THE FINE STRUCTURE OF THE NUCLEAR MATERIAL OF A BLUE-GREEN ALGA, *ANABAENA CYLINDRICA* LEMM.

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ABSTRACT

The chromatinic material of the blue-green alga *Anabaena cylindrica* has complex configurations in the central regions of the cells. The distribution of the chromatin within the cells varies in different filaments, probably in response to variations in the disposition of other cellular components. In electron micrographs of thin sections of organisms fixed by the method of Kellenberger, Ryter, and Séchaud (1958) the centroplasm contains fibrillar and possibly granular components which can be identified as the nuclear material by comparison with stained preparations viewed in the light microscope. The fibrils in the nuclear regions have diameters in the range of 5 to 7 mμ and are embedded in a matrix of lower density. The nuclear regions are not greatly different from the cytoplasm in their electron density. Reducing the calcium content of the fixative results in coagulation of the fibrils to form coarser structures. The significance of the observations is discussed in relation to observations on the fine structure of other classes of algae and of bacteria.

INTRODUCTION

It has long been realised that the Myxophyceae (Cyanophyceae) differ from all other classes of algae in many characteristics (Fritsch, 1945). In other algae the photosynthetic pigments are located in bodies which are large enough to be easily visible in the light microscope, the nuclei contain chromosomes, and all, except the Rhodophyceae, possess flagella. The blue-green algae lack these features and have a simpler structure resembling that of bacteria; they also resemble certain bacteria in that diaminopimelic acid is present in their cell walls (Work and Dewey, 1953). Cohn (1853) suggested that the Myxophyceae are closely related to the bacteria and this view has been generally accepted, although Pringsheim (1949) stressed the differences between the two groups and concluded that they are not related. In view of this difference of opinion it was of interest to study the fine structure of a blue-green alga by a method that has proved rewarding in the study of bacteria (Kellenberger, Ryter, and Séchaud, 1958; Ryter and Kellenberger, 1958; Glauert and Hopwood, 1960; Glauert, Brieger, and Allen, in press).

The fine structure of the nuclear material of bacteria differs from that of all other organisms so far studied, including green, brown, red, and yellow-green algae. This investigation, therefore, has been mainly concerned with the nuclear material of the blue-green alga, *Anabaena cylindrica*, to see whether its fine structure resembles that of bacteria, or of other algae, or whether it differs from both.

MATERIALS AND METHODS

Organism: *Anabaena cylindrica* Lemm., No. 1403/2 of the Botany School culture collection of algae and

813

protozoa, University of Cambridge, kindly supplied by Mr. E. A. George.

Method of Growth: The organism was grown in pure culture on an agar medium containing soil extract and mineral salts. The cultures were illuminated by light from daylight fluorescent tubes at an intensity of approximately 350 foot-candles for periods of 16 hours each day.

Light Microscopy: Filaments were removed from the culture with a platinum loop, spread on the surface of thin coverslips in drops of distilled water, and fixed in the vapour of a 2 per cent solution of osmium tetroxide for 4 minutes. The coverslips were rinsed briefly in distilled water and then immersed in N-HCl at 60°C. for 8 minutes. After another brief wash in distilled water, the organisms were stained in azure A-SO_2 (DeLamater, 1951) for 3 to 4 hours and mounted in distilled water. Photomicrographs were taken on 35 mm. Ilford microneg film with a 1.4 N.A. apochromatic objective at a magnification of about 300.

Fixation for Electron Microscopy: Samples of growing organisms, consisting of bundles of parallel filaments, were removed from the surface of the culture medium with a platinum loop and placed in the fixative; the filaments are surrounded by a layer of mucilaginous material which causes them to remain together during the fixation and embedding procedures. The fixation schedule of Kellenberger, Ryter, and Séchaud (1958) was followed exactly, except that the prefixation was omitted. In one experiment the content of calcium chloride in the fixative was reduced; the fixative contained 0.005 M $CaCl_2$ instead of 0.01 M. After fixation for about 16 hours, the specimens were immersed for 1 hour in a 0.5 per cent aqueous solution of uranyl acetate.

Embedding: The specimens were dehydrated in ethanol and embedded in *n*-butyl methacrylate by the standard procedure (Newman, Borysko, and Swerdlow, 1949).

Thin Sectioning: The blocks were sectioned on an A. F. Huxley microtome with a glass knife. The sections were collected on the surface of a 10 per cent solution of acetone in water, spread by treatment with xylene vapour (Satir and Peachey, 1958) and mounted on grids coated with thin collodion or carbon films.

Staining of Sections: Some sections were stained with

Light microscope photographs of the blue-green alga *Anabaena cylindrica* fixed in the vapour of 2 per cent osmic acid, and stained with azure A-SO_2 (DeLamater, 1951) for chromatin. Magnification 2,600. The scale mark represents 10 microns.

FIGURE 1

Two trichomes in which the chromatinic material is mainly concentrated in the central regions of the cells. Stages in division of the chromatinic bodies can be seen. The cells are of uniform diameter, except for those at the growing tip of the left-hand trichome.

FIGURE 2

Trichomes similar to those in Fig. 1, in which some cells have nearly completed division and contain dumb-bell–shaped chromatinic bodies (arrow).

FIGURE 3

Trichomes in which the chromatinic material is more widely dispersed than in those in Figs. 1 and 2. One trichome contains a heterocyst (*H*).

FIGURE 4

Electron micrograph of thin section of *Anabaena cylindrica*. An unstained section of a dividing cell fixed by the method of Kellenberger, Ryter, and Séchaud (1958), but with less calcium in the fixative. The nuclear material (*N*) is slightly coagulated and appears denser than the rest of the cell. The chromatoplasm contains gas vacuoles (*G*), lamellae (*L*), dense granules (*D*), and cyanophycin granules (*CY*). A septum is forming at *S*. × 53,000. The scale mark represents 0.1 micron.
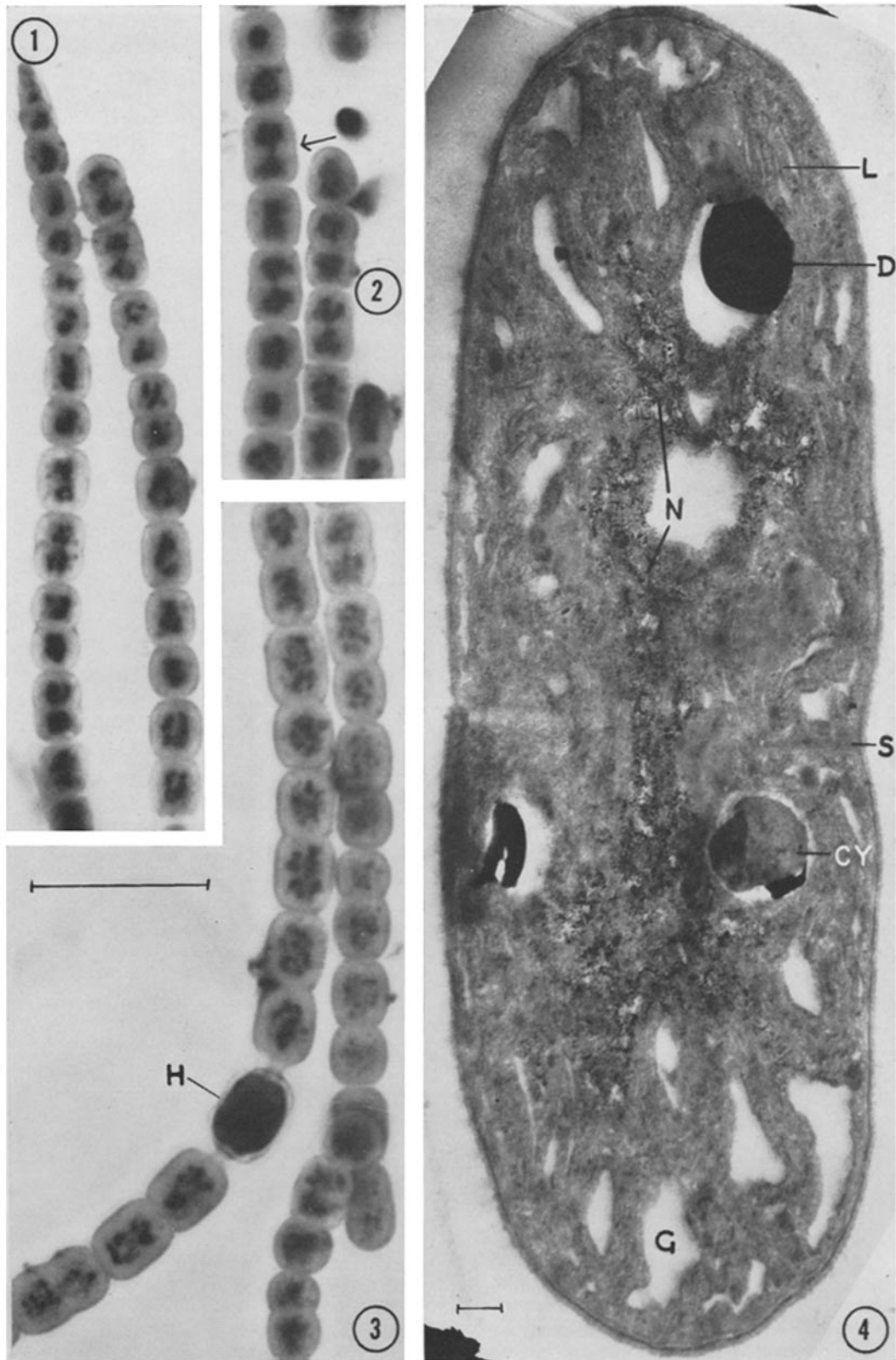
lead hydroxide (Watson, 1958) in a plastic syringe (Peachey, 1959), or with aqueous uranyl acetate (Gibbons and Bradfield, 1956).

Electron Microscopy: The sections were examined in a Siemens Elmiskop I, operating at 80 kv. with a 50μ objective aperture, and photographs were taken at instrumental magnifications of 10,000 and 20,000 on Ilford special contrasty lantern plates.

RESULTS

A culture of *Anabaena cylindrica* consists of long chains of cells or "trichomes" (Figs. 1 to 3 and 5) surrounded by mucilaginous material (Figs. 5 and 6, M) which often holds adjacent filaments together in a parallel array (Fig. 5). Each trichome consists of cells of uniform diameter, staining properties, and fine structure, but the cells of different filaments vary considerably. The cells at the growing tips are narrower than those in the rest of the trichome (Fig. 1). Cell division occurs along the whole length of the trichome in a plane at right angles to the axis of the filament, so that the filament merely elongates during growth, without branching; many developing septa are visible (Fig. 5). At intervals specialised cells, the heterocysts, appear (Fig. 3, H), the function and significance of which are unknown (Fritsch, 1945; Fogg, 1951).

Light Microscopy

In the light microscope the protoplasm of living cells of *Anabaena cylindrica*, like that of other blue-green algae, is seen to have a peripheral chromatoplasm, containing the photosynthetic pigments, and a central, colourless centroplasm. The chromatinic material, stained with azure A-SO₂, has complex configurations, mainly within the centroplasm but with ramifications into the chromatoplasm. Each non-dividing cell appears to have a single complex chromatinic body, while

cells in the process of division have a dumb-bell–shaped body (Fig. 2). The distribution of the chromatinic material varies in different filaments; in its most condensed state the chromatin appears as a dense central body (Fig. 2), while in other trichomes it appears as a "spongy" network (Fogg, 1951) which ramifies throughout much of the cell (Fig. 3). This difference in distribution of the chromatin is probably due to differences in the age or physiological state of the cells.

Electron Microscopy

General Organisation of the Cells: On the basis of observations made with the light microscope, the cells of blue-green algae have been described as lacking the rigid cell wall that is present in bacteria and as being bounded instead by two layers, the "cell-sheath" which appears to be a firmer portion of the mucilaginous envelope, and the "inner investment," which completely surrounds the cell and forms the septa between adjacent cells (Fritsch, 1945). In thin sections of *Anabaena cylindrica* these two layers are clearly seen. The outer layer is the less dense of the two and appears to be fibrous; it thus resembles the sheaths of other blue-green algae (Metzner, 1955; Niklowitz and Drews, 1956; Drawert and Metzner-Küster, 1958). In *A. cylindrica* the sheath has many fine connections with the mucilaginous envelope, which also appears to have a fibrous structure (Fig. 6). The connections are barely visible in unstained sections, but are clearly revealed by staining with lead hydroxide (Fig. 7). The inner investment is continuous with the septa; its nature is uncertain, but it may have some properties in common with the cell walls of bacteria, since in *Oscillatoria amoena* it has been found to be sensitive to treatment with lysozyme (Fuhs, 1958 c).

The peripheral chromatoplasm of the cells contains lamellae (Figs. 4 to 7, L) which are associated

FIGURE 5

Thin section of *Anabaena cylindrica*. A low power electron micrograph of a bundle of parallel trichomes. After fixation exactly according to the formula of Kellenberger, Ryter, and Séchaud (1958) the centroplasm (C) has a similar density to the rest of the cell and the outlines of the nuclear regions are not distinguishable at this low magnification. The peripheral chromatoplasm contains gas vacuoles (G) and lamellae (L). The trichomes are surrounded by mucilaginous material (M). Section stained with uranyl acetate. × 16,000. The scale mark represents 1.0 micron.
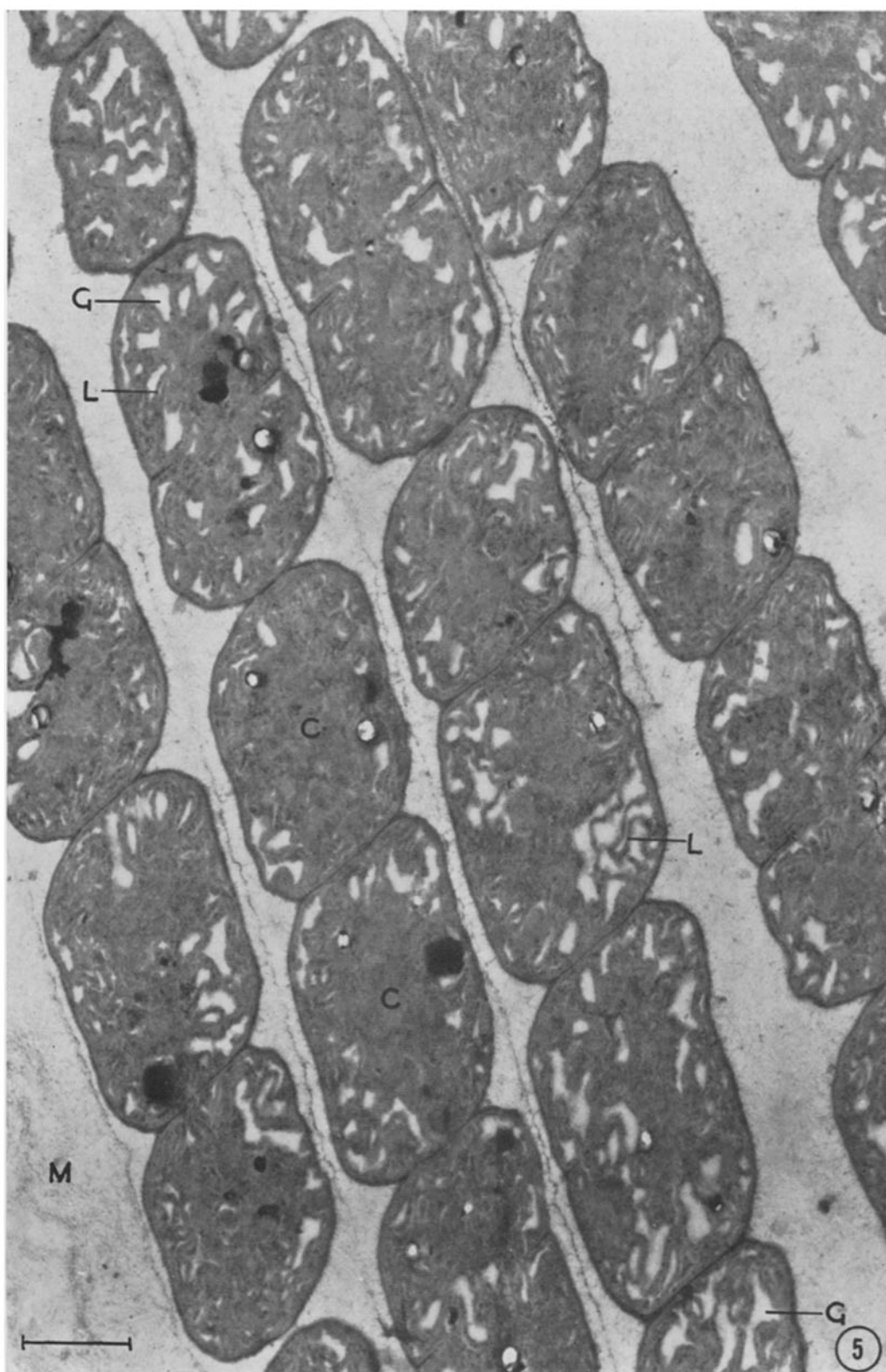
with dense granular material. In some regions the lamellae are separated from one another by irregular lacunae (Figs. 4 to 7, G), which, on the basis of their size, shape, and distribution can be identified with the gas vacuoles observed in the light microscope (Fritsch, 1945). Large dense granules (Figs. 4 and 7, D) probably correspond to volutin because they have the same appearance in electron micrographs as the granules which have been identified as the metachromatic volutin granules of bacteria (Glauert and Brieger, 1955). Granules of low density (Fig. 4, CY), which may be cyanophycin granules, are also present in the chromatoplasm. Similar low density granules have been described in thin sections of other blue-green algae (Drews and Niklowitz, 1956, 1957; Fuhs, 1958 b), where they have been called mitochondrial equivalents.

The Nuclear Material: In electron micrographs at low magnifications (about 16,000, Fig. 5) the nuclear material is not easily distinguished from other structures within the cell. The centroplasm has a uniformly granular appearance and has the same density as the lamellae and granular material in the chromatoplasm. However, when the content of calcium in the fixative is half that in the formula of Kellenberger, Ryter, and Séchaud (1958), some very dense material is visible in the centroplasm (Fig. 4). By comparison with the configurations of the chromatinic material in stained preparations viewed in the light microscope (Fig. 2, arrow), this dense material can be identified as the nuclear material. Under these conditions of fixation, the nuclear material in electron micrographs of unstained sections is considerably denser than the rest of the cell and consequently the boundaries of the nuclear regions are easily recognised (Fig. 4, N). The nuclear material is seen in the centre

of the cell, and also extends within the lamellae between the gas vacuoles of the inner regions of the chromatoplasm. Staining with uranyl acetate increases the density of the cytoplasm so that the contrast between the nuclear material and the cytoplasm is reduced (Fig. 6).

In organisms fixed exactly according to the formula of Kellenberger, Ryter, and Séchaud, the boundaries of the nuclear regions are much less distinct (Figs. 5 and 7), but irregular areas, with a different fine structure from that of other parts of the cell, are discernible in the central regions (Fig. 7, N). These areas contain dense round profiles, 5 to 7 mμ in diameter, and elongated less dense profiles, about 5 to 7 mμ wide, within a finely granular matrix of lower density (Fig. 8). Since the distribution of such areas is similar to that of the dense material in organisms fixed with a lower concentration of calcium (Figs. 4 and 6) and to that of the chromatinic material seen in the light microscope (Figs. 1 to 3), these areas may be identified as the nuclear regions.

DISCUSSION

The configurations of the chromatinic material of *Anabaena cylindrica* stained with azure A-SO$_2$ resemble those described in cells stained by the HCl-Giemsa method by Cassel and Hutchinson, (1954). As these authors point out, the staining properties and configurations of the chromatinic bodies of blue-green algae are similar to those of the chromatinic bodies of bacteria. Robinow (1956) emphasised that "the chromatin structures of both classes of organisms share the lack of a limiting membrane, divide in a simple way not involving spindles and recognizable chromosomes

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Figure 6

Electron micrograph of thin section of *Anabaena cylindrica*. A dividing cell fixed by the method of Kellenberger, Ryter, and Séchaud (1958), but with less calcium in the fixative. Section stained with uranyl acetate. The nuclear material (N) is slightly coagulated and after staining with uranyl acetate it does not appear much denser than the rest of the cell. The chromatoplasm contains gas vacuoles (G) and lamellae (L). The cell is bounded by two layers; an inner dense layer which is continuous with the septa (S) and an outer less dense layer. The fibrous nature of the mucilaginous envelope (M) surrounding the trichome is emphasised by the staining with uranyl acetate. X 59,000. The scale mark represents 0.1 micron.

and have no distinct resting stages." Figs. 1 to 3 of this paper show that the shapes assumed by the chromatinic material of *A. cylindrica*, like those of bacterial chromatinic bodies, vary from cell to cell. It is probable that the chromatin occupies spaces of varying shape between and within the lamellae of the inner chromatoplasm, and may thus be forced to assume different shapes in the same way that the configurations of bacterial chromatin may be modified by the presence of lipid inclusions (Delaporte, 1939/1940; Robinow, 1956). Thus the exact shapes of the chromatinic material of blue-green algae may not be very significant, and attempts to determine the number of units of hereditary material present in a cell by cytological observations (Fuhs, 1958 *a*) in the absence of genetical data are hazardous.

This study has shown that the fine structure of the nuclear material of *Anabaena cylindrica* differs from that of the nuclei of the other classes of algae that have been examined in the electron microscope. Members of the Chlorophyceae (Sager and Palade, 1957), Xanthophyceae (Greenwood, Manton, and Clarke, 1957), Phaeophyceae (Manton and Clarke, 1956; Manton, 1957), and Rhodophyceae (Brody and Vatter, 1959) have nuclei that are bounded by membranes and resemble those of higher organisms. In contrast, no nuclear membrane can be detected either in *A. cylindrica* or in *Oscillatoria formosa* (Bradfield, 1956); in this the Myxophyceae resemble the bacteria.

The fine structure of bacterial nuclear material

varies with the method of fixation (Ryter and Kellenberger, 1958). After fixation by the method of Palade (1952), it appears as dense structures within a transparent "vacuole," whereas after fixation by Kellenberger, Ryter, and Séchaud's method (1958) the nuclear regions are of lower average density than the cytoplasm and contain fibrils with diameters of 3 to 6 mμ. Ryter and Kellenberger have reasoned that the finely fibrillar nuclear material is the nearest approximation to the real structure so far obtained, and that dense structures within a "vacuole" are produced by coagulation after imperfect fixation. Giesbrecht (1959), however, has taken the opposite view and has interpreted the dense structures in the nuclear regions of *Bacillus megaterium* as sections of a chromosome built up from a fine fibril by repeated coiling; he considers the finely fibrillar nuclear material to be produced by artificial uncoiling of the chromosome during fixation and embedding. It is not yet possible to decide objectively between these two opposing interpretations, but the picture revealed after fixation by Kellenberger, Ryter, and Séchaud's method seems to be more satisfactory than that produced by other methods of fixation since it is difficult to believe that the bacterial nucleus consists of a chromosome suspended in a completely electron-transparent space. Moreover, this method of fixation also results in better preservation of complex membranous systems in the cytoplasm of bacteria (Glauert and Hopwood, 1960; Glauert, Brieger, and Allen, in press).

The nuclear regions of bacteria fixed by the

FIGURE 7

Electron micrograph of thin section of *Anabaena cylindrica*. A cell fixed exactly according to the formula of Kellenberger, Ryter, and Séchaud (1958). Section stained with lead hydroxide. The nuclear material (*N*) occupies the central region of the cell and ramifies among the lamellae (*L*) and gas vacuoles (*G*) of the chromatoplasm. The two layers of the cell boundary are clearly seen; the inner, dense layer is continuous with the septum (*S*) and the outer, less dense layer appears to be fibrous and is connected with the mucilaginous envelope (*M*) by numerous fine fibrils which are emphasised by staining with lead hydroxide. × 50,000. The scale mark represents 0.1 micron.

FIGURE 8

Electron micrograph of thin section of *Anabaena cylindrica*. Part of the nuclear region of a cell. The nuclear material appears to consist of fine fibrils (arrow), and possibly granules, about 5 to 7 mμ in diameter, within a matrix of lower density. Section stained with uranyl acetate. × 150,000. The scale mark represents 0.1 micron.
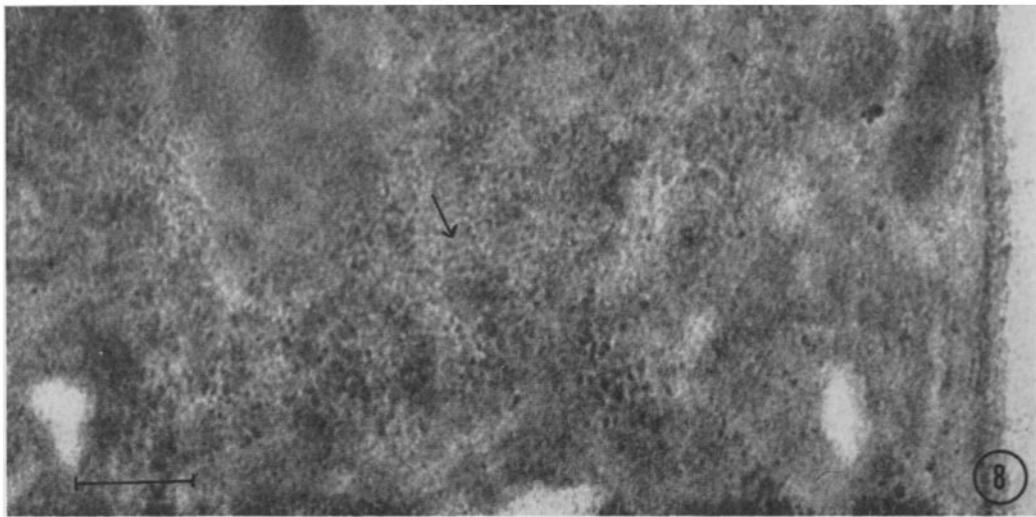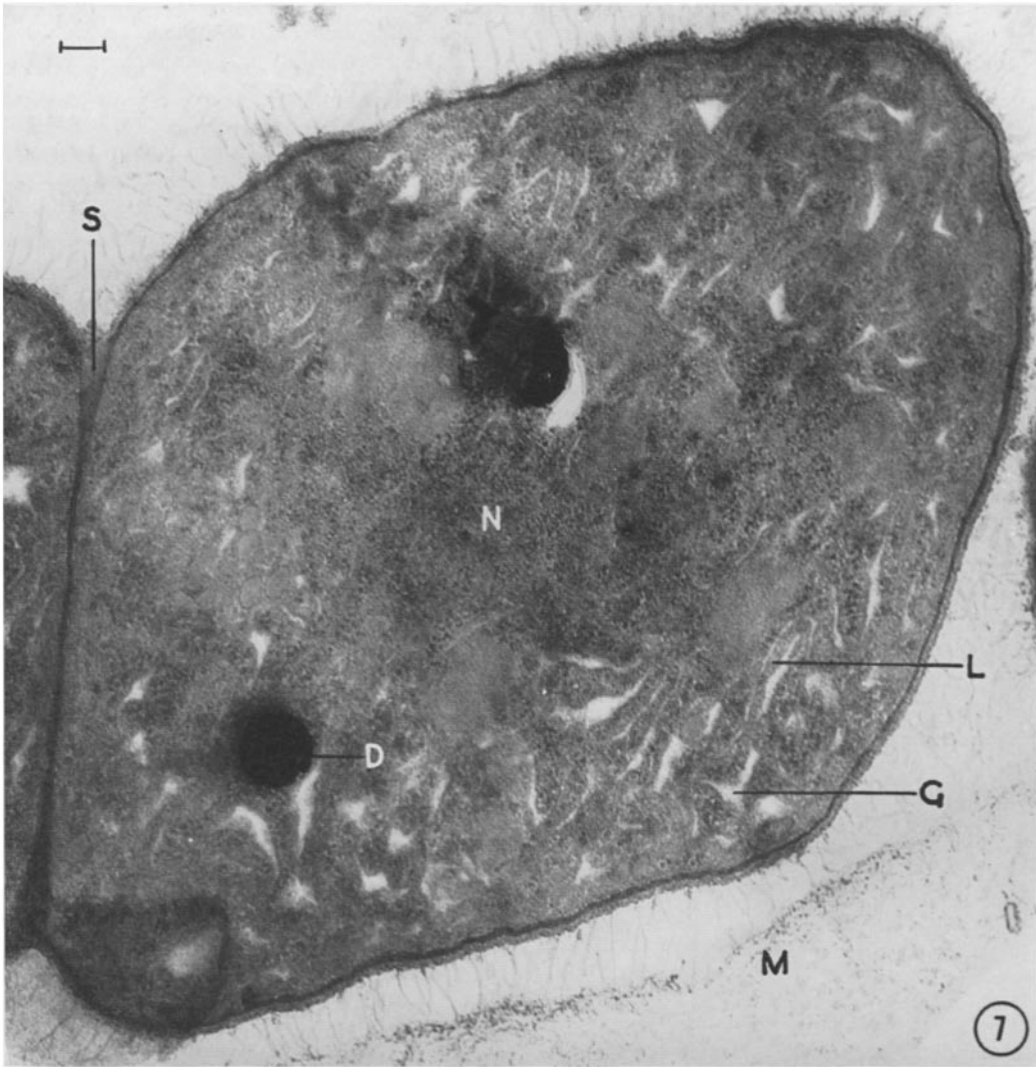
method of Kellenberger, Ryter, and Séchaud have a general resemblance in that they all appear to contain fine fibrils and possibly granules within a matrix of low density. These regions, although they are not bounded by a membrane, are readily distinguishable from the rest of the cell as a result of their lower average density. Differences in the concentration and arrangement of the fibrils of the nuclear regions of different species are evident in the published micrographs (Ryter and Kellenberger, 1958; Glauert, Brieger, and Allen, in press; van Iterson and Ruys, 1960; Hopwood and Glauert, 1960), but perhaps these differences should not be stressed until further work has been done on a greater range of organisms in different physiological states.

The nuclear fine structure of Anabaena cylindrica has a general resemblance to that of bacteria, and appears to be most like that of the actinomycete Streptomyces coelicolor (Hopwood and Glauert, 1960). Moreover the nuclear material of A. cylindrica also resembles that of bacteria in its response to slight changes in the composition of the fixative, particularly its content of calcium ions (Ryter

and Kellenberger, 1958). This similarity of response is also suggested by the observations of Drews and Niklowitz (1956) on Phormidium uncinatum and of Fuhs (1958 b) on Oscillatoria amoena fixed by Palade's method. In their preparations irregular dense masses in "vacuoles" in the centroplasm were identified as the nuclear material, which thus resembled the nuclear material of bacteria fixed by the same method.

The nuclear regions of Anabaena cylindrica differ from those of bacteria in not having a lower average electron opacity than the cytoplasm. This observation agrees with the fact that the nuclear material of A. cylindrica has a higher ultraviolet absorption than the cytoplasm (Fogg, 1951), while in rapidly growing bacteria the reverse is true (Dr. A. Cosslett, personal communication). In bacteria the high ultraviolet absorption of the cytoplasm has been attributed to a high content of ribonucleoprotein, and possibly one of the main differences between blue-green algae and bacteria is in the composition of the cytoplasm rather than in a basically different organisation of the nuclear material.

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