of *Escherichia coli* infected with the bacteriophage T2 did not exhibit crystalline arrays, but showed an increase in the number of 2 m μ fibers observed in the nucleoplasm compared to uninfected cells. The phage heads only became visible later (Kellenberger, Ryter and Sechaud 1958). However, Schwartz and Zinder (1963) have described a crystalline array composed of dense granules spaced 17 m μ apart in *Escherichia coli* cells 50 minutes after infection with the RNA-containing bacteriophage t2.

Engelbrecht and Esau (1963) related a cross-hatched crystal having a 7 m μ lattice with beet yellows infection, but Cronshaw, Hoefert and Esau (1966) considered that such crystals can be found in the chloroplasts of both infected and uninfected plants. Cronshaw, *et al.* (1966) and Esau, Cronshaw and Hoefert (1967) have shown micrographs of tissue sections of *Beta vulgaris* heavily infected with beet yellows virus which revealed somewhat irregularly-packed, cytoplasmic "flexuous rods" 10 m μ in diameter, each with a 3-4 m μ transparent core. Profiles of these masses bear some resemblance to those of the "convoluted inclusions" we have observed in *Nostoc muscorum* (ISU J15), but they are more like the 7 m μ fine filamentous inclusions we have shown in several species. Arnott and Smith (1967) reported three types of cytoplasmic inclusions in sunflower leaves infected with a virus. One of these, "circular inclusions of lamellate construction", resembles the membrane spheres observed in *Nostoc linckia*. They suggest that these inclusions may represent the protein portion of the virus.

Preliminary efforts have been made to "infect" several crystal-free collections of *Nostoc* with the lamellar bodies of *Nostoc pruniiforme* (ISU J11). In these experiments, crystal-free, actively growing cultures of *N. calcicola*, *N. muscorum* (ICC 486), *N. ellipsosporum*, *N. punctiforme*, and *N. pruniiforme* (ICC LB756) were liberally inoculated with cell-free homogenates of *N. pruniiforme* (ISU J11). After 27 days of growth, the inoculated cultures were examined for crystals with the electron microscope. To date we have had no success.

A number of other kinds of cells known to be infected with viruses have shown intracellular crystalline aggregates; however, many intracellular crystals are not of viral origin. The crystals described by Geselius (1959) in the slime mold, *Dictyostelium*, and by Schnepf (1964) in the cytoplasm of leaf parenchyma cells of *Lathraea* had 9 m μ and 7 m μ periodicities respectively and were generally similar to the crystals described here. Membranous vesicles containing crystalloids have been seen in the cytoplasm of cells from *Avena* coleoptiles (Thornton and Thimann 1964) and in phloem parenchyma cells of *Acer* as well as cultured cells of *Eucalyptus* (Cronshaw 1964).

These crystals also resemble those described here except that the substructural spacing is larger, 15, 16 and 20 m μ for *Avena*, *Eucalyptus* and *Acer* crystals respectively. DeDuve (1960) pointed out, and numerous other workers have confirmed, that microbodies of a variety of animal and plant cells often contain similar crystals, presumably proteinaceous (Vigil 1969). Although crystals resembling the peripheral crystals of the blue-green algae have frequently been called proteinaceous in the literature, we would prefer to be cautious until they have been better characterized by cytochemical procedures or fractionation followed by microchemical analysis.

The membranous inclusions, in particular the scrolls and membranous spheres, are puzzling. At the present time, we can only speculate as to their role in the cell. Are these structures normal elements evolved over long periods and characteristic of hitherto unrecognized taxa? On the other hand, as previously suggested for the total of inclusions described here, these may be reaction products of some non-normal element in the environment, most likely infection by a symbiont. The most commonly observed biological membranes, the so-called unit membranes, have no edges nor do they bifurcate in healthy, normal cells. Certainly such processes as pinocytosis, the "reverse pinocytosis" of secretion, formation of vesicles from Golgi dictyosomes, and development of thylakoids in chloroplasts must involve breakage and rejoining of edges of membranes. Apparently, such events are never observed as actual breaks in the profiles of membranes in sections of well-fixed cells. Such membrane disruption must occur very rapidly—much like the breakage and rejoining of the soap film in the formation of a bubble from a bubble pipe. In addition to exhibiting breaks and bifurcations, profiles of the inclusions described here were quite different in appearance from the typical "unit" membranes of the plasma membrane and the thylakoids.

Despite the ubiquity of 12 to 27 m μ -wide microtubules in eukaryotic cells, until recently no microtubular components except for the bacterial flagellum have been described in prokaryotic cells. Pfennig and Cohen-Bazire (1967) reported intracytoplasmic fibrillar organelles in the green bacterium *Pelodictyon clatratiforme*. These organelles bear a superficial resemblance to the plate-filament arrays observed in *Nostoc pruniiforme* (ICC LB756). Van Iterson, Hoeniger and Van Zanten (1967) have described microtubules in the cytoplasm of *Proteus vulgaris*. These were 20 m μ in width with a 6 m μ core and ranged up to 0.45 μ in length. They were slightly larger than the 13 m μ diameter flagella of this species. These workers were in doubt whether these microtubules represented a normal cell constituent or "the consequence of induction by a stimulus of foreign origin".

Summary

The fine structure of some sixty different collections of blue-green algae representing at least thirty different species was studied. In addition to the

usual components of blue-green algal cells, a number of different, unique, intracellular structures were observed. Membranous "stacks", and "spheres", and "scrolls" in which the membranes are not typical unit membranes are described. "Spheroids", 25 to 30 $m\mu$ in diameter, were found in intrathylakoidal spaces of several collections. Other inclusions, including a variety of filamentous and crystalline arrays, are described. It is suggested that some of these inclusions are intracellular symbionts.

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