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WILDMAN, Ruth Bowman, 1924-

STUDIES ON THE NUCLEOPLASM AND THE PHOTOSYNTHETIC PIGMENT SYSTEM OF BLUE-GREEN ALGAE.

Iowa State University, Ph.D., 1969
Botany

University Microfilms, Inc., Ann Arbor, Michigan

STUDIES ON THE NUCLEOPLASM AND THE PHOTOSYNTHETIC PIGMENT SYSTEM OF BLUE-GREEN ALGAE

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Cell Biology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Chairman, Advisory Committee
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Signature was redacted for privacy.

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Signature was redacted for privacy.

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1969

TABLE OF CONTENTS

INTRODUCTION

The classification of organisms has long presented a challenge to biologists. Many systems have been proposed, a few have been granted temporary acceptance, but none has enjoyed complete approval by the scientific community. Perhaps the most useful delineation to the cytologist is the simplest--the distinction between procaryotic and eucaryotic organisms. At the ultrastructural level, this separation is unambiguous. The concept of procaryotic and eucaryotic cell types based on the pattern of cellular organization was introduced in 1937, but not until the advent of electron microscopy and other instrumental tools and techniques for ultrastructure research was this classification strengthened and widely accepted.

The procaryotes encompass the bacteria and the blue-green algae. They lack structural separation of the subunits of cellular function. There are no membrane-bounded nuclei, plastids, or mitochondria. The flagellum, if present, is a single fibril without a 9 + 2 arrangement. DNA is diffuse, and no mitotic apparatus is found. The eucaryotes include the fungi, all other algae, protozoa, and higher plants and animals. They are characterized by membrane-bounded nuclei and organelles, a 9 + 2 arrangement of flagellar fibrils, and genetic material which is organized into complex nucleoprotein chromosomes.

The Cyanophyta (blue-green algae) are almost as ubiquitous as their fellow procaryotes, the bacteria. Few ecological niches are known without some species of blue-green algae adapted to exist there. Growths have been reported in a wide range of habitats including desert (Forest and Weston, 1966), saline, and fresh water environments, parasitic and symbiotic associations with other organisms, and such temperature extremes as hot springs and ice-covered lakes (Reif and Fine, 1967).

Like the bacteria, blue-green algae are both a boon and a burden to mankind. They show some promise for improving soil fertility in marginal areas due to the ability of many species to fix nitrogen. Pilot experiments have been undertaken in Japan and India to study this potential. In the United States, the binding ability of blue-green algae in retarding erosion and loss of water from soil has been demonstrated (Echlin, 1966). Better known, however, are the troublesome features. Subaerial species colonize and discolor roof shingles from Mexico to British Columbia (Brook, 1968). Blue-green algae often "bloom" in great profusion during warm summer months, fouling recreational lakes and water supplies and even causing the death of water fowl and animals in isolated, unusual cases of toxic growths (Gorham, 1960). More common than the sporadic occurrence of death factors is the alarming rise in populations of blue-

green algae associated with the increasing pollution of lakes and streams. Although the health problems traceable to bacteria may be more serious and widespread than the inconveniences resulting from blue-green algal growths, the long-range implications can no longer be ignored.

The advantages of short generation time and ease of culturing have made bacteria desirable tools for metabolic and genetic research, and extensive use has been made of them. The geneticists' workhorse, Escherichia coli, has been invaluable in research leading to an understanding of genetic mapping, transcription, translation, and control of inheritance. In comparison, the blue-green algae have been far less tractable and, as a result, less studied and understood. Species which bloom profusely in nature often fail to grow in unialgal culture in the laboratory. Axenic cultures are almost impossible to attain and maintain (Vance, 1966).

Blue-green algae are believed to divide asexually only. Two cases of apparent genetic recombination have been reported but not conclusively confirmed (Kumar, 1962; Singh and Sinha, 1965). From the geneticist's viewpoint, almost nothing is known about the Cyanophyta, and only a modest amount of work has been aimed toward the physiology of these organisms. There are many good descriptions of individual species at the ultrastructural level. Several research

groups have carried out biochemical studies of the photosynthetic pigments in the Cyanophyta and their relationships under varying conditions of light intensity and wave length. However, a large gap remains between description of fine structure and knowledge of macromolecular constituents of the blue-green algal cell. A vast area of understanding of chemical composition and function in relation to morphology needs to be elucidated.

The research reported in this dissertation is intended to be a contribution to our understanding of the nucleoplasm and the photosynthetic pigment systems in some representative blue-green algae. The plan of attack was to carry out exploratory surveys in these areas, including a search for methods of isolating and characterizing high molecular-weight blue-green algal DNA, an effort to correlate the morphology and ultrastructure of the thylakoid system with physico-chemical information on the component photosynthetic pigments, and studies of the cytological and biochemical effects on the pigment system of varying intensity and wavelength of illumination.

LITERATURE REVIEW

Organization of the Procaryotic Cell

Comparisons of bacteria and blue-green algae were published as early as 1853 and have been the subject of many publications (Pringsheim, 1949; van Neil, 1955; Stanier and van Neil, 1941, 1962; Stanier et al., 1963; Echlin and Morris, 1965). Over the years, taxonomic grouping together of the blue-green algae and bacteria has fallen in and out of favor and has been both staunchly defended and decried. The accumulation of evidence during the past ten years has stressed the structural similarities of these two groups of organisms. Because of these similarities, the bacteriologists' tools and techniques have resulted in a body of information that provides a valuable background for discussion of the blue-green algal cell.

Bacteria and blue-green algae are bounded by outer layers of varying complexity but roughly comparable in structure and composition. Most blue-green algae are surrounded by a mucilaginous sheath composed of fibrils (Lang, 1968a). Four types of comparable gelatinous layers have been recognized to surround bacteria. The cell wall or envelope gives rigidity to the procaryotic cell and protects the protoplasm from damage. Perkins (1963), Martin (1966), and Salton (1964, 1967) have reviewed research on bacterial

cell walls, and Lang (1968a) has discussed work on blue-green algae in the same area. The chemical composition of the walls of bacteria and blue-green algae has been studied and found to be similar (Frank et al., 1962a, 1962b; Drews and Meyer, 1964; Höcht et al., 1965). A macromolecular mucopolymer (murein, mucopolysaccharide, mucopeptide) composed of a polysaccharide backbone cross-linked by short peptide chains gives mechanical strength to the procaryotic cell. It comprises a major part (40-90%) of the Gram-positive wall, 4-21% of the Gram-negative wall, and about 50% of the wall of several blue-green algae (Frank et al., 1962a, 1962b; Höcht et al., 1965). Diaminopimelic acid (Work and Dewey, 1953) and muramic acid, components of the mucopolymer, appear to be unique to procaryotic cells and absent from eucaryotic cells. Echlin and Morris (1965) discussed the structural similarity of the wall layers of blue-green algae and Gram-positive bacteria but noted that chemically the blue-green and Gram-negative walls showed greater likeness. In fine structure, the blue-green algal cell wall and the Gram-negative bacterial wall parallel each other closely (Claus and Roth, 1964; Cohen-Bazire et al., 1964; Steed and Murray, 1966). The mucopolymer had been thought to occupy the electron-transparent zone immediately peripheral to the plasma membrane (Frank et al., 1962a). More recently Allen (1968b) proposed that the electron-transparent

zone may be artifact. Based on analogy to the location of the mucopolymer layer in Escherichia coli as described by Murray et al. (1965), Allen (1968b) concluded that the blue-green algal mucopolymer layer is electron dense and lies next to the plasma membrane, either appressed to it or separated from it by a transparent zone in some electron micrographs.

The plasma membrane of bacteria and blue-green algae gives the typical image of a "unit membrane" measuring 70-80 A in width.

The mitochondrion has been established as the site of respiration in the eucaryotic cell, and some of its ultrastructural organization has been correlated with segments of biochemical respiratory pathways. However, the same metabolic processes in procaryotes appear to require the intact cell. The location of enzymes of the electron transport system and the tricarboxylic acid cycle of several bacteria has been traced to the plasma membrane. Drews and Niklowitz (1956, 1957) suggested that intracytoplasmic inclusions in blue-green algae which they called structured granules might be mitochondrial equivalents. Complex vesicular, lamellar, or tubular packets of membrane enclosed by invaginations of the plasma membrane and called mesosomes (Fitz-James, 1960), plasmalemmosomes (Edwards and Stevens, 1963), or chondrioids (van Iterson, 1965) have been found in

Gram-positive bacteria and less frequently and in simpler form in Gram-negative organisms. Salton (1967) has reviewed the current status of the mesosome, including the functions attributed to it but not yet conclusively established: a center of respiration (mitochondrial equivalent), anchors in cell division for separation after replication of the bacterial chromosomes, a role in new wall formation, a site of formation and secretion of exocellular proteins, and a site of fatty acid biosynthesis and formation of new membranes. Mesosomes usually appear close to septa and may change with the physiological condition of the organism. Similar invaginations which appear to be derived from the plasma membrane or thylakoid membranes have been reported in Anacystis nidulans and called lamellasomes by Echlin (1964a).

The ultrastructure of the blue-green algal cell with descriptions of the diverse inclusions has been discussed by Ris and Singh, 1961; Chapman and Salton, 1962; Wildon and Mercer, 1963a, 1963b; Pankratz and Bowen, 1963; Lang, 1968a, 1968b. In general, blue-green algae are richer in cytoplasmic inclusions than bacteria. Procaryotic cells lack true membrane-bounded vacuoles but expanded intrathylakoidal spaces and pseudovacuoles (gas vacuoles) have been described in a number of species of blue-green algae (Bowen and Jensen, 1965; Jost, 1965; Jost and Matile, 1966;

Smith and Peat, 1967a, 1967b). De novo production of gas vacuoles can be induced in Nostoc muscorum (Waaland and Branton, 1969). Many inclusions are species- or collection-specific but those which appear to be common to all Cyanophyta include α-granules, β-granules, structured granules, and polyhedral bodies. Lefort (1960b), Fuhs (1963), Leak and Wilson (1965), and Edwards et al. (1968) have described ribosomes in the nucleoplasm of blue-green algae. Ribosomes are found in all living organisms, but those of bacteria and blue-green algae are smaller than the cytoplasmic ribosomes of eucaryotes (Ris and Singh, 1961; Wildon and Mercer, 1963; de Ley, 1964). Craig and Carr (1967, 1968b) compared the physical properties of ribosomes isolated from blue-green algae and bacteria.

The genetic material of the procaryotic cell is not organized into nucleoprotein chromosomes which undergo mitotic division nor is it contained within a membrane-bounded area but is distributed throughout a diffuse, roughly central region called the nucleoplasm (central body, centroplasm, chromatinic body, nucleoid, nuclear region). Robinow (1956, 1962) reviewed work on bacterial chromatin. Since the nucleoplasm of the blue-green algal cell is a major concern of this dissertation, pertinent literature is reviewed later in detail.

The photosynthetic structures of the blue-green algae

and the three groups of photosynthetic bacteria have been reviewed recently by Echlin and Morris (1965), Pfennig (1967) and Lang (1968a). In blue-green algae, the photosynthetic process takes place on membranous sacs called thylakoids. The membranes composing these lamellae have the dimensions of unit membranes and may be either so closely appressed that a myelin-like configuration is observed or separated (occasionally widely) by an intrathylakoidal space. The thylakoids have been observed to range in location and number, depending on species, age, and growth conditions. The chromatophores in photosynthetic bacteria exhibit greater diversity in structure and shape, and frequently are extensions of the plasma membrane (Echlin and Morris, 1965; Pfennig, 1967). The end product of the photosynthetic light reaction in blue-green algae is oxygen, while in bacteria there is no oxygen evolution and photosynthesis occurs only under anaerobic conditions, utilizing reduced sulfur compounds or organic compounds as electron donors. However, the "dark reaction" resulting in reductive assimilation of carbon dioxide appears to be identical in bacteria and blue-green algae. The photosynthetic thylakoid system is reviewed in detail in a later section.

Nucleoplasm of the Blue-green Algal Cell

In earlier reviews, Cassel and Hutchinson (1954) and Robinow (1956, 1962) discussed cytological studies of the

genetic systems of procaryotes at the light microscope level. Fuhs (1965c) described the ultrastructural organization of the bacterial nucleoid as parallel arrangements of DNA fibers in bundles. Ris (1961), Reese (1967), and Lang (1968a) have reviewed research on the fine structure of the blue-green algal nucleoplasm. Ris and Singh (1961) described the nucleoplasm as a "polymorphous structure of low density containing fibrils of about 25 A thickness" which agglutinate into coarse complexes unless osmium fixation is followed by uranyl acetate before dehydration. They equated the ultrastructure of the blue-green algal nucleoplasm with that of the chromatin bodies in bacteria.

Studies on the nucleoplasm of individual species of Cyanophyta have been reported by Hopwood and Glauert (1960b), Leak and Wilson (1960), and Jensen and Bowen (1961). Cultures of Anabaena and Lyngbya species were exposed to tritiated thymidine, and the resulting incorporation of label was studied (Leak 1965, 1966). Leak (1967) carried out a comparative cytochemical and ultrastructural investigation on the nucleoplasm of Anabaena variabilis.

When DNA was isolated from Escherichia coli, Zubay and Watson (1959) reported that the extract contained 50% by weight protein but showed that it was not the histone-type of protein that is known to be in combination with DNA of eucaryotic cells (Wilkins and Zubay, 1959). They suggested

that the biologically active form of DNA in bacteria is free of protein. It has been generally assumed that the DNA of the procaryotic nucleoplasm is not bound to protein (Kubai and Ris, 1969). Biswas (1957) carried out cytochemical studies on the "central body" of several blue-green algal species as well as chemical studies of isolated DNA and RNA (Biswas 1956, 1960, 1961; Biswas and Myers, 1959, 1960b) and was convinced that the nucleic acids of blue-green algae are bound by structural proteins. Ris and Chandler (1963) and De and Ghosh (1965) concluded that blue-green algal DNA lacks associated protein.

Photosynthetic Pigment System of the Blue-green Algal Cell

Photosynthesis

The literature on the mechanisms of the photosynthetic process is voluminous, and a thorough review of it is beyond the scope and purpose of this dissertation. Despite much excellent research in the field of photosynthesis, the roles of accessory pigments and chlorophyll *a* in the overall process have not yet been conclusively defined. Blue-green algae have been valuable research organisms in the assembling of knowledge in this area.

In a review, Haxo (1960) discussed investigations which established the importance of light absorption by accessory pigments and possible mechanisms for their participation in

photosynthesis. The accessory pigments of the blue-green algae occur in the form of water-soluble pigment-protein complexes known as biliproteins or phycobiliproteins. The chromophores (prosthetic groups) of the conjugated biliproteins are called phycobilins. Quanta absorbed by the dominant accessory pigments of the red and blue-green algae are able to excite chlorophyll fluorescence, and workers concluded that accessory pigments transferred their absorbed energy to chlorophyll *a*. In more recent work, an additional role for the accessory pigments has been shown; they extend the absorption range of chlorophyll toward the far-red and may be essential for the efficient functioning of chlorophyll *a* (Haxo, 1960).

Among the blue-green algae, the photosynthetic action spectrum is strikingly depressed at both ends of the spectrum where chlorophyll *a* absorption is great. Carotenoid absorption also appears to have a low efficiency in photosynthesis. Action spectra for blue-green algae show high photosynthetic activity at wavelengths absorbed by the phycobilins. The conclusion reached by Duysens *et al.* (1961) was that two photochemical pigment systems occur in red and blue-green algae, one containing a photosynthetically active and fluorescent chlorophyll *a* and much phycobilin, and one containing a photosynthetically inactive and non-fluorescent chlorophyll *a* and less phycobilin. Energy ab-

sorbed by the phycobilins was thought to be transferred specifically to the active chlorophyll a.

Emerson et al. (1957) accumulated evidence that the quantum efficiency of photosynthesis begins to decline at wavelengths where absorption by chlorophyll a still is strong. This suggested that a form of chlorophyll which absorbs in the far red is ineffective in photosynthesis. They discovered the Emerson enhancement effect by which high quantum yields could be extended to longer wavelengths when supplementary light of wavelengths absorbed by accessory pigments was utilized. The rate of photosynthesis was larger than the sum of the rates for each wavelength separately. Enhancement appeared to follow the absorption spectrum of the accessory pigments present (Emerson, 1958). According to Emerson, the path of transfer was from pigments with absorption bands at shorter wavelengths to those of longer wavelengths, and overlapping of the fluorescence band of the donor pigment with the absorption band of the accepting pigment increased the probability of transfer. Subsequent studies confirmed the enhancement role of accessory pigments.

From this accumulation of evidence and work by Kok (Kok and Hoch, 1961) and Duysens et al. (1961), the existence of two photochemical systems each mediated by a different pigment system was established. These oxidation-reduction reactions were hypothesized to function in series. In system

1, carbon dioxide is reduced *via* triphosphopyridine nucleotide and a cytochrome is oxidized. System 2 reduces the cytochrome and oxidizes water, yielding oxygen (Duysens and Amesz, 1962; Amesz and Duysens, 1962). Both pigment systems were found to contain chlorophyll *a* and phycoerythrin in *Porphyridium cruentum*, a red alga, and chlorophyll *a* and phycocyanin in the blue-green *Anacyctis nidulans*. The activity of system 1 was found to be greater at 680 mμ and smaller at 560 mμ than that of system 2. In a review, Vernon and Avron (1965) discussed the light-induced electron transfer and associated phosphorylation reactions of photosynthesis in terms of two coupled photoreactions based on two pigment systems.

In developing a "unitary concept" of photosynthesis, Stanier (1961) noted that the one common denominator of all photosyntheses is that they are metabolic processes resulting in conversion of radiant energy into chemical energy. He stressed excitation of chlorophyll and expulsion of electrons as the one common physical event in all types of photosynthesis, resulting in one universal chemical consequence--transfer of electrons through a closed carrier system coupled to chlorophyll and the generation of ATP in the process.

In marine red algae, the action spectrum for photosynthetic enhancement in the blue was found to be similar to that for enhancement in the far red, following the absorp-

tion spectrum of phycoerythrin in both cases (Haxo, 1960). This work contradicted the hypothesis that the Emerson enhancement effect depends on excitation of a pigment with an energy level higher than the first excited state of chlorophyll a and made more attractive the alternate explanation that efficient photosynthesis requires simultaneous excitation of chorophyll a and an accessory pigment. The enhancement effect can function when the two wavelengths are given alternately in periods lasting several seconds, suggesting an intermediate with a lifetime of several seconds that is common to both reactions and disproving the idea that two light quanta combine in a photochemical event (Vernon and Avron (1965).

The primary photochemical reactions of photosynthesis are thought to occur in molecular complexes ("reaction centers") each consisting of a chlorophyll-like molecule associated with molecules that can be oxidized or reduced (Duysens, 1966). Chlorophyll a has been reported to exist in at least six states in the living cell (Thomas, Kleinen Hammans, and Arnold, 1965). Holm-Hansen (1968) has reviewed the physiology and biochemistry involved in the photosynthetic process in the blue-green algae. Plastoquinone, plastocyanin, and a cytochrome c_{554} are thought to serve in the series of electron transfers (Holm-Hansen, 1968). Light energy that has been absorbed by accessory photosynthetic

pigments is transferred through a number of chlorophyll a molecules to a specialized chlorophyll a (or chlorophyll a in a specialized environment) of the reaction center where it is trapped for use in photochemical reactions (Clayton, 1963; Duysens, 1966).

Photosynthetic pigments

Divergence in photosynthetic pigments represents one of the two major exceptions in the long list of structural and chemical similarities between bacteria and blue-green algae. Blue-green algae and eucaryotic plants possess several types of chlorophyll a. The accessory chlorophylls of eucaryotes are not found in the blue-green algae. Photosynthetic bacteria lack chlorophyll a, but there are at least four major types of bacterial chlorophylls (Pfennig, 1967; Klein and Cronquist, 1967). The bacterial chlorophylls have the basic chemical structure of chlorophyll a but show minor variations in hydrogenation and side chains.

The major carotenoid pigment of the blue-green algae is β-carotene which also is the major carotenoid of eucaryotic plants. In addition, there are other carotenoids including echinenone (myxoxanthin), myxoxanthophyll (myxorhodin, phycoxanthin), flavicine, and small amounts of zeaxanthin, lutein, and several unidentified xanthophylls (Strain, 1951; Goodwin, 1960a, 1960b; Klein and Cronquist, 1967). Bacteria

(except Rhodomicrobium) lack β-carotene but contain a variety of carotenoids which often mask the color of the bacteriochlorophyll (Pfennig, 1967; Klein and Cronquist, 1967). Carotenoids are always found associated with chlorophylls in chloroplasts or chromatophores and are postulated to protect cells against lethal photosensitized oxidations (Krinsky, 1966). They appear to have very little efficiency in blue-green algal photosynthesis as evidenced by action spectra (Blinks, 1954).

The efficiency of the photosynthetic process in the blue-green algae is dependent upon the biliproteins or phycobiliproteins which absorb light and pass the energy to chlorophyll a molecules. The biliproteins absorb wavelengths in the middle of the spectrum that chlorophyll a cannot absorb. The action spectrum of photosynthesis closely corresponds to the absorption spectra of the biliproteins and not to the absorption spectra of chlorophyll a and the carotenoids (Blinks, 1954). In Chroococcus, phycobilins and chlorophyll a were reported to have almost equal quantum efficiency, but in Oscillatoria, Anabaena, and Phormidium, chlorophyll a and carotenoids showed very low efficiency in photosynthesis. Smith and French (1963) contended that two pigment systems, each with separate reactions essential to the overall process, are involved simultaneously in the photochemical process of photosyn-

thesis. In the blue-green algae, one system made up of the phycobilins as primary absorbers transfers energy to chlorophyll $\underline{a}_{673}$; the other pigment system is believed to be chlorophyll $\underline{a}_{683}$ which feeds the energy sink P_{700}. Brody and Brody (1965) considered P_{700} an aggregated form of chlorophyll _a_ rather than a special chlorophyll chromoprotein.

The chemistry of the biliproteins has been reviewed by Ó hEocha (1960, 1965a, 1966) and Bogorad (1965). Biliproteins also are found in two groups of eucaryotic algae, the red algae (Rhodophyta) and the cryptomonad algae (Cryptophyta). Nomenclature among the biliproteins is confusing. Originally it was thought that only four occurred in algae, and these were designated C-phycoerythrin and C-phycocyanin for the two found in the Cyanophyta and R-phycoerythrin and R-phycocyanin for those occurring in the Rhodophyta. More recently, B-phycoerythrin (Airth and Blinks, 1956) and allophycocyanin (Haxo _et al._, 1955) and several different cryptomonad biliprotein types (Ó hEocha, 1965a) have been reported as well as a less restricted distribution of C- and R-phycocyanin and phycoerythrin. Hattori and Fujita (1959b, 1959c) isolated and purified the phycocyanin, phycoerythrin, and allophycocyanin from _Tolypothrix tenuis_, several other blue-green algae, and three red algae. They concluded that phycocyanin from red and blue-green algae is

the same but that spectral properties of R- and C-phycoerthyrin differ as do those of phycoerythrins within the same algal class (Rhodophyta). Berns (1967) extracted biliproteins from representative Cyanophyta, Rhodophyta, and Cryptophyta, used them to stimulate rabbit antibody production, and discovered that C-phycocyanins from all sources were antigenically and immunologically related and also related to allophycocyanin but not to any phycoerythrins. Larger antigenic differences were observed among the phycoerythrins from different algal groups but all were related. Berns' (1967) data showed little if any antigenic difference between B-phycoerythrin and R-phycoerythrin.

Phycoerythrin and phycocyanin have been isolated and studied by a number of workers. These algal pigment-protein complexes are reasonably stable in solution (Ó hEocha, 1965a). Since strong mineral acids are required to free the chromophores from the protein, many of the resulting pigments probably are artifacts attributable to the isolation procedure, although a few may be native.

The colored prosthetic groups of the conjugated biliproteins (phycobilins) are linear tetrapyrroles which are structurally related to animal bile pigments (Bogorad, 1965). The phycobilins have been reviewed by Ó hEocha (1958, 1962, 1965b). Absorption spectrophotometry has been the method of choice in studying the phycobilins and

phycobilin-protein interactions. Variations in absorption spectra with species and method of handling have complicated the issue.

The prosthetic groups of algal biliproteins are thought to be attached to the apoproteins by covalent bonds since they are liberated only by digestion with concentrated acids. Light absorption and fluorescence spectra of the native biliproteins are altered by protein denaturing agents. Phycocyanins are more easily dissociated and denatured than phycoerythrins but both are readily released from ruptured algal cells. C-phycocyanin and allophycocyanin both contain phycocyanobilin as the prosthetic group, as evidenced by similar absorption spectra of the denatured biliproteins, but the absorption maxima of the native biliproteins differ by 35 mμ, indicating different states of the pigment (Ó hEocha, 1963; Chapman et al., 1967, 1968). Procedures for isolation were shown to produce new, artifactual pigments (Ó hEocha, 1963). Spectrally different C-phycoerythrins all appear to possess only one type of pigment, phycoerythrobilin, as the prosthetic group (Ó hEocha, 1958), although R- and B-phycoerythrin also contain phycourobilin as a prosthetic group (Ó hEocha, 1966).

Most blue-green algae contain phycocyanin as the major or sole biliprotein; the major biliprotein of red algae is phycoerythrin. However, when Hattori and Fujita (1959c)

isolated and purified the biliproteins from red and blue-green algal species, they discovered that the phycoerythrin content exceeded that of phycocyanin not only in all the red algae as expected but in the blue-green algae Tolypothrix tenuis, Anabaena variabilis, Nostoc muscorum, and Aphanothece sacrum as well. Berns and co-workers (Berns et al., 1964; Berns and Edwards, 1965; Berns and Morgenstern, 1966; Berns and Scott, 1966), Boucher et al. (1966), Fujimori and Pecci (1966), Hattori et al. (1965), and Craig and Carr (1968a) have conducted physico-chemical investigations on C-phycocyanin and have discovered that it is an aggregating system. Berns proposed that the hexamer state probably is the most important in vivo. Craig and Carr (1968a) purified C-phycocyanin and allophycocyanin from Anabaena variabilis and developed an assay procedure for determining the proportion of the two pigments when present as a mixture. They found that the ratio of the two pigments remained essentially constant throughout the growth cycle and during growth at reduced illumination. Allophycocyanin was found to be widely distributed throughout the red and blue-green algae (Hattori and Fujita, 1959c).

Hattori and Fujita (1959a, 1959b, 1959d) and Fujita and Hattori (1960a, 1960b, 1962a, 1962b, 1963) have studied Tolypothrix tenuis, a filamentous blue-green alga containing phycocyanin, phycoerythrin, and allophycocyanin in addition

to chlorophyll a and carotenoids. Cells which had been illuminated but deprived of nitrogen subsequently could form biliproteins in the dark with a nitrogen source, but synthesis was suppressed by inhibitors of protein synthesis. These investigators proposed a scheme for the biosynthesis of the biliproteins based on formation of a phycocyanin precursor during illumination in the presence of carbon dioxide. Conversion of the phycocyanin precursor into a phycoerythrin precursor could be induced by brief exposure to green light and the reverse by red light. The action spectra of these interconversions were different from the absorption spectra of chlorophyll a and carotenoids. Carbon dioxide was not required during the period of the chromatic illumination (Fujita and Hattori, 1960b). When grown in red light, cells of Tolypothrix tenuis had increased amounts of phycocyanin and decreased amounts of phycoerythrin; in blue or green light, mostly phycoerythrin was produced. The biosynthesis of phycocyanobilin in Cyanidium caldarium has been studied by Troxler and Lester (1967). Bogorad (1963) discussed possible routes of biogenesis of biliproteins.

Blue-green algae, red algae, and photosynthetic purple bacteria share with green plants the phenomenon of emitting light for seconds after illumination (Arnold and Thompson, 1956). The action spectra for production of this delayed light by blue-green algae show a maximum at 620 mμ, the

absorption maximum for phycocyanin; the emission spectra of the blue-green, red, and green algae are the same. Both action and emission spectra are quite different for the bacteria.

Thylakoid system

Evidence presented by many groups has established the thylakoid system as the site of photosynthetic capability. Photophosphorylating activity, ability to carry out the Hill reaction, and photoreduction of triphosphopyridine nucleotide were identified with the membranous fractions from Anabaena variabilis (Shatkin, 1960; Petrack and Lipmann, 1961; Susor et al., 1964; Susor and Krogmann, 1964, 1966) and Anacystis nidulans (Fredricks and Jagendorf, 1964). The crucial task of connecting in vitro studies of pigments and in vivo location and mechanism of function has frequently involved comparison of the blue-green thylakoid system with chloroplast grana of higher plants. Lefort (1960a) compared blue-green algal thylakoids to chloroplast structures and proposed that the membranes are lipoprotein in composition and support chlorophyll a. Cell-free preparations have been used to prove the presence of chlorophyll and carotenoids in thylakoid fractions from ruptured cells of Synechococcus cedorum (Calvin and Lynch, 1952) and Anabaena variabilis (Shatkin, 1960).

Refinements in high-resolution electron microscopy and

freeze-etching have led to numerous reports of sub-unit structure in and on the membranes of chloroplasts and blue-green algal thylakoids. Several models have been proposed for the structure of the chloroplast lamellae. The idea of a symmetrical tripartite "unit membrane" as seen in low-magnification electron micrographs of sectioned material has been displaced in current thinking. Park and Pon (1961, 1963) and Park and Biggins (1964) coined the term "quantosome" for spherical particles which they observed on thylakoid membranes of spinach chloroplasts and later, based on freeze-etched material, revised their model to that of a micellar membrane with particles of two sizes (100 X 90 A; 175 X 90 A, corresponding to the quantosome and found only on membranes containing chlorophyll or bacteriochlorophyll) embedded in the matrix (Park and Branton, 1966). In chloroplasts that were extracted with acetone before permanganate fixation, thylakoid staining was greatly reduced. Based on the assumption that lipid is the major reductant of permanganate during fixation, Park and Branton (1966) pictured a membrane with the external surfaces composed of polar ends of lipids which are bound to non-polar micelles within the membrane.

Muhlethaler (1966) and Muhlethaler et al. (1965) used freeze-etching and described the chloroplast thylakoid membrane as a 40-A central layer (probably lipid) covered with protein particles (60 A in diameter) on both sides. The

particles seemed to be embedded to a depth of about 20 A, and removal left a perforated appearance of the lipid layer. On the outer surface of the membrane, the particles appeared to be grouped in fours (presumably multi-enzyme complexes) measuring 120 X 60 A.

Kreutz (1966) and Menke (1966) concluded that thylakoids of bacteria and chloroplasts are similar in molecular structure and are built up of a number of layers (aliphatic lipid, porphyrin rings of chlorophyll, and protein) symmetrically arranged in pairs on either side of a central aqueous layer. According to this model, the protein layer is on the outside and is composed of 40-A particles of structural protein arranged in quadratic lattice layers. If additional groups of four particles are added to certain lattice points, particles fitting the dimensions of the quantosome could result.

From observations of fixed material, Weier et al. (1965, 1966) proposed a thylakoid membrane for higher plants composed of spherical sub-units with dark rims (28 A in width) and light cores (37 A in diameter) which would result in the unit membrane image at lower magnifications. Fuhs (1966) and Jost (1965) have presented electron microscopic evidence that the thylakoids of blue-green algae contain spherical sub-unit structure.

Although the location of chlorophyll and carotenoids in

or on the thylakoid membranes is widely accepted, the site of accessory pigments has not been clearly established. Brody and Vatter (1959) concluded that the phycobilins as well as chlorophyll of the red alga Porphyridium cruentum are associated with the chloroplast lamellae. In studying the same species and Porphyridium aerugineum, Gantt and Conti decided that the phycobiliproteins are located in granules (phycobilisomes) attached to the lamellae (Gantt and Conti, 1965, 1966a, 1966b; Gantt et al., 1968).

Effects of environment

Environmental effects on the morphology and ultrastructure of the blue-green algae have been investigated. Peat and Whitton (1967) observed marked differences in arrangement of the thylakoids of Chlorogloea fritschii with age and light intensity. Bowen and Pankratz (1963) followed the recovery of blue-green algal cells in which chlorosis had been induced by prolonged exposure to high light. Under low illumination, vesicular remnants of photosynthetic elements formed typical lamellae, and new lamellar elements appeared to arise from elaborations of the plasma membrane. Nultsch (1961, 1962) reported on the influence of light on movement of blue-green algae. Allen (1968a) studied changes in pigment content and fine structure of Anacystis nidulans under varied conditions of light and temperature. Relative ratios of biliproteins can be varied with light intensity and wavelength during

culture (ó hEocha, 1965b; Holm-Hansen, 1968).

An interesting study by Cook (1963) on Euglena revealed widely divergent light intensity optima for pigment production, photosynthesis, and respiration. In investigating the red alga Porphyridium cruentum, Brody and Brody (1962) found that variation in light intensity had more influence on the content of phycoerythrin than that of chlorophyll a. At very low levels of light, pigmentation was found to fall although at higher levels the amount of pigment varied inversely with the light intensity. Brown and French (1961) observed a significant shift to lower wavelengths of the absorption and fluorescence maxima when Euglena was grown at low light intensity. Algae grown at low light intensities with a resultant accumulation of pigmentation developed a potential for higher rates of photosynthesis which could be utilized on transfer to high light intensities. Studies concerning the effect of light intensity and quality on the physiology of algae have been discussed by Brown et al. (1967) and Brown and Richardson (1968). These workers have measured growth, pigmentation, photosynthesis, respiration, quinone Hill reaction, cell morphology, and structure as a function of the light intensity for growth in 18 algae representing ten taxonomic divisions.

Kratz and Myers (1955b) studied photosynthesis and respiration in three blue-green algal species and examined

the relationships between pigment content and light intensity during growth in Anacystis nidulans (Myers and Kratz, 1955). Echlin (1964b, 1964c) observed the effects on fine structure of Anacystis montana resulting from changes in environment, including light intensity and growth in the dark. Fujita and Hattori (1960b, 1962a), Hattori and Fujita (1959a), and Jones and Myers (1965) varied the wavelength of incident light for growth of blue-green algae. The absorption and fluorescence properties of Anacystis nidulans grown in lights of different wavelength and intensity were studied by Ghosh and Govindjee (1966). Lazaroff (1966), Lazaroff and Schiff (1962), Lazaroff and Vishniac (1961, 1962, 1964), and Holohan and Moore (1967) have described the non-photosynthetic photoinduction (at 650 mμ) of development in a dark-grown Nostoc muscorum strain from aseriate to filamentous stages. Peat and Whitton (1967) examined Chlorogloea fritschii which had been grown in the dark for three years.

Genetic Studies

Although exchange of genetic information by means of conjugation, transduction, and transformation has been well studied and verified in a number of bacterial genera, the only evidence of apparent sexual or parasexual phenomena among the blue-green algae is in brief accounts by Kumar (1962) and Singh and Sinha (1965). Mutations among the

Cyanophyta are known but not common. Some mutations have been induced by treatment with ultraviolet light (Singh and Singh, 1964), but the rate of spontaneous mutation among blue-green algae is low. A number of blue-greens exhibited considerably more resistance to gamma radiation than the resistant bacterium, Micrococcus radiodurans (Kraus, 1968). Kumar (1962) isolated two types of antibiotic-resistant mutants of Anacystis nidulans and Singh and Sinha (1965) isolated sporulating and non-sporulating drug-resistant mutants of Cylindrospermum majus. In each study, rare recombinants were reported. Kumar was unable to grow clones of his recombinants. The lack of a sufficient number of suitable mutant strains is a great handicap in studying the genetics of the blue-green algae.

Blue-green Algae in Evolution

The role of the blue-green algae in evolution has prompted much speculation. Pringsheim (1949) attributed morphological similarities between bacteria and blue-green algae to convergence and not to descent from a common ancestor. Concluding that the cell underwent no major evolutionary changes once the characteristic organization of the present-day eucaryotic cell was achieved, Stanier et al. (1963) suggested that blue-green algae and bacteria represent vestiges of a stage in the evolution of that cell. Stanier and van

Niel (1962) envisioned the procaryotic cell as providing the structural framework for evolutionary diversification to give a wide variety of microorganisms differing in gross organization (unicellular, multicellular, and coenocytic), manner of locomotion, cell division, and metabolism (chemotrophs and autotrophs), while parallel modes of evolutionary diversification occurred at the eucaryotic level of organization.

In an extensive review, Klein and Cronquist (1967) have attempted to assemble and correlate newer information on thallophytes in respect to taxonomy and phylogeny. They present a strong case for derivation of blue-green algae from photosynthetic bacteria roughly at the evolution level of Rhodomicrobium, emphasizing that the only qualitative differences of phylogenetic significance (obligate reduction vs. oxygen evolution and pigment differences) are overshadowed by the many chemical, structural, and physiological similarities. Biosynthesis of chlorophyll a (and resulting oxygen-evolving photosynthesis), phycobilins, and cyclic carotenoids would have been possible through addition or substitution of relatively few enzymes. Therefore, Klein and Cronquist concluded that the blue-green algae must be derived from the same line from which the photosynthetic bacteria converged. Modern bacteria and blue-green algae would then be derived from archaic bacteria.

Echlin and Morris (1965) suggested a common ancestor

for bacteria and blue-green algae with differences in photosynthetic pigments evolving later. Independent evolution of so many features common to blue-green algae and bacteria seemed to them to be unlikely.

Theories proposed many years earlier were revived by Ris and Plaut (1962) in presenting evidence to support the idea that chloroplasts evolved from endosymbiotic blue-green algae. Their claim was based on the similarities between the DNA-containing region of the chloroplast in the green alga Chlamydomonas and the nucleoplasm of blue-green algae, as well as on similarities in photosynthetic thylakoid systems in the two groups. The main difference was in the pigment composition. Hall and Claus (1967) suggested that the gap could be bridged by the unicellular alga Cyanidium which has a form of pigmentation intermediate between that of blue-green algae and true chloroplasts. Bisalputra and Bisalputra (1967) reported that the similarity between the chloroplast of Laurencia spectabilis, a red alga, and a blue-green alga is even more striking. Weier (1963) proposed that grana of higher plant chloroplasts evolved from the single-disk type of thylakoid in the blue-green and red algae through double- and multiple-disk associations. The suggestion that the red algae evolved from the blue-green algae or some common ancestral stock is supported by Berns' (1967) discovery that biliprotein antigens from the red and blue-green algae are closely related.

MATERIALS AND METHODS

Isolation and Culture Techniques

The species of Cyanophyta chosen for study represent both unicellular and filamentous genera. In addition to the algae listed in Table 1, Aphanizomenon flos-aquae (L.) Ralfs (collected from Silver Lake, Milford, Iowa in August 1968) and Spirulina major Kütz. (Culture Collection of Algae at Indiana University, No. 552) were fixed for electron microscopic examination but were not maintained in culture. All cultures were unialgal. Several methods were utilized to achieve the unialgal condition: repeated transfers of single filaments with careful washing in several changes of distilled water or medium, growth in soft agar-medium and transfer of filament tips as they emerged from the agar, and dispersion (DeVilbiss atomizer) of a thin suspension of culture on agar-medium plates followed by transfer of the desired species to fresh medium. Anacystis nidulans and Nostoc muscorum were grown in bacteria-free condition, based on absence of bacterial growth on agar-0.1% Bactotryptone plates. It did not prove possible or practical for the purposes of this study to maintain the heavily-sheathed species in axenic culture, but transfers were made frequently enough to keep the bacterial growth minimal. All media and culture flasks were autoclaved for 20 min at 121°C and 15 psi, and

Table 1. Collections and culture conditions

Alga	Source	Medium	Temperature	Light[a]
Anacystis nidulans[b]	CCAIU[c] 625	Kratz-Myers Medium C (Kratz and Myers, 1955a)	21-22°C	200-300 ft-c
Arthrospira Jenneri (Hassall) Stizenberger	Lake Okoboji, Milford, Iowa; July, 1967	Modified Chu 10 plus 40 ml/l soil extract (Gerloff *et al.*, 1950)	21-22°C	30-35 ft-c
Microcoleus vaginatus (Vauch.) Gom. (per Drouet, 1968)[d]				
Type A[d]	Lake Okoboji, Milford, Iowa; July 1967	Modified Chu 10 plus 40 ml/l soil extract (Gerloff *et al.*, 1950)	21-22°C	30-35 ft-c
Type B[d]	CCAIU[c] 1270	"	21-22°C	30-35 ft-c
Nostoc muscorum[b,d]	Kaiser Research Foundation, Strain M-12.4.1	"	21-22°C	200 ft-c, 10 days; then 100 ft-c
Symploca muscorum (Ag.) Gom.	CCAIU[c] 617	Kratz-Myers Medium C	21-22°C	100-200 ft-c
Tolypothrix distorta var. *symplocides* Hansgirg	CCAIU[c] 424	"	21-22°C	30-35 ft-c

[a]Fluorescent (200 watts) and incandescent (100 watts), 12-hr alternating light and dark. Intensity was measured with a Tri-lux ft-c meter, P. Gossen and Co., Erlangen, West Germany.
[b]Bacteria-free.
[c]Culture Collection of Algae at Indiana University, Bloomington, Indiana (Starr, 1964).
[d]Identified by Dr. Francis Drouet, The Academy of Natural Sciences of Philadelphia, Penna.

aseptic techniques were employed.

After evaluation of various media and culture conditions for each alga, those summarized in Table 1 were adopted as standard. Nostoc muscorum was grown in 2,800-ml Fernbach culture flasks containing 1 liter of medium per flask and inoculated with 100 ml of a three-week culture. All other species were grown in 125-ml and 250-ml Erlenmeyer flasks containing 75 ml and 100 ml of medium, respectively. Symploca muscorum grew best in flasks containing a layer of 2% agar-medium solidified on the bottom and covered with 50 ml of liquid medium. Anacystis nidulans also was grown in large-scale culture in a sterile 40-l carboy surrounded by three vertical 15-watt fluorescent lamps, with filtered 4% carbon dioxide in air bubbled slowly through a sintered glass sparger. After a six-week growth period, the crop was harvested in a Sharples Supercentrifuge. For miscellaneous testing, all species were grown in screw-top test-tubes containing 10 ml of medium per tube. Stock cultures were maintained on agar-medium slants at 21°C, 30 ft-c.

The species of Anacystis, Nostoc, Spirulina, and Tolypothrix were harvested in a Sorvall RC2-B refrigerated centrifuge at 4°C, 7,000-9,000 g for 5-10 minutes. Other species could be aggregated on a transfer needle or glass rod. All algae were washed with distilled water before further treatment.

Electron Microscopy of Sectioned Material

Fixation, dehydration, and embedding

Several fixation methods were employed for comparison of subcellular structures, including 1% osmium tetroxide (veronal acetate buffer, pH 6.1) according to Kellenberger et al. (1958) and as modified by Pankratz and Bowen (1963), 3-4% glutaraldehyde (Sabatini et al., 1963) (0.1 M and 0.05 M phosphate buffer at pH 6.2, 6.8, or 7.3) followed by 1% osmium tetroxide (veronal acetate buffer, pH 6.1), unbuffered 1.5% potassium permanganate, 2.5% glutaraldehyde - 0.7% picric acid in 0.1 M cacodylate buffer (pH 6.1), and 4% glutaraldehyde - 0.7% picric acid in 0.05 M phosphate buffer (pH 6.1) (Stefanini et al., 1967; Zamboni and De Martino, 1967). The fixatives and procedures used are outlined in Appendix A. Fixation times were varied from 1 to 16 hours, and temperatures of 4°C or 23°C were tested to study the effect on preservation of detail. Specimens were washed thoroughly with buffer (three changes over 1-12 hours) between glutaraldehyde and osmium tetroxide fixations and once following fixation before dehydration. Cells were pelleted by low-speed centrifugation between steps of fixation and washing. Pellets of Anacystis, Aphanizomenon, Nostoc, Spirulina, and Tolypothrix were embedded in 3% agar (held at 45°C in a water bath) after fixation (or occasionally before fixation). On cooling, the solidified agar was cut into 1-mm cubes for

dehydration.

The standard procedure described in Appendix A was followed for dehydration, infiltration, and embedding. However, several formulations for embedding media were used. Considerable difficulty was encountered with cells of the Microcoleus species and Arthrospira Jenneri pulling out of the embedding medium on sectioning when Epon 812 was employed. Mixtures of Epon-Araldite alleviated the problem, and the formulation listed in Appendix A proved most satisfactory. Araldite 502 (Glauert and Glauert, 1958) gave inferior results on sectioning.

Sectioning and staining

Sections 40-70 mμ in thickness were cut using a DuPont diamond knife in an LKB Ultrotome III or a Reichert Om U 2 ultramicrotome. The sections were collected on 400-mesh etched copper grids or 150-mesh formvar coated grids (with or without carbon coating). The supported grids were essential in examining Arthrospira, Spirulina, and the two Microcoleus strains.

The sections were stained 10 minutes in 10% uranyl acetate in methanol on formvar-supported grids or 5 minutes in 20% methanolic uranyl acetate on 400-mesh grids (Stempak and Ward, 1964). A 2-10 minute post-stain in lead citrate solution (Venable and Coggeshall, 1965) was employed frequently.

Observation and electron micrography

Specimens were viewed in an RCA EMU-3F or Hitachi HU-11C electron microscope at 50 or 75 KV. Micrographs were taken on DuPont Cronar litho film, developed in Kodak Dektol developer, and printed on Kodak F2-F5 Kodabromide paper.

Freeze-etching

Harvested cells, unfixed or fixed in 3% glutaraldehyde (0.1 M phosphate buffer, pH 7.3) for 11 hr, 4°C, were washed with 0.1 M phosphate buffer, pelleted by low-speed centrifugation, and infiltrated with 30% ethylene glycol at 23°C for 1.5 hr to reduce ice damage. A thick suspension of cells was packed into a rolled 400-mesh, 3-mm copper grid. The filled grid was frozen rapidly in liquid propane, blotted quickly, and transferred to liquid nitrogen for insertion into the specimen holder of a Berkeley Freeze-etch Device (C. W. French). The device (also held in liquid nitrogen) was transferred quickly to a Varian VE-30M vacuum evaporator equipped with modified Ladd Industry electrodes (previously cleaned and aligned) and immediately pumped down. The electrodes were degassed when a pressure of 10^{-5} Torr was reached, and at 4×10^{-6} Torr (10-min pump-down time) the lid of the device was rotated to fracture the specimen. An etching time of 15 minutes was permitted utilizing the heat sink before evaporating C/Pt on the specimen at an angle of 30°, followed by evaporation of pure carbon from above to

form the replica. On removal from the device, the replica was gently freed from the specimen in 50% glycerol and then was cleaned in hot 90% nitric acid for one hour, washed in four changes of distilled water, and picked up on a 400-mesh copper grid for examination in the electron microscope.

Gram-staining

The Gram stain procedure according to Smith (1965) was repeated several times for each species tested.

Effects of Treatments on Ultrastructure

Plasmolysis

The effect on Nostoc muscorum of a series of sucrose solutions ranging in concentration from 1 to 30% and of 25% sucrose in 0.03 M Tris buffer (pH 8) was studied. Cells were examined for plasmolysis under phase and polarized light after 1, 2, and 8 hr of treatment.

Protoplast formation and lysis

Following a modification of the procedure of Fujita and Sato (1966), cells of Nostoc muscorum suspended in 0.03 M Tris buffer (pH 8.0) or in 25% sucrose- 0.03 M Tris buffer (pH 8.0) at 28°C for 2 hr were treated with 1mM EDTA (disodium salt) and 50 µg/ml lysozyme (Calbiochem, egg-white) and incubated at 28°C for 8 hr. Aliquots were examined under phase and polarized light at 2, 3, and 8 hr, before and after

dilution with water (1:5) to promote lysis. The procedure was repeated using 2 mM Na_2EDTA and 100 µg/ml of lysozyme at 28°C for 10 hr before microscopic examination. Samples were fixed (3% glutaraldehyde followed by 1% osmium tetroxide) for electron microscopy after treatment with 25% sucrose - 0.03 M Tris and following a 22-hr exposure to Na_2EDTA and lysozyme. Another procedure for lysing (Noller and Hartsell, 1961a) exposed cells in 0.027 M phosphate buffer (pH 7.0) to 80 µg/ml lysozyme, 40 µg/ml trypsin (Calbiochem, bovine pancreas), and 5% (v/v) n-butanol at 27°C. Examination under phase optics after 40 min and 6 hr was followed by fixation in 3% glutaraldehyde and 1% osmium tetroxide for electron microscopy.

Effect of Tris-EDTA

Cells of Nostoc muscorum were washed with 0.01 M Tris buffer (pH 8.2) and suspended in 0.03 M Tris-0.001 M EDTA (pH 8.0). Aliquots were removed at 0.5, 1, 2, and 12 hr, centrifuged, and washed with distilled water. Half of each resulting aliquot was fixed in 1% osmium tetroxide for electron microscopy, and the other half was resuspended in fresh medium to test viability and growth (by turbidity at 625 mµ) over a 7-day period.

Enzyme digestions

Cells of Nostoc muscorum that had been treated with Tris-EDTA for 1 hr and washed were hydrolyzed in N hydro-

chloric acid for 10 min at 60°C, digested with DNase (Worthington, 0.3 mg/ml in 0.001 M magnesium sulfate at pH 6.0) for 4 hr at 37°C, and treated with RNase (Calbiochem, bovine pancreas, 0.3 mg/ml in 0.01 M phosphate buffer at pH 7.6) for 3 hr at 37°C. In another series, cells which had been treated with Tris-EDTA for 1 hr and washed were exposed to RNase (Calbiochem, 1 mg/ml), DNase (Worthington, 1 mg/ml), lipase (General Biochemicals, 2 mg/ml), acid phosphatase (Worthington, wheat germ, 2 mg/ml), and pronase (Calbiochem, 1 mg/ml) for 12 hr at 45°C. The RNase- and DNase-treated cells were subjected to extraction with 5% trichloroacetic acid for 20 min at 4°C and washed. All samples were fixed in 1% osmium tetroxide for electron microscopy.

DNA Studies

Isolation procedures

Several methods were employed in efforts to isolate DNA from Anacystis nidulans, Microcoleus vaginatus (Type B), and Nostoc muscorum. A compromise between complete rupture of the cells and minimal degradation of the DNA proved to be the greatest obstacle. Lysis was accomplished by the use of lysozyme in saline-EDTA (Marmur, 1961) or in BPES buffer (Massie and Zimm, 1965), freeze-thawing in saline-EDTA containing 2% sodium lauryl sulfate with or without the addition of lysozyme (Smith and Halvorson, 1967), or by employing the lytic enzymes prepared from cultures of Streptomyces albidoflavus (transfer obtained through the generosity of Dr. M.

Mandel, University of Texas, Houston). Streptomyces albidoflavus was grown on lyophilized cells of the alga to be lysed, according to the procedure of Tabata and Terui (1962), on a New Brunswick gyrotory shaker (low speed) at 31°C for 88 hr (culture medium in Appendix B). Utilizing the procedure recommended by M. Mandel (University of Texas, Houston, private communication, February 16, 1968), filtration followed by centrifugation gave a clear yellow supernatant from which 70% ammonium sulfate salted out the lytic enzyme as a brown precipitate. The enzyme was dissolved in 0.1 M phosphate buffer (pH 6.1) and dialyzed against 0.1 M phosphate buffer. Centrifugation removed a brown sediment and provided a clear brown supernatant containing the enzyme. When cells of Nostoc muscorum or Anacystis nidulans suspended in saline-EDTA were incubated with the enzyme solution at 30°C for 21 hr on the New Brunswick gyrotory shaker (low speed), a reddish fluorescence was evident due to released phycocyanin and most cells had lysed. The DNA was isolated according to the procedure of Marmur (1961) or Massie and Zimm (1965).

The isolated and purified DNA was dissolved in standard saline citrate solution for estimation of DNA concentration (Burton, 1956) and stored at -20°C.

Characterization of DNA

A monolayer of DNA was spread at an air-liquid interface following the Kleinschmidt (1968) technique. The DNA in

saline citrate was added to an equal volume of 1 M ammonium acetate containing 0.01% cytochrome c, and 0.25 ml was carefully introduced onto the surface of 0.1 M ammonium acetate in a Langmuir trough. The spread material was picked up on carboned formvar-coated 150-mesh copper grids, washed in ethanol, and air dried. The grids were shadowed with platinum at an angle of 7° during rotation at 120 rpm in a vacuum evaporator (Mikros VE 10) and examined in the electron microscope.

CsCl equilibrium centrifugations were carried out on a Spinco Model E Analytical Ultracentrifuge fitted with ultraviolet absorption optics, using DNA from Proteus vulgaris as the reference. Ultraviolet absorbance photographs were traced on a Beckman Analytrol Densitometer.

Autoradiography

The incorporation of tritiated thymidine into Anacystis nidulans, Nostoc muscorum, Microcoleus vaginatus, and Symploca muscorum was undertaken for the purpose of locating the labelled DNA at the electron microscopy level. A 2.4-ml suspension of each alga in appropriate medium (Table 1) was transferred to a sterile 25-ml Erlenmeyer flask and tritiated thymidine (Schwarz BioResearch, sp. act. 6.0 c/mmole) was added to give 20 µc/ml. Each culture was incubated under the appropriate growth conditions (Table 1). Aliquots were withdrawn after 3 and 7 days. The cells were harvested,

washed with cold 0.1 M phosphate buffer (pH 7.3), embedded in 3% agar, and fixed for electron microscopy in 3% phosphate-buffered glutaraldehyde (pH 7.3) for 16 hr at 4°C, followed by washing and post-fixation in 1% osmium tetroxide (Appendix A). Control samples were identical except for the omission of tritiated thymidine. Sections were cut and collected on 100-mesh graphite or 150-mesh copper Formvar-coated grids and stained 30 min with lead citrate. Grids were affixed to glass microscope slides with double-sided Scotch tape and coated with Ilford L_4 nuclear emulsion (Ilford Ltd., Ilford, Essex, England) using a 4-cm copper loop. All work was done in a darkroom under a Wratten Series A0 (yellow-green) safety light. After thorough air drying, the slides were packed in slide boxes containing Drierite and sealed from light with black tape. Exposure at 4°C was continued for seven weeks, but duplicate grids were removed at two and five weeks, developed in Kodak Microdol-X, and examined in the electron microscope. Post-staining with uranyl acetate or lead citrate improved image contrast in the biological material.

Tritiated actinomycin D (Schwarz BioResearch, sp. act. 3.38 c/mmole) was incorporated into the same four species. Since actinomycin D is strongly adsorbed on glass, all glassware was pre-rinsed with a solution (12 µg/ml) of unlabelled actinomycin D (generously supplied by Merck, Sharp

and Dohme Research Lab, West Point, Pa.) to prevent loss of useful radioactivity. The cells were harvested, washed with cold 0.01 M Tris buffer (pH 8.2), and suspended in 0.03 M Tris buffer containing 0.001 M EDTA (pH 8.0) for 1 hr at 23°C to increase permeability to actinomycin D. Preliminary tests of viability, growth, and normal cell appearance in electron microscopy were used to establish the concentration and duration of treatment. After washing with distilled water, the cells were incubated with tritiated actinomycin D (41 μc/ml of medium) under the appropriate growth conditions for 24 hr. Two sets of controls were run under the same conditions but employing unlabelled actinomycin D in one group and omitting actinomycin D in a second set. Aliquots were withdrawn at 7 and 24 hr. All samples were washed with distilled water, fixed 10 hr in 1% osmium tetroxide (Appendix A) at 4°C, washed with distilled water, embedded in 3% agar, dehydrated, and embedded in Araldite-Epon (Appendix A). Sections were stained with 10% uranyl acetate in methanol and prepared for autoradiography as previously described. Exposure at 4°C was continued for 4 weeks, but duplicate grids were removed at 2, 3, and 4 weeks for examination.

Cell Rupture and Fractionation

Cells of Nostoc muscorum suspended in cold NET buffer (0.1 M sodium chloride, 0.001 M EDTA, 0.001 M Tris, pH 8.1)

containing 20% sucrose were disrupted in an Aminco French press at pressures of 2,000, 8,000, and 18,000 psi. In another series, glutaraldehyde (to 2%) was added to cold suspensions of Nostoc muscorum in 0.05 M phosphate buffer or NET buffer, each containing 20% sucrose, just before rupture at 2,000 psi.

Differential centrifugation

The thick suspensions resulting from treatment in the French press were subjected to differential centrifugation in a Sorvall RC2-B refrigerated centrifuge at 4°C and speeds of 30, 121, 480, and 1935 g. The pellets at each speed were fixed in 1% osmium tetroxide for electron microscopy.

Density gradient centrifugation

Pellets from differential centrifugation were further fractionated on sucrose density gradients (50 to 70%) in a Beckman L4 ultracentrifuge at 3,200 g for 45 minutes. The gradient tubes were illuminated from above to permit visual differentiation for removal of diffuse layers via a hypodermic needle. Each fraction was centrifuged, and the resulting pellets were fixed in 1% osmium tetroxide for electron microscopy.

Cells of Nostoc muscorum which had been ruptured in a French press at 4,000 psi were exposed to deoxycholate at 4°C for 15 min. The resulting material was fixed with 4%

glutaraldehyde and centrifuged at 7,700 g for 30 min. The pellets were post-fixed in 1% osmium tetroxide for electron microscopy, and the supernatants were fractionated on sucrose (10-70%) density gradients.

Absorption Spectra of Pigments

In vivo absorption spectra were obtained on cell suspensions using a Cary Model 14 spectrophotometer with a 700-watt projection lamp as the modified light source and opal glass plates between the cuvettes and the detectors or on a Cary Model 14R spectrophotometer with lead sulfide detectors to extend the usable range into the infrared region. The absorption spectrum of cells filtered or spread on a Gelman (GF/C A) glass fiber filter disk and balanced against a moist reference disk without cells was obtained on a Beckman DB-G spectrophotometer. The scanning range extended from 340 mu to 750 mμ.

Unfixed cells of Nostoc muscorum and cells which had been fixed in 4% glutaraldehyde (0.1 M phosphate buffer, pH 7.0) for 2.5 hr at 23°C and washed with buffer were extraced repeatedly with 80% methanol-20% acetone, employing centrifugation at 4°C between extractions. Pigment extractions were monitored by running absorption spectra on extracts and on extracted material. Absorption spectra also were recorded for the fractions obtained by density gradient centrifugation of the supernatant after deoxycholate treatment of ruptured cells.

Effect of Illumination on Ultrastructure

Variation in light intensity

Cultures of Nostoc muscorum were grown at 20, 100, 200, and 900-1,000 ft-c for three weeks. Aliquots from all cultures were prepared for electron microscopy and in vivo absorption spectra were obtained on equal volumes of cell suspension.

Variation in wavelength

A 3-ml culture of Nostoc muscorum was grown in orange light produced by focusing with a spherical mirror the image of the filament of a GE DWY tungsten halogen lamp on the entrance slit of a Bausch and Lomb monochromator which was set at 620 mμ. The culture was centered in the exiting beam, and the intensity of the incident illumination was measured from 380 mμ to 750 mμ with an ISCO spectroradiometer. At the end of three weeks, an aliquot was withdrawn for electron microscopy and the in vivo absorption spectrum of the remaining suspension was recorded. The experiment was repeated for growth in red light with the monochromator set at 675 mμ.

Growth in darkness

Cultures of Nostoc muscorum and Microcoleus vaginatus (Type A) with 1% sucrose or glucose added to the medium were incubated in screw-top test-tubes at 21°C in a light-tight box for five weeks and harvested and fixed under green light for electron microscopy.

RESULTS

Electron Microscopy of Sectioned Material

All of the fixation procedures employed gave satisfactory results although the quality of preservation varied with the species for a given fixative. The pH of the buffer had no appreciable effect in the range from pH 6.1 to 7.2. Temperatures of 4° and 23°C proved equally acceptable when the fixation time was adjusted accordingly. The best general appearance of cell components was achieved with 1% osmium tetroxide in veronal acetate buffer at room temperature (23°C) for 3 hr. In general, pre-fixation with 3-4% glutaraldehyde did not appear to enhance the quality of the image. In several cases depending on the inclusion or area to be studied, one fixative provided more information than another and these will be discussed in the following sections.

The choice of stains also contributed specific information and differentiation. The heavily-sheathed Microcoleus vaginatus ecophenes and Symploca muscorum stain much more quickly than other species with both uranyl acetate and lead citrate, so the staining time and the concentration of the stain were reduced for these species, especially when the double stain was employed. A compromise in staining had to be accepted in these cases. Optimal staining for wall structure resulted in over-staining of phycobilisomes. The specific area under consideration dictated the staining time

and concentration.

Because the Microcoleus vaginatus ecophenes and Symploca muscorum tended to pull out of the embedding medium on sectioning, Formvar-coated grids were essential for supporting these species. The Araldite-Epon mixture was considerably more satisfactory than Epon in coping with this difficulty, and weighing of the components of the embedding medium rather than measuring by volume gave more consistent results in block hardness. Methanolic uranyl acetate has a destructive effect on Formvar which required the use of a lower concentration of the stain (10% uranyl acetate in methanol).

Although emphasis in this study has been on the nucleoplasm and the photosynthetic pigment system, a general consideration of other cell components is in order since several of the species have not been described at the ultrastructural level. Features common to all species studied include a multi-layered wall, a plasma membrane, nucleoplasmic and cytoplasmic regions, a thylakoid system, polyhedral bodies, α-granules, β-granules, and ribosomes (Figure 1). The contrast of the α-granules is enhanced by post-staining with lead citrate following uranyl acetate (Figure 2).

Local areas of convoluted membrane elaborations were observed frequently in Nostoc muscorum (Figures 1, 3, 4, and

5), apparently corresponding to the lamellasomes described by Echlin (1964a). They were found most often adjacent to a crosswall at the outer wall (Figures 3 and 4) but occasionally appeared along the outer wall alone (Figure 5). Only occasionally was continuity with the plasma membrane clearly observed. Structured granules were found in Nostoc muscorum, the Microcoleus vaginatus ecophenes, Symploca muscorum, and Tolypothrix distorta. The internal pattern is particularly evident in Tolypothrix distorta (Figures 6 and 34). Tubular elements, differing in dimensions and staining characteristics from the photosynthetic thylakoids, were observed occasionally in Nostoc muscorum (Figures 7, 8, and 10) and Spirulina major (Figures 9 and 33). They parallel each other and appear to be associated with the nucleoplasm. Profiles of these elements appear to resemble those of microtubules, but since no clear-cut tubular cross-sections could be identified, the geometry remains in question. Gas vacuoles are a prominent feature of Aphanizomenon flos-aquae (Figure 11) and Arthrospira Jenneri (Figure 12). They are difficult to fix and stain without overstaining other cell components.

Cylindrical bodies were found in Microcoleus vaginatus (Type A) and Symploca muscorum. They usually are located near the cross-walls (Figures 13 through 16) of these filamentous algae and often appear in clusters (Figures 13, 15, 16, 17, and 18). The structure consists of two concentric

electron-opaque layers and a central core that in longitudinal section (Figures 14, 17, and 18) appears to be composed of electron-dense sub-units. Cross-sections of the core (Figures 15, 16, and 19) show a dense rim and centerpoint with a lighter zone between. Measuring centripetally, the outermost dense layer of the cylindrical body is approximately 17 mu in diameter, followed by a light zone of 13 mμ, a narrower dense zone of 13 mμ, a light zone of 13 mμ, and the core which measures 39 mμ. The overall diameter of each cylindrical body is approximately 148 mμ, and the lengths observed in this study range up to 350 mμ.

Studies of the Cell Wall

To lay the groundwork for gentle rupture of the cell wall in order to isolate intact high molecular-weight DNA and polyhedral bodies, as well as for maximum uptake of labelled precursors in autoradiographic studies, the character and properties of the blue-green algal cell wall were investigated. The different species varied considerably in their response to conditions for plasmolysis, protoplast formation, and lysis.

Gram-staining

Anacystis nidulans, *Arthrospira Jenneri*, the *Microcoleus vaginatus* ecophenes, *Nostoc muscorum*, *Spirulina major*, *Symploca muscorum*, and *Tolypothrix distorta* stained

an opaque, deep blue-purple, apparently indicating a Gram-positive wall. Empty walls appeared red, and cross-walls of vegetative cells were unstained or faintly red. The sheaths of the Microcoleus vaginatus ecophenes, Symploca muscorum, and Tolypothrix distorta did not stain.

Plasmolysis

Cells of Nostoc muscorum showed no signs of plasmolysis in solutions of sucrose concentration up to 20%. Evidence of plasmolysis was observed with both phase and polarization microscopy in solutions of 20% and higher sucrose concentration. Figure 20 shows the distortion of the protoplasts after 2 hr in 30% sucrose solution. The cells of the trichome have not separated. Plasmolysis was severe after 8 hr in 30% sucrose solution. A 25% solution of sucrose also effected plasmolysis but slower and to a lesser extent. Cells which had been partially plasmolyzed in 25% sucrose-0.03 M Tris buffer (pH 8.0) for 2 hr, washed, and fixed in 3% glutaraldehyde followed by 1% osmium tetroxide for electron microscopy showed good preservation of cell components but a pulling away of the protoplasm from the cell wall (Figure 22) while the wall remained intact.

Protoplast formation and lysis

Lysis of cells of Nostoc muscorum was achieved using lysozyme, trypsin, and n-butanol, according to the procedure of Noller and Hartsell (1961a) for lysing Gram-negative bacteria. Figure 21 shows that lysis was almost complete after 6 hr at 27°C. A few cells remained intact but detached from a trichome. When this preparation was fixed for electron microscopy, breakdown of the cell walls and lysis was evident (Figure 23). Few cellular inclusions can be recognized to have escaped deterioration.

A more gentle method (designed to form protoplasts) involved treatment of cells of Nostoc muscorum with lysozyme and EDTA at 28°C after plasmolysis in 25% sucrose-0.03 M Tris buffer (pH 8.0). The resulting cells were studied with the electron microscope (Figures 24 and 26). Considerable destruction and rearrangement of cell contents is evident, but strands of DNA, β-granules, and thylakoids can be discerned. The normal angular outline of polyhedral bodies is seen although the electron-opaque contents are missing. No cell wall can be seen. Figure 25 shows the effect of the same treatment on a spore with a complex, thickened envelope. In Figures 25 and 26 the tripartite nature of the plasma membrane and of the thylakoid membranes is preserved, but the wall material and polyhedral body contents are missing. Further lysing of these cells was observed on dilution (1:5) with water.

Studies on the Nucleoplasm

Isolation and characterization of DNA

All of the four methods outlined for isolation of the DNA from *Anacystis nidulans*, *Microcoleus vaginatus* (Type A), and *Nostoc muscorum* gave disappointingly low yields. The poor recovery could be attributed to incomplete lysis of cells under the gentle conditions employed, coupled with considerable loss of DNA during the purification steps to ensure removal of RNA and protein. The concentration of the purified DNA, dissolved in standard saline citrate (SSC) was determined by the Burton method (1956).

Cesium chloride density gradient analysis of two isolates of DNA from *Anacystis nidulans* and one isolate from *Nostoc muscorum* was performed using *Proteus vulgaris* DNA of known buoyant density (1.698 g/ml) as the reference. Two DNA bands appeared for each isolate in addition to the reference band. Densitometer tracings of the ultraviolet absorption photographs are shown in Figures 27, 28, and 29. Equilibrium centrifugation after treatment of the *Anacystis* DNA with DNase gave only the band for the reference DNA. The buoyant densities were calculated by the method of Mandel *et al.* (1968) to be 1.726 g/ml for the major band and 1.714 g/ml for the secondary band in both isolates from *Anacystis nidulans*. The GC content of the major DNA fraction was calculated to be 67.3% and that of the minor fraction was 55.1%.

The major band from Nostoc muscorum had a buoyant density of 1.723 g/ml with a corresponding GC content of 64.3%. The secondary peak was sufficiently over-shadowed by the strong reference peak to make calculations of the buoyant density and GC content questionable.

Electron microscopy of isolated DNA

The DNA of Anacystis nidulans and Nostoc muscorum (from the same isolations as the DNA characterized in the preceding section) was spread on 0.1 M ammonium acetate in a Langmuir trough, picked up on carboned Formvar-coated grids, dehydrated, shadowed with platinum, and examined. The image appeared as a long, anastomosing continuum with very few discernible ends or short lengths. The diameter of the spread DNA was not uniform; although the strand measured 29 Å in diameter along most of its length, thickened or clumped areas were observed at random along the strand.

Electron microscopy of sectioned material

The region termed "nucleoplasm" varies in extent and degree of differentiation with the fixative and stain employed as well as from species to species. The Ryter-Kellenberger fixative that has proven so successful for preservation of bacterial DNA gives the same image in blue-green algae of fine fibrils diffusely spread throughout the nucleoplasm. In Figure 31, the nucleoplasm of Nostoc muscorum which was

fixed in 1% osmium tetroxide fills much of the cell including some peripheral areas and appears less electron dense than the cytoplasm. Patches of the same electron density as the cytoplasm are interspersed throughout the nucleoplasm. Fibrils of DNA are evident throughout the nucleoplasm. Figure 32 shows a cell of Nostoc muscorum after Ryter-Kellenberger fixation. The nucleoplasm of the cell of Anacystis nidulans shown in Figure 37 occupies a smaller area in proportion to the cytoplasm in the plane of section and appears to be confined to a central region with the thylakoid system and cytoplasm on the periphery. A similar arrangement is observed in Spirulina major (Figure 33). In Tolypothrix distorta (Figure 34), there is less definite distinction in staining between nucleoplasm and cytoplasm. In contrast to the electron transparency of the nucleoplasm, the ribosomes and structured granules are deeply stained by uranyl acetate and lead citrate. Cells of Microcoleus vaginatus (Type A) are so filled with parallel arrays of thylakoids (Figure 35) that the less dense nucleoplasm is not clearly visualized after fixation with osmium tetroxide. Potassium permanganate followed by uranyl acetate stain gives a better differentiation of the DNA-containing regions in this species, although the DNA fibrils appear coarser (Figure 36). Under these conditions the thylakoids are sharply contrasted, and the α-granules show distinctly between thylakoids.

Polyhedral bodies have been observed to occur in all of the species studied. They always appear to be associated with an area of the nucleoplasm but are not always central. They frequently occur in clusters and individually measure up to 500 mμ in diameter. Following osmium fixation and staining with uranyl acetate, their angularity is outlined by a dark boundary with a light zone between the limiting line and the interior of the inclusion (Figures 38 and 39). After osmium fixation, polyhedral bodies stain deeply and relatively homogeneously with uranyl acetate and lead citrate (Figure 40) but very lightly with lead citrate alone (Figure 41). Sometimes the dark boundary is seen to completely surround the polyhedral body (Figure 42), but more commonly part or all of the edge appears diffuse (Figures 43 through 45). The boundary occasionally has the tripartite appearance of a unit membrane for a considerable distance (Figures 46 through 50). In Figure 50, the outer layer of the "sandwich" appears to be more dense and is wider (25 A) than the inner layer (20 A) with an electron transparent zone of 20 A between them. There are occasional suggestions of an association between the polyhedral bodies and the thylakoid system (Figures 51 through 55).

When cells of Nostoc muscorum were ruptured in a French press at low pressure and the resulting suspension was fractionated by differential centrifugation, the integrity of the

polyhedral bodies appeared to be maintained when protected by sucrose or sucrose-Ficoll (Figure 56). Various degrees of electron density were exhibited by released polyhedral bodies (Figures 56 through 58), and frequently the contents were not homogeneous in density. The electron-dense boundary generally was retained and the membrane-like image was observed (Figure 58). Polyhedral bodies pelleted along with whole cells and thylakoids at speeds of centrifugation as low as 30 g and rarely were observed in high-speed fractions. This prevented recovery of a pure fraction of polyhedral bodies by differential centrifugation or by density gradient centrifugation although enriched fractions could be obtained.

Enzyme digestions of whole cells of Nostoc muscorum were carried out in an effort to chemically characterize the nature of the polyhedral bodies. Pre-treatment with Tris-EDTA for 1 hr was utilized for the purpose of possibly increasing permeability to the enzymes (see Discussion). After washing with water, cells were exposed to 1 N hydrochloric acid for 10 min at 60°C, DNase for 4 hr at 37°C, and RNase for 4 hr at 37°C and then were fixed in 1% osmium tetroxide for electron microscopy. Figure 59 shows a cluster of electron-dense polyhedral bodies which appear to have survived digestion, although the sharp boundary is missing. In a series of digestions involving the action of individual enzymes on cells of Nostoc muscorum (pre-treated with Tris-EDTA) for 12 hr at 45°C, RNase failed to remove the poly-

hedral bodies (Figure 60). DNase treatment resulted in deterioration of the polyhedral body image but most of the electron opacity of the contents remained (Figure 61). A control cell of Nostoc muscorum in water, treated with Tris-EDTA for 1 hr but without enzyme treatment, is shown in Figure 62. Polyhedral bodies are missing from cells treated with lipase (Figure 63), although remnants of their presence are indicated in the spore pictured in Figure 64. Acid phosphatase appears to have caused a differential or partial digestion of polyhedral bodies in Figure 65. Pronase caused general destruction of wall material and of the sharp boundary of the polyhedral bodies but did not remove the electron-dense contents (Figure 66).

Autoradiography

Tritiated thymidine was incorporated into blue-green algal DNA, but the number of silver grains observed in an electron micrograph was limited by the relatively slow growth of the algae and by thinness of sections. Labelling was considerably greater in the faster-producing bacteria associated with the heavy sheaths of Microcoleus vaginatus and Symploca muscorum. Within the algal cell, the label appeared to be associated with the nucleoplasm but was not confined to the central region. Representative micrographs are shown in Figures 67 and 68. Staining before and after coating of the sections on the grid was essential to give an

image of sufficient contrast to see the biological material through the emulsion.

Labelling of DNA with tritiated actinomycin D was employed to avoid the problem of biosynthesis associated with a slow growth state, since actinomycin D should attach immediately to the algal DNA if permeability is achieved (Simard, 1967; Reich and Goldberg, 1964). A somewhat higher density of labelling was attained (Figures 69 through 72), indicating the presence of DNA in locations throughout the cell.

Studies on the Photosynthetic Pigment System

Arrangement and fine structure of thylakoids

The membranous system which provides the site for the photosynthetic reactions was found to vary in number, location, and arrangement of thylakoids with species, age, and growth conditions. Very young cells displayed fewer thylakoids, and they usually appeared to be peripheral in location. Under the conditions employed in this study (Table 1), the patterns characteristic of thylakoid arrangement in vegetative cells can be summarized as follows.

In Anacystis nidulans, the thylakoids usually are peripheral, few in number, and regularly spaced parallel to each other (Figure 73). A similar pattern was observed in Spirulina major (Figures 9, 33, and 38), although the plane of section in Figure 52 shows thylakoids occupying interior

regions. The thylakoids of Nostoc muscorum showed greater undulation and distribution throughout the cell, although the tendency for parallel arrangement appeared to be strong (Figure 74). The thylakoid pattern of the vegetative cell is quite different from that of the akinete; in the latter, intrathylakoidal spaces often are expanded (Figure 75) or the lamellae form elaborate reticulate arrangements (Figure 77). The lamellar remnants in the heterocyst pictured in Figure 75 appear grossly altered from the thylakoids of a vegetative cell. The thylakoids in Microcoleus vaginatus (Type A) are unusually numerous and regularly parallel, often appearing to occupy most of the cell volume (Figure 78). Frequently a group of thylakoids lies in a plane tangential or perpendicular to an adjacent group (Figure 79). Fixation of the Microcoleus vaginatus ecophenes with glutaraldehyde followed by osmium tetroxide gives a different image (Figures 80 and 81), but the same pattern is evident. All intrathylakoidal spaces are greatly expanded in Aphanizomenon flos-aquae (Figure 11), and the thylakoids are extremely irregular in shape and pattern. An intrathylakoidal spacing sometimes is observed in Arthrospira Jenneri (Figure 12), but the thylakoids are parallel and regularly arranged in layers. The thylakoids of Tolypothrix distorta are elaborately swirled and looped throughout the cell (Figures 6 and 34).

At low magnifications, the thylakoid image in cross-

section consists of two tripartite unit membranes either closely appressed or separated by an intrathylakoidal space. Occasionally a five-layered, myelin-like configuration is observed (Figure 6). At high magnifications, the image of the thylakoid membranes gives the impression of sub-unit structure in the form of spherical particles 20-30 A in diameter. This bead-like configuration may be due to diffraction phenomena. It seems unlikely that units of such small size would appear as discrete particles when viewed in a section that is 600 mμ in thickness. However, they correspond in dimensions to sub-unit structures reported by Weier et al. (1966) for thylakoids of a green alga.

In freeze-etched material, globular units measuring 125-175 A in diameter appear closely associated with thylakoidal elements of Nostoc muscorum (Figures 103 and 104).

Phycobilisomes

In material fixed in osmium tetroxide (with or without pre-fixation in glutaraldehyde or picric acid-glutaraldehyde) and stained with uranyl acetate or uranyl acetate-lead citrate, but not in material fixed in potassium permanganate, periodically arranged, diffuse granules of moderate electron density frequently are observed to be associated with the thylakoids. Double staining accentuates these granules which appear only on the exterior surfaces of the thylakoids. The granules are in rows and are either attached to or partially embedded in

the thylakoid membrane. In one plane of sectioning, such that the row is viewed "face on", their profiles are approximately semicircular, flattened at the region of association with the thylakoid. In another plane of sectioning, such that the flattened granules are viewed "edge on", the image at low magnification is one of rows of roughly parallel elements, oriented perpendicular to and in contact with the thylakoid. Figures 12, 51, and 85 through 93. The geometric form of these granules is best described as discoid (Figure 85b). The granules have been observed in every species studied, although they are most conspicuous in micrographs of Arthrospira Jenneri, Microcoleus vaginatus, Nostoc muscorum, and Symploca muscorum.

An accurate measurement of the size of the granules is difficult to obtain because they do not appear to have precise boundaries, but the average diameter of the semicircular profile is 350 A. The thickness of individual granules in "edge view" ranges from 60 to 110 A. Rows of granules are generally parallel to each other with a center-to-center spacing (in "face view") of approximately 450 A. Granules on adjacent thylakoids appear to interdigitate in Microcoleus vaginatus (Type A) but more commonly appear to touch head-to-head in Nostoc muscorum.

Occasionally the image of the granule appears to have sub-unit structure (Figures 94 through 97). When many of

these images were subjected to the technique of rotational reinforcement (Markham et al., 1963), a periodicity of six was encountered most frequently, suggesting an organization of six sub-units per granule (Figures 98 and 99).

The appearance of these granules is so similar to that of the pigment-containing phycobilisomes associated with the thylakoids of red algae (Gantt and Conti, 1966a, 1966b; Gantt et al., 1968) that further studies were undertaken to establish the location of the biliproteins in blue-green algae. Cells of Nostoc muscorum were suspended in phosphate or NET buffer containing 17% sucrose and 20% Ficoll, chilled, and ruptured at 4,000 psi in a French press. An aliquot of the resulting thick suspension was treated with 0.2% deoxycholate at 4° for 15 min, centrifuged, and washed. The pellet was fixed in 4% glutaraldehyde followed by 1% osmium tetroxide. The granules which normally are seen associated with the thylakoids were absent from deoxycholate-treated material (Figure 100) but present in the untreated control (Figure 101). The absorption spectrum of the supernatant from deoxycholate treatment in NET buffer showed a maximum at 618 mμ (phycocyanin) and a shoulder at 460-490 mμ (carotenoids) but very little absorption due to chlorophyll a. In phosphate buffer, some chlorophyll was extracted as indicated by small absorption peaks at 677 and 443 mμ. Absorption spectra of the untreated control material indicated the presence of most

of the phycocyanin in the pelleted, ruptured cells and very little in the supernatant. Therefore, treatment with deoxycholate removed the phycocyanin pigment from the cells, and this was manifested by the loss of the granules from the outer surfaces of the thylakoids, and the presence of phycocyanin in the extract.

Whole cells of Nostoc muscorum were fixed in 4% glutaraldehyde, washed, and extracted with 80% methanol-20% acetone until no visible color appeared in the extracts. The absorption spectrum of the first extract showed a small peak at 664 mμ (chlorophyll a) and a larger peak at 478 mμ (carotenoids) and was golden yellow in color. Additional extracts were yellow-green, and the extraction of chlorophyll a and carotenoids was indicated by the absorption spectra. The remaining blue-grey pellet of extracted cells was fixed in 1% osmium tetroxide for electron microscopy, and an absorption spectrum of the cells spread on glass fiber disks indicated the absence of chlorophyll and the presence of phycocyanin. Examination of this extracted material in the electron microscope showed a major loss in the image of the normally conspicuous thylakoid system but a retention of many of the granules associated with the surfaces of the thylakoids (Figure 102). The combined spectroscopic and electron microscopic evidence was accepted as establishing the granules as the location of phycobilin pigments. Since the term

"phycobilisome" has been used for granules of similar structure and function in the Rhodophyta, the name will be adopted in further discussion of the Cyanophyta.

In vivo absorption spectra

In vivo absorption spectra in the visible and near infrared regions were desired to better correlate the pigment content of each alga studied with the ultrastructural image of the thylakoids and associated phycobilisomes. The difficulties involved in obtaining a well-resolved spectrum on a reasonably heavy cell suspension are well known, and numerous procedures have been suggested to minimize the loss due to light scattering, sedimentation, and the sieve effect. Absorption spectra on moderately thin suspensions were obtained using a Cary Model 14 spectrophotometer with opal glass plates placed in the light paths between the specimen and reference cuvettes and the detectors (Shibata et al., 1954). Increase in incident light intensity by using a 700-watt projector lamp as the source was an additional aid. However, the most successful technique, especially for spectra of heavily-sheathed, filamentous species which could not be evenly suspended in solution, was spreading of the sample on a Gelman glass fiber filter disk (Truper and Yentsch, 1967) which is sufficiently translucent to permit passage of some light and can be balanced against a similar, moistened disk lacking specimen. Anacystis nidulans and Nostoc muscorum

could be filtered onto the disks for even spreading. Filamentous species were spread as evenly as possible with a spatula. The absorption spectra of the eight Cyanophyta under study are shown in Figures 105 through 112. Symploca muscorum and Microcoleus vaginatus (Type B) were found to possess both phycocyanin and phycoerythrin. The spectra of the other species indicated phycocyanin as the only biliprotein present, although peaks representing very small quantities of other biliproteins might be obscured by the lack of sharp resolution between maxima in several of the spectra.

Effect of variation in light intensity

The in vivo absorption spectra of samples of Nostoc muscorum which were grown at 20, 100, 200, and 900-1,000 ft-c were obtained each week for three weeks. An aliquot of 5 ml of each culture was filtered through a glass fiber disk so the amounts of each pigment could be compared (Figure 113a). The absorption peaks for chlorophyll a at 678 mμ were practically superimposable in wavelength and height for all light intensities. The amount of phycocyanin varied somewhat, but the absorption maximum remained constant at 625 mμ (in water). The carotenoid peak at 490 mμ increased in height as light intensity increased. The ratios of chlorophyll to phycocyanin at the four light intensities were calculated by the method of Jones and Myers (1965) which takes into consid-

eration the slight absorption of phycocyanin at 678 mμ and of chlorophyll a at 625 mμ (Table 2).

There is no obvious difference at the ultrastructural level between cells grown at 100 or 200 ft-c (Figures 114 and 115). In cells grown at 900-1,000 ft-c, structured granules are numerous and large, and α-granules are prominent (Figure 116). Cells grown at low light intensity fre-

Table 2. Effects on in vivo pigment ratios of varying light intensity or wavelength

Incident illumination	Intensity of illumination	$\frac{678}{625}$[a]	Absorption $\frac{\text{chlorophyll}}{\text{phycocyanin}}$[b]	$\frac{490}{625}$
White light (200 watts fluor., 100 watts incand.)	900 ft-c	1.09	1.33	1.22
	200	1.14	1.43	1.12
	100	1.12	1.39	1.01
	20	1.09	1.30	0.87
Orange light (610-640 mμ)	8.1 uw/cm^2	1.24	1.62	0.91
Red light (660-690 mμ)	8.5 uw/cm^2	0.99	1.17	0.90

[a]Ratio based on peak heights (Figures 113a, 113b).

[b]Ratio corrected for absorption of chlorophyll a at 625 mμ and phycocyanin at 678 mμ using simultaneous equations, according to Jones and Myers (1965).

quently show expanded intrathylakoidal spaces and a more random orientation of thylakoids (Figure 118).

Effect of variation in wavelength of light

More dramatic than the effect of varying light intensity was the result of exposing Nostoc muscorum for three weeks to light of a narrow wavelength band. In orange light (610-640 mμ), which is absorbed mainly by phycocyanin and very slightly by chlorophyll a, a change in thylakoid arrangement to circular but parallel configurations was characteristic of all cells examined, and phycobilisomes were present but inconspicuous (Figure 119). The in vivo absorption spectrum was in agreement with these observations (Figure 113b). The chlorophyll/phycocyanin ratio was 1.62, an increase from the ratio of 1.30 in low-intensity light of a full wavelength range (incandescent and fluorescent light) (Table 2). A striking increase in size and number of phycobilisomes was evident in cells grown in red light (660-690 mμ) (Figure 120). The in vivo absorption spectrum showed peaks of almost equal height for chlorophyll (678 mμ) and phycocyanin (628 mμ) absorption (Figure 113b). The chlorophyll/phycocyanin ratio decreased to 1.17 (Table 2).

Growth in darkness

Cells of Nostoc muscorum and Microcoleus vaginatus (Type A) were able to survive in the dark for five weeks with 1%

sucrose or glucose added to the medium. Aliquots were harvested, washed, and fixed under green light in 4% glutaraldehyde and 1% osmium tetroxide for electron microscopy. Growth was extremely limited, but cells appeared green at the end of the experimental period. Cell structure did not appear to be altered appreciably, although the nucleoplasmic volume seemed greatly reduced in proportion to cytoplasm (Figures 121 and 122). The intrathylakoidal spaces of most cells appeared to be deeply stained or the inner membrane layers were appressed to give a five-parted, myelin-like image (Figures 122 and 123). Thylakoids of Microcoleus vaginatus frequently were appressed in myelin- or grana-like stacks (Figure 123).

DISCUSSION

Nucleoplasm

Properties of the cell wall; lysis

Many species of blue-green algae are remarkably resistant to mechanical rupture as well as to enzymatic and chemical lysis. In order to release DNA with a minimum of shearing and to isolate intact intracellular inclusions, a method was sought for opening the blue-green algal cell as gently but thoroughly as possible. To achieve this goal, a study of the properties of the cell wall seemed essential. Conditions of plasmolysis, protoplast formation, and lysis were investigated for Anacystis nidulans, Nostoc muscorum, and Microcoleus vaginatus (Type A).

Evidence presented in the literature does not permit a clear-cut analogy of the blue-green algal cell wall with the wall of either Gram-positive or Gram-negative bacteria. Several investigators have quantitatively analyzed cell walls isolated from blue-green algae and demonstrated the presence of a mucopolymer component. Drews and Meyer (1964) analyzed isolated walls of Anacystis nidulans and Chlorogloea fritschii and found proteins and lipids in addition to the mucopolymer. Mucopolymer was a major component (about 50%) of isolated walls of Phormidium foveolarum and Tolypothrix tenuis (Höcht et al., 1965). The molar ratio pattern of glucosamine:muramic acid:glutamic acid:diaminopimelic acid:

alanine (1:1:1:1:2) in the mucopolymer component is also characteristic of Gram-negative bacteria. They recorded a 2:1 ratio of glucosamine to muramic acid in Anacystis nidulans, suggesting a unique organization of the polysaccharide backbone in the mucopeptide of this species. However, Drews and Meyer (1964) reported the ratio to be approximately 1:1 for Anacystis nidulans. Mannose appeared to be a persistent component of the mucopeptide in Anacystis nidulans (Höcht et al., 1965). On analysis of isolated walls of Phormidium uncinatum, Frank et al. (1962a, 1962b) found 52% of the dry wall weight to be mucopolymer with a molar ratio corresponding to that of Gram-negative bacteria (Martin and Frank, 1962). The investigators qualified this observation by mentioning that outer layers of the wall probably were lost in the isolation procedure. Even when this possibility is taken into consideration, the values for the amount of mucopolymer in the cell wall in these independent studies seem unusually high to be equated with the amount found in Gram-negative bacteria and more nearly in line with the 40-90% range of mucopolymer in the Gram-positive bacterial wall.

Based on electron microscopic observations, Frank et al. (1962a) suggested that the mucopolymer occupied the electron-transparent zone immediately peripheral to the plasma membrane. Allen (1968b), noting the variation in width and occasional absence of this electron-transparent zone, pro-

posed that it is an artifact of preparation and that the true site of the mucopolymer is in the adjacent (peripherally) thin, electron-dense layer. The latter view agrees with the location given to the mucopolymer in Escherichia coli by Murray et al. (1965). Discussing wall layers in Jost's terminology (see Figure 74), Allen concluded that since this dense layer, corresponding to Jost's L_{II} layer, invaginates with the plasma membrane to form the new septum in cell division, it is logical that it should contain components imparting strength and shape. Steed and Murray (1966) observed formation of a true septum (which appeared to consist of mucopolymer layer only) in division of Gram-negative bacterial cells and concluded that division of Gram-negative and Gram-positive bacteria is remarkably similar. Later the septum of the bacteria or blue-green algae is split by the growth of outer wall to complete cell separation. Allen (1968b) attributed to synthesis the thickening of the L_{II} layer at the point of initial septum formation. In the current investigation, the electron transparent zone (L_I according to Jost) immediately peripheral to the plasma membrane appears uniform in thickness and occurrence in the species examined. Since the L_I layer, as well as the L_{II} layer, invaginates with the plasma membrane to form the septum in the early stages of cell division, there is no reason to eliminate the L_I layer from consideration as the strength-imparting mucopolymer zone.

Moreover, Jost (1965) reported granules in different quantities on layers of freeze-etched Oscillatoria which he interpreted as mucopolymer and middle lamella, implying that there are two distinct cross-wall layers.

When the blue-green algal species employed in the current study were subjected to Gram-staining, the general appearance was that of cells opaquely stained a deep purple, indicative of a Gram-positive reaction. The test was repeated many times because the results of Gram-staining are not always definite or reproducible. In Arthrospira Jenneri, Microcoleus vaginatus, Symploca muscorum, and Tolypothrix distorta, the wall appeared to stain red around purple cells. Empty walls were stained red by the counterstain. In many cases, the cross-walls appeared unstained or faintly red. The sheath material did not stain. Currently, it is thought that the crystal violet-iodine lake is not washed out of a Gram-positive organism during the decolorizing step with ethanol due to the nature of the wall, and consequently the blue-purple color of the primary stain is retained. Classification of the blue-green algal cell wall as Gram-positive on the basis of these results is contradictory to chemical and ultrastructural analogies between the blue-green algal and Gram-negative bacterial cell wall. The literature contains very few reports

of Gram-staining on blue-green algae. Echlin (1964a) mentioned that Anacystis nidulans is Gram-positive.

Lysozyme is a glycosidase which catalyzes hydrolysis of bonds in the structurally vital N-acetylglucosamine-N-acetylmuramic acid backbone of mucopolymer in the procaryotic cell wall. The walls of different blue-green algal species vary widely in their resistance to the lytic action of lysozyme just as do bacterial walls. Drews and Meyer (1964) reported that lysozyme lysed isolated walls of Anacystis nidulans and Chlorogloea fritschii but whole cells could be transformed into spheroplasts only by including EDTA in the medium with lysozyme. Höcht et al. (1965) found that the walls of Anacystis nidulans and Phormidium foveolarum were degraded by lysozyme but that the normal cell shape was retained in cells of Tolypothrix tenuis. The production of protoplasts (wall entirely removed) and spheroplasts (wall partially removed) has been reported. Protoplasts were prepared from Oscillatoria amoena (Fuhs, 1958a), Fremyella diplosiphon, Plectonema calothricoides, Phormidium luridum, and Oscillatoria tenuis, but Oscillatoria formosa, Synechococcus lividus, and Cyanidium caldarium were little affected by 24-hr treatment with lysozyme (Crespi et al., 1962). The protoplasts were maintained in osmotic balance in the presence of sucrose and Ficoll but lysed easily on dilution with water, releasing the biliproteins. When the marine blue-green species

Lyngbya Lagerheimii, Microcoleus chthonoplastes, Plectonema terebrans, and Agmenellum quadruplicatum, and fresh-water species Anacystis nidulans, Anabaena variabilis, Nostoc muscorum, and Oscillatoria sp. were treated with lysozyme, filaments were disrupted and cells rounded but none burst on dilution with water (Fulco et al., 1967). The investigators interpreted these results as indicating spheroplast rather than protoplast formation. The marine species required longer than the fresh-water species to form spheroplasts and possessed higher internal osmotic pressures. Surprisingly, the highest osmotic pressure of the algae included in the study was attributed to the fresh-water species Nostoc muscorum, but no explanation was advanced. This observation is important in connection with the difficulty encountered in lysing cells of Nostoc muscorum during the current study.

The range in sensivity of blue-green algae to lysozyme treatment may reflect varying amounts of mucopeptide in the wall or varying composition and arrangement of the components of the mucopolymer. In reviewing the mechanisms of enzymatic bacteriolysis, Strominger and Ghuysen (1967) pointed out that tightness of the peptidoglycan net, the presence of non-peptidoglycan components, and the chemical properties of the mucopeptide (i.e., size, extent of cross-linking, frequency of substitution, and net electrical charge) can affect and even prevent the action of lysozyme. If polysaccharides,

often associated with lipids and proteins, are present as non-mucopeptide components (as in most Gram-negative and some Gram-positive bacteria), lysozyme is inactive or limited in activity.

Several ancillary procedures, including heat treatment, incubation at alkaline pH, and freezing and thawing, have been employed to potentiate the action of lysozyme and improve the yield of protoplasts or spheroplasts (Martin, 1963). The use of a chelating agent such as ethylenediamine tetraacetic acid (EDTA) (Repaske, 1956; Fujita and Sato, 1966; Birdsell and Cota-Robles, 1967) and pre-treatment with lipase (Ghosh and Murray, 1967) have been successful in dissolving or penetrating the outer wall layers and exposing the inner mucopolymer layer to enzymatic attack. Noller and Hartsell (1961a, 1961b) described four methods for rendering Gram-negative bacteria susceptible to lysozyme digestion, all of which alter the outer layers of the wall chemically or structurally and thus expose the mucopolymer layer to attack. Birdsell and Cota-Robles (1967) found that plasmolysis in sucrose-Tris buffer enabled lysozyme to degrade the wall of Escherichia coli, with or without the addition of EDTA. Divalent cations are thought to play an important part in maintaining the lipopolysaccharides of the outer layer of Gram-negative bacteria, and treatment with EDTA results in a release of cell wall lipopolysaccharides (Leive, 1965d). Apparent spheroplasts may be formed by lysozyme treatment of cells which retain their shape

unless EDTA is employed to weaken the outer layers of the wall and permit rounding of the cell to the protoplast configuration. In this respect, lipoproteins are thought by Noller and Hartsell (1961b) to contribute to the rigidity of the normal cell in addition to the strength supplied by the mucopolymer.

Plasmolysis of Nostoc muscorum in sucrose-Tris buffer proved to be a helpful preliminary to lysozyme digestion in the presence of EDTA and was less destructive to cell contents than lysis with lysozyme, trypsin, and n-butanol, based on observation of sectioned material (Figures 20 through 26). This treatment appeared to have no effect on the thickened, complex envelope of the spore pictured in Figure 25. Of the three species lysed for DNA isolation, the heavily sheathed Microcoleus vaginatus (Type B) proved most resistant to lysozyme digestion in contradiction to the suggestion of Crespi et al. (1962) that heavily-sheathed species may contain more lysozyme substrate than those with less sheath material. Since the lysozyme substrate, mucopolymer, is a wall component, the relationship to sheath thickness, if it exists at all, would be indirect. Next most resistant to lysozyme was Nostoc muscorum, while Anacystis nidulans lysed rather easily on treatment with lysozyme and EDTA without the necessity of plasmolyzing the cells in advance.

It is dangerous to generalize concerning the Gram-posi-

tive nature of the blue-green algal wall based on the accumulated evidence from Gram-staining and protoplast formation in Nostoc muscorum. At best, protoplast formation and lysis were incomplete and required lengthy induction times and high concentrations of lysozyme, but several micrographs indicate wall removal (Figures 23, 24, and 26). Lytic enzymes were isolated from cultures of Streptomyces albidoflavus which had been grown in the presence of walls of Nostoc muscorum or Anacystis nidulans. These enzymes were used to rupture cells of the respective algae. Lysis proved to be slow and incomplete, but the method was retained as an alternative for the isolation of algal DNA.

Isolation and characterization of DNA

Working with the handicap of limited cell lysis, isolations of DNA were restricted to low yields. Although the first crude precipitation of DNA with ethanol provided a viscous product which could be spooled on a glass rod, purification to remove protein and RNA involved considerable loss of DNA. The final precipitation of pure DNA with isopropanol as recommended by Marmur (1961) was unsuccessful, and treatment with five volumes of cold 95% ethanol followed by centrifugation was necessary to recover the product.

The base composition of DNA can be determined from its buoyant density in CsCl (Schildkraut et al., 1962; Mandel et al., 1968) or its thermal denaturation temperature (Marmur and

Doty, 1962; Mandel and Marmur, 1968). The DNA isolated from Anacystis nidulans and Nostoc muscorum was subjected to CsCl density gradient analysis to determine the buoyant densities and base ratios. The appearance of two bands for each DNA sample in addition to the reference band was surprising. Edelman et al. (1967) characterized the DNA isolated from a number of blue-green algae by the method of Marmur (1961) following cell breakage in a French press and found only unimodal distributions in CsCl equilibrium density gradients. A second band in many of the isolated DNA fractions was identified as a carbohydrate polymer. They reported buoyant densities of 1.715 g/ml (56% GC) for Anacystis nidulans and 1.703 g/ml (44% GC) for Nostoc muscorum. In the current study employing different methods of isolation, the buoyant densities and corresponding GC (guanine plus cytosine) contents for the two DNA bands were found to be 1.726 g/ml (67.3% GC) and 1.714 g/ml (55.1% GC) in Anacystis nidulans and 1.723 g/ml (64.3%) and approximately 1.709 g/ml (50% GC) in Nostoc muscorum. The minor band of Anacystis nidulans corresponds in buoyant density to the single band reported by Edelman et al. (1967). Subsequent treatment of each DNA sample with RNA-free DNase, followed by CsCl equilibrium centrifugation, resulted in the disappearance of the blue-green algal DNA bands, leaving only the reference DNA band. These results eliminated consideration of a non-nucleic acid contaminant such as a polysaccharide. The presence of DNA from a contaminating organism would be suspected

as the logical explanation for two DNA bands, but the culture appeared unialgal and bacteria-free on electron microscopic examination and when checked for growth on agar-0.1% Bacto-tryptone plates. DNA isolated from Anacystis nidulans by two different isolation and purification procedures gave identical buoyant densities for each of the two bands. The possibility of two strains of Anacystis nidulans is unlikely based on the wide divergence of GC contents (67.3% and 55.1%) corresponding to the two bands.

The DNA isolated from blue-green algal species and degraded for chemical analysis has not been reported to contain appreciable amounts of unusual bases. Biswas and Myers (1959, 1960b) reported a value of 0.82 for the AT/GC ratio of the DNA isolated from Anacystis nidulans. The same ratio calculated from the two bands obtained on CsCl gradients in the current research gave a value of 0.81 for the minor band (55.1% GC) and 0.49 for the major band (67.3%) of Anacystis nidulans. Biswas (1961) reported that the DNA in Nostoc muscorum is heterogeneous and can be divided into two fractions with different constituent bases; one fraction was soluble in alkali and the other in acids. He ascribed different metabolic patterns to the two types of DNA, but no further work in this area has appeared in the literature.

Although only one isolate of DNA from Nostoc muscorum was available for density gradient analysis in the current

study, the presence of two DNA bands from Nostoc muscorum as well as Anacystis nidulans represents a most interesting observation and poses the long-range problem of location and function. Isolations of DNA should be repeated, preferably on cultures grown from single cell isolates to eliminate the possibility of a contaminating organism. The fine fibrils observed in the nucleoplasm in electron micrographs have been accepted as representing strands of DNA. At this time, suggesting a site for the second DNA species can be no more than speculative, but Kaye et al. (1967) proposed that a satellite DNA which they isolated from Plectonema boryanum might be thylakoidal. Another possible site might be the enigmatic polyhedral bodies. DNase treatment affects but fails to completely digest the contents of the polyhedral bodies. However, other enzymatic digestions indicate that they are composed of more than a single chemical component. Any resident DNA might be protected from attack by DNase under the conditions of whole-cell treatment. The autonomous genophore of the bacterial episome (Cuzin and Jacob, 1967) might be considered as a possible model.

The presence of DNA and RNA in mitochondria and chloroplasts of eucaryotic cells has been well established and these nucleic acids have been characterized in a number of studies (Gibor and Granick, 1964; Nass and Nass, 1965; Brawerman, 1966). Differences in base ratios between chloro-

plast and nuclear DNA are available for a number of plant and algal sources (Kirk, 1966; Brawerman, 1966). As an example, it is interesting to compare the buoyant densities and corresponding GC values that Swift (1965) obtained for the DNA species from Swiss chard: nuclear DNA, 1.689, GC 31%; mitochondrial DNA, 1.705, GC 47%; chloroplast DNA, 1.700, 42%. The buoyant density of the nuclear DNA is not always lower than that of mitochondrial or plastid DNA. In some cases, plastid DNA has two components of different buoyant densities (Kirk, 1966; Brawerman, 1966). These observations are in line with an early hypothesis which has been revived and supported by Ris (1961, 1962) and Ris and Plaut (1962). Mitochondria and plastids possess genetic systems which determine many of their own properties. Since endosymbiotic relationships of blue-green algae and bacteria with host cells are well known, Ris suggested that cell organelles such as plastids and mitochondria may have evolved from endosymbionts during the evolution of plant and animal cells.

Electron microscopy

Although absence of a nuclear membrane in procaryotic cells has been well established, there clearly are cytoplasmic and nucleoplasmic regions. The nucleoplasmic region is generally characterized by a lower electron density than the cytoplasm, a random distribution of fine filaments of DNA,

and numerous ribosomes. The relative immobility of procaryotic cell contents which permits this separation of regions without an evident boundary suggests a gel state in vivo. However, such a state would complicate rapid replication of a randomly distributed genophore.

The nucleoplasm in all blue-green algae examined in the current study is irregular in outline and proportion of total cell volume and only roughly can be considered central in relation to the cytoplasmic areas. Ris and Singh (1961) recognized the occasional occurrence of DNA material in the peripheral regions in addition to the major accumulation in the central nucleoplasm. The ratio of nucleoplasm to cytoplasm appears to vary with species and conditions of growth, although the plane of sectioning undoubtedly contributes to proportioning in any given micrograph. In Nostoc muscorum (Figure 31), areas with the same electron density as cytoplasm are interspersed throughout the central region of nucleoplasm with the nucleoplasm appearing as an anastomosing, three-dimensional net in a relatively continuous cytoplasm. The terms "chromatoplasm" and "centroplasm" have been abandoned with good reason, and "nucleoplasm" and "cytoplasm" are valid only as expressions of chemical composition and properties but not in the sense of designating rigid locations.

Ribosomes stain deeply with uranyl acetate and lead citrate. They are observed throughout the blue-green algal cell but are especially numerous and evident in nucleoplasmic

regions where their electron opacity contrasts sharply with the less dense background. They are comparable in size (90-100 A) to bacterial ribosomes (Glauert, 1962) and ribosomes found in plastids and mitochondria (100-150 A) and are smaller than cytoplasmic ribosomes of eucaryotes (150-350 A) (Webster and Whitman, 1963; Jacobson et al., 1963; Swift, 1965). Edwards et al. (1968) reported that at high magnifications the ribosomes of Synechococcus lividus appeared to be in rosettes connected to filaments of DNA.

When fixed for electron microscopy by the Ryter-Kellenberger method (Kellenberger et al., 1958), the DNA of bacteria, dinoflagellates, actinomycetes, and blue-green algae appears as a mass of fine fibrils whose diameter (20-60 A) approximates that of the double-stranded DNA molecule (Hopwood and Glauert, 1960a, 1960b; Ris and Singh, 1961; Kellenberger, 1962). In discussing the electron microscopic image of DNA following fixation, Fuhs (1965a, 1965b) argued that osmium tetroxide does not react with or have any effect on DNA. Using viscosimetric measurements, Fuhs (1965a) showed that the conformation of native DNA is not changed by treatment with osmium tetroxide at pH 6-7 or by treatment with uranyl ions. He concluded that uranyl treatment does not alter the physical or organizational state of intracellular DNA in bacteria and, in fact, by complexing with intracellular nucleic acids reduces shrinking of cells as a whole and pre-

vents contraction of the bacterial nucleoid during dehydration. Post-treatment of osmium-fixed cells with aqueous uranyl acetate (Ryter-Kellenberger fixation) gives the now familiar image of fine molecular fibrils of DNA throughout the nuclear region which Fuhs (1964a, 1965b) considers the normal state in developing cells. Consequently, condensed states of DNA fixed by other methods should be considered artifacts (Ryter and Kellenberger, 1958).

Reports in the literature claim that the DNA of blue-green algae shows the same lability toward osmium fixation as bacterial DNA and only under conditions of the Ryter-Kellenberger fixation does the DNA appear as fine fibrils (Kellenberger, 1962). Hopwood and Glauert (1960a, 1960b) reported the resemblance of the nucleoplasmic fine structure of Anabaena cylindrica to that of bacteria, noting the response to slight changes (particularly in content of calcium ions) in the composition of the fixative. Dense masses within "vacuoles" were observed when Phormidium uncinatum (Drews and Niklowitz, 1956) and Oscillatoria amoena (Fuhs, 1958b) were fixed according to Palade's (1952) method. Admittedly, the modified osmium fixation (Appendix A; Pankratz and Bowen, 1963) that was employed in the current study compromises to some extent the very fine fibrillar image of DNA that is obtained with the Ryter-Kellenberger fixation (Figure 32). However, the Ryter-Kellenberger fixation causes

such dense staining of other cellular components that treatment with uranyl acetate before dehydration usually was omitted. Severe clumping of DNA into dense masses could be avoided by using the modified method.

Mention should be made that the fine DNA fibrils observed in the bacterial nucleoid appear to lie roughly parallel and arranged in loose bundles (Fuhs, 1965c), although the pattern varies from cell to cell and changes continuously during DNA replication and nucleoid division. An analogous organization is less apparent in most blue-green algae, although the nucleoplasm of Anacystis nidulans sometimes appears comparable to the bacterial nucleoid.

Ris (1961) proposed the term "genophore" for the physical entity corresponding to a linkage group in the procaryotes to distinguish it from the chemically and morphologically distinct "chromosome" of the eucaryotes. Although a few workers have suggested changes in organization and distribution of chromatin analogous to mitosis (Leak and Wilson, 1960), it is generally believed that no mitotic apparatus is present in the blue-green algal cell.

It is not yet possible to visualize the blue-green algal genophore. The genetic material of Escherichia coli is known to be organized as a single linkage group in the form of a long, circular macromolecule of DNA. The Kleinschmidt technique (Kleinschmidt, 1968) for spreading a monolayer of DNA

at an air-water interface has been utilized to study the genophores of bacteria and phages. Because blue-green algae are so resistant to lysis, the technique of lysing at the air-liquid interface, followed by spreading of the DNA, could not be utilized in the current study. The DNA spread in this work was isolated by the method of Massie and Zimm (1965) and represents the same isolate on which buoyant density determinations were made. The electron microscopic image of the DNA, which was spread according to the method of Zahn et al. (1962), indicates great continuous length with few ends in evidence. This observation strengthens the probability that the genetic material of the blue-green algal cell also is composed of one linkage group contained in a long macromolecule of DNA. The strand width measures about 29 A along most of the length, in agreement with measurements of DNA in sectioned material.

On the basis of cytochemical studies on Calothrix and Oscillatoria species and chemical studies on Nostoc muscorum, Biswas (1957, 1961) concluded that the nucleic acids of blue-green algae are bound by proteins. Zubay and Watson (1959) and Wilkins and Zubay (1959) found non-histone proteins associated with DNA isolated from Escherichia coli when deproteinization steps were omitted. Ris and Chandler (1963) attributed the aggregation of DNA fibers, unless complexed with a heavy metal such as uranyl acetate following fixation and preceding dehydration, to lack of associated basic proteins.

De and Ghosh (1965) reported a lack of positive staining for histones in four genera of Cyanophyta and considered this absence an explanation for the diffuse nature of the genetic material. They reasoned that since nuclear histones are known to be specific repressors of genetic material and important in chromosome coiling, their absence in bacteria and blue-green algae (which lack organized chromosomes) is understandable.

The occasionally uneven diameter of the spread DNA in the current study is difficult to understand since treatment with RNase and pronase were employed in the isolation procedure to digest RNA and protein, followed by chloroform-isoamyl alcohol treatment to remove protein before precipitation of the DNA with ethanol. Any protein should have been removed by these purification steps. However, the possibility remains that associated protein may be present.

Polyhedral bodies

Although polyhedral bodies have been described in many studies of blue-green algae, their chemical composition and function remain unresolved. They always appear to be associated with nucleoplasm, usually but not always in the central region of the cell. Frequently they are seen in clusters. The characteristic angular outline of the inclusions is retained when they are released from their cellular environment if care is taken to protect them from osmotic shock

(Figures 56 through 58) and can be considered inherent rather than imposed by compression or other effects of their surroundings in the cell. The boundary appears electron-dense after osmium (but not permanganate) fixation and staining with uranyl acetate or uranyl acetate-lead citrate. Less contrast is observed with lead citrate alone. Frequently the image of the boundary appears to be that of a tripartite membrane (Figures 46 through 51) of dimensions slightly narrower (55 A) than those of the thylakoid and plasma membranes (70-80 A). The dense layers of this boundary image sometimes appear asymmetric with the external layer wider and more electron-dense than the internal layer (Figures 45 and 50). This difference may account for the fact that the internal layer usually is inconspicuous to the point of not being noticed at low magnifications against the electron-dense contents of the polyhedral body. The appearance of an internal layer may simply be an interface effect with the molecules of the body of the granule specially oriented or packed at the interface and may result from an orientation such that this region adsorbs or binds more osmium. The staining characteristics of the polyhedral body boundary differ from those of the thylakoid and plasma membrane. The latter have electron-dense profiles in permanganate-fixed material while the boundary of the polyhedral body shows almost no contrast to the moderate density of the contents under these conditions. In this respect, the staining characteristics of the poly-

hedral body boundary resemble those of the outer membrane (Jost's L_{IV} layer) of the wall. Peat and Whitton (1967) reported a single case of a polyhedral body surrounded by a membrane in over 2,000 examined after permanganate fixation but mentioned structures "resembling polyhedral bodies ... found in intimate association with the photosynthetic lamellae." Some of the latter were membrane-bounded.

The contents of the polyhedral body are rather homogeneously and densely stained by uranyl acetate after osmium fixation. Permanganate fixation results in a less dense image. Isolated polyhedral bodies frequently exhibit heterogeneous density, and this condition occasionally is observed within normal vegetative cells as well. Peat and Whitton (1967) attributed the heterogeneous staining that they observed in some polyhedral bodies of Chlorogloea fritschii to internal structure. In some cases, the dense boundary of the polyhedral body retains its angular shape although the contents is missing. Occasionally the contents appear to have a diffuse edge rather than a sharp boundary, but such an image is understandable in terms of plane of sectioning just as the thylakoid image often appears blurred in tangential section. The thickness of the section (about 600 A) must be kept in mind.

Edwards et al. (1968) distinguished between the polyhedral bodies and polyphosphate granules in Synechococcus lividus on the basis of staining and stability. They reported

a moderate electron density and stability in the electron beam for polyhedral bodies as opposed to strong electron density and instability of polyphosphate bodies. Jensen (1968) observed spherical, electron-dense bodies in Nostoc pruniforme. These inclusions usually were in close association with the nucleoplasm and the less electron-dense polyhedral bodies and sometimes appeared to be membrane-bounded. Cells which had been grown in a medium enriched with inorganic phosphate contained an abundance of these inclusions which he identified as polyphosphate bodies on the basis of their appearance after lead sulfide staining, according to the procedure of Ebel and Muller (1958). These polyphosphate bodies were clearly distinct from polyhedral bodies in electron density and readily disintegrated under the electron beam while polyhedral bodies remained unchanged in appearance.

Enzyme digestions seem to indicate a complex chemical composition for the polyhedral bodies. Reese (1967) reported apparent removal of polyhedral bodies from fixed cells of Nostoc muscorum with RNase, although the conditions of the digestion were severe (60°C for 4 hr). Polyhedral bodies could be detected in the control. In the current study, RNase under milder conditions of treatment did not remove polyhedral bodies from unfixed cells of Nostoc muscorum (Figures 59 and 60). DNase treatment at 45°C for 12 hr appeared to cause partial digestion, including general erosion of the boundary

(Figure 61). Acid phosphatase caused partial digestion of the contents of the polyhedral bodies, leaving a mottled appearance (Figure 65). A possible protein component of the electron-dense boundary was indicated by changes in the electron microscopic image after pronase digestion (Figure 66). Of the enzymes employed in the series of digestions, lipase seemed most effective in removing the polyhedral bodies (Figures 63 and 64). Digestion of the polyhedral bodies in the akinete pictured in Figure 64 seems to be less complete than in the vegetative cell for the same reaction time, probably resulting from resistance to enzyme permeability by the thick and perhaps chemically different spore wall. The results of this series indicate probable lipid, phosphate, and protein components of the polyhedral bodies, and, despite the lack of convincing results in the nuclease experiments, the possibility of nucleic acid constituents cannot be dismissed.

Speculations of the function of the polyhedral body have included a nucleolar role. The possibility of a DNA component (based on partial digestion by DNase) and an RNA component (Reese, 1967) in the polyhedral bodies would support this suggestion.

Almost nothing is known concerning the mechanism of replication and distribution of genetic material in cell division of blue-green algae. The constant association of

polyhedral bodies with DNA-containing regions makes them possible candidates for a role in this unexplained process. In studies on replication of the bacterial genophore, Hanawalt and Ray (1964) found that DNA had to be disaggregated from complexes with lipase and proteolytic enzymes to free the "growing point" of the replicating DNA. The polyhedral bodies might serve as chemically similar complexes which anchor "growing points" for replication of blue-green algal DNA.

Ryter (1968) recently reviewed the mounting evidence that there is an association between bacterial DNA and the cytoplasmic membrane (either directly or via mesosomes). It is not known whether the point of attachment and the point of replication are identical or whether DNA is anchored and the site of replication moves along the length of the genophore. Cuzin and Jacob (1967) demonstrated that the genophore and episome of Escherichia coli segregate together, implying that both replicons are attached to some common structure. The possibility of an episomic replicon (suggested by the observation of a second DNA species) in blue-green algae, coupled with the possible need for some means of separating a replicated genophore in cell division, leads to speculation that the polyhedral bodies might be involved. The model proposed by Jacob, Brenner, and Cuzin (1964) for a mechanism of distribution of sister genophores hypothesizes attachment of

the genophore to the plasma membrane and synthesis of new membrane material at the point of attachment to bring about separation. The attachment of the blue-green genophore to a polyhedral body or a cluster of polyhedral bodies appears to be morphologically feasible and might serve in lieu of attachment to the plasma membrane, although there is no electron microscopic evidence to date for regulated separation of polyhedral bodies in a cluster. Since there are many polyhedral bodies or clusters in most blue-green cells, the possibility of multiple copies of the genophore would have to be anticipated. Resistance to mutation among the blue-green algae is a characteristic which supports the concept of multiple copies of the genophore.

Enough observations are available of polyhedral bodies in contact with thylakoid membranes (Figures 51 through 55) to support the possibility of a relationship between these cellular components. The indication of lipid and protein constituents of the polyhedral bodies might be the basis for thylakoid synthesis on cell division. Figures 40 and 51 suggest that thylakoids could be formed in association with polyhedral bodies. Chapman and Salton (1962) reported centrally located membranes of Nostoc muscorum which were similar in appearance to photosynthetic thylakoids but more densely stained by post-treatment of osmium-fixed cells with uranyl acetate (no additional staining). These membranes seemed to be prevalent in

cells undergoing division, and it was suggested that they might be newly synthesized lamellae. Such a differential staining was not encountered in the current study with uranyl acetate following osmium fixation. Echlin (1964b) discussed the possibility of thylakoid proliferation in the "nuclear region" followed by movement to the periphery of the cell.

All of these suggestions for functions of the polyhedral bodies are recognized as speculations, and this problem will require much more investigation. However, the ubiquitous nature of these inclusions in blue-green algae argues for some vital role.

Autoradiography

Leak (1966, 1967) demonstrated the incorporation of tritiated thymidine in actively dividing cultures of Anabaena and Lyngbya species. Silver grains appeared over central and peripheral nucleoplasmic areas. Comparable results were obtained in the current study for uptake of tritiated thymidine in DNA synthesis during log-phase growth of Anacystis nidulans, Nostoc muscorum, Microcoleus vaginatus (Type A), and Symploca muscorum. The relatively slow rate of cell division and thinness of sections limits the number of silver grains observed. Rapidly-dividing bacteria associated with the sheaths of Microcoleus vaginatus and Symploca muscorum incorporated the label at a much greater rate.

Incorporation of tritiated actinomycin D was demonstrated

on the same four species following modifications of the methods used by Camargo and Plaut (1967) and Ebstein (1967) to cytochemically label small amounts of DNA. Simard (1967) carried out electron microscopic studies of the binding of tritiated actinomycin D to heterochromatin in animal tissue. In the current study, silver grains always appeared in areas identified as nucleoplasm. The rate of incorporation was improved over that of tritiated thymidine, probably due to direct attachment of the label to cellular DNA without the synthesis-dependent step.

EDTA in Tris buffer was employed in an effort to enhance permeability of the cell to actinomycin D. Leive (1965a, 1965b, 1965c) found that sensitivity of Escherichia coli to actinomycin D could be achieved by treatment of the normally resistant bacterium with EDTA. Salton (1967) suggested that an additional barrier in the Gram-negative wall excluded actinomycin D which would otherwise pass through the membrane and enter the cell. As discussed earlier, EDTA breaks down the lipopolysaccharide outer layer of Gram-negative bacteria (Leive, 1965d). By adjusting the conditions of the treatment, the growth rate and viability of the cells could be maintained and still permit the increased permeability to actinomycin D. Preliminary experiments with Nostoc muscorum established the concentration of EDTA in Tris buffer and the duration of exposure compatible with subsequent growth in medium without EDTA and normal appearance of cell components on examination

in the electron microscope. These conditions then were adopted for pre-treatment of cells before exposure to tritiated actinomycin D.

Photosynthetic Pigment System

Morphology and ultrastructure of thylakoids

The thylakoid system of the blue-green algal cell does not conform to a rigid arrangement but varies widely in the number of membranous units and in pattern with species, age, and environmental conditions for any given species. Shatkin (1960) reported that young cells have fewer thylakoids, and they are more widely separated than in older cells. The reasonably simple pattern that is characteristic of Anacystis nidulans (Figure 73) has relatively few, parallel thylakoids arranged peripherally. This pattern appeared to be less subject to alteration than the arrangement of thylakoids in Nostoc muscorum. Allen (1968a) noted the obvious continuity of thylakoid double membranes with the plasma membrane. Similar connections were observed but not so conspicuously in the current study. Allen (1968a) reported that the total amount of the thylakoid system was proportional to the chlorophyll content of the cell.

Although Smith and Peat (1967b), Jost (1965), Fuhs (1966), Echlin and Morris (1965), Bowen and Pankratz (1963), and Allen (1968a) have reported probable formation of blue-green algal lamellae by invagination of the plasma membrane and con-

tinuity between the plasma membrane and lamellae, Allen (1968b) reported invagination of the thylakoids in advance of the new septum formation in Anacystis nidulans but not in Gleocapsa alpicola.

Echlin (1964a) described membranous inclusions in Anacystis nidulans which he termed lamellasomes in analogy with the bacterial mesosomes. The lamellasomes appeared to him to be derived from the plasma membrane. Iterson (1965) stated that mesosomes are extensions of the plasma membrane bounded by one unit membrane or an even thinner layer and are open toward the cell wall. Intracellular reduction of potassium tellurite (which can be identified in an electron micrograph by its electron opacity) occurs in the mesosomes, supporting the suggestion that they are centers of respiration equivalent to mitochondria of eucaryotic cells (Ryter, 1968). Evidence has not been presented to define a similar function for lamellasomes. As in bacteria, they usually are seen adjacent to the cell boundary and frequently near the junction of a cross-wall and lateral wall. Figure 4 shows two lamellasomes flanking such a junction. In the many micrographs observed in this study, invagination of the plasma membrane to form the lamellasome was never clearly evident. Elaboration of the thylakoid system to form the lamellasome image is just as possible. In no case has a lamellasome appeared to have contents which open out toward the cell

wall as in bacteria.

The ultrastructure of chloroplast thylakoids in higher plants has been studied extensively. As indicated in the literature review, many models of sub-unit structure have been proposed. Fuhs (1966) considered the "unit membrane" image of the thylakoid membrane an optical artifact. On electron microscopic examination of 250-A sections of Oscillatoria amoena and Pseudoanabaena catenata (permanganate fixation), he observed the thylakoid membrane as a two-dimensional packing of 80-A spheres. The 80-A dimension corresponded to the thickness of individual lamellae. Jost (1965) utilized freeze-etching of Oscillatoria rubescens in formulating his model of the thylakoid membrane. Since the contents of the intrathylakoidal space was lost on sublimation during freeze-etching, he assumed that it was aqueous. He described a layer with a supportive function and a lamellar layer. Spherical particles 50-70 A in diameter were associated with the former, and particles 100-200 A in diameter, which he equated with the quantosomes of Park and Pon (1961, 1963), were associated on the inner side of the membrane. Menke (1961, 1965, 1966) and Kreutz and Menke (1962) studied negatively stained thylakoids from Oscillatoria chalybea. Cross-sections of thylakoids appeared to have monolayers of particles which agreed in dimension (30-40 A diameter) with the protein particles measured in small-angle x-ray studies. They favored

a model of the thylakoid membrane with spherical protein sub-units on the outer side and a homogenous layer of lipids on the inner side.

In freeze-etched Anabaena cylindrica, Muhlethaler (1966) noted that the intrathylakoidal space seemed empty due to sublimation of its aqueous contents. The thylakoid membrane appeared to be covered on both sides with particles. He attributed the narrower dimensions of fixed (75 A) than frozen (120 A) lamellae to uncoiling of protein molecules during fixation. This uncoiling would result in thin films on either side of the central lipid layer, giving the unit membrane image after osmium or permanganate fixation. In the current study, the particles (125-175 A in diameter) seen on the thylakoid membranes of Nostoc muscorum after freeze-etching (Figures 103 and 104) agree in size and appearance with those of Jost. On the basis of limited experience in evaluating micrographs of freeze-etched material, no attempt is made to judge whether the particles are located on the inner, outer, or split faces of the thylakoid membrane. Each location has been advocated by one of the proposed models.

In sectioned material at higher magnifications (Figures 82 through 84, 94), the thylakoid membrane appears beaded due to rows of spherical densities (about 30 A in diameter) sandwiched on each side of an electron-transparent zone (about

24 A wide). Occasionally beads opposite each other appear to join in the shape of a ring with a dense rim and a light core, corresponding to the image reported by Weier *et al.* (1966). His dimensions for the ring in chloroplast membranes of the green alga *Scenedesmus quadricanta* were 39 A for the dark rim and 23 A for the light core. In higher plant chloroplasts, the dark rim measured 28 A in diameter and the core was 37 A. In the thylakoid membranes of *Nostoc muscorum* and *Microcoleus vaginatus* (Type A), the ring structure was not sufficiently evident or frequent in appearance to be endorsed. Although the beaded image is suggestive of regular sub-units, the thickness of the section (600 A) must be kept in mind in relation to the dimension of the particles (30 A) and the unlikelihood of viewing 20 such particles perfectly aligned in depth to appear as a single sphere. It is also possible that diffraction effects might complicate images of particulate, closely-parallel layers in "edge on" view.

Photosynthetic pigments

Evidence has accumulated to support the theory that two photochemical reactions powered by separate pigment systems are required for photosynthesis. The accessory pigments (e.g., phycocyanin and phycoerythrin in the blue-green algae) absorb light quanta and transfer the energy to one of the two photoreactions.

The structure of the aggregating C-phycocyanin system has been described by Berns and Edwards (1965). Earlier physicochemical studies (Berns et al., 1964; Scott and Berns, 1965) of purified phycocyanin had indicated that the quaternary structure of phycocyanin involves monomer (3S), trimer (7S), hexamer (11S), and dodecamer (19S) states of aggregation with pH affecting the equilibrium. Electron micrographs were taken of phycocyanin aggregates in buffers at different pH values. Many round structures about 130 A in diameter with a central hole were observed at pH 5.0, 6.3, 7.0, and 7.4. Based on size and predominance these ring-shaped aggregates were judged to be the hexamers. At higher magnifications, a sub-unit structure of six globular particles arranged in a regular hexagon was detected in the hexamer. The experimental evidence from chemical, physical, and microscopic data was in agreement for an oblate or prolate ellipsoidal hexamer. The investigators selected the hexamer as the important species in vivo because it was the aggregate favored close to the isoelectric point and was present in large amounts in fresh algal extracts.

The site of the biliproteins in the blue-green algae has not been clearly established. Bergeron's (1963) measurements based on pigment concentrations and cell volume of Anacystis nidulans indicated that all of the chlorophyll molecules could be accommodated on the thylakoid membranes, but uniform

spacing of phycocyanin molecules would leave them less than a molecular distance apart. He concluded that phycocyanin molecules probably exist in the interthylakoidal spaces in a state permitting molecular interaction. Giraud's (1963) calculations also indicated insufficient space on the thylakoid surface for a layer of biliproteins within a distance from chlorophyll permitting efficient transfer of energy in photosynthesis. Berns (1967) postulated a "stroma" location where biliproteins might also function as structural proteins. Fuhs (1964b) concluded that the thylakoid membrane is composed of closely packed, proteinaceous, spherical units about 80 A in diameter which in part, at least, are phycocyanin molecules. He noted that negatively-stained pure phycocyanin showed spherical molecules about 80 A in diameter.

Since the efficiency of energy transfer to chlorophyll molecules would be difficult to achieve with phycocyanin aggregates freely dispersed between the thylakoids, a suggestion of Scott and Berns (1965) and Berns and Edwards (1965) is more acceptable. They postulated a random array of tetrapyrrole chromophores in the phycocyanin hexamer with the possibility of a chlorophyll molecule associated with each monomer unit in the hexamer and oriented with the porphyrin heads situated on the surface of the phycocyanin sub-units and the phytol tails in the hydrophobic center of

the hexamer or in lipid of the thylakoid membrane. Such an arrangement would permit optimal energy transfer and be consistent with the known semi-ordered arrangement of chlorophyll molecules (Goedheer, 1957). Aqueous extraction would be expected to free the phycocyanin molecules while the chlorophyll molecules (if embedded in the thylakoid membrane) would remain associated with the hydrophobic lipid fraction. The investigators carefully stated that they did not necessarily consider the chlorophyll-phycocyanin complex the only arrangement in which chlorophyll molecules are involved in the blue-green algae.

Gantt and Conti (1965, 1966a, 1966b) and Gantt et al. (1968) have located the biliproteins of two red algae in granules (phycobilisomes) which are loosely associated with the thylakoid membranes on the external surface. These granules are about 350 A in diameter and are regularly arranged in rows with a center-to-center distance of 400-500 A. They are resistant to hydrolysis by RNase. Treatment with deoxycholate removed the phycobilisomes and the biliprotein content of the cells. Extraction of glutaraldehyde-fixed material with methanol-acetone removed chlorophyll and carotenoids, but phycobilisomes remained in position when examined electron microscopically. Preservation of phycobilisomes required glutaraldehyde before osmium fixation. Lefort's (1965) electron micrographs of the endosymbionts

Glaucocystis nostochinearum and Cyanophora paradoxa after fixation in osmium clearly showed granules similar in appearance to the phycobilisomes of red algae, but permanganate fixation did not reveal them. A recent ultrastructural study of the cyanelles of Glaucocystis nostochinearum in which both fixations were used failed to mention or show phycobilisomes (Hall and Claus, 1967). Edwards et al. (1968) noted granules on the thylakoids of Synechococcus lividus and termed them phycobilisomes because of their similarity in appearance to the pigment-containing granules in red algae.

Reese (1967) reported the occurrence of phycobilisome-like granules in Nostoc muscorum. However, the granules have not been generally reported as characteristic features of the blue-green algal thylakoid system, and no evidence has appeared in the literature to support a function for the granules comparable to that reported for Rhodophytan phycobilisomes. The observations and data accumulated in this study are considered evidence that moderately dense granules which are loosely associated with the outer surfaces of the thylakoid membranes in blue-green algae serve as locations of photosynthetically active phycobiliproteins and should be called phycobilisomes. The phycobilisomes are labile and easily dissociated from the thylakoids on cell rupture in aqueous solution or on treatment with deoxycholate to yield the phycobiliproteins in solution. Extraction with methanol-

acetone removes chlorophyll but phycobilisomes can still be discerned in the electron microscopic image.

From the accumulated observations, a model of the blue-green algal phycobilisome can be proposed. The form suggested is discoid. The side ("edge on") view measures about 60-80 A wide when grown in white light or 100-110 A wide when grown in red light. In face view, the diameter is about 320 A when grown in white light or about 340 A when grown in red light. Since the rotational reinforcement pattern of the discoid in face view suggests a periodicity of six for the sub-unit structure, the phycobilisome might be considered a hexamer of hexamers. The width of the profile in side view could vary, depending on the number of hexamer units stacked in parallel (and perpendicular to the surface of the thylakoid). Each hexamer unit averages 25 A in thickness, according to Berns and Edwards (1965) who reported a spherical size of about 25 A in diameter for the monomer unit. A schematic representation of the model is presented in Figure 85b.

Chlorophyll molecules might be attached to the outer surfaces of the phycocyanin molecules, permitting absorption by the biliproteins and transfer to adjacent chlorophyll molecules that would be well within the 50-A range which Forster (1951) and Giraud (1963) considered the maximum allowable separation for efficient resonance transfer of excitation energy between fat- and water-soluble pigments.

Resonance transfer of energy from chlorophyll to chlorophyll could pass toward the thylakoid membrane where additional chlorophyll molecules undoubtedly are located. Goedheer (1957) concluded from his experiments on dichroism, anomalous dispersion of birefringence, and fluorescence polarization that chlorophyll forms a monolayer about 4 A thick at the lamellar surface.

This proposed distribution of phycobiliproteins and chlorophyll molecules over a surface area much larger than that of the thylakoid membranes alone would eliminate any concern about lack of sufficient space to account for the estimated amount of pigments. Phycobilisomes of increased size which were observed after growth of Nostoc muscorum in red light would be interpreted as enlarged aggregations resulting from the further addition of hexamers to the stacks, (widening the discoid profile in "end on" view), in agreement with spectroscopic evidence of increased phycocyanin content. According to the observations in this study and the proposed model, both the side- and face-view profiles of the phycobilisome can be identified with one pigment, phycocyanin. Gantt's (Gantt et al., 1968) suggestion that the shape is determined by the content of two pigments (predominantly phycoerythrin for the "spherical" and phycocyanin for the "disk" shape) may be unnecessary to account for the two profiles.

Variation in illumination

The blue-green algae can absorb a broad range of wavelengths and grow well at low light intensities but in many cases cannot tolerate a high light intensity. Bowen and Pankratz (1963) demonstrated disintegration of the thylakoids and chlorosis of several blue-green algal species on exposure to high light and recovery in low light with reassembly and new synthesis of thylakoidal elements from membranous vesicles and by invagination of the plasma membrane. In the current study, 100-200 ft-c of illumination was found to be optimal for growth of Nostoc muscorum. The slower growing Microcoleus vaginatus (Type A) and Arthrospira Jenneri thrived only at lower intensities (30-50 ft-c) and quickly yellowed under higher illumination. Anacystis nidulans can tolerate considerably higher light intensities and temperatures for rapid growth. However, Allen (1968a) found that pigment concentration and lamellar content varied inversely with light intensity in cells of Anacystis nidulans grown in 100 and 1,000 ft-c of fluorescent light.

In attempting to understand the relationships between the accessory pigments and chlorophyll a in blue-green algae, Myers and his colleagues carried out detailed studies on the ratios of these pigments under varied conditions of illumination. Kratz and Myers (1955b) grew Anacystis nidulans at 25°C and 39°C in light intensities of 80-960 input watts and

studied the ratios of pigments extracted by acetone (chlorophyll and carotenoids) and water (phycocyanin) after sonic disintegration of the cells. The chlorophyll/phycocyanin ration remained relatively constant inspite of a 3-fold range in chlorophyll content and a 4-fold variation in the amount of phycocyanin, which they interpreted as support for closely related mechanisms of synthesis of the two pigments. More recent studies on the action spectra of biosynthesis of biliproteins indicate that the biosynthetic pathways for chlorophyll and the biliproteins are different (Ó hEocha, 1965b). The carotenoid content was found to show small variation (Kratz and Myers, 1955b). Since input watt ratings of lamps rather than direct intensity readings were recorded in their work, it is difficult to make direct comparisons with light intensity studies reported in this dissertation.

When cultures of Nostoc muscorum were grown for three weeks at light intensities of 20, 100, 200, and 900-1,000 ft-c, almost no variation in the total in vivo content of chlorophyll was observed although the phycocyanin content varied somewhat (Figure 113a). The greatest effect was on carotenoid concentration which increased directly with light intensity. This increase in amount of carotenoids may reflect the cells' need for the protective function of carotenoids against photosensitized oxidations (Krinsky, 1966) at the

higher light intensities. The pigment ratio of chlorophyll/ phycocyanin showed a definite but not dramatic shift with change in light intensity. The measurement of pigments *in vivo* has an advantage in accuracy and true representation of the natural situation over the method of sonication and pigment extraction.

Jones and Myers (1965) grew *Anacystis nidulans* under red photographic safe light (640-740 mμ) which is predominantly absorbed by chlorophyll *a* and observed a dramatic lowering of the chlorophyll content to 1/4 that under low intensity white light. Small changes occurred in the amounts of phycocyanin and carotenoids. Pigment analyses were made *in vivo* on cell suspensions, on sonicates, and on methanol extracts. They calculated that over the range of 540-640 mμ, 75-85% of the quanta absorbed by cells grown in low white light is absorbed by phycocyanin, but in red light, over 95% is absorbed by phycocyanin. At intensities so low that little growth occurred, they detected little change in pigment ratios, and only on increase in intensity to a level supportive of moderate growth was the displacement in pigment ration observed. To achieve the desired growth rate, they compromised the specificity of wavelength by adding a 10-watt incandescent lamp to the red light source. In the current study, when a very low intensity of red light without the addition of any supplementary incandescent illumination was supplied to cells

of Nostoc muscorum, growth was definitely slow. However, a definite displacement in the pigment ratio was found (Figure 113b; Table 2), perhaps because Nostoc muscorum normally requires a lower light intensity level for growth than does Anacystis nidulans.

In explaining their data, Jones and Myers (1965) referred to the work of Fujita and Hattori (1960b, 1962a) and Hattori and Fujita (1959a, 1959d) on Tolypothrix tenuis. These investigators found that the relative amounts of phycocyanin and phycoerythrin produced were under direct chromatic control and not sensitive to intensity. There was a preferential increase in amount of the pigment absorbing best at the wavelength of highest intensity (e.g., growth in green light caused an increase in phycoerythrin and growth in red light caused an increase in phycocyanin without changes in chlorophyll or carotenoids). Phycoerythrin and phycocyanin are accessory pigments linked to the same photoreaction (system 2) in photosynthesis.

In contrast to direct chromatic control, light intensity control is inverse, resulting in a decrease in active pigments such as chlorophyll and biliproteins in response to increase in intensity, especially above light saturation of photosynthesis (Jones and Myers, 1965). Jones and Myers (1965) considered the pigment control in their study with red light to be an inverse chromatic control, resulting in decrease of

that pigment which absorbs best at the incident wavelength of highest intensity. The difference between this inverse chromatic control and the direct chromatic control in Hattori and Fujita's work is that the pigments involved in the former are chlorophyll and phycocyanin which are preferentially associated with two different photoreactions. Phycocyanin is the major absorbing pigment for system 2, and chlorophyll a for system 1. In red light, quanta are absorbed chiefly by chlorophyll a and in excess of their use in system 1 with the result that the inverse pigment control operates to reduce the concentration of chlorophyll a and adjust to the low rate of system 2 at that wavelength.

The results obtained by Jones and Myers (1965) are confirmed by the data of the current study on a different organism. When Nostoc muscorum was exposed to red light for three weeks, the concentration of chlorophyll a in relation to phycocyanin was decreased over that observed in low white light (Figure 113b; Table 2). The incident red light was restricted in effective wavelength band width to 30 mμ (total intensity 42 $\mu w/cm^2$) by use of the monochromator.

Jones and Myers (1965) did not detect a decrease in the amount of phycocyanin relation to chlorophyll a when Anacystis nidulans was grown at 540-640 mμ. They could not explain this failure of their hypothesis for inverse chromatic control except to note that phycocyanin serves as an energy

absorber for both photosystems 1 and 2, so there would not be a serious imbalance in rates of the two photoreactions in orange light which is strongly absorbed by phycocyanin and not by chlorophyll. When Nostoc muscorum was grown for three weeks in light adjusted to a band width of 610-640 mμ by use of a monochromator, the predicted decrease in phycocyanin in relation to chlorophyll (compared with pigment ratios for low white light) was observed. This is reflected in the increased value for the ratio chlorophyll/phycocyanin in Table 2. These data substantiate the hypothesis of inverse chromatic control for growth of Nostoc muscorum at wavelengths absorbed largely by phycocyanin (610-640 mμ) and for wavelengths absorbed largely by chlorophyll a (660-690 mμ).

The spectral evidence for changes in pigment ratios with wavelength of incident light was accompanied by compatible changes in the morphology of the pigment system. Growth of Nostoc muscorum in red light with a demonstrated decrease in the chlorophyll/phycocyanin ratio, resulted in the appearance of numerous, enlarged phycobilisomes. Increased production of phycocyanin must be at least partially responsible for the decrease in chlorophyll/phycocyanin ratio. Therefore the hypothesis of inverse chromatic control (which defines the ratio in terms of reduction in chlorophyll production) may be only a partial explanation. If efficient photosynthesis in blue-green algae is dependent upon absorption by

phycocyanin, which absorbs very poorly at 660-690 mμ, increased production of the biliprotein (as evidenced by increased size and numbers of phycobilisomes in Figure 120) probably occurs to compensate for the handicap. In light of 610-640 mμ, where phycocyanin absorbs strongly, less is required for photosynthesis, the ratio of chlorophyll/phycocyanin is increased, and the phycobilisomes appear small and inconspicuous (Figure 119).

Ghosh and Govindjee (1966) published spectral data but no electron micrographs for Anacystis nidulans grown in lights of different wavelengths and intensities. Strong orange light caused an increase in the ratio of chlorophyll a/phycocyanin, and strong red light caused a decrease in chlorophyll a/phycocyanin. They reported a decrease in efficiency of energy transfer from phycocyanin to chlorophyll a whenever the ratio of chlorophyll a/phycocyanin deviated from the normal, as was the case when algae were grown in high intensity white light (2.0×10^7 ergs/cm^2/sec). Total pigment concentrations were low in all cells grown at high light intensities. They stated that the color of weak light did not significantly affect the pigment ratio. Although it would be desirable to combine the specificity of wavelength (that is possible using a monochromator) with a higher intensity of illumination to increase the displacement of pigment ratio, the results reported here are considered significant in conjunction with

the observed changes in phycobilisomes at the electron microscopic level.

Wildon and Mercer (1963) reported that cells of Nostoc muscorum which had been grown in the dark in a medium containing 1% glucose showed thylakoids of the same dimensions and tripartite (dark-light-dark) image as light-grown cells but with a reduction in number of thylakoids. In the work reported here, when Nostoc muscorum was grown in the dark for five weeks on modified Chu 10 medium supplemented with 1% glucose or sucrose as the energy and carbon source, considerable rearrangement of thylakoids was observed from a peripheral, parallel pattern to disorganized anastomosing throughout the cell. The most obvious change was intense staining of the intrathylakoidal space or an appression of the two interior layers of thylakoidal membrane, resulting in a myelin-like image of five layers. The appearance was similar to negatively stained material. Electron-transparent α-granules (or spaces formerly filled by α-granules) were prominent in the interthylakoidal regions. Microcoleus vaginatus (Type A) was able to survive under the same conditions, but growth was very slight. An interesting stacking of appressed thylakoids in a myelin-like configuration was observed (Figure 123).

Lazaroff and Vishniac (1961, 1962, 1964) conducted a series of investigations on the developmental cycle of the

Allison strain of Nostoc muscorum. When this strain was cultivated in darkness on glucose, slow growth resulted in a mass of large, undifferentiated cells (aseriate stage). Exposure to light or addition of an extract from light-grown cells permitted development of typical filaments. The investigators concluded that a factor essential to completion of the developmental cycle was formed only in the light. The action spectrum for non-photosynthetic photoinduction of development had a single, sharp peak at 650 mμ, the absorption maximum for allophycocyanin (Lazaroff and Schiff, 1962). Phycoerythrins may act as photoreceptors for reversal of induction in green light (Lazaroff, 1966). Large, undifferentiated cells typical of the aseriate stage were not observed in the current study after five weeks of growth in the dark and fixation under green light.

SUMMARY

Culture conditions were established for seven blue-green algal species: Anacystis nidulans, Arthrospira Jenneri, Microcoleus vaginatus (two ecophenes), Nostoc muscorum, Symploca muscorum, and Tolypothrix distorta.

The fine structure of the above species and of Aphanizomenon flos-aquae and Spirulina major was studied in sectioned material, using several methods of fixation and staining. Freeze-etching was employed to study Nostoc muscorum and Symploca muscorum. Sub-unit structure in or on the thylakoid membranes was observed in freeze-etched preparations.

Numerous cylindrical bodies were observed in Microcoleus vaginatus and Symploca muscorum. They usually are located near the cross-walls and frequently occur in clusters. In cross-section, the cylindrical body appears as two concentric, dense rings separated by electron-transparent zones; a central core appears to have a dense rim and center point separated by a light zone. Local areas of convoluted membrane elaborations adjacent to the longitudinal wall were observed frequently in Nostoc muscorum. Tubular elements, differing from thylakoids in dimensions and staining characteristics, were seen in Nostoc muscorum and Spirulina major and appeared to be associated with the nucleoplasm. Structured granules were prominent in Nostoc muscorum, Microcoleus vaginatus, Symploca muscorum, and Tolypothrix distorta. Features common to all species

studied include a multi-layered wall, a plasma membrane, nucleoplasmic and cytoplasmic areas, a thylakoid system, α-granules, β-granules, ribosomes, and polyhedral bodies.

The fine structure of the polyhedral bodies was examined in detail. Alterations in the polyhedral bodies of Nostoc muscorum after digestion with RNase, DNase, lipase, acid phosphatase, and pronase were studied. Based on these observations, it is concluded that polyhedral bodies are chemically complex and probably have lipid, protein, and phosphate constituents. The possibility of a nucleic acid component was not eliminated. Fixed and unfixed cells of Nostoc muscorum in an osmotically protected medium were ruptured in a French press and subjected to differential and density gradient centrifugation. The fractions were examined in the electron microscope for enrichment of polyhedral bodies and for the effect of isolation on fine structure.

The properties of the blue-green algal wall as characterized by Gram-staining, plasmolyzing conditions, and treatment with lysozyme and Tris-EDTA were investigated. The results were useful in developing several methods of lysis for isolation of DNA from Anacystis nidulans and Nostoc muscorum. The isolated DNA was characterized by CsCl density gradient analysis and electron microscopic examination. Locations of DNA within the cell were identified by autoradiography, using tritiated thymidine and tritiated actinomycin D as radioactive labels.

In vivo absorption spectra were recorded for each alga that was maintained in culture. The effects on pigment ratios and ultrastructure of varying intensity and wavelength of incident illumination were studied on *Nostoc muscorum*. *In vivo* absorption spectra and electron micrographs were employed. For cells grown in red light, the chlorophyll/phycocyanin ratio was decreased, and in orange light it was increased over the ratio for cells grown in low-intensity white light. The results of extraction with methanol-acetone and treatment with deoxycholate were followed by absorption spectra and electron microscopic examination. Evidence is presented for the location of phycocyanin in granules (phycobilisomes) associated with the outer surfaces of the thylakoids. A model is proposed for the geometry of the phycobilisome in a discoid form. Phycobilisomes were observed in all species examined. They were enlarged and numerous in cells of *Nostoc muscorum* which had been grown in red light and inconspicuous in cells grown in orange light. Cells of *Nostoc muscorum* and *Microcoleus vaginatus* were grown in the dark with 1% glucose or sucrose for five weeks, and the resulting alterations in ultrastructure were observed. The intrathylakoidal space or appressed inner membranes of the thylakoids stained intensely and α-granules were prominent. Stacking of thylakoids in a myelin-like configuration was observed in cells of dark-grown *Microcoleus vaginatus*.

LITERATURE CITED

Airth, R. L., and L. R. Blinks. 1956. A new phycoerythrin from Porphyra naiadum. Biological Bulletin 111: 321-327.

Allen, M. M. 1968a. Photosynthetic membrane system in Anacystis nidulans. Journal of Bacteriology 96: 836-841.

_____. 1968b. Ultrastructure of the cell wall and cell division of unicellular blue-green algae. Journal of Bacteriology 96: 842-852.

Amesz, J., and L. N. M. Duysens. 1962. Action spectrum, kinetics and quantum requirement of phosphopyridine nucleotide reduction and cytochrome oxidation in the blue-green alga, Anacystis nidulans. Biochimica et Biophysica Acta 64: 261-278.

Arnold, W., and J. Thompson. 1956. Delayed light production by blue-green algae, red algae and purple bacteria. Journal of General Physiology 39: 311-318.

Bergeron, J. A. 1963. Studies on the localization, physicochemical properties, and action of phycocyanin in Anacystis nidulans. Photosynthetic mechanisms of green plants. National Academy of Science-National Research Council Publication 1145: 527-536.

Berns, D. S. 1967. Immunochemistry of biliproteins. Plant Physiology 42: 1569-1586.

_____, and M. R. Edwards. 1965. Electron micrographic investigations of C-phycocyanin. Archives of Biochemistry and Biophysics 110: 511-516.

_____, and A. Morgenstern. 1966. Ultracentrifuge investigation of protein aggregation in dilute solutions of C-phycocyanin. Biochemistry 5: 2985-2990.

_____, and E. Scott. 1966. Protein aggregation in a thermophilic protein: phycocyanin from Synechococcus lividus. Biochemistry 5: 1528-1533.

_____, _____, and K. T. O'Reilly. 1964. C-phycocyanin: minimum molecular weight. Science 145: 1054-1056.

Birdsell, D. C., and E. H. Cota-Robles. 1967. Production and ultrastructure of lysozyme and ethylenediaminetetraacetate-lysozyme spheroplasts of Escherichia coli. Journal of Bacteriology 93: 427-437.

Bisalputra, T., and A. A. Bisalputra. 1967. The occurrence of DNA fibrils in chloroplasts of Laurencia spectabilis. Journal of Ultrastructure Research 17: 14-22.

Biswas, B. B. 1956. Chemical nature of nucleic acids in Cyanophyceae. Nature 177: 95-96.

_____. 1957. Cytochemical studies on the central body of the Cyanophyceae. Cytologia 22: 90-95.

_____. 1960. Comparative studies on DNA of Chlorella ellipsoidea and Anacystis nidulans. Plant Physiology Supplement 35: xxx.

_____. 1961. Studies on the nucleoproteins of Nostoc muscorum. Bose Research Institute, Transactions 24: 25-30.

_____, and J. Myers. 1959. Characterization of desoxyribonucleic acid from Anacystis nidulans. 9th International Botanical Congress, Proceedings 2: 34-35.

_____, and _____. 1960a. A methyl cytidine from the ribonucleic acid of Anacystis nidulans. Nature 186: 238-239.

_____, and _____. 1960b. Characterization of deoxyribonucleic acid from Anacystis nidulans. Nature 188: 1029-1030.

Blinks, L. R. 1954. The photosynthetic function of pigments other than chlorophyll. Annual Review of Plant Physiology 5: 93-114.

Bogorad, L. 1963. The biogenesis of heme, chlorophylls, and bile pigments, p. 183-231. In P. Bernfield (ed.), Biogenesis of natural compounds. Pergamon Press, Oxford, England.

_____. 1965. Studies of phycobiliproteins. Record of Chemical Progress 26: 1-12.

Boucher, L. J., H. L. Crespi, and J. J. Katz. 1966. Optical rotatory dispersion of phycocyanin. Biochemistry 5: 3796-3802.

Bowen, C. C., and T. E. Jensen. 1965. Blue-green algae: fine structure of the gas vacuoles. Science 147: 1460-1461.

_____, and H. S. Pankratz. 1963. Formation of photosynthetic lamellae in the blue-green algae. Journal of Cell Biology 19: 8A.

Brawerman, G. 1966. Nucleic acids associated with the chloroplasts of Euglena gracilis, p. 301-317. In T. W. Goodwin (ed.), Biochemistry of chloroplasts. Academic Press, New York.

Brody, M., and S. Brody. 1962. Induced changes in the photosynthetic efficiency of Porphyridium cruentum. Archives of Biochemistry and Biophysics 92: 354-359.

_____, and _____. 1965. An experiment showing that P_{700} can be an aggregated form of chlorophyll a. Archives of Biochemistry and Biophysics 110: 583-585.

_____, and A. E. Vatter. 1959. Observations on cellular structures of Porphyridium cruentum. Journal of Biophysical and Biochemical Cytology 5: 289-294.

Brook, A. J. 1968. The discoloration of roofs in the United States and Canada by algae. Journal of Phycology 4: 250.

Brown, J. S. and C. S. French. 1961. The long wavelength forms of chlorophyll a. Biophysical Journal 1: 539-550.

Brown, T. E., and F. L. Richardson. 1968. The effect of growth environment on the physiology of algae: light intensity. Journal of Phycology 4: 38-54.

_____, _____, D. F. Stoltz, M. Vaughn, and M. N. Woodruff. 1967. Effect of growth environment on the physiology of algae: light quality. Journal of Phycology 3: Supplement p. 9.

Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemical Journal 62: 315-323.

Calvin, M., and V. Lynch. 1952. Grana-like structures of Synechococcus cedorum. Nature 169: 455-456.

Camargo, E. P., and W. Plaut. 1967. The radioautographic detection of DNA with tritiated actinomycin D. Journal of Cell Biology 35: 713-716.

Cassel, W. A., and W. G. Hutchinson. 1954. Nuclear studies on the smaller Myxophyceae. Experimental Cell Research 6: 134-150.

Chapman, D. J., W. J. Cole, and H. W. Siegelman. 1967. Chromophores of allophycocyanin and R-phycocyanin. Biochemical Journal 105: 903-905.

_____, _____, and _____. 1968. Phylogenetic implications of phycocyanobilin and C-phycocyanin. American Journal of Botany 55: 314-316.

Chapman, J. A., and M. R. J. Salton. 1962. A study of several blue-green algae in the electron microscope. Archiv für Mikrobiologie 44: 311-322.

Claus, G. W., and L. E. Roth. 1964. Fine structure of the Gram-negative bacterium Acetobacter suboxydans. Journal of Cell Biology 20: 217-233.

Clayton, R. K. 1963. Photosynthesis: primary physical and chemical processes. Annual Review of Plant Physiology 14: 159-180.

Cohen-Bazire, G., N. Pfennig, and R. Kunisawa. 1964. The fine structure of green bacteria. Journal of Cell Biology 22: 207-225.

Cook, J. R. 1963. Adaptations in growth and division in Euglena effected by energy supply. Journal of Protozoology 10: 436-444.

Craig, I. W., and N. G. Carr. 1967. Ribosomes from the blue-green alga, Anabaena variabilis. Biochemical Journal 103: 64P.

_____, and _____. 1968a. C-phycocyanin and allophycocyanin in two species of blue-green algae. Biochemical Journal 106: 361-366.

_____, and _____. 1968b. Ribosomes from the blue-green alga Anabaena variabilis. Archiv für Mikrobiologie 62: 167-177.

Crespi, H. L., S. E. Mandeville, and J. J. Katz. 1962. The action of lysozyme on several blue-green algae. Biochemical and Biophysical Research Communications 9: 569-573.

Cuzin, F., and F. Jacob. 1967. Bacterial episomes as a model of replicon, p. 39-50. In V. V. Koningsberger and L. Bosch (ed.), Regulation of nucleic acid and protein biosynthesis. Elsevier Publishing Company, Amsterdam, Holland.

De, D. N., and S. N. Ghosh. 1965. Cytochemical evidence for the apparent absence of histone in the cells of Cyanophyceae. Journal of Histochemistry and Cytochemistry 13: 298.

Drews, G., and M. Meyer. 1964. Untersuchungen zum chemischen Aufbau der Zellwände von Anacystis nidulans und Chlorogloea fritschii. Archiv für Mikrobiologie 48: 259-267.

_____, and W. Niklowitz. 1956. Beiträge zür Cytologie der Blaualgen. II. Zentroplasm und granuläre Einschlüsse von Phormidium uncinatum. Archiv für Mikrobiologie 24: 147-162.

_____, and _____. 1957. Beiträge zür Cytologie der Blaualgen. III. Untersuchungen über die granulären Einschlüsse der Hormogonales. Archiv für Mikrobiologie 25: 333-351.

Drouet, F. 1968. Revision of the classification of the Oscillatoriaceae. Monograph 15. The Academy of Natural Sciences of Philadelphia. Fulton Press, Lancaster, Pennsylvania.

Duysens, L. N. M. 1966. Primary photosynthetic reactions in relation to transfer of excitation energy, p. 71-80. In Energy conversion by the photosynthetic apparatus. Brookhaven Symposia in Biology, No. 19. Brookhaven National Laboratory, Upton, New York.

_____, and J. Amesz. 1962. Function and identification of two photochemical systems in photosynthesis. Biochimica et Biophysica Acta 64: 243-260.

_____, _____, and B. M. Kamp. 1961. Two photochemical systems in photosynthesis. Nature 190: 510-511.

Ebel, J. P., and S. Muller. 1958. Recherches cytochimiques sur les polyphosphates inorganiques contenus dans les organismes vivants. Experimental Cell Research 15: 21-42.

Ebstein, B. S. 1967. Tritiated actinomycin D as a cytochemical label for small amounts of DNA. Journal of Cell Biology 35: 709-713.

Echlin, P. 1964a. Intra-cytoplasmic membranous inclusions in the blue-green alga, Anacystis nidulans. Archiv für Mikrobiologie 49: 267-274.

_____. 1964b. The fine structure of the blue-green alga Anacystis montana f. minor grown in continuous illumination. Protoplasma 58: 439-457.

_____. 1964c. The fine structure of certain unicellular blue-green algae. British Phycological Bulletin 2: 389.

_____. 1966. The blue-green algae. Scientific American 214: 74-81.

_____, and I. Morris. 1965. The relationship between blue-green algae and bacteria. Biological Reviews 40: 143-187.

Edelman, M., D. Swinton, J. A. Schiff, H. T. Epstein, and B. Zeldin. 1967. Deoxyribonucleic acid of the blue-green algae (Cyanophyta). Bacteriological Reviews 31: 315-331.

Edwards, M. R., D. S. Berns, W. C. Ghiorse, and S. C. Holt. 1968. Ultrastructure of the thermophilic blue-green alga, Synechococcus lividus Copeland. Journal of Phycology 4: 283-298.

_____, and R. W. Stevens. 1963. Fine structure of Listeria monocytogenes. Journal of Bacteriology 86: 414-428.

Emerson, R. 1958. The quantum yield of photosynthesis. Annual Review of Plant Physiology 9: 1-24.

_____, R. Chalmers, and C. Cederstrand. 1957. Some factors influencing the long-wave limit of photosynthesis. National Academy of Sciences, Proceedings 43: 133-143.

Fitz-James, P. C. 1960. Participation of the cytoplasmic membrane in the growth and spore formations of bacilli. Journal of Biophysical and Biochemical Cytology 8: 507-528.

Forest, H. S., and C. R. Weston. 1966. Blue-green algae from the Atacama Desert of northern Chile. Journal of Phycology 2: 163-164.

Forster, T. 1951. Fluoreszenz organisher Verbindungen. Vandenhoeck and Ruprecht, Gottingen, Germany.

Frank, H., M. Lefort, and H. H. Martin. 1962a. Elektronenoptische und chemische Untersuchungen an Zellwänden der Blaualge Phormidium uncinatum. Zeitschrift für Naturforschung 17b: 262-268.

_____, _____, and _____. 1962b. Chemical analysis of a mucopolymer component in the cell walls of the blue-green alga Phormidium uncinatum. Biochemical and Biophysical Research Communications 7: 322-325.

Fredricks, W. W., and A. T. Jagendorf. 1964. A soluble component of the Hill reaction in Anacystis nidulans. Archives of Biochemistry and Biophysics 104: 39-49.

Fuhs, G. W. 1958a. Enzymatischer Abbau der Membranen von Oscillatoria amoena (Kütz.) Gomont mit Lysozym. Archiv für Mikrobiologie 29: 51-52.

_____. 1958b. Untersuchungen an Ultradünnschnitten von Oscillatoria amoena (Kütz.) Gomont. Protoplasma 49: 523-540.

_____. 1963. Cytochemisch-elektronenmikroskopische Lokalisierung der Ribonukleinsäure und des Assimilats in Cyanophyceen. Protoplasma 56: 178-187.

_____. 1964a. Die Wirkung von Uranylsalzen auf die Struktur des Bakteriennucleoid. Archiv für Mikrobiologie 49: 383-404.

_____. 1964b. A new structure in photosynthetic lamellae of Cyanophyceae and the localization of phycocyanin. Bacteriological Proceedings, 64th Annual Meeting, p. 15.

_____. 1965a. Der physikalische Status der Deoxyribonucleinsäure im Bakteriennucleoid. Antonie van Leeuwenhoek Journal of Mikrobiology and Serology 31: 25-44.

_____. 1965b. Symposium on the fine structure and replication of bacteria and their parts. I. Fine structure and replication of bacterial nucleoids. Bacteriological Reviews 29: 277-298.

_____. 1965c. Grundzüge der Nucleoidfeinstruktur. Archiv für Mikrobiologie 50: 25-51

_____. 1966. Spherical subunits in photosynthetic membranes of two Cyanophyceae and the bacterium Rhodospirillum rubrum. Archiv für Mikrobiologie 54: 253-265.

Fujimori, E., and J. Pecci. 1966. Dissociation and association of phycocyanin. Biochemistry 5: 3500-3508.

Fujita, Y., and A. Hattori. 1960a. Formation of phycoerythrin in pre-illuminated cells of Tolypothrix tenuis with special reference to nitrogen metabolism. Plant and Cell Physiology 1: 281-292.

_____, and _____. 1960b. Effect of chromatic lights on phycobilin formation in a blue-green alga Tolypothrix tenuis. Plant and Cell Physiology 1: 293-303.

_____, and _____. 1962a. Photochemical interconversion between precursors of phycobilin chromoproteids in Tolypothrix tenuis. Plant and Cell Physiology 3: 209-220.

_____, and _____. 1962b. Changes in composition of cellular material during formation of phycobilin chromoproteids in a blue-green alga, Tolypothrix tenuis. Journal of Biochemistry 52: 38-42.

_____, and _____. 1963. Effects of second chromatic illumination on phycobilin chromoprotein formation in chromatically preilluminated cells of Tolypothrix tenuis. In Studies on microalgae and photosynthetic bacteria. University of Tokyo Press, Tokyo, Japan.

Fujita, T., and R. Sato. 1966. Studies on soluble cytochromes in Enterobacteriaceae. III. Localization of cytochrome c-552 in the surface layer of cells. Journal of Biochemistry 60: 568-577.

Fulco, L., P. Karfunkel, and S. Aaronson. 1967. Effect of lysozyme (muramidase) on marine and freshwater blue-green algae. Journal of Phycology 3: 51-52.

Gantt, E., and S. F. Conti. 1965. The ultrastructure of Porphyridium cruentum. Journal of Cell Biology 26: 365-381.

_____, and _____. 1966a. Granules associated with the chloroplast lamellae of Porphyridium cruentum. Journal of Cell Biology 29: 423-434.

_____, and _____. 1966b. Phycobilin localization in algae, p. 393-405. In Energy conversion by the photosynthetic apparatus. Brookhaven Symposia in Biology, No. 19. Brookhaven National Laboratory, Upton, New York.

_____, M. R. Edwards, and S. F. Conti. 1968. Ultrastructure of Porphyridium aerugineum a blue-green colored Rhodophytan. Journal of Phycology 4: 65-71.

Gerloff, G. C., G. P. Fitzgerald, and F. Skoog. 1950. The isolation, purification, and nutrient solution requirements of blue-green algae. In J. Brunel, G. W. Prescott, and L. H. Tiffany (ed.), The culturing of algae. Charles F. Kettering Foundation, Yellow Springs, Ohio.

Ghosh, A. K., and Govindjee. 1966. Transfer of the excitation energy in Anacystis nidulans (Cyanophyta) grown to obtain different pigment ratios. Biophysical Journal 6: 611-619.

Ghosh, B. K., and R. G. E. Murray. 1967. Fine structure of Listeria monocytogenes in relation to protoplast formation. Journal of Bacteriology 93: 411-426.

Gibor, A., and S. Granick. 1964. Plastids and mitochondria: inheritable systems. Science 145: 890-897.

Giraud, G. 1963. La structure les pigments et les caractéristiques fonctionnelles de l'appareil photosynthétique de diverse algues. Physiologie Vegetale 1: 203-255.

Glauert, A. M. 1962. The fine structure of bacteria. British Medical Bulletin 18: 245-250.

_____, and R. H. Glauert. 1958. Araldite as an embedding medium for electron microscopy. Journal of Biophysical and Biochemical Cytology 4: 191-194.

Goedheer, J. C. 1957. Optical properties and in vivo orientation of photosynthetic pigments. Thesis, University of Utrecht, Holland. Original not available; cited in Brody, M., and A. E. Vatter. 1959. Observations on cellular structures of Porphyridium cruentum. Journal of Biophysical and Biochemical Cytology 5: 289-294.

Goodwin, T. W. 1960a. Algal carotenoids, p. 1-10. In M. B. Allen (ed.), Comparative biochemistry of photoreactive systems. Academic Press, New York.

_____. 1960b. Chemistry, biogenesis, and physiology of the carotenoids, p. 394-443. In W. Ruhland (ed.), Handbuch der Pflanzenphysiologie. Springer-Verlag, Berlin, Germany.

Gorham, P. R. 1960. Toxic waterblooms of blue-green algae. Canadian Veterinary Journal 1: 235-245.

Hall, W. T., and G. Claus. 1967. Ultrastructural studies on the cyanelles of Glaucocystis nostochinearum Itzigsohn. Journal of Phycology 3: 37-51.

Hanawalt, P. C., and D. S. Ray. 1964. Isolation of the growing point in the bacterial chromosome. National Academy of Sciences, Proceedings 52: 125-132.

Hattori, A., H. L. Crespi, and J. J. Katz. 1965. Association and dissociation of phycocyanin and the effects of deuterium substitution on the processes. Biochemistry 4: 1225-1238.

_____, and Y. Fujita. 1959a. Formation of phycobilin pigments in a blue-green alga, Tolypothrix tenuis, as induced in illumination with colored lights. Journal of Biochemistry 46: 521-524.

_____, and _____. 1959b. Crystalline phycobilin chromoproteids obtained from a blue-green alga, Tolypothrix tenuis. Journal of Biochemistry 46: 633-644.

_____, and _____. 1959c. Spectroscopic studies on the phycobilin pigments obtained from blue-green and red algae. Journal of Biochemistry 46: 903-909.

_____, and _____. 1959d. Effect of pre-illumination on the formation of phycobilin pigments in a blue-green alga, Tolypothrix tenuis. Journal of Biochemistry 46: 1259-1261.

Haxo, F. T. 1960. The wavelength dependence of photosynthesis and the role of accessory pigments, p. 339-360. In M. B. Allen (ed.), Comparative biochemistry of photoreactive systems. Academic Press, New York.

_____, C. Ó hEocha, and P. Norris. 1955. Comparative studies of chromatographically separated phycoerythrins and phycocyanins. Archives of Biochemistry and Biophysics 54: 162-173.

Höcht, H., H. H. Martin, and O. Kandler. 1965. Zur Kenntnis der Zusammensetzung der Zellwand der Blaualgen. Zeitschrift für Pflanzenphysiologie 53: 39-57.

Holm-Hansen, O. 1968. Ecology, physiology, and biochemistry of the blue-green algae. Annual Review of Microbiology 22: 47-70.

Holohan, P. D., and R. B. Moore. 1967. Chemical changes accompanying photomorphogenesis of a blue-green alga. Journal of Phycology 3: Supplement p. 8.

Hopwood, D. A., and A. M. Glauert. 1960a. The fine structure of Streptomyces coelicolor. II. The nuclear material. Journal of Biophysical and Biochemical Cytology 8: 267-278.

_____, and _____. 1960b. The fine structure of the nuclear material of a blue-green alga, Anabaena cylindrica Lemm. Journal of Biophysical and Biochemical Cytology 8: 813-823.

Iterson, W. van. 1965. Symposium on the fine structure and replication of bacteria and their parts. II. Bacterial cytoplasm. Bacteriological Reviews 29: 299-325.

Jacob, F., S. Brenner, and F. Cuzin. 1964. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symposia on Quantitative Biology 28: 329-348.

Jacobson, A. B., H. Swift, and L. Bogorad. 1963. Cytochemical studies concerning the occurrence and distribution of RNA in plastids of Zea mays. Journal of Cell Biology 17: 557-570.

Jensen, T. E. 1965. The fine structure of certain members of the order Nostocales. Unpublished Ph.D. thesis. Library, Iowa State University of Science and Technology, Ames, Iowa.

_____. 1968. Electron microscopy of polyphosphate bodies in a blue-green alga, Nostoc pruniforme. Archiv für Mikrobiologie 62: 144-152.

_____, and C. C. Bowen. 1961. Organization of the centroplasm in Nostoc pruniforme. Iowa Academy of Science, Proceedings 68: 86-89.

Jones, L. W. and J. Myers. 1965. Pigment variations in Anacystis nidulans induced by light of selected wavelengths. Journal of Phycology 1: 7-14

Jost, M. 1965. Die Ultrastruktur von Oscillatoria rubescens D. C. Archiv für Mikrobiologie 50: 211-245.

_____, and P. Matile. 1966. Zur Charakterisierung der Gasvacuolen der Blaualge Oscillatoria rubescens. Archiv für Mikrobiologie 53: 50-58.

Kaye, A. M., R. Salomon, and B. Fridlender. 1967. Base composition and presence of methylated bases in DNA from a blue-green alga Plectonema boryanum. Journal of Molecular Biology 24: 479-483.

Kellenberger, E. 1962. The study of natural and artificial DNA-plams by thin sections, p. 233-245. In R. J. C. Harris (ed.), The interpretation of ultrastructure. Academic Press, New York.

_____, A. Ryter, and J. Sechaud. 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. Journal of Biophysical and Biochemical Cytology 4: 671-678.

Kirk, J. T. O. 1966. Nature and function of chloroplast DNA, p. 319-340. In T. W. Goodwin (ed.), Biochemistry of chloroplasts. Academic Press, New York.

Klein, R. M., and A. Cronquist. 1967. A consideration of the evolutionary and taxonomic significance of some biochemical, micromorphological, and physiological characters in the thallophytes. Quarterly Review of Biology 42: 105-296.

Kleinschmidt, A. K. 1968. Monolayer techniques in electron microscopy of nucleic acid molecules, p. 361-377. In L. Grossman and K. Moldave (ed.), Methods in enzymology. Vol. 12B. Academic Press, New York.

Kok, B., and G. Hoch. 1961. Spectral changes in photosynthesis, p. 397-423. In W. D. McElroy and B. Glass (ed.), Light and life. Johns Hopkins Press, Baltimore, Maryland.

Kratz, W. A., and J. Myers. 1955a. Nutrition and growth of several blue-green algae. American Journal of Botany 42: 282-287.

_____, and _____. 1955b. Photosynthesis and respiration in three blue-green algae. Plant Physiology 30: 275-280.

Kraus, M. P. 1968. Radiation resistance in blue-green algae. Journal of Phycology 4: Supplement p. 9.

Kreutz, W. 1966. The structure of the lamellar system of chloroplasts, p. 83-88. In T. W. Goodwin (ed.), Biochemistry of the chloroplasts. Academic Press, New York.

_____, and W. Menke. 1962. Strukturuntersuchungen an Plastiden. Zeitscrift für Forschung 17b: 675-683.

Krinsky, N. I. 1966. The role of carotenoid pigments as protective agents against photosensitized oxidations in chloroplasts, p. 423-430. In T. W. Goodwin (ed.), Biochemistry of chloroplasts. Academic Press, New York.

Kubai, D. F., and H. Ris. 1969. Division in the dinoflagellate Gyrodinium cohnii (Schiller). A new type of nuclear reproduction. Journal of Cell Biology 40: 508-528.

Kumar, H. D. 1962. Apparent genetic recombination in a blue-green alga. Nature 196: 1121-1122.

Lang, N. J. 1968a. The fine structure of the blue-green algae. Annual Review of Microbiology 22: 15-45.

_____. 1968b. Ultrastructure of blue-green algae, p. 235-248. In D. F. Jackson (ed.), Algae, Man, and the Environment. Syracuse University Press, Syracuse, New York.

Lazaroff, N. 1966. Photoinduction and photoreversal of the Nostocacean developmental cycle. Journal of Phycology 2: 7-17.

_____, and J. Schiff. 1962. Action spectrum for developmental photoinduction of the blue-green alga Nostoc muscorum. Science 137: 603-604.

_____, and W. Vishniac. 1961. The effect of light on the developmental cycle of Nostoc muscorum, a filamentous blue-green alga. Journal of General Microbiology 25: 365-374.

_____, and _____. 1962. The participation of filament anastomosis in the developmental cycle of Nostoc muscorum, a blue-green alga. Journal of General Microbiology 28: 203-210.

_____, and _____. 1964. The relationship of cellular differentiation to colonial morphogenesis of the blue-green alga, Nostoc muscorum A. Journal of General Microbiology 35: 447-457.

Leak, L. V. 1965. Electron microscopic autoradiography incorporation of H^3-thymidine in a blue-green alga, Anabaena sp. Journal of Ultrastructure Research 12: 135-146.

_____. 1966. The ultrastructure of nucleoplasmic areas in some prokaryotic cells. Journal of Cell Biology 31: 67A.

_____. 1967. Studies on the preservation and organization of DNA-containing regions in a blue-green alga, a cytochemical and ultrastructural study. Journal of Ultrastructure Research 20: 190-205.

_____, and G. B. Wilson. 1960. The distribution of chromatin in a blue-green alga Anabaena variabilis Kütz. Canadian Journal of Genetics and Cytology 2: 320-324.

_____, and _____. 1965. Electron microscopic observations on a blue-green alga, Anabaena sp. Canadian Journal of Genetics and Cytology 7: 237-249.

Lefort, M. 1960a. Structure inframicroscopique du chromatoplasma de quelques Cyanophycées. Comptes Rendus Academie Sciences 250: 1525-1527.

_____. 1960b. Nouvelles recherches sur l'infrastructure du chromatoplasma des Cyanophycées. Comptes Rendus Academie Sciences 251: 3046-3048.

_____. 1965. Sur le chromatoplasma d'une Cyanophycée endosymbiotique: Glaucocystis nostochinearum Itzigs. Comptes Rendus Academie Sciences 261: 233-236.

Leive, L. 1965a. Actinomycin sensitivity in Escherichia coli produced by EDTA. Biochemical and Biophysical Research Communications 18: 13-17.

_____. 1965b. A nonspecific increase in permeability in Escherichia coli produced by EDTA. National Academy of Science, Proceedings 53: 745-750.

_____. 1965c. RNA degradation and the assembly of ribosomes in actinomycin-treated Escherichia coli. Journal of Molecular Biology 13: 862-875.

_____. 1965d. Release of lipopolysaccharide by EDTA treatment of Escherichia coli. Biochemical and Biophysical Research Communications 21: 290-296.

Ley, J. de. 1964. On the unity of bacterial ribosomes. Journal of General Microbiology 34: 219-227.

Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA, p. 195-206. In L. Grossman and K. Moldave (ed.), Methods in enzymology. Vol. 12B. Academic Press, New York.

_____, C. L. Schildkraut, and J. Marmur. 1968. Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA, p. 184-195. In L. Grossman and K. Moldave (ed.), Methods in enzymology. Vol. 12B. Academic Press, New York.

Markham, R., S. Frey, and G. J. Hills. 1963. Methods for the enhancement of image detail and accentuation of structure in electron microscopy. Virology 20: 88-102.

Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. Journal of Molecular Biology 3: 208-218.

_____, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. Journal of Molecular Biology 5: 109-118.

Martin, H. H. 1963. Bacterial protoplasts--a review. Journal of Theoretical Biology 5: 1-34.

_____. 1966. Biochemistry of bacterial walls. Annual Review of Biochemistry 35: 457-484.

_____, and H. Frank. 1962. Quantitativ Bausteinanalyse der Stützmembran in der Zellwand von Escherichia coli B. Zeitschrift für Naturforschung 17b: 190-196.

Massie, H. R., and B. H. Zimm. 1965. Molecular weight of the DNA in the chromosomes of E. coli and B. subtilis. National Academy of Science, Proceedings 54: 1636-1641.

Menke, W. 1961. Über das Lamellarsystem des Chromatoplasma von Cyanophyceen. Zeitschrift für Naturforschung 16b: 543-546.

_____. 1965. Versuche zur Aufklärung der molekularen Strukture der Thylakoidmembran. Zeitschrift für Naturforschung 20b: 802-805.

_____. 1966. The molecular structure of photosynthetic lamellar systems, p. 328-340. In Energy conversion by the photosynthetic apparatus. Brookhaven Symposia in Biology, No. 19. Brookhaven National Laboratory, Upton, New York.

Muhlethaler, K. 1966. The ultrastructure of the plastid lamellae, p. 49-64. In T. W. Goodwin (ed.), Biochemistry of chloroplasts. Academic Press, New York.

_____, H. Moor, and J. K. Szarkowski. 1965. The ultrastructure of the chloroplast lamellae. Planta (Berlin) 67: 305-323.

Murray, R. G. E., P. Steed, and H. E. Elson. 1965. The location of the mucopeptide in sections of the cell wall of Escherichia coli and other gram-negative bacteria. Canadian Journal of Microbiology 11: 547-560.

Myers, J., and W. A. Kratz. 1955. Relations between pigment content and photosynthetic characteristics in a blue-green alga. Journal of General Physiology 39: 11-22.

Nass, M. M. K., and S. Nass. 1965. The general occurrence of mitochondrial DNA. Journal of Cell Biology 19: 613-629.

Niel, C. B. van. 1955. Classification and taxonomy of the bacteria and blue-green algae, p. 89-114. In A century of progress in the natural sciences, 1853-1953. California Academy of Sciences, San Francisco, California.

Noller, E. C., and S. E. Hartsell. 1961a. Bacteriolysis of Enterobacteriaceae. I. Lysis by four lytic systems utilizing lysozyme. Journal of Bacteriology 81: 482-491.

_____, and _____. 1961b. Bacteriolysis of Enterobacteriaceae. II. Pre- and co-lytic treatments potentiating the action of lysozyme. Journal of Bacteriology 81: 492-499.

Nultsch, W. 1961. Der Einfluss des Lichtes auf die Bewegung der Cyanophyceen. I. Photo-topotaxis von Phormidium autumnale. Planta (Berlin) 56: 632-647.

_____. 1962. Der Einfluss des Lichtes auf die Bewegung der Cyanophyceen. II. Photokinesis bei Phormidium autumnale. Planta (Berlin) 57: 613-623.

Ó hEocha, C. 1958. Comparative biochemical studies of the phycobilins. Archives of Biochemistry and Biophysics 73: 207-219.

_____. 1960. Chemical studies of phycoerythrins and phycocyanins, p. 181-203. In M. B. Allen (ed.), Comparative biochemistry of photoreactive systems. Academic Press, New York.

_____. 1962. Phycobilins, p. 421-435. In R. A. Lewin (ed.), Physiology and biochemistry of algae. Academic Press, New York.

_____. 1963. Spectral properties of the phycobilins. I. Phycocyanobilin. Biochemistry 2: 375-382.

_____. 1965a. Biliproteins of algae. Annual Review of Plant Physiology 16: 415-434.

_____. 1965b. Phycobilins, p. 175-196. In T. W. Goodwin (ed.), Chemistry and biochemistry of plant pigments. Academic Press, New York.

_____. 1966. Biliproteins, p. 407-421. In T. W. Goodwin (ed.), Biochemistry of chloroplasts. Academic Press, New York.

Palade, G. E. 1952. A study of fixation for electron microscopy. Journal of Experimental Medicine 95: 285-298.

Pankratz, H. S., and C. C. Bowen. 1963. Cytology of blue-green algae. I. The cells of Symploca muscorum. American Journal of Botany 50: 387-399.

Park, R. B., and J. Biggins. 1964. Quantosome: size and composition. Science 144: 1009-1011.

_____, and D. Branton. 1966. Freeze-etching of chloroplasts from glutaraldehyde-fixed leaves, p. 341-352. In Energy conversion by the photosynthetic apparatus. Brookhaven Symposia in Biology, No. 19. Brookhaven National Laboratory, Upton, New York.

_____, and N. G. Pon. 1961. Correlation of structure with function in Spinacea oleracea chloroplasts. Journal of Molecular Biology 3: 1-10.

_____, and _____. 1963. Chemical composition and the substructure of lamellae isolated from Spinacea oleracea chloroplasts. Journal of Molecular Biology 6: 105-114.

Peat, A., and B. A. Whitton. 1967. Environmental effects on the structure of the blue-green alga, Chlorogloea fritschii. Archiv für Mikrobiologie 57: 156-180.

Perkins, H. R. 1963. Chemical structure and biosynthesis of bacterial cell walls. Bacteriological Reviews 27: 18-55.

Petrack, B., and F. Lipmann. 1961. Photophosphorylation and photohydrolysis in cell-free preparations of blue-green algae, p. 621-630. In W. D. McElroy and B. Glass (ed.), Light and life. Johns Hopkins Press, Baltimore, Maryland.

Pfennig, N. 1967. Photosynthetic bacteria. Annual Review of Microbiology 21: 285-324.

Pringsheim, E. G. 1949. The relationship between bacteria and myxophyceae. Bacteriological Reviews 13: 47-98.

Reese, J. A. 1967. A study of the fine structure of the blue-green alga, Nostoc muscorum. Unpublished Ph.D. thesis. Library, Iowa State University of Science and Technology, Ames, Iowa.

Reich, E., and I. H. Goldberg. 1964. Actinomycin and nucleic acid function, p. 184-234. In J. N. Davidson and W. E. Cohn (ed.), Progress in nucleic acid research and molecular biology. Academic Press, New York.

Reif, C. B., and D. L. Fine. 1967. Two winter blue-green algal populations. Phycologia 6: 105-106.

Repaske, R. 1956. Lysis of gram-negative bacteria by lysozyme. Biochimica et Biophysica Acta 22: 189-191.

Ris, H. 1961. Ultrastructure and molecular organization of genetic systems. Canadian Journal of Genetics and Cytology 3: 95-120.

_____. 1962. Ultrastructure of certain self-dependent cytoplasmic organelles. 5th International Congress for Electron Microscopy. Academic Press, New York.

_____, and B. L. Chandler. 1963. The ultrastructure of genetic systems in prokaryotes and eukaryotes. Cold Spring Harbor Symposia on Quantitative Biology 28: 1-8.

_____, and W. Plaut. 1962. Ultrastructure of DNA-containing areas in the chloroplast of Chlamydomonas. Journal of Cell Biology 13: 383-391.

_____, and R. N. Singh. 1961. Electron microscope studies on the blue-green algae. Journal of Biophysical and Biochemical Cytology 9: 63-80.

Robinow, C. F. 1956. The chromatin bodies of bacteria. Bacteriological Reviews 20: 207-242.

_____. 1962. Morphology of the bacterial nucleus. British Medical Bulletin 18: 31-35.

Ryter, A. 1968. Association of the nucleus and the membrane of bacteria: a morphological study. Bacteriological Reviews 32: 39-54.

_____, and E. Kellenberger. 1958. Étude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. Zeitschrift für Naturforschung 13: 597-605.

Sabatini, D. D., K. Bensch, and R. J. Barrnett. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. Journal of Cell Biology 17: 19-58.

Salton, M. R. J. 1964. The bacterial cell wall. Elsevier Publishing Co., Amsterdam, Holland.

_____. 1967. Structure and function of bacterial cell membranes. Annual Review of Microbiology 21: 417-442.

Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. Journal of Molecular Biology 4: 430-443.

Scott, E., and D. S. Berns. 1965. Protein-protein interaction. The phycocyanin system. Biochemistry 4: 2597-2606.

Shatkin, A. J. 1960. A chlolophyll-containing cell fraction from the blue-green alga, Anabaena variabilis. Journal of Biophysical and Biochemical Cytology 7: 583-584.

Shibata, K., A. A. Benson, and M. Calvin. 1954. The absorption spectra of suspensions of living micro-organisms. Biochimica et Biophysica Acta 15: 461-470.

Simard, R. 1967. The binding of actinomycin D-^{3}H to heterochromatin as studied by quantitative high resolution radioautography. Journal of Cell Biology 35: 716-722.

Singh, R. N., and H. N. Singh. 1964. Ultra-violet induced mutants of blue-green algae. I. Anabaena cycadeae Reinke. Archiv für Mikrobiologie 48: 109-117.

_____, and R. Sinha. 1965. Genetic recombination in a blue-green alga, Cylindrospermum majus Kuetz. Nature 207: 782-783.

Smith, A. L. 1965. Principles of microbiology. 5th ed. The C. V. Mosby Co., St. Louis, Missouri.

Smith, D., and H. O. Halvorson. 1967. The isolation of DNA from yeast, p. 538-541. In L. Grossman and K. Moldave (ed.), Methods in enzymology. Vol. 12A. Academic Press, New York.

Smith, J. H. C., and C. S. French. 1963. The major and accessory pigments in photosynthesis. Annual Review of Plant Physiology 14: 181-224.

Smith, R. V., and A. Peat. 1967a. Comparative structure of the gas-vacuoles of blue-green algae. Archiv für Mikrobiologie 57: 111-122.

_____. 1967b. Growth and gas-vacuole development in vegetative cells of Anabaena flos-aquae. Archiv für Mikrobiologie 58: 117-126.

Stanier, R. Y. 1961. Photosynthetic mechanisms in bacteria and plants: development of a unitary concept. Bacteriological Reviews 25: 1-17.

_____, M. Doudoroff, and E. A. Adelberg. 1963. The microbial world. 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

_____, and C. van Niel. 1941. The main outlines of bacterial classification. Journal of Bacteriology 42: 437-466.

_____, and _____. 1962. The concept of a bacterium. Archiv für Mikrobiologie 42: 17-35.

Starr, R. C. 1964. The culture collection of algae at Indiana University. American Journal of Botany 51: 1013-1044.

Steed, P., and R. G. E. Murray. 1966. The cell wall and cell division of gram negative bacteria. Canadian Journal of Microbiology 12: 263-270.

Stefanini, M., C. De Martino, and L. Zamboni. 1967. Fixation of ejaculated spermatozoa for electron microscopy. Nature 216: 173-174.

Stempak, J. G., and R. T. Ward. 1964. An improved staining method for electron microscopy. Journal of Cell Biology 22: 697-701.

Strain, H. H. 1951. The pigments of algae, p. 243-262. In G. M. Smith (ed.), Manual of phycology. Chronica Botanica, Waltham, Massachusetts.

Strominger, J. L., and J. M. Ghuysen. 1967. Mechanisms of enzymatic bacteriolysis. Science 156: 213-221.

Susor, W. A., W. C. Duane, and D. W. Krogmann. 1964. Studies on photosynthesis using cell-free preparations of blue-green algae. Record of Chemical Progress 25: 197-208.

_____, and D. W. Krogmann. 1964. Hill activity in cell-free preparations of a blue-green alga. Biochimica et Biophysica Acta 88: 11-19.

_____, and _____. 1966. Triphosphopyridine nucleotide photoinduction with cell-free preparations of Anabaena variabilis. Biochimica et Biophysica Acta 120: 65-72.

Swift, H. 1965. Nucleic acids of mitochondria and chloroplasts. American Naturalist 99: 201-227.

Tabata, S., and G. Terui. 1962. Microbial enzyme active in hydrolyzing yeast cell wall. I. Isolation of strain and culture conditions for the enzyme formation. Journal of Fermentation Technology 40: 366-373. Original not available for examination; cited in Smith, D., and H. O. Halvorson. 1967. The isolation of DNA from yeast, p. 538-541. In L. Grossman and K. Moldave (ed.), Methods in enzymology. Vol. 12A. Academic Press, New York.

Thomas, J. B., J. W. Kleinen Hammans, and W. J. Arnolds. 1965. Studies on the red absorption band of chlorophyll a in vivo. Biochimica et Biophysica Acta 102: 324-332.

Troxler, R. F. and R. Lester. 1967. Biosynthesis of phycocyanobilin. Biochemistry 6: 3840-3846.

Trüper, H. G., and C. S. Yentsch. 1967. Use of glass fiber filters for the rapid preparation of in vivo absorption spectra of photosynthetic bacteria. Journal of Bacteriology 94: 1255-1256.

Vance, B. D. 1966. Sensitivity of Microcystis aeruginosa and other blue-green algae and associated bacteria to selected antibiotics. Journal of Phycology 2: 125-128.

Venable, J. H., and R. Coggeshall. 1965. A simplified lead citrate stain for use in electron microscopy. Journal of Cell Biology 25: 407-408.

Vernon, L. P., and M. Avron. 1965. Photosynthesis. Annual Review of Biochemistry 34: 269-296.

Waaland, J. R., and D. Branton. 1969. Gas vacuole development in a blue-green alga. Science 163: 1339-1341.

Webster, G., and S. L. Whitman. 1963. Structure and function of ribosomes, p. 30-39. In O. Lindberg (ed.), Functional biochemistry of cell structures. Vol. 2. 5th International Congress of Biochemistry, Proceedings, Macmillan, New York.

Weier, T. E. 1963. Changes in the fine structure of chloroplasts and mitochondria during phylogenetic and ontogenetic development. American Journal of Botany 50: 604-611.

_____, T. Bisalputra, and A. Harrison. 1966. Subunits in chloroplast membranes of Scenedesmus quadricanda. Journal of Ultrastructure Research 15: 38-56.

_____, A. H. P. Engelbrecht, A. Harrison, and E. B. Risley. 1965. Subunits in the membranes of chloroplasts of Phaseolus vulgaris, Pisum sativum, and Aspidistra sp. Journal of Ultrastructure Research 13: 92-111.

Wildon, D. C., and F. V. Mercer. 1963. The ultrastructure of the vegetative cell of blue-green algae. Australian Journal of Biological Science 16: 585-596.

Wilkins, M. H. F., and G. Zubay. 1959. The absence of histone in the bacterium Escherichia coli. II. X-ray diffraction of nucleoprotein extract. Journal of Biophysical and Biochemical Cytology 5: 55-58.

Work, E., and D. L. Dewey. 1953. The distribution of α-ε-diaminopimelic acid among various micro-organisms. Journal of General Microbiology 9: 394-409.

Zahn, R. K., E. Tiesler, A. K. Kleinschmidt, and D. Lang. 1962. Ein Konservierungs- und Darstellungs- Verfahren für Desoxyribonucleinsäuren und ihre Ausgangsmaterialen. Biochemische Zeitschrift 336: 281-298.

Zamboni, L., and C. De Martino. 1967. Buffered picric acid-formaldehyde: a new, rapid fixative for electron microscopy. Journal of Cell Biology 35: 148A.

Zubay, G., and M. R. Watson. 1959. The absence of histones in the bacterium Escherichia coli. I. Preparation and analysis of nucleoprotein extract. Journal of Biophysical and Biochemical Cytology 5: 51-54.

ACKNOWLEDGMENTS

The candidate wishes to express her appreciation to Dr. C. C. Bowen for proposing the research area, for encouragement and support throughout the program, and for permitting almost unlimited freedom in the course of the study. The assistance of Dr. H. T. Horner, Jr. in technical advice and discussions has been invaluable, and Mr. Paul Elsner deserves much credit for his time and efforts in training and trouble-shooting.

Rarely is a doctoral research program accomplished single-handedly by the candidate, and in this case it would have been impossible without the indulgence and assistance of many people in several disciplines at Iowa State University. It is a pleasure to acknowledge the generosity of Drs. J. D. Dodd, R. E. Ford, C. E. LaMotte, and D. J. Nevins in Botany, Drs. S. Aronoff and C. L. Tipton in Biochemistry, Drs. J. G. Holt, P. A. Pattee, and F. D. Williams in Bacteriology, and Dr. D. W. Lynch in Physics and members of their respective research groups for cheerfully sharing facilities. Dr. Pattee deserves special recognition for carrying out the CsCl equilibrium centrifugations.

The financial assistance of a pre-doctoral fellowship from the Department of Interior, Federal Water Pollution Control Administration, from August 1967 to June 1969 is

gratefully acknowledged.

Last but not least, the candidate thanks her long-suffering husband for his patience and sacrifices toward the cause.

APPENDIX A: FIXATION, DEHYDRATION, AND EMBEDDING

Fixation

Osmium tetroxide:

The Ryter-Kellenberger fixative for bacteria was modified by Pankratz and Bowen (1963) for blue-green algae as follows:

Michaelis buffer:

1.94 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)

2.94 g sodium veronal (sodium barbital)

3.40 g sodium chloride

distilled water to 100 ml

Fixing buffer:

5 ml stock Michaelis buffer

7 ml 0.1 N hydrochloric acid

13 ml distilled water

0.25 ml 1 M calcium chloride

pH 6.1 - 6.2

Bacto-tryptone solution:

1 g Bacto-tryptone

0.5 g sodium chloride

distilled water to 100 ml

Fixative:

Mix fixing buffer 1:1 with 2% aqueous osmium tetroxide

Add 1 ml Bacto-tryptone solution/ml of fixative

Fix specimens 3 hr at room temperature

Glutaraldehyde (Sabatini et al., 1963):

Phosphate buffer:

0.1 M KH_2PO_4	0.1 M Na_2HPO_4	pH
38 ml	12 ml	6.2
24 ml	26 ml	6.8
10 ml	40 ml	7.3

Fixative:

Mix 50% glutaraldehyde with above buffer of desired pH to obtain a solution of 2-4% glutaraldehyde

Fix 1 hr at room temperature or 2-12 hr at 4°C

Wash three times in buffer over 1-12 hr

Post-fix in 1% osmium tetroxide (above) 1-3 hr at room temperature

Glutaraldehyde-picric acid:

The method of Zamboni and De Martino (1967) was modified as follows:

Cacodylate buffer:

0.1 M sodium cacodylate

1 N hydrochloric acid to pH 6.7

Phosphate buffer:

24 ml 0.05 M KH_2PO_4

26 ml 0.05 M Na_2HPO_4

pH 7.3

Fixatives:

25 ml 0.1 M cacodylate buffer

1.25 ml 50% glutaraldehyde (2.5%)

Fixatives: (continued)

0.175 g picric acid (0.7%)

pH 6.1

---

25 ml 0.05 M phosphate buffer

2 ml 50% glutaraldehyde (4%)

0.175 g picric acid (0.7%)

pH 6.1

Fix 4 hr at 4°C

Wash three times in buffer over 12 hr

Post-fix in 1% osmium tetroxide in same buffers for 1-2 hr at room temperature

Potassium permanganate:

Fix 30 min in unbuffered, aqueous 1.5% potassium permanganate

Wash with distilled water

Dehydration and Infiltration

The following protocol was carried out at room temperature:

5 min each in 25%, 50%, 70%, and 95% ethanol

3 changes of 5 min each in 100% ethanol

3 changes of 5 min each in propylene oxide

15 min in 1 part embedding mixture: 3 parts propylene oxide

30 min in 1 part embedding mixture: 1 part propylene oxide

60 min in 3 parts embedding mixture: 1 part propylene oxide

8-12 hr in 100% embedding mixture on a rotating disk

Embedding and Polymerization

Epon:

Mixture A:

Epon 812

DDSA (dodecanyl succinic anhydride)

Mixture B:

Epon 812

NMA (nadic methyl anhydride)

Mix 3 parts by weight A, 2 parts B, and 0.2 ml DMP-30 (catalyst) per 10 ml of Epon mixture

Stir thoroughly

Epon-Araldite:

10.0 g Araldite 502

12.0 g Epon 812

30.0 g DDSA

1.5 ml DMP-30

Mix thoroughly

Embedding:

Pour infiltrated specimens into shallow aluminum foil forms

Polymerization:

8-12 hr at 35°C

8-12 hr at 45°C

3-5 days at 60°C

APPENDIX B: CULTURE MEDIUM FOR *STREPTOMYCES ALBIDOFLAVUS*

The culture medium for the best lytic enzyme production in *Streptomyces albidoflavus*, according to Tabata and Terui (1962), was generously supplied by Dr. M. Mandel, University of Texas, Houston.

3 g/l crude cell wall

2 g K_2HPO_4

0.5 g yeast extract

0.5 g KCl

10 g glucose

1 g $MgSO_4 \cdot 7H_2O$

1 g peptone

0.5 NaCl

1 g $NaNO_3$

Culture on a shaker for 70 hr at $30^{\circ}C$

APPENDIX C: FIGURES

Key to all Figures

A - α-granule

AK - akinete

B - β-granule

C - cytoplasm

Car - carotenoids

CB - cylindrical body

Chl - chlorophyll

D - DNA fibrils

GV - gas vacuole

H - heterocyst

IT - intrathylakoidal space

L - lamellasome-like membranous convolution

N - nucleoplasm

PB - polyhedral body

Phy - phycocyanin

PM - plasma membrane

PS - phycobilisome

R - ribosomes

S - sheath

SG - structured granule

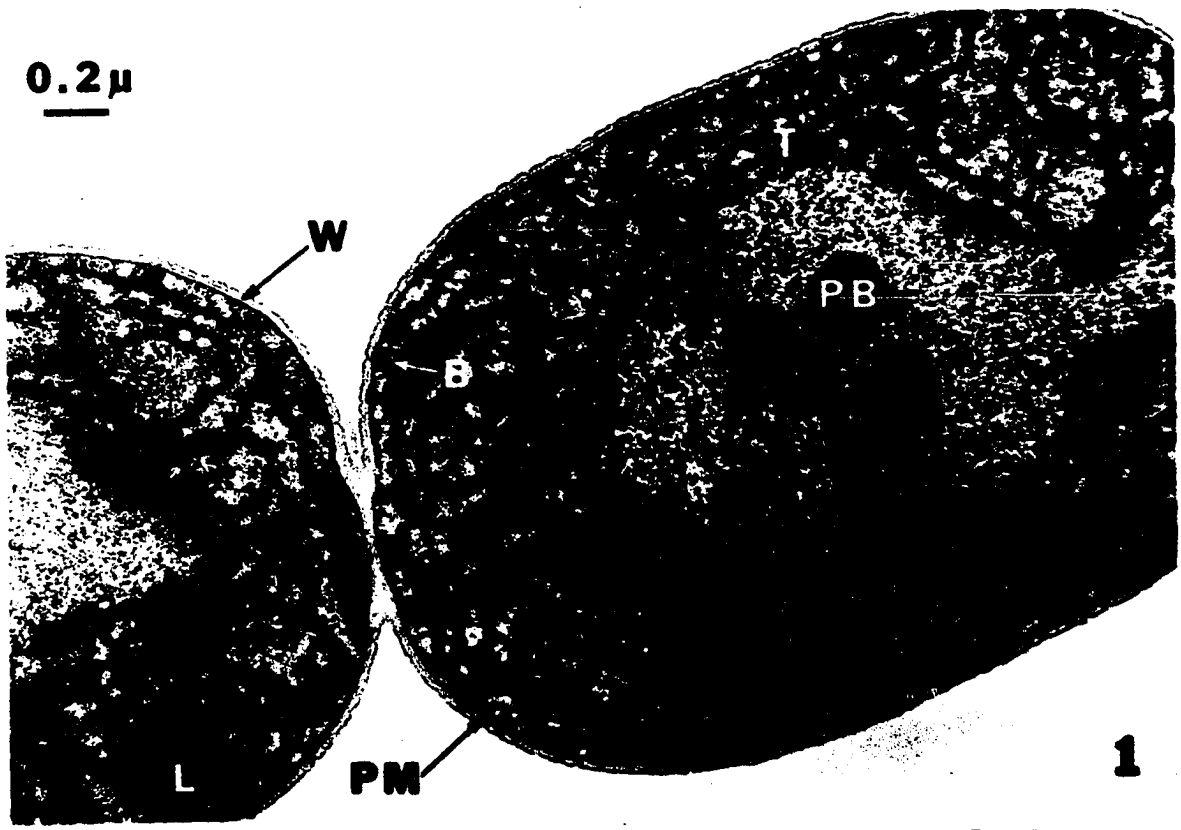
T - thylakoid

TE - tubular element

W - wall [layers L_I, L_{II}, L_{III}, and L_{IV}, according to Jost's (1965) terminology]

Figure 1. Cells of Nostoc muscorum, fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate, showing the multi-layered cell wall (W), plasma membrane (PM), thylakoid system (T), polyhedral bodies (PB), β-granules (B), and a membranous convolution resembling a lamellasome (L). 42,500 X.

Figure 2. Cell of Nostoc muscorum, fixed in glutaraldehyde-osmium tetroxide. The α-granules are clearly differentiated by staining with uranyl acetate followed by lead citrate. 75,500 X.


0.2µ
W
B
PB
L
PM
1

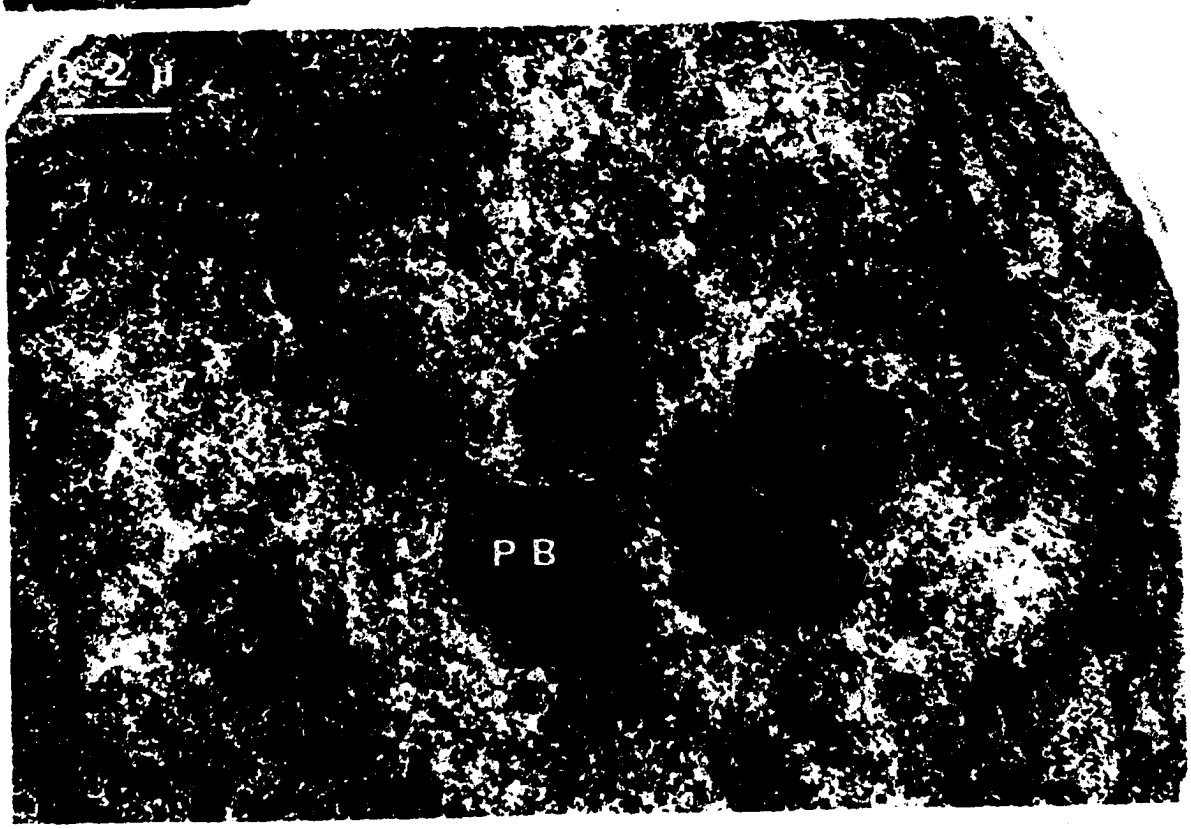
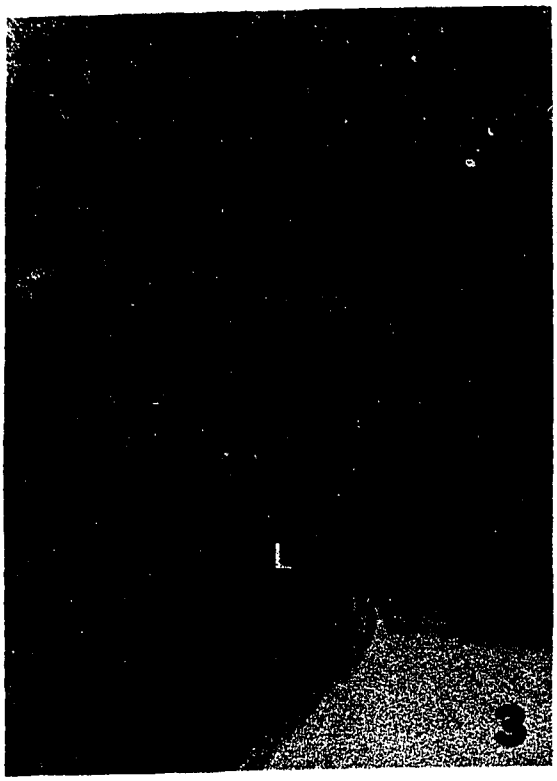

0.2 µ
PB

Figure 3. Dividing cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. A membranous convolution resembling a lamellasome (L) is adjacent to a cross wall. 75,500 X.

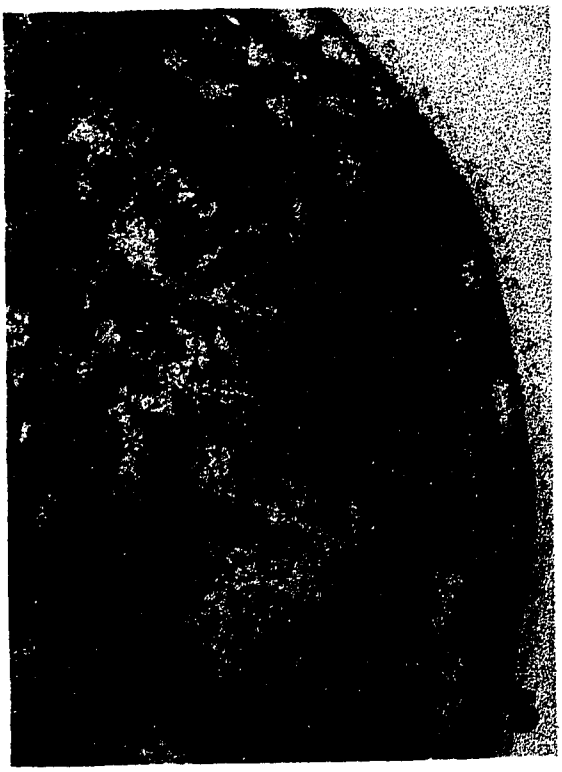
Figure 4. Portions of cells of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Lamellasome-like configurations flank the cross wall at the longitudinal wall. 75,500 X.

Figure 5. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The membranous convolution appears to be continuous with the thylakoid system. 75,500 X.

Figure 6. Portion of a cell of Tolypothrix distorta, fixed in osmium tetroxide and stained with uranyl acetate. The structured granules show an internal pattern of dark and light striations. 75,500 X.


L
3


4



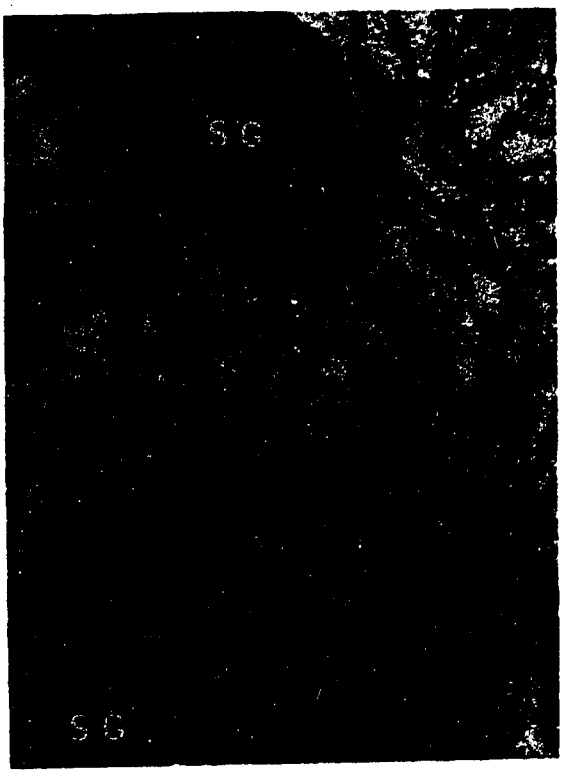

SG
SG

Figure 7. Portion of a cell of Nostoc muscorum, fixed in in glutaraldehyde-osmium tetroxide and stained with uranyl acetate. Tubular elements (TE) are distinct from the thylakoids (T) but appear to lie parallel to them. 75,500 X.

Figure 8. Cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate. Tubular elements (TE) appear to be associated with the nucleoplasm. 75,500 X.

Figure 9. Cell of Spirulina major, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Tubular elements (TE) appear to run from the cytoplasm into the nucleoplasm. 75,500 X.

Figure 10. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate. Parallel tubular elements (TE) appear in the nucleoplasm. 116,000 X.

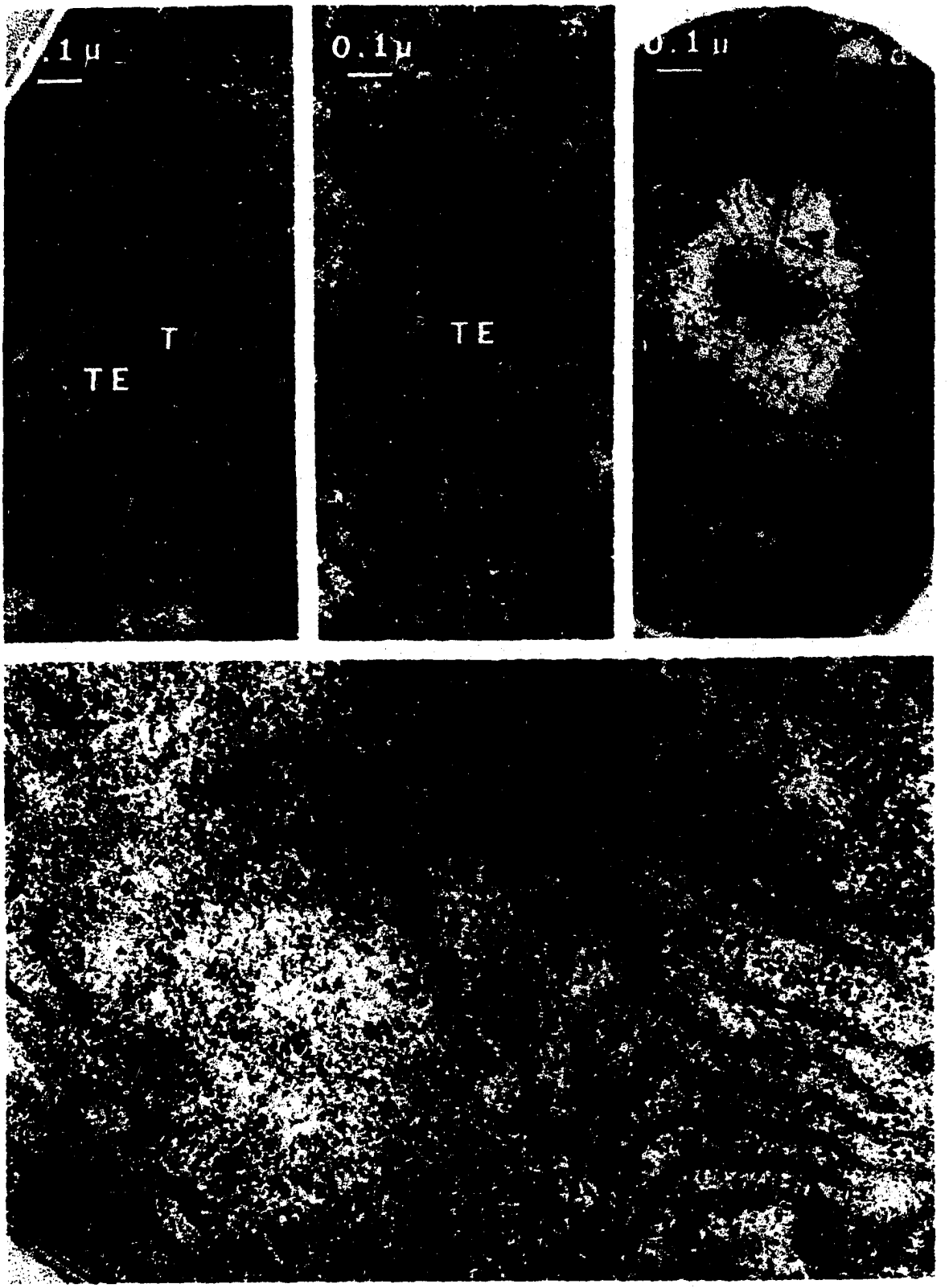

.1 μ
T
TE
0.1μ
TE
0.1 μ

Figure 11. Portion of a cell of Aphanizomenon flos-aquae, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Gas vacuoles (GV) appear in longitudinal and transverse view. Large intrathylakoidal spaces (IT) are characteristic of this species. 42,500 X.

Figure 12. Portion of a cell of Arthrospira Jenneri, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Gas vacuoles (GV) are scattered throughout the cell. 55,500 X.

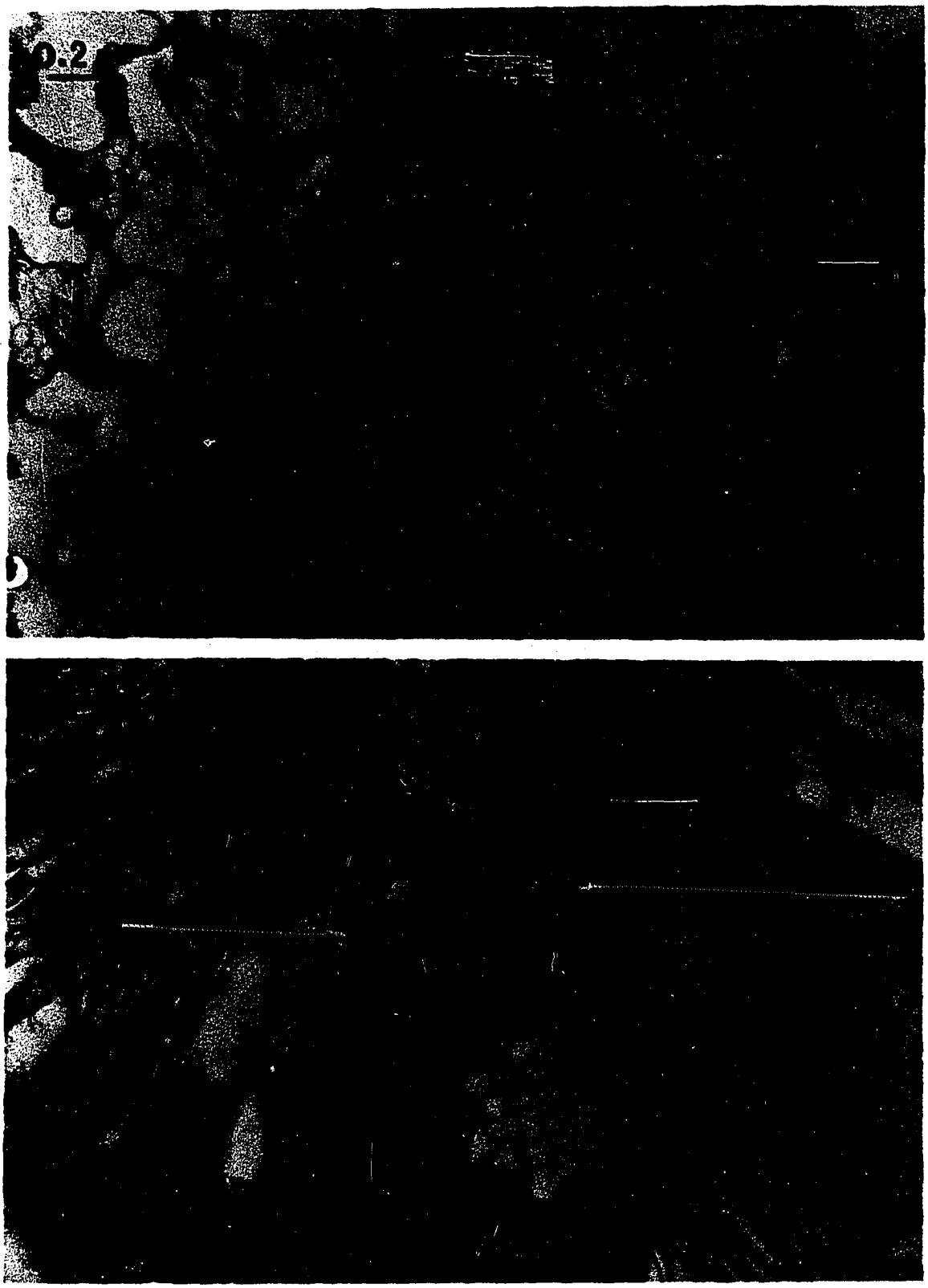


Figure 13. Portion of a cell of Microcoleus vaginatus (Type A), fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate. A cluster of cylindrical bodies (CB) lies adjacent to a cross-wall and has been sectioned transversely. 46,000 X.

Figure 14. Portion of a cell of Microcoleus vaginatus (Type A), fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate. A cylindrical body (CB) in longitudinal view is seen perpendicular to a cross-wall. 46,000 X.

Figure 15. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. A cluster of cylindrical bodies (CB) is oriented parallel to a cross-wall. 46,000 X.

Figure 16. Enlargement of the cylindrical bodies in Figure 15, showing sub-unit structure of the core. 230,000 X.

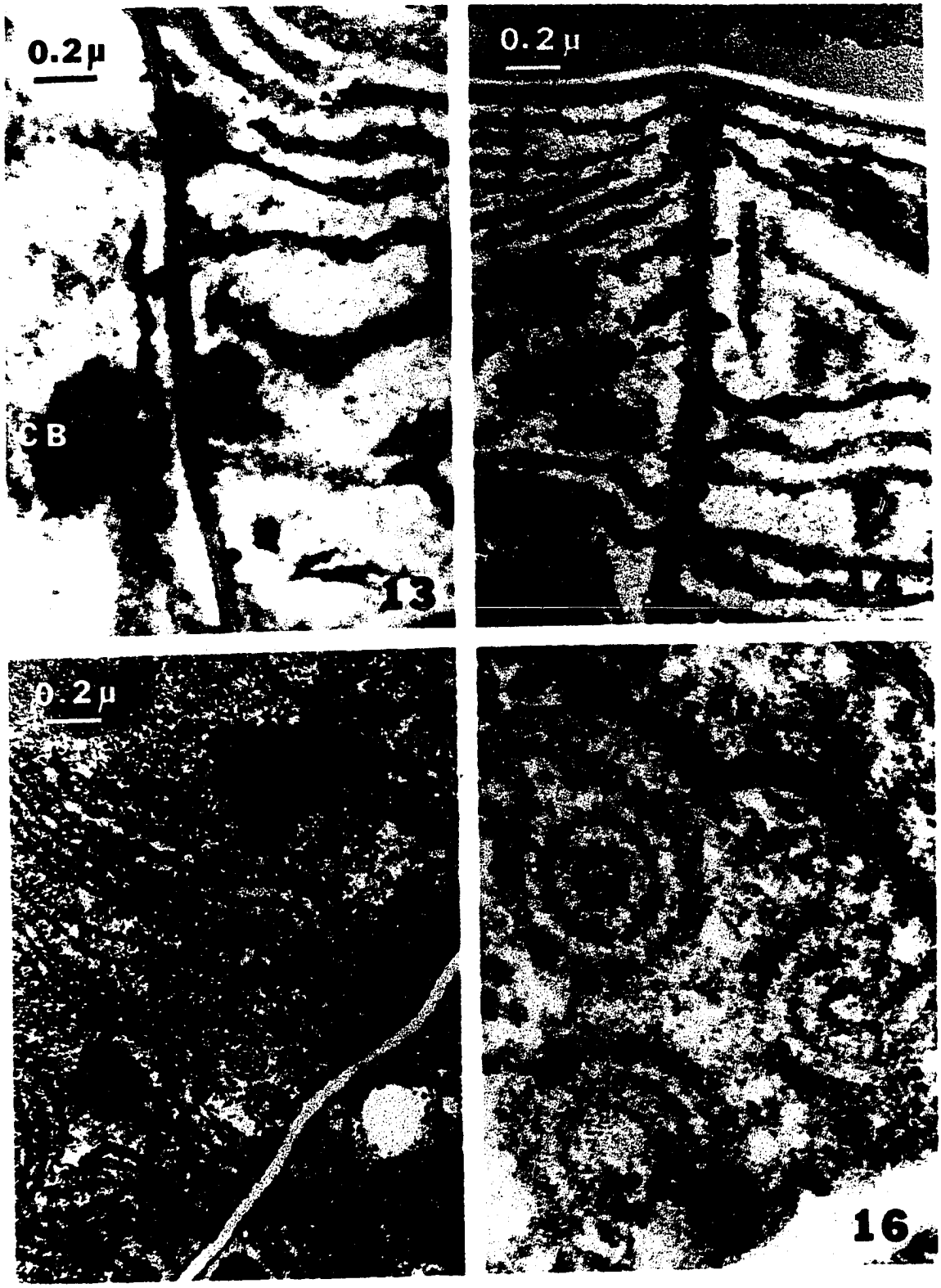

0.2μ
CB
13
0.2μ
0.2μ
16

Figure 17. Portion of a cell of Microcoleus vaginatus (Type A), fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate. 42,500 X.

Figure 18. An enlargement of the cylindrical bodies in Figure 17. Sub-unit structure is evident in the electron-dense layers. 110,000 X.

Figure 19. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. A cylindrical body (CB), showing sub-unit structure, appears between the thylakoids. Ribosomes (R) are prominent with the lead staining. 96,500 X.

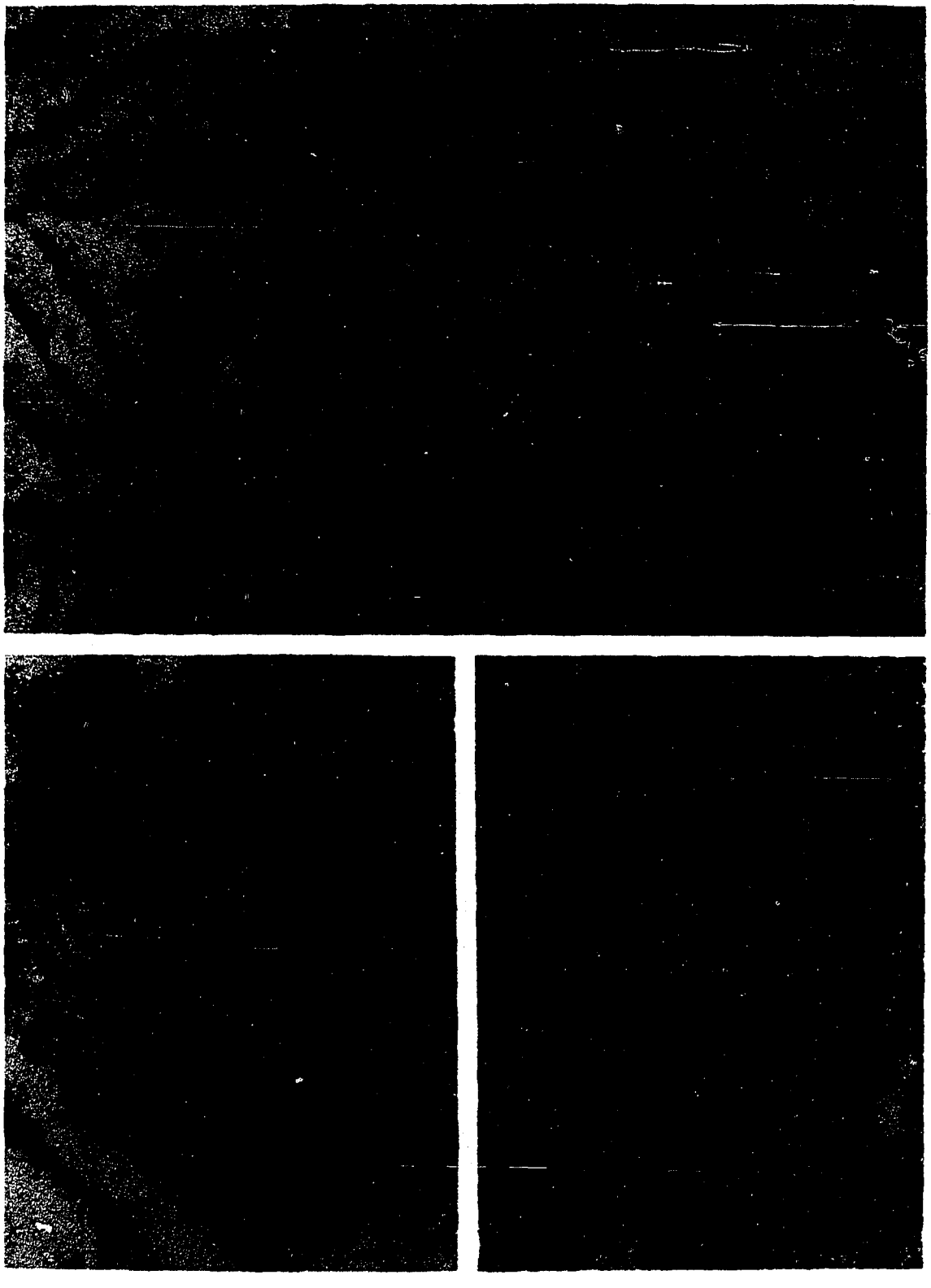
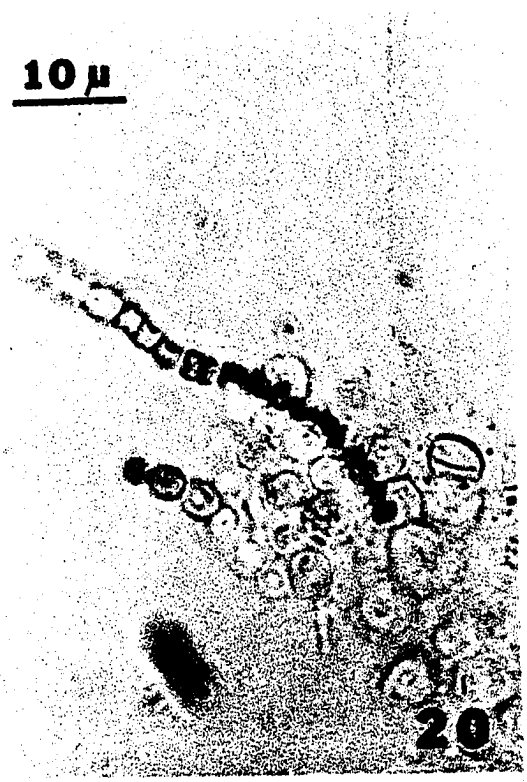
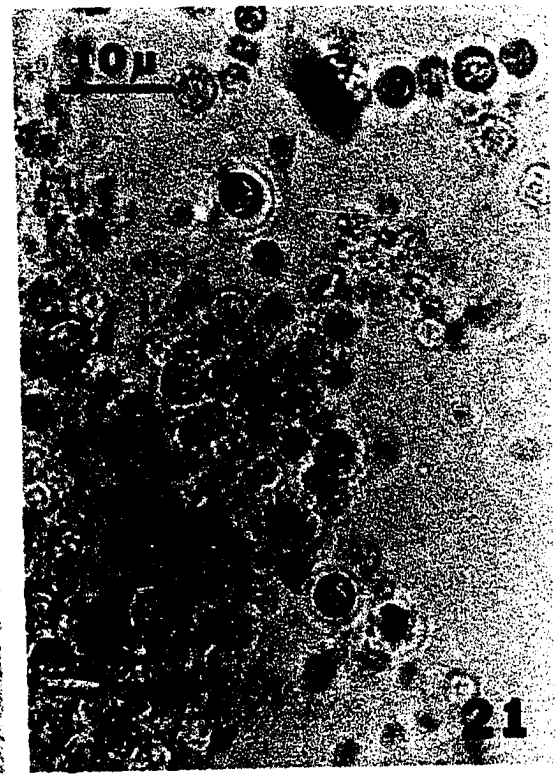


Figure 20. Plasmolyzed cells of Nostoc muscorum after 2 hr in 30% sucrose solution. 1,520 X.

Figure 21. Cells of Nostoc muscorum, treated with lysozyme, trypsin, and n-butanol. A few cells remain intact but no longer in a trichome. Most cells have been lysed. 1,520 X.

Figure 22. Portion of a cell of Nostoc muscorum, plasmolyzed in 25% sucrose-Tris buffer (pH 8.0), fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate. The protoplast has begun to pull away from the wall (arrow). 55,500 X.


10 µ
20


10µ
21

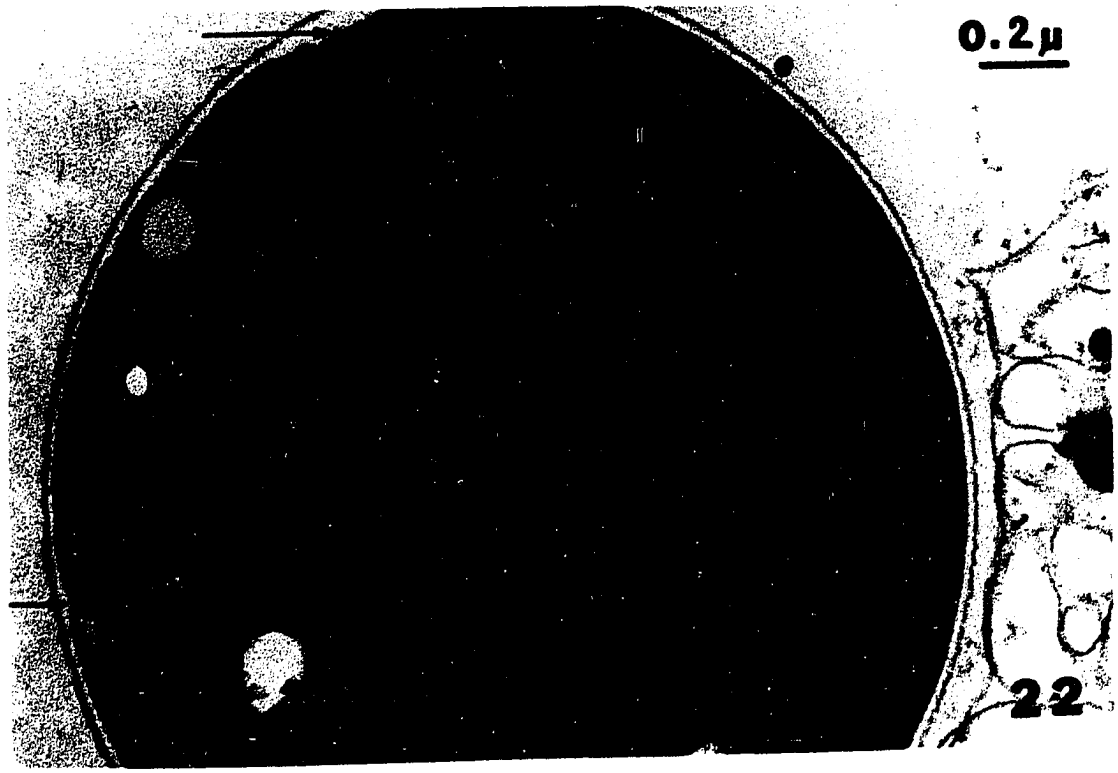
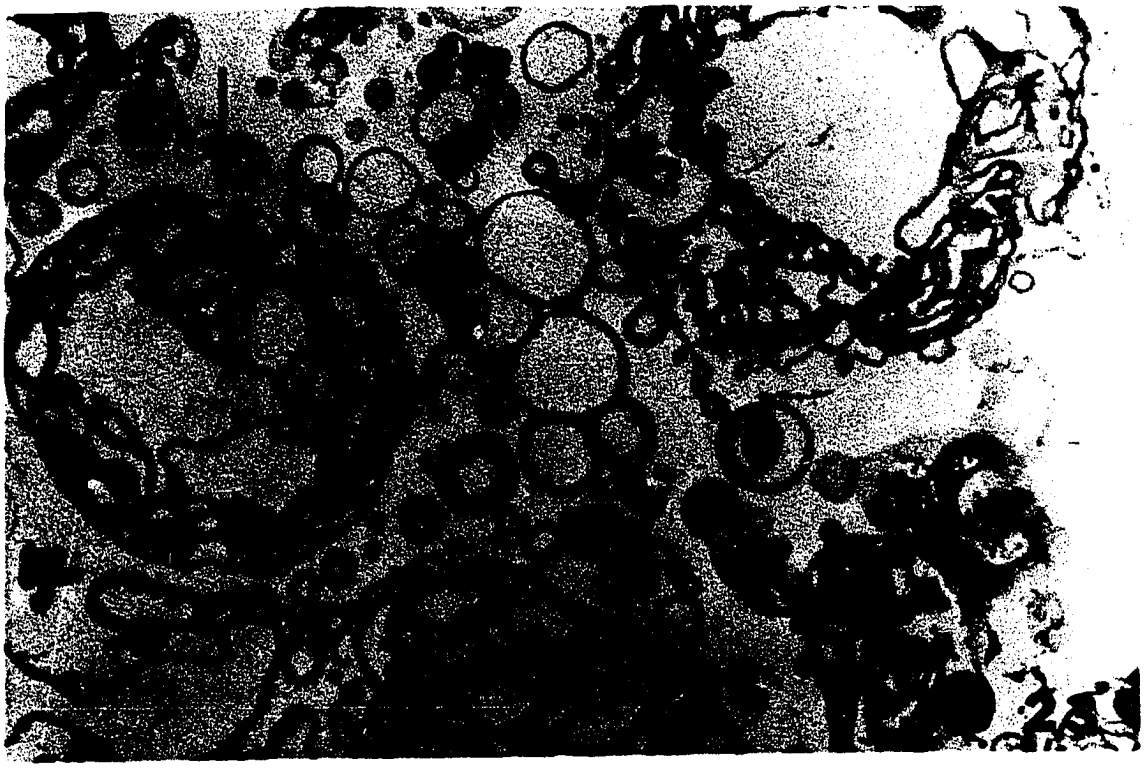

0.2µ
22

Figure 23. Cells of Nostoc muscorum, treated with lysozyme, trypsin, and n-butanol, fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate. Cell wall material is gone and most cells have been lysed. One cell (arrow) gives the appearance of a protoplast, devoid of wall but retaining the plasma membrane. 28,500 X.

Figure 24. Cell of Nostoc muscorum, produced by treatment of plasmolyzed cells with lysozyme and EDTA, fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate. Strands of DNA (D), abnormal thylakoid arrangements, β-granules, and the former sites of polyhedral bodies (PB) can be identified. 19,000 X.

Figure 25. Spore of Nostoc muscorum, treated as in Figure 24. The complex wall and sheath appear undisturbed. Sections of plasma membrane can be observed surrounding the remnants of cell contents. 28,500 X.




0.5

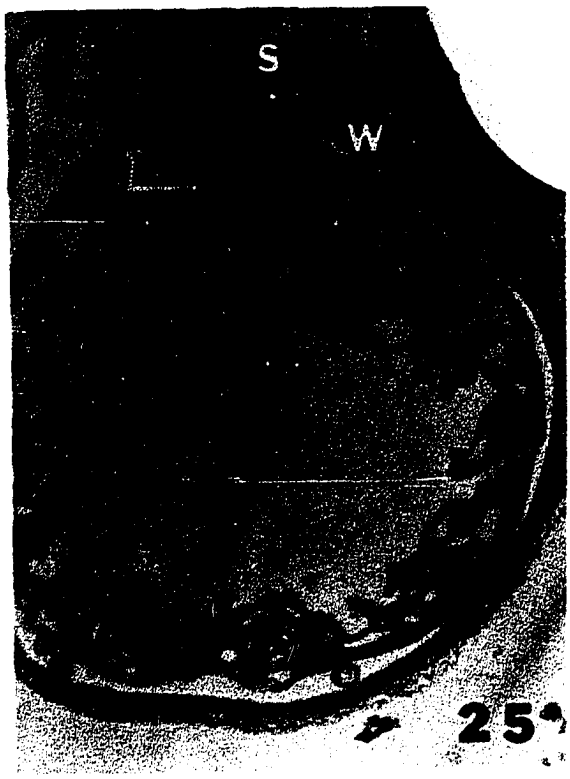
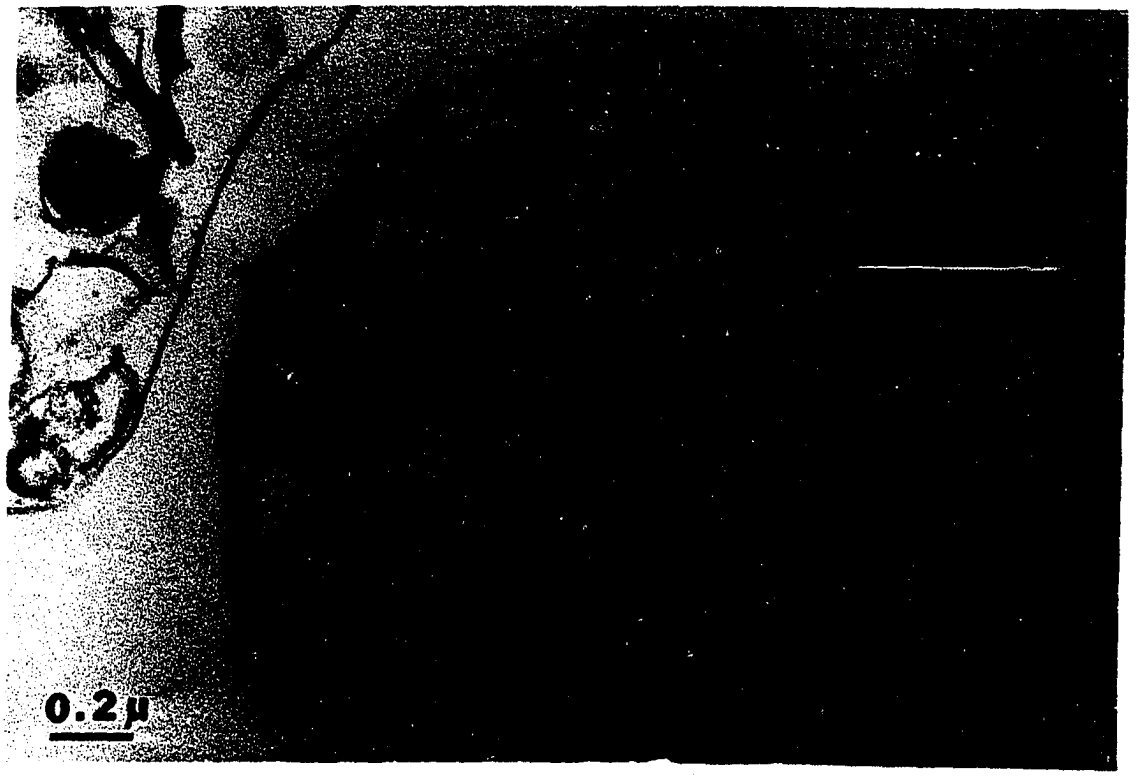

S
W
25

Figure 26. Cell of Nostoc muscorum, treated as in Figure 24. The plasma membrane and thylakoid membranes can be resolved, but wall material is missing. Angular outlines of polyhedral bodies (PB) devoid of contents can be discerned. 55,500 X.

Figure 27. Microdensitometer tracing of an ultraviolet absorption photograph of DNA isolated from Nostoc muscorum by the method of Smith and Halvorson (1967) and reference DNA from Proteus vulgaris. Centrifugation on CsCl for 24 hr at 25°C, 47,700 rev/min.


0.2μ

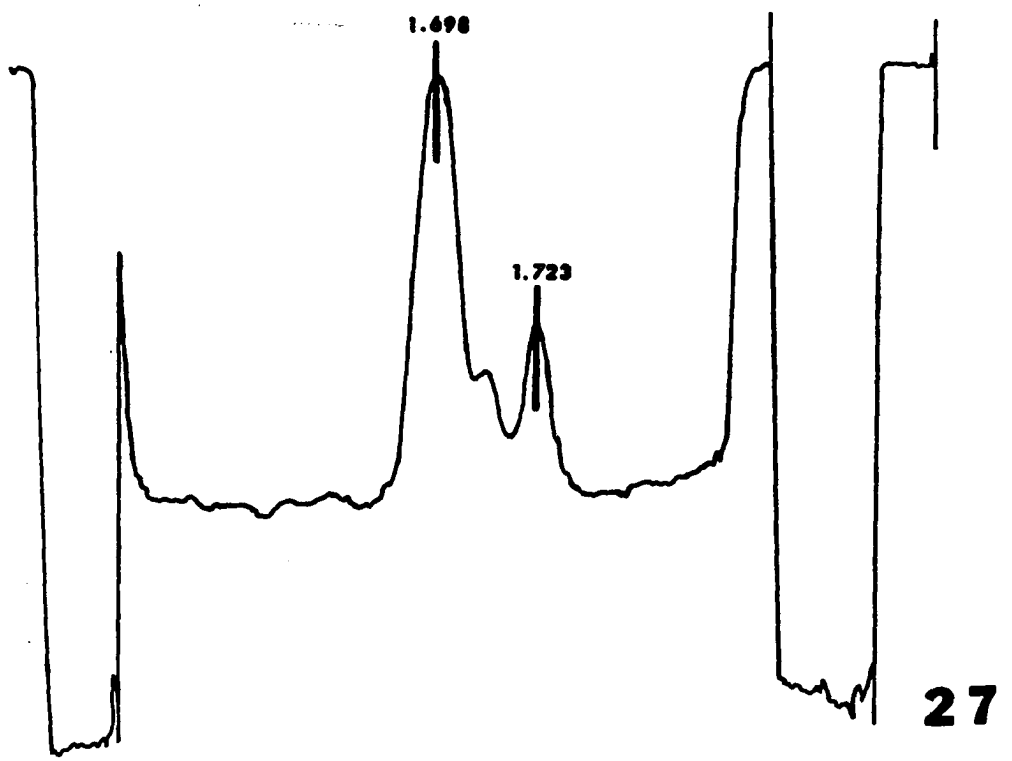
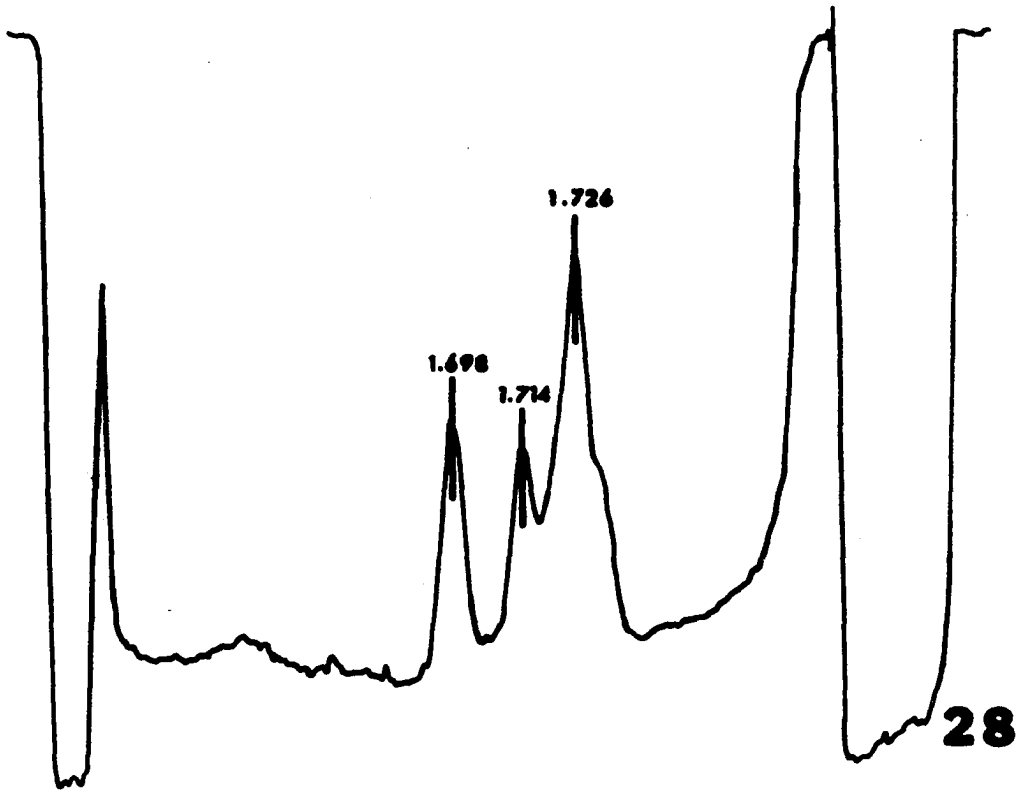
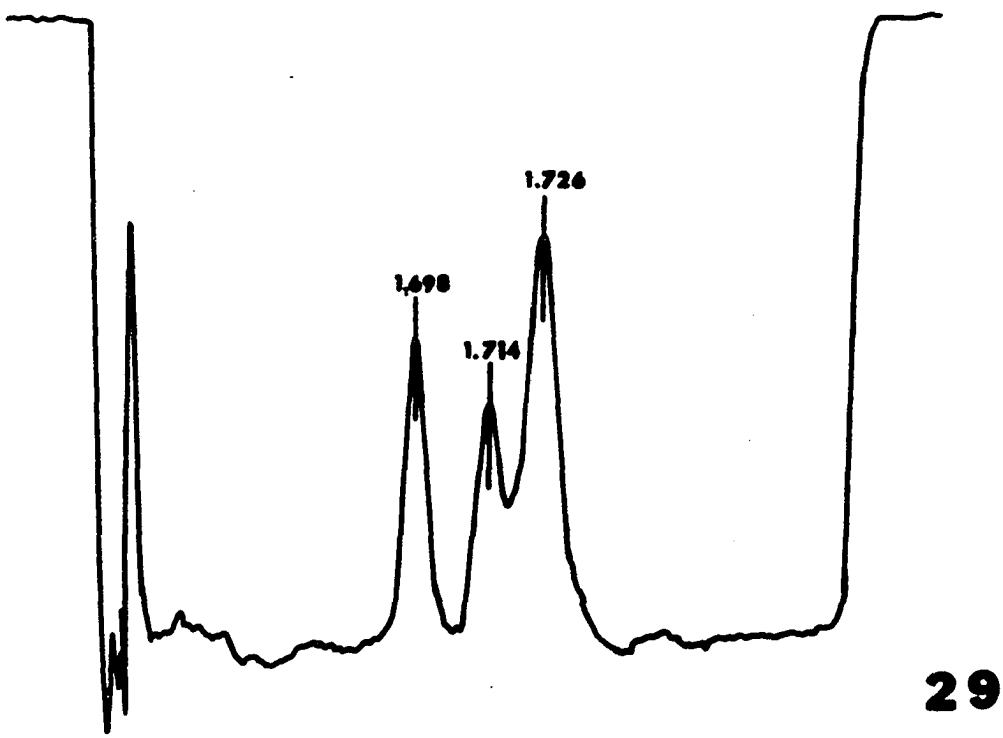

1.698
1.723
27

Figure 28. Microdensitometer tracing of an ultraviolet absorption photograph of DNA isolated from Anacystis nidulans by the method of Massie and Zimm (1965) and reference DNA from Proteus vulgaris. Centrifugation on CsCl for 24 hr at 25°C, 47,700 rev/min.

Figure 29. Microdensitometer tracing of an ultraviolet absorption photograph of DNA isolated from Anacystis nidulans by the method of Smith and Halvorson (1967) and reference DNA from Proteus vulgaris. Centrifugation on CsCl for 24 hr at 25°C, 47,700 rev/min.



28



29

Figure 30. Portion of a cell of Nostoc muscorum, fixed in glutaraldehyde-picric acid (cacodylate buffer) and osmium tetroxide; stained with uranyl acetate and lead citrate. The nucleoplasm occupies a large area extending to the peripheral thylakoids. Fibrils of DNA can be seen throughout the nucleoplasm and frequently appear as coarser, aggregated strands (arrows) under the conditions of this fixation. 95,000 X.

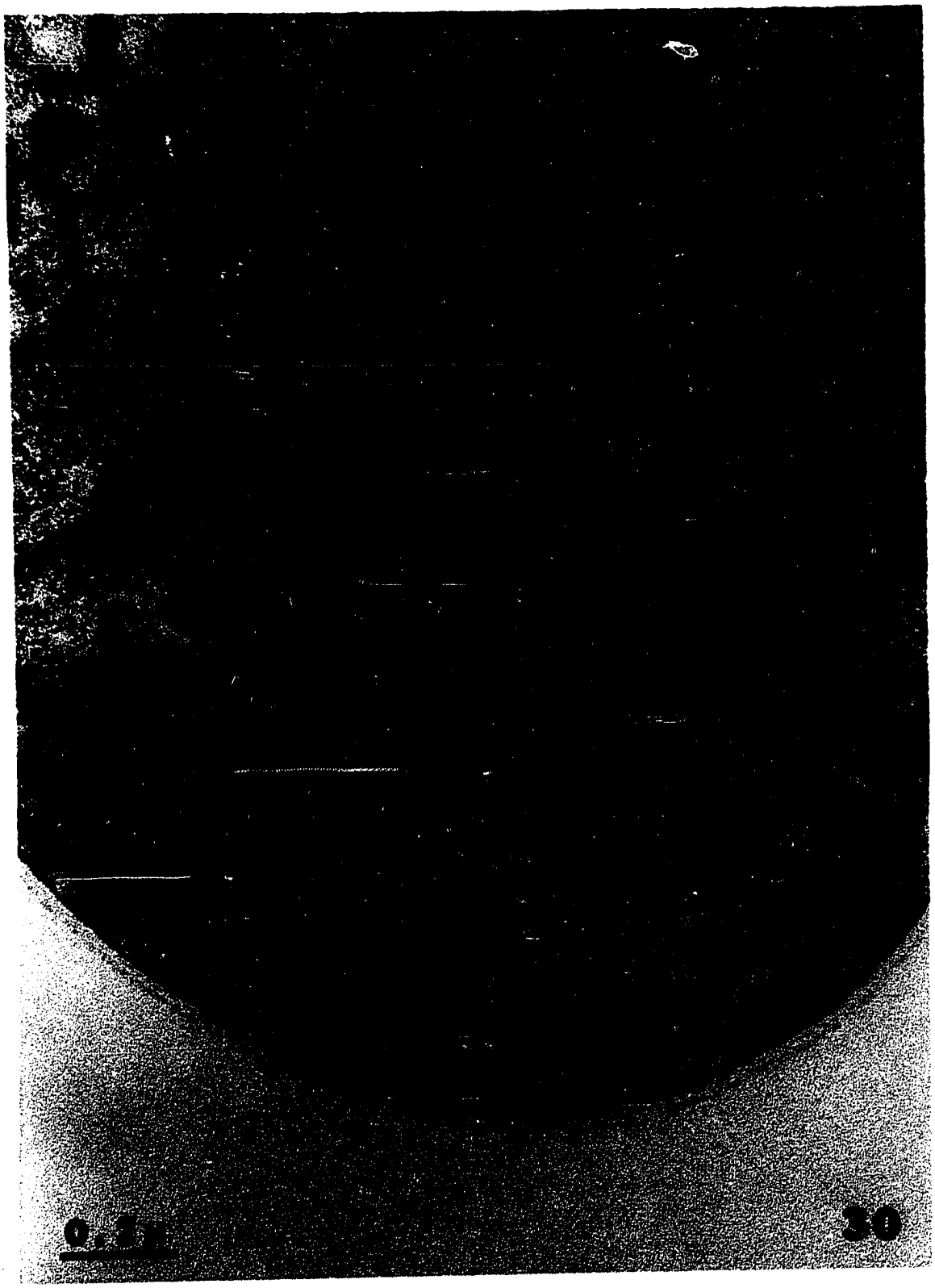


Figure 31. Cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate. Fibrils of DNA (D) diffuse throughout the nucleoplasm (N) which is less electron-dense than the cytoplasm (C). The polyhedral body (PB) appears to lack a sharp boundary. 71,000 X.

31

Figure 32. Portion of a cell of Nostoc muscorum, fixed by the Ryter-Kellenberger method and stained with uranyl acetate. Fine fibrils of DNA can be seen in a central region of low electron density. 75,500 X.

Figure 33. Portion of a cell of Spirulina major, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The nucleoplasm appears to be interior in relation to the peripheral cytoplasm and thylakoid system. DNA fibrils (D) and tubular elements (TE) can be identified. 75,500 X.

Figure 34. Portion of a cell of Tolypothrix distorta, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The nucleoplasm is difficult to discern, although small areas of lower electron density appear to contain strands of DNA. Ribosomes (R) and structured granules (SG) are heavily stained. Polyhedral bodies (PB) exhibit intermediate electron density. 75,500 X.

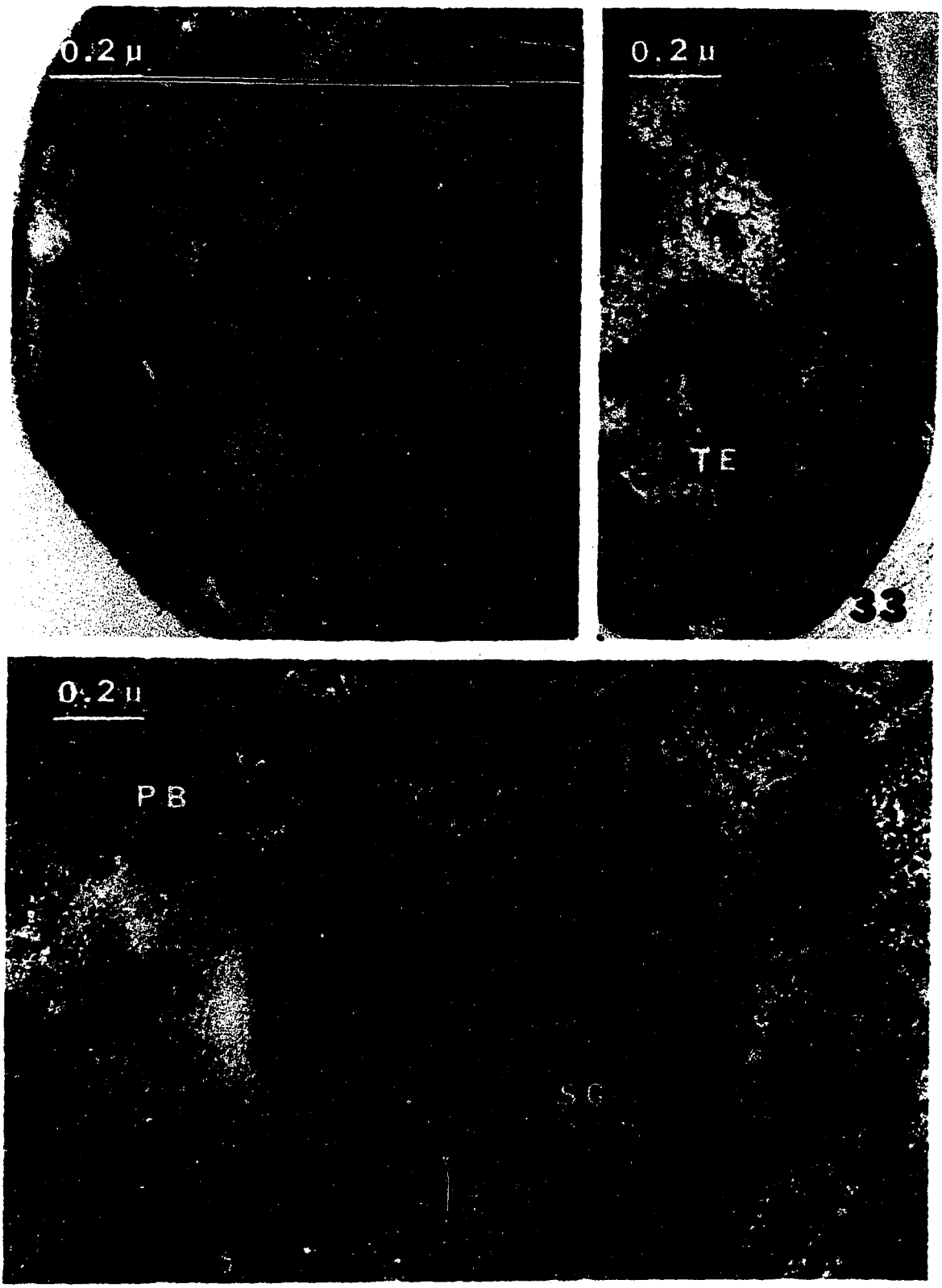
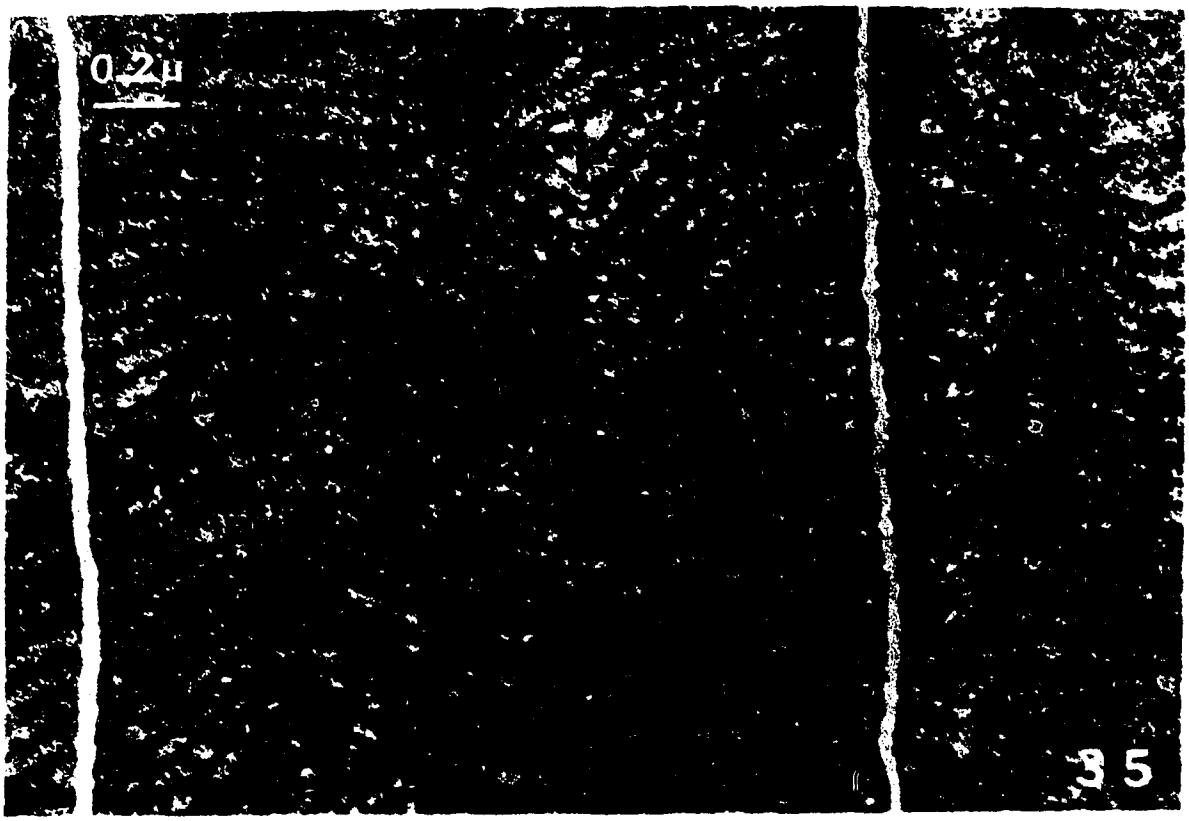

0.2 µ
0.2 µ
TE
33
0.2 µ
PB
SG

Figure 35. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Cells are so filled with thylakoids that the nucleoplasmic and cytoplasmic areas are difficult to differentiate. 55,500 X.

Figure 36. Portion of a cell of Microcoleus vaginatus (Type A), fixed in potassium permanganate and stained with uranyl acetate. Under these conditions, small areas of lower electron density, containing DNA fibrils, can be seen throughout the cell. α-granules are evident between the deeply stained thylakoids. 55,500 X.


0.2μ
3.5


0.2μ
P
A
3.6

Figure 37. Cell of Anacystis nidulans, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The nucleoplasm (N) appears to be confined to a central location and surrounded by peripheral cytoplasm (C) and thylakoids (T). 75,500 X.

Figure 38. Portion of a cell of Spirulina major, fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate and lead citrate. Polyhedral bodies (PB) exhibit electron density and appear to be outlined by a dark boundary. 75,500 X.

Figure 39. Portion of a cell of Aphanizomenon flos-aquae, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Polyhedral bodies (PB) are located in areas limited by the greatly expanded intrathylakoidal cavities. A dark boundary is evident around the polyhedral bodies. 75,500 X.

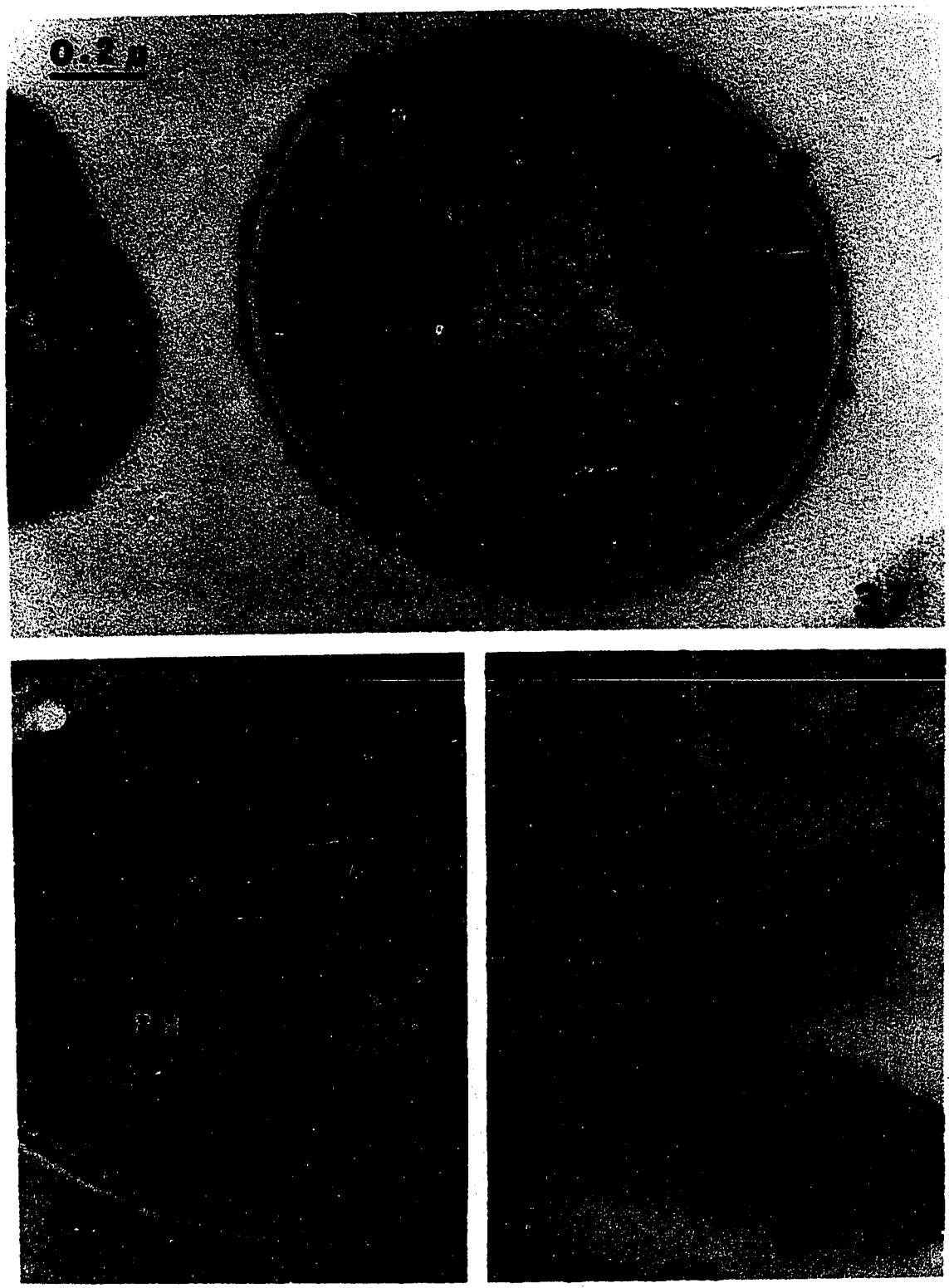
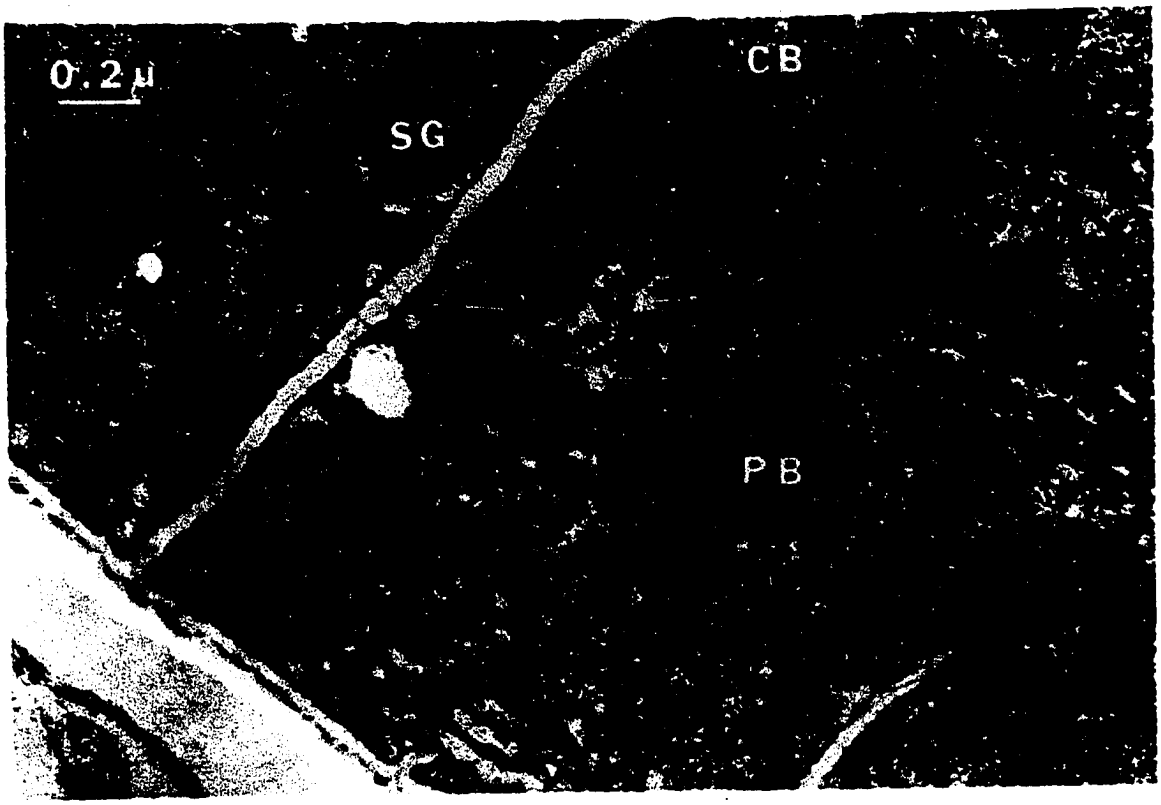

0.2 μ

Figure 40. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The polyhedral bodies (PB) appear to be associated with thylakoids as well as the nucleoplasm. Structured granules (SG) and cylindrical bodies (CB) can be seen. 55,500 X.

Figure 41. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with lead citrate. The polyhedral bodies and cylindrical bodies are stained much less intensely without uranyl acetate. The dark boundary of the polyhedral body can barely be discerned. 55,500 X.


0.2μ
SG
CB
PB

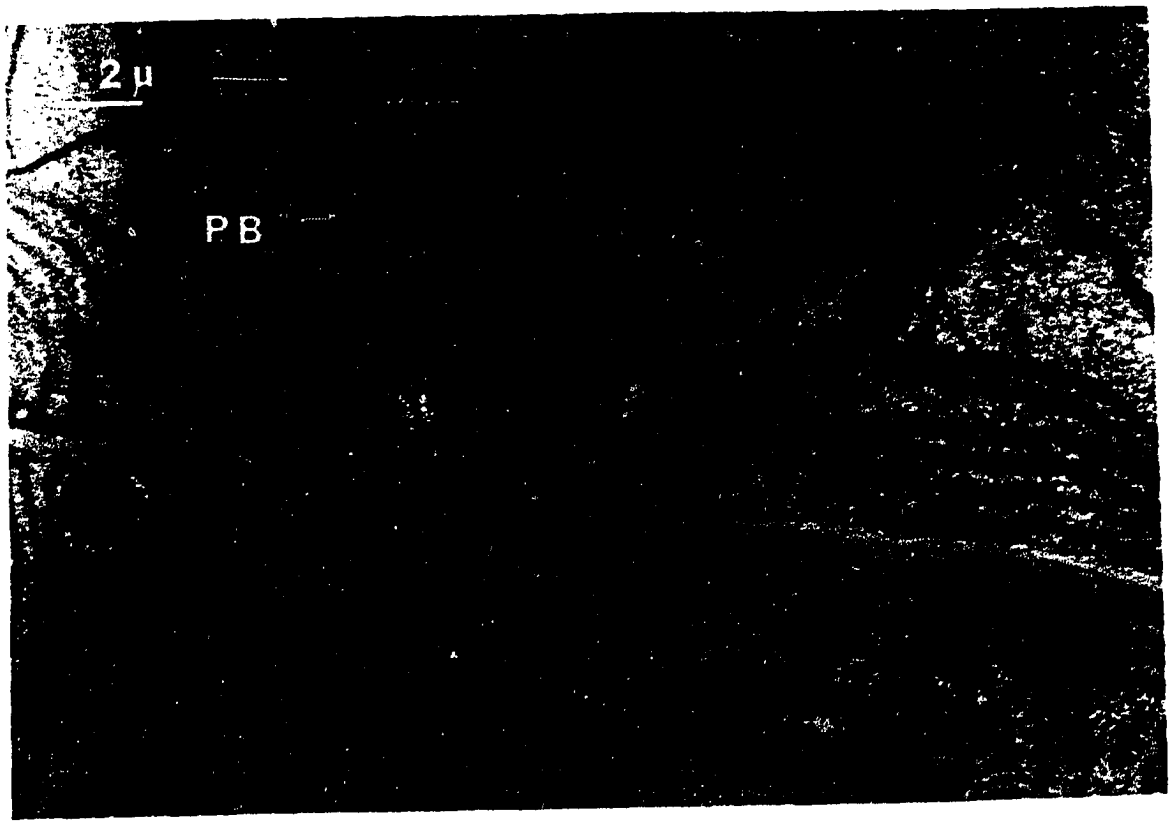

2μ
PB

Figure 42. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. An electron-dense boundary appears to completely surround the polyhedral body in this plane of sectioning. 75,500 X.

Figures 43 and 44. Portions of cells of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. In some regions, the contents of the polyhedral bodies appear to diffuse into the less-dense, surrounding nucleoplasm; in other regions, a dense boundary delimits the contents. Figure 43, 75,500 X; Figure 44, 116,000 X.

Figure 45. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. An electron-transparent region is evident between the contents and the boundary of a polyhedral body in one region; in another region, the contents appear to diffuse into the nucleoplasm. 250,000 X.

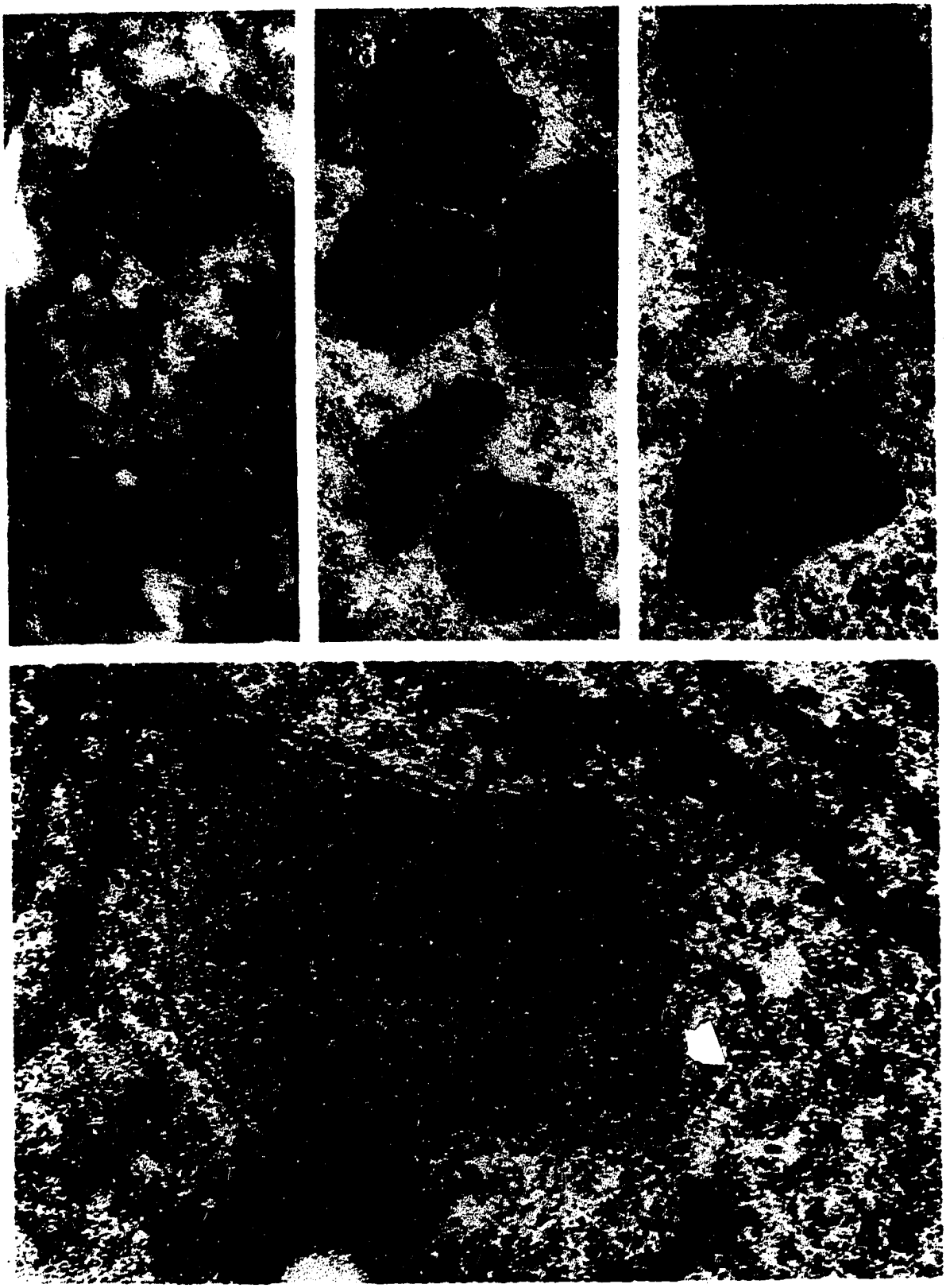
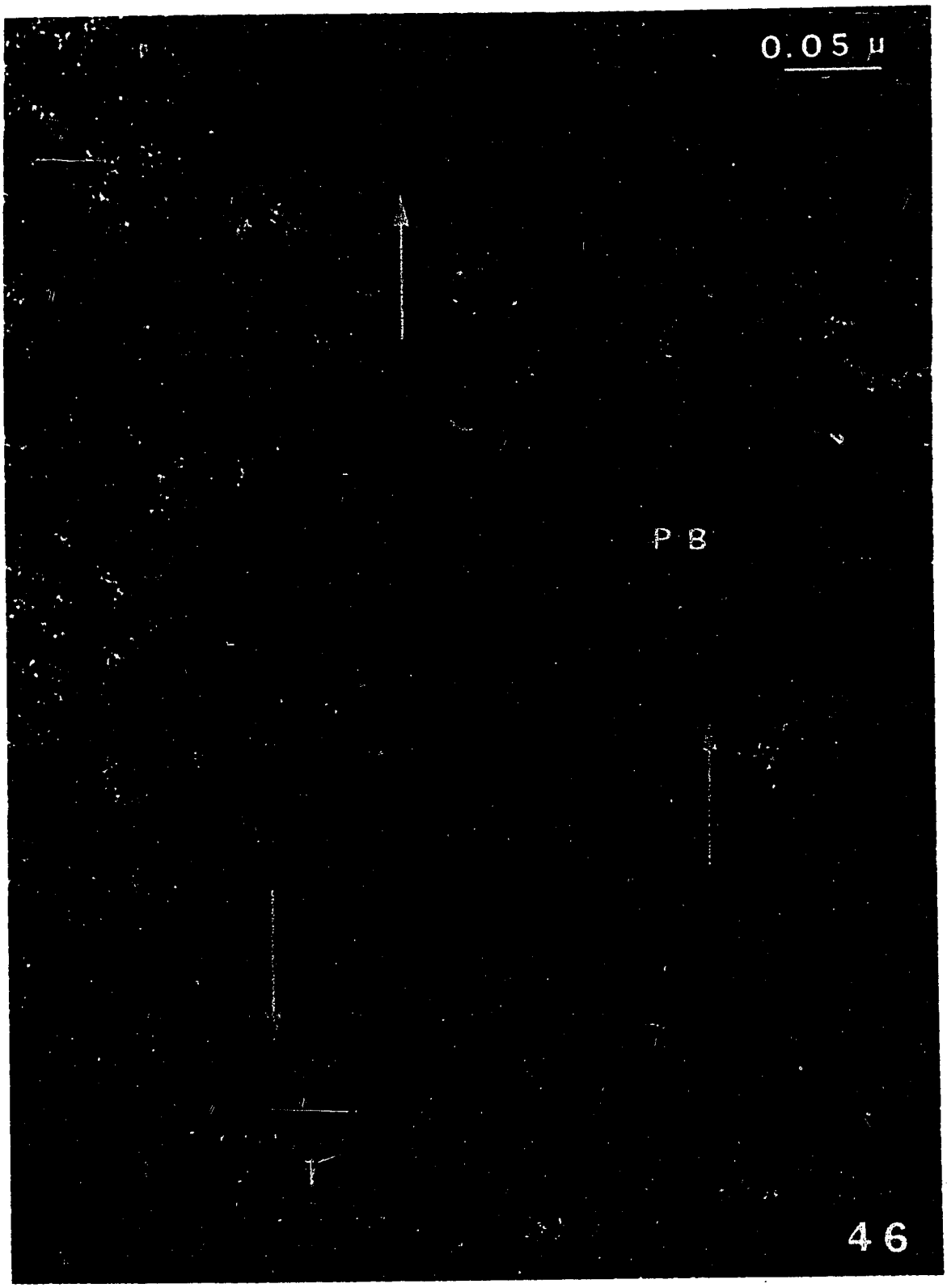


Figure 46. Portion of a cell of Nostoc muscorum, fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate and lead citrate. Sections of the boundary of the polyhedral bodies appear to have a tripartite image (arrows). 320,000 X.


0.05 μ
P B
T
46

Figures 47 and 48. Portions of cells of Nostoc muscorum, fixed and stained as in Figure 46. The tripartite image (arrow) is seen to enclose two polyhedral bodies in the same cell. 500,000 X.

Figure 49. Portion of a cell of Nostoc muscorum, fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate and lead citrate. Regions of the edge of the polyhedral bodies appear as a tripartite boundary (arrow). 600,000 X.

Figure 50. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The boundary of a polyhedral body appears to show differential staining of two electron-opaque layers (arrows). 500,000 X.

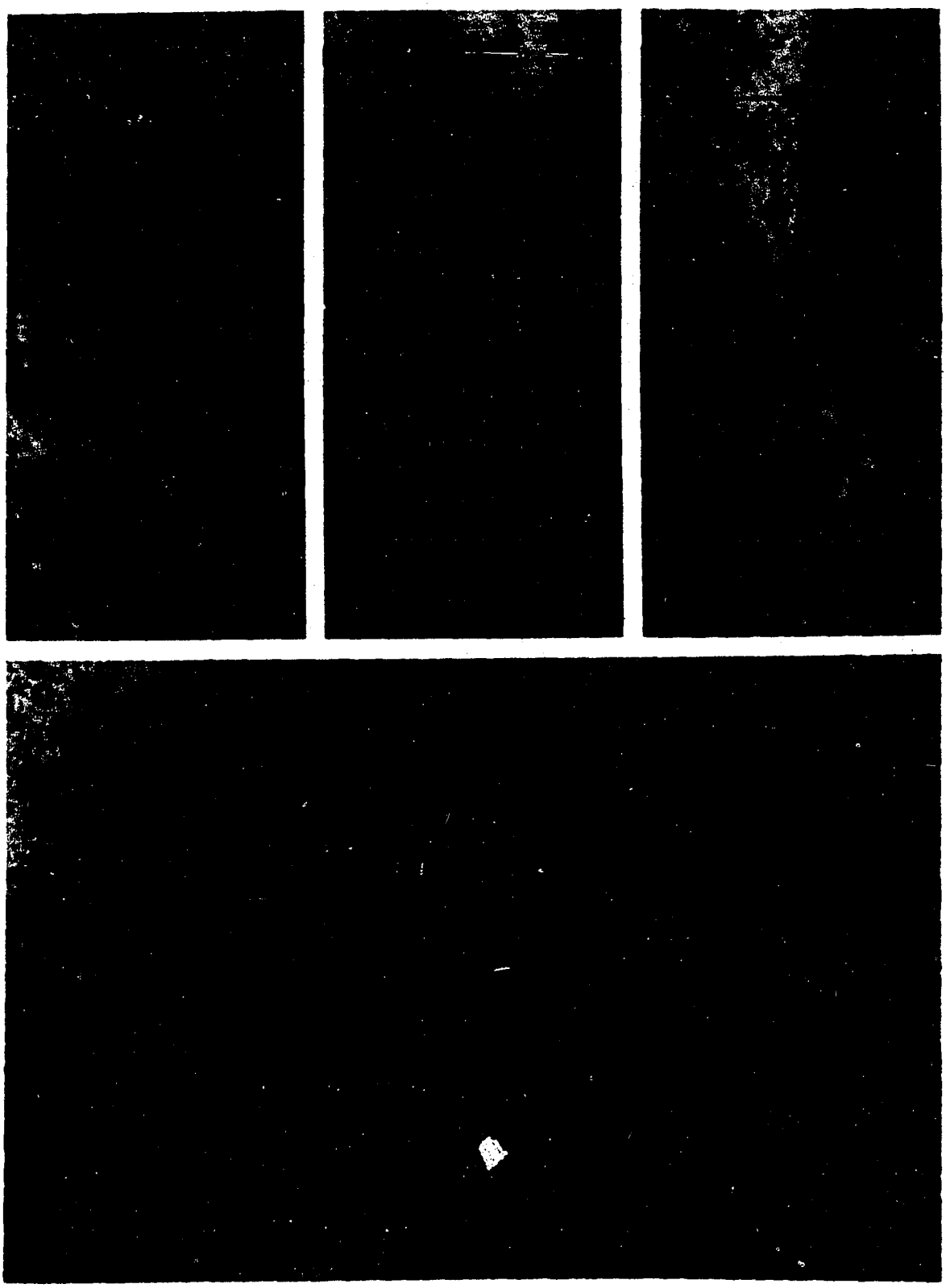


Figure 51. Portion of a cell of Nostoc muscorum, fixed in glutaraldehyde-picric acid and osmium tetroxide and stained with uranyl acetate and lead citrate. A polyhedral body appears to be associated with a membranous element (arrow). 96,500 X.

Figure 52. Portion of a cell of Spirulina major, fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate and lead citrate. There appears to be an association between the polyhedral bodies and the thylakoid system. 75,500 X.

Figure 53. An enlargement of Figure 51. 140,000 X.

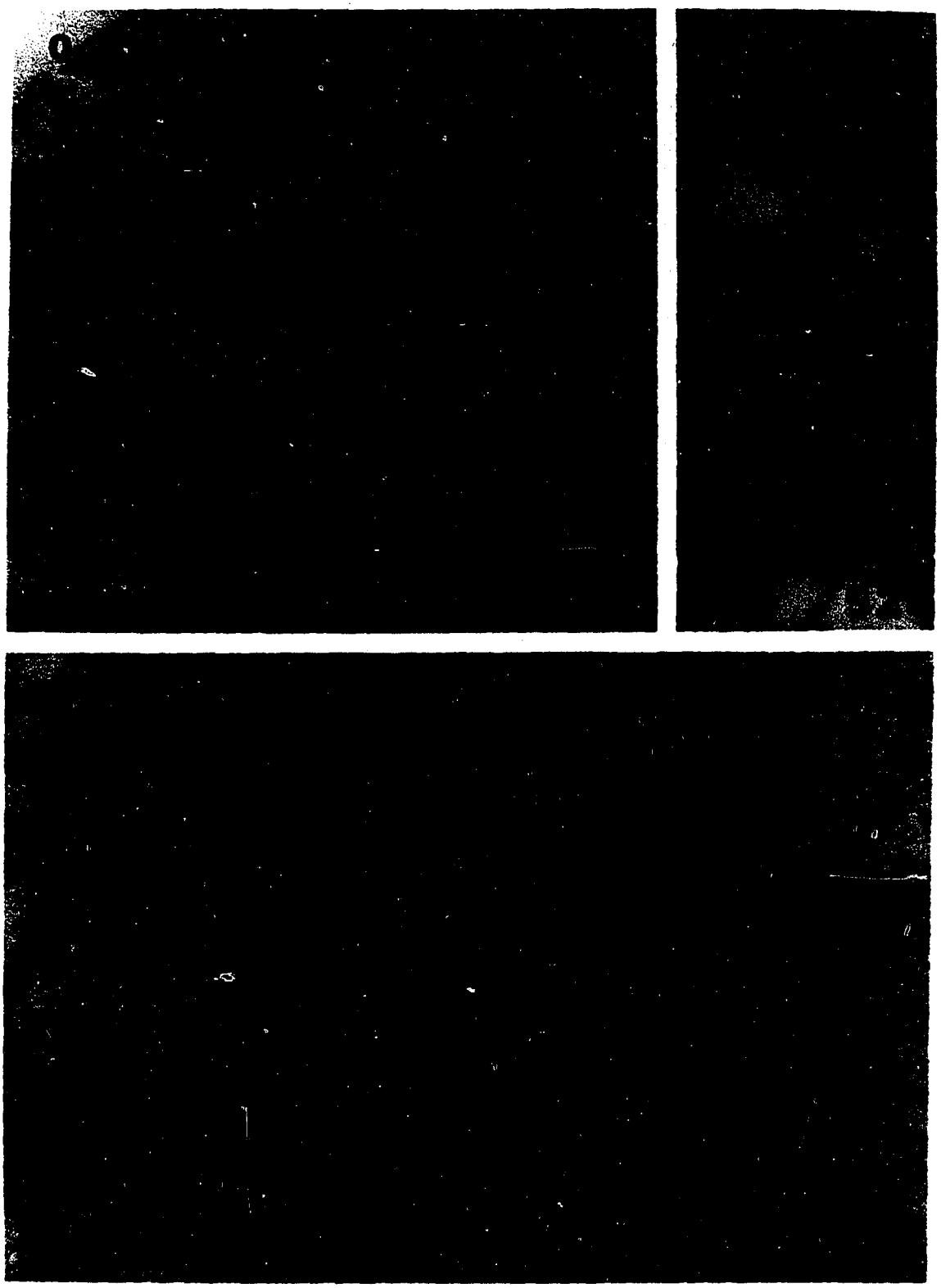
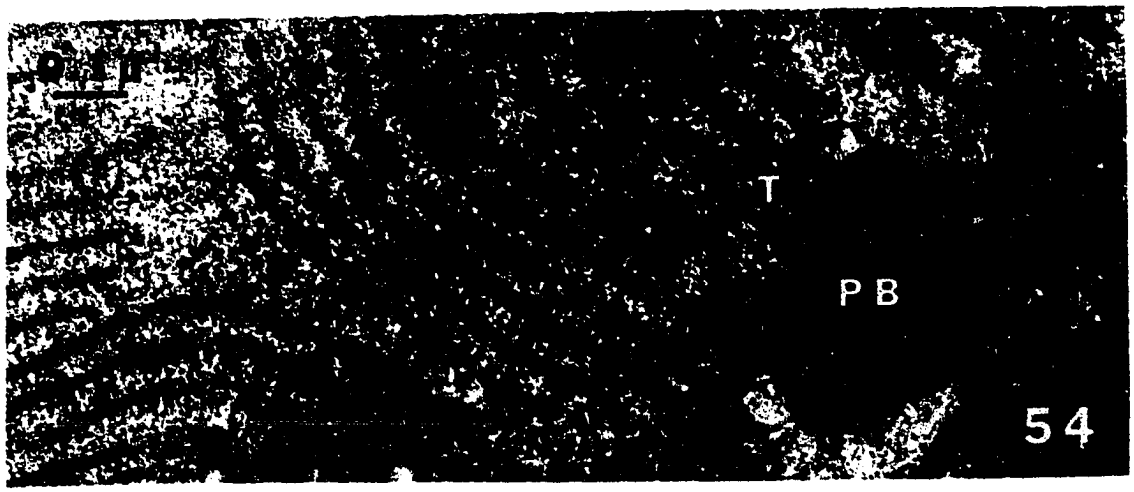


Figure 54. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The polyhedral body appears to be associated with thylakoids in several places. 96,500 X.

Figure 55. An enlargement of Figure 54. 410,000 X.


T
PB
54

Figures 56 to 58. Polyhedral bodies released from cells of Nostoc muscorum during rupture in a French press retain their angular profile. Phycobilisomes (PS) can be seen attached to released thylakoids. 42,500 X.

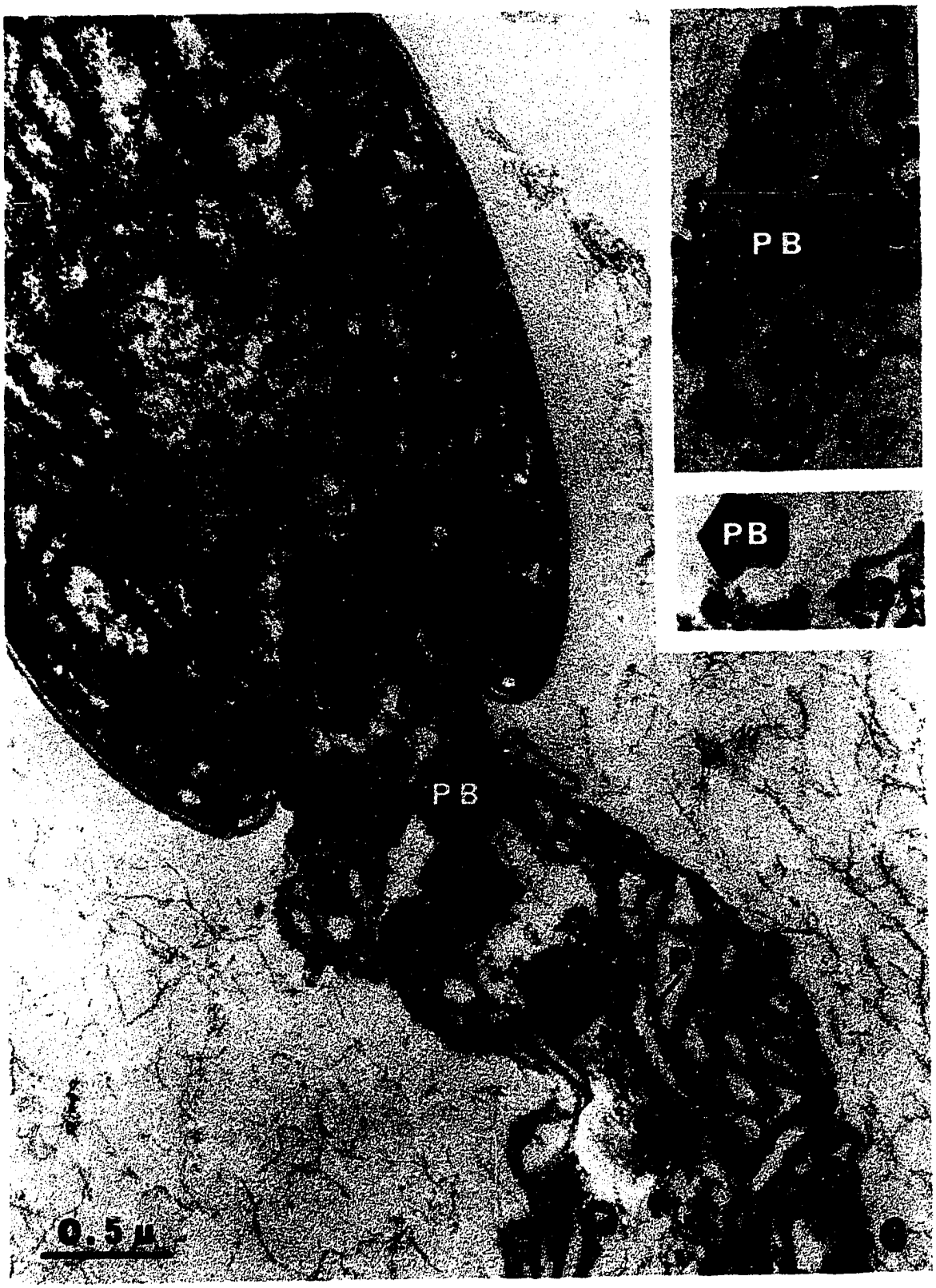
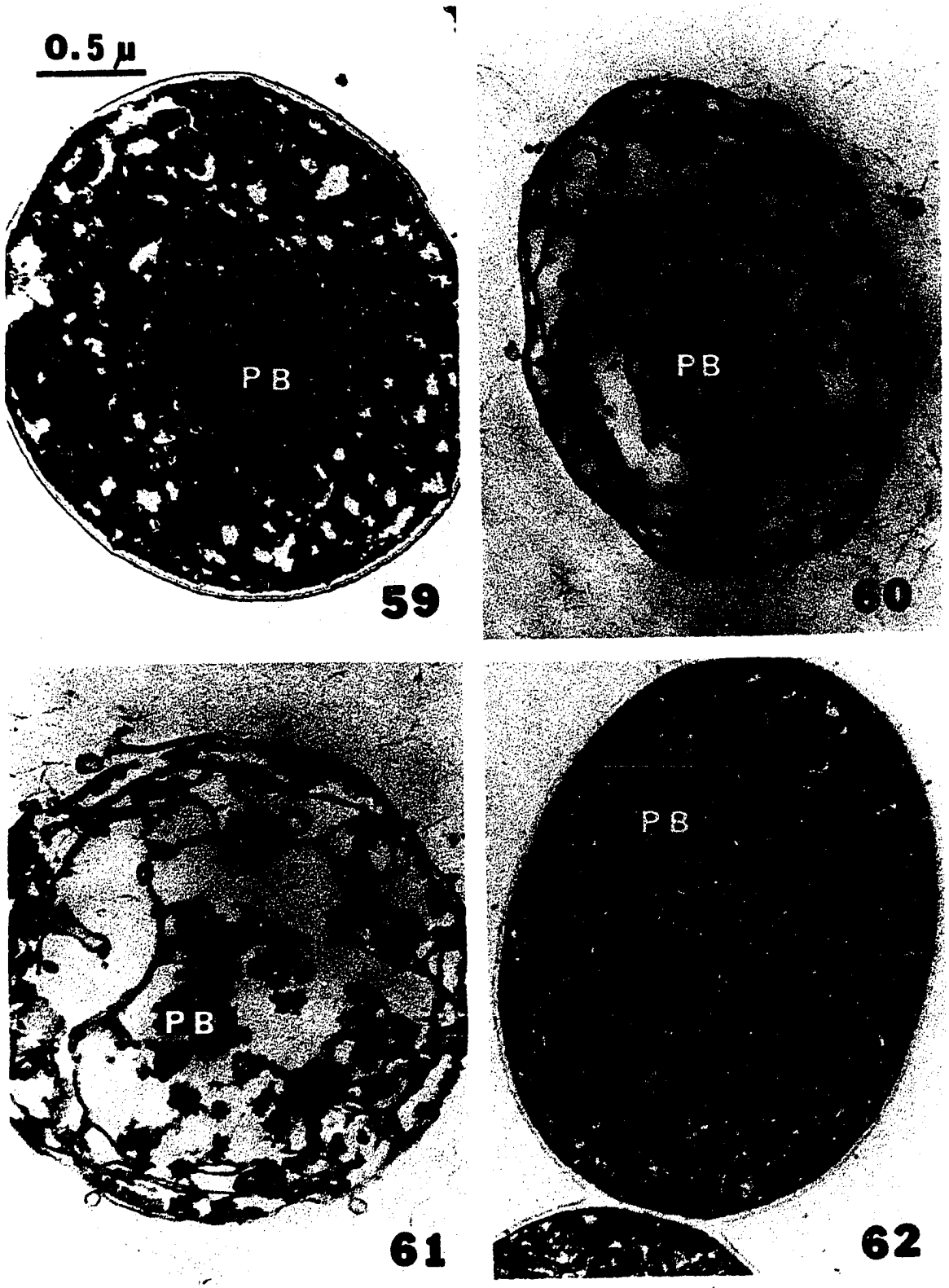

PB
PB
PB
0.5 μ

Figure 59. Cell of Nostoc muscorum, exposed to 1N hydrochloric acid, DNase, and RNase before fixation in osmium tetroxide; stained with uranyl acetate and lead citrate. Polyhedral bodies retain their electron-dense appearance but have lost the sharp boundary. 35,500 X.

Figure 60. Cell of Nostoc muscorum, treated with RNase for 12 hr at 45°C, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Polyhedral bodies retain their electron-opacity. 35,500 X.

Figure 61. Cell of Nostoc muscorum, treated with DNase for 12 hr at 45°C; fixed and stained as in Figure 60. The contents of the polyhedral bodies appears to have deteriorated to some extent but remains dense. 35,500 X.

Figure 62. Cell of Nostoc muscorum which serves as a control for Figures 61 through 66; treated in the same manner but without enzyme. 35,500 X.


0.5 μ
PB
59
PB
60
PB
61
PB
62

Figures 63 and 64. Cells of Nostoc muscorum, treated with lipase for 12 hr at 45°C, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Polyhedral bodies are missing from vegetative cells (Figure 63) and only remnants of them appear in the akinete (Figure 64, arrow). 35,500 X.

Figure 65. Cell of Nostoc muscorum, treated with acid phosphatase for 12 hr at 45°C; fixed and stained as in Figures 63 and 64. A differential or partial digestion of the contents of the polyhedral bodies appears to have occurred. 35,500 X.

Figure 66. Cell of Nostoc muscorum, treated with pronase for 12 hr at 45°C; fixed and stained as in Figures 63 and 64. The cell wall and the sharp boundary of the polyhedral bodies have been affected, but the contents of the polyhedral bodies remains electron-opaque. 35,500 X.

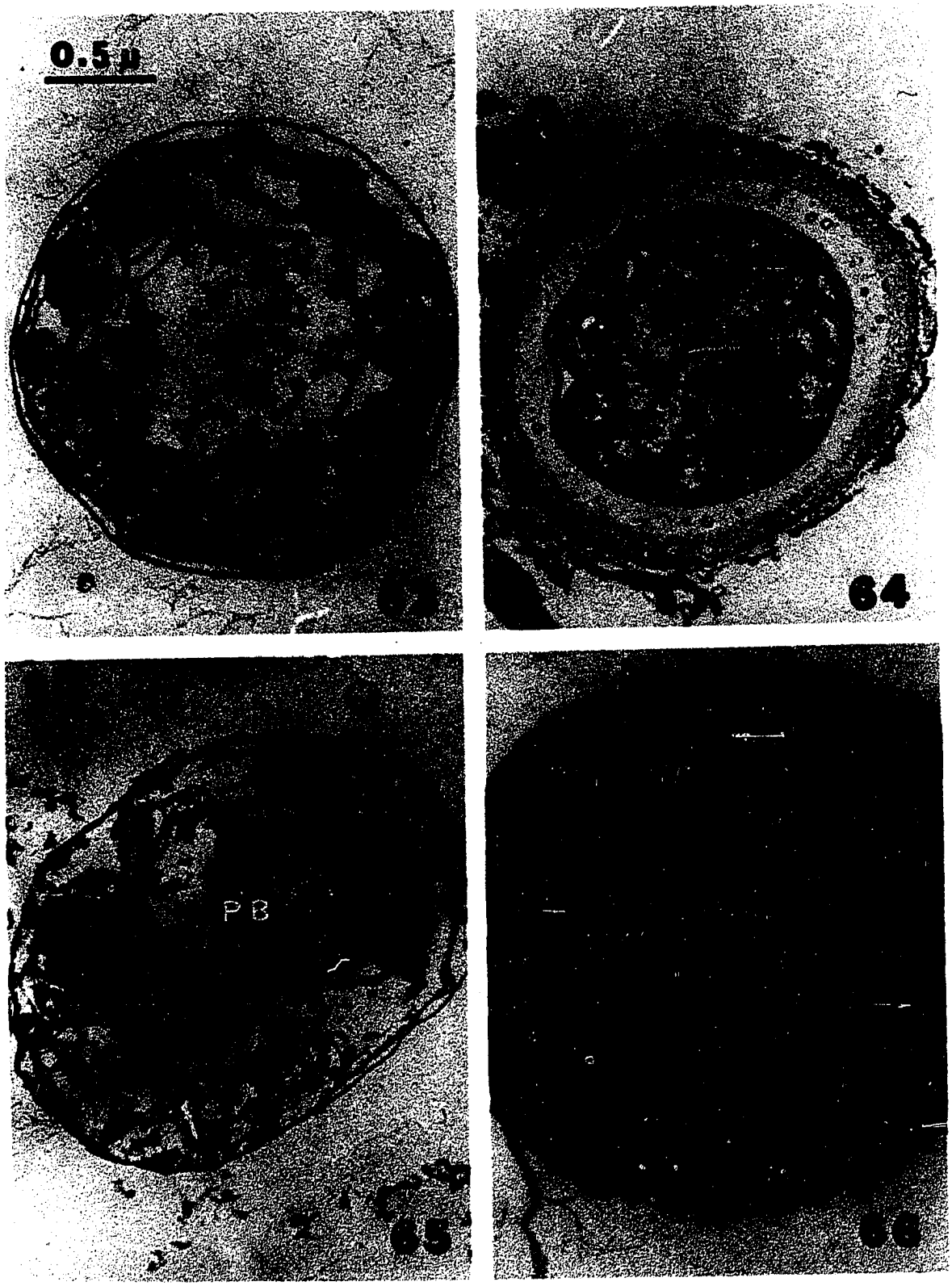

0.5 μ
PB

Figure 67. Portion of a cell of Nostoc muscorum, exposed to tritiated thymidine for 7 days, fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate and lead citrate. 55,500 X.

Figure 68. Portion of a cell of Microcoleus vaginatus (Type A), exposed to tritiated thymidine for 7 days, fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate and lead citrate. DNA-containing regions are indicated throughout the cell. 28,500 X.

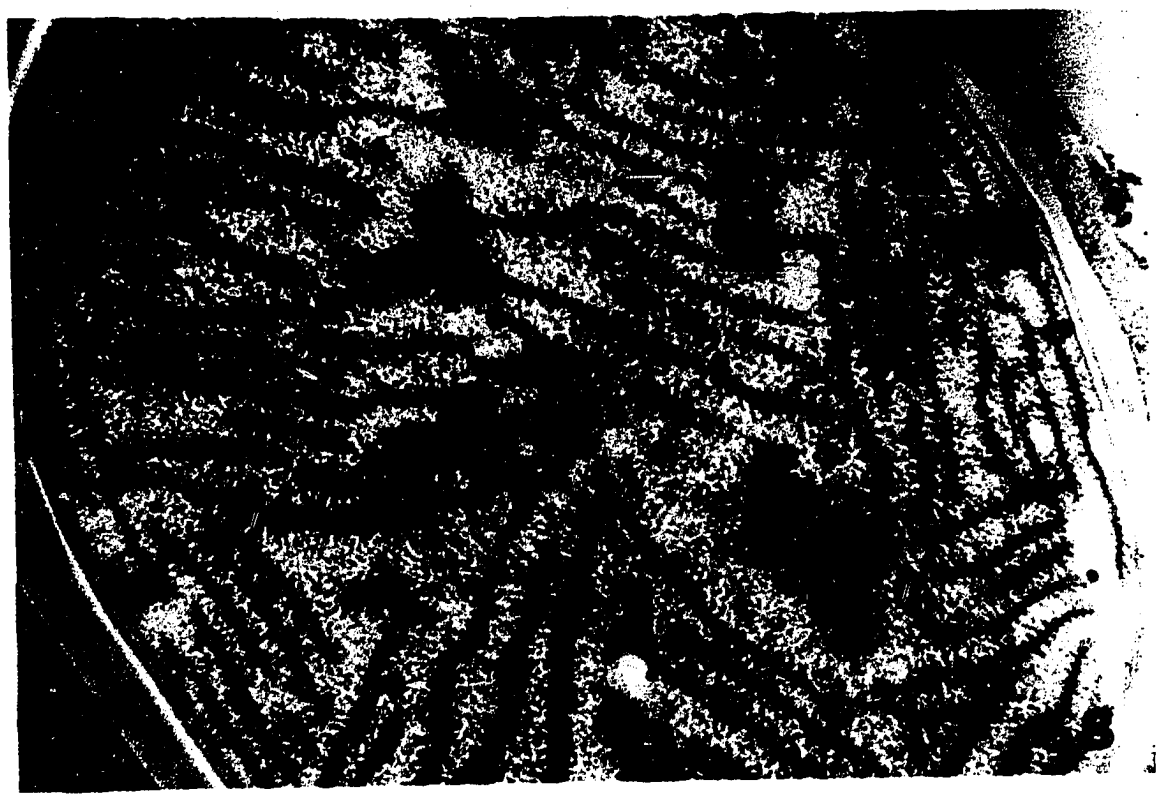
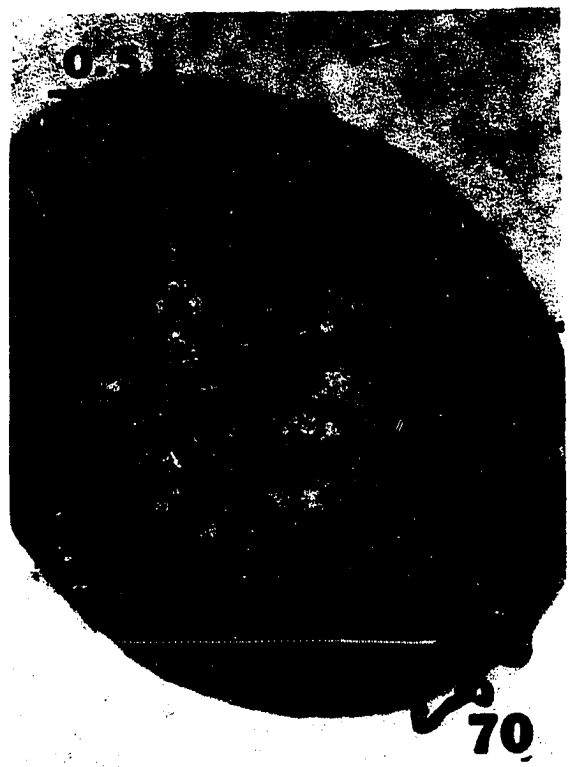


Figure 69. Portion of a cell of Anacystis nidulans, labelled with tritiated actinomycin D for 24 hr, fixed in osmium tetroxide, and stained with uranyl acetate. 42,500 X.

Figure 70. Portion of a cell of Nostoc muscorum, labelled with tritiated actinomycin D for 24 hr, fixed in osmium tetroxide, and stained with uranyl acetate. The nucleoplasm is largely central, but a peripheral location of DNA is also suggested. 35,500 X.

Figures 71 and 72. Portions of cells of Microcoleus vaginatus (Type A), labelled with tritiated actinomycin D for 24 hr, fixed in osmium tetroxide, and stained with uranyl acetate. Locations of DNA throughout the cell are indicated by silver grains. 19,000 X.

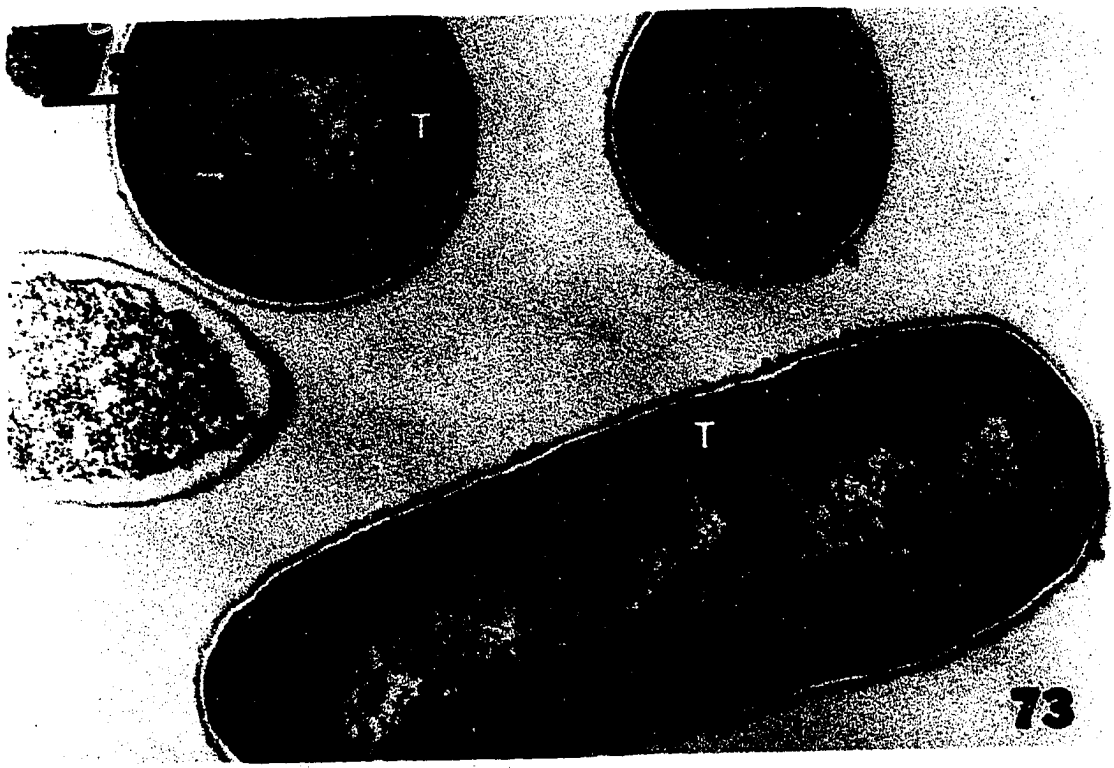

0.2
69


0.5
70


0.5
71

Figure 73. Cells of Anacystis nidulans, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. The thylakoids are peripheral and parallel each other with uniform spacing. 42,500 X.

Figure 74. Cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Thylakoids are generally evenly spaced and parallel but located throughout the cell. The wall layers are labelled according to the terminology of Jost (1965). 47,000 X.

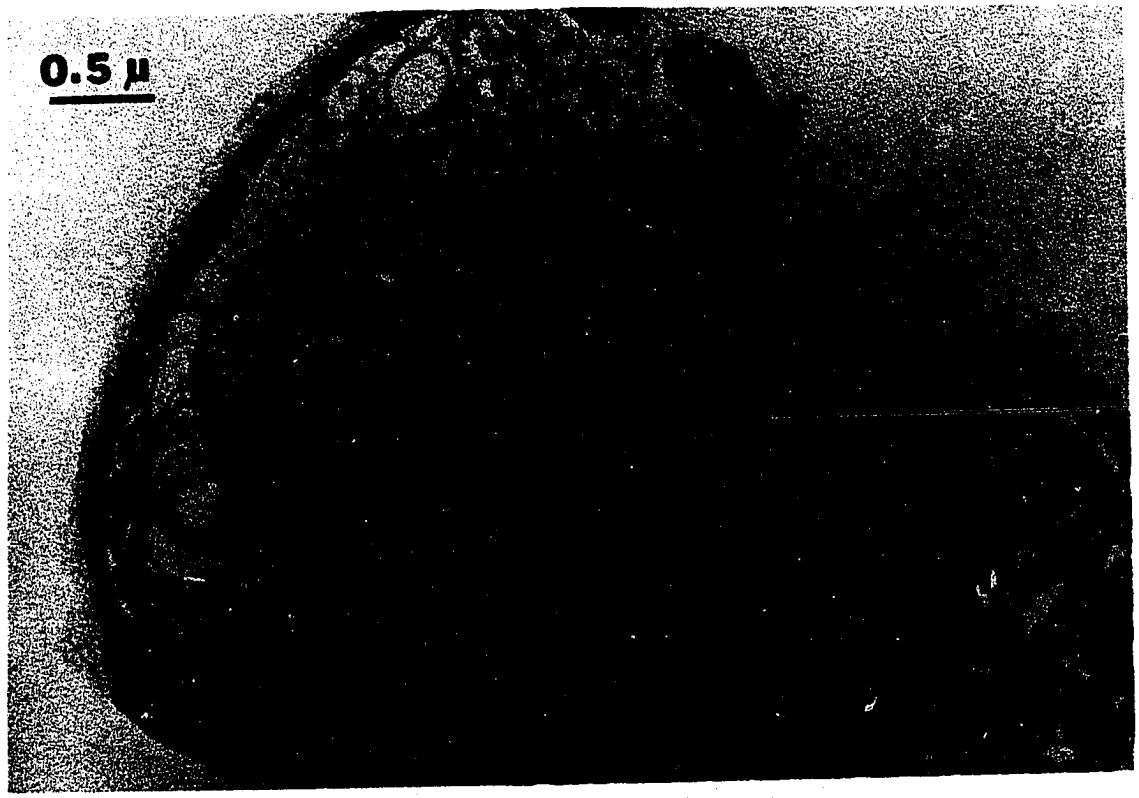


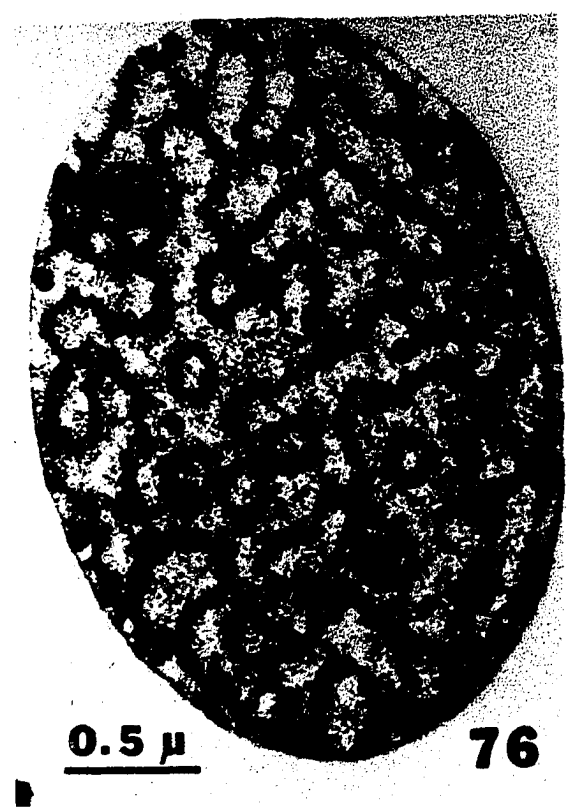
73

74

Figure 75. A heterocyst (H) and an akinete (AK) of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate. The thylakoid patterns differ from those of a vegetative cell. Intrathylakoidal spaces are enlarged in the akinete. 27,000 X.

Figures 76 and 77. Probable early stages in development of akinetes of Nostoc muscorum, fixed in glutaraldehyde-picric acid and osmium tetroxide, and stained with uranyl acetate and lead citrate. Reticulate patterning of the thylakoids is observed, and the lamellae no longer are parallel. 35,000 X.


0.5 µ


0.5 µ
76

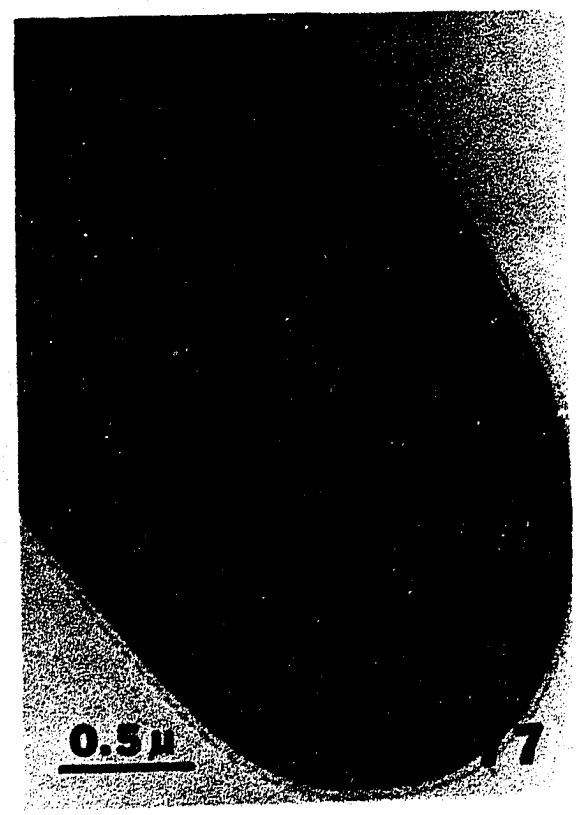

0.5µ
7

Figure 78. Portion of a cell of Microcoleus vaginatus (Type A), fixed in potassium permanganate and stained with uranyl acetate. Thylakoids are numerous and parallel with uniform interthylakoidal spacing. α-granules (A) are evident. 75,000 X.

Figure 79. Portion of a cell of Microcoleus vaginatus (Type A), fixed in potassium permanganate and stained with uranyl acetate. Thylakoids frequently are observed in a group, lying in a plane perpendicular or tangential to the plane of an adjacent group. 42,500 X.

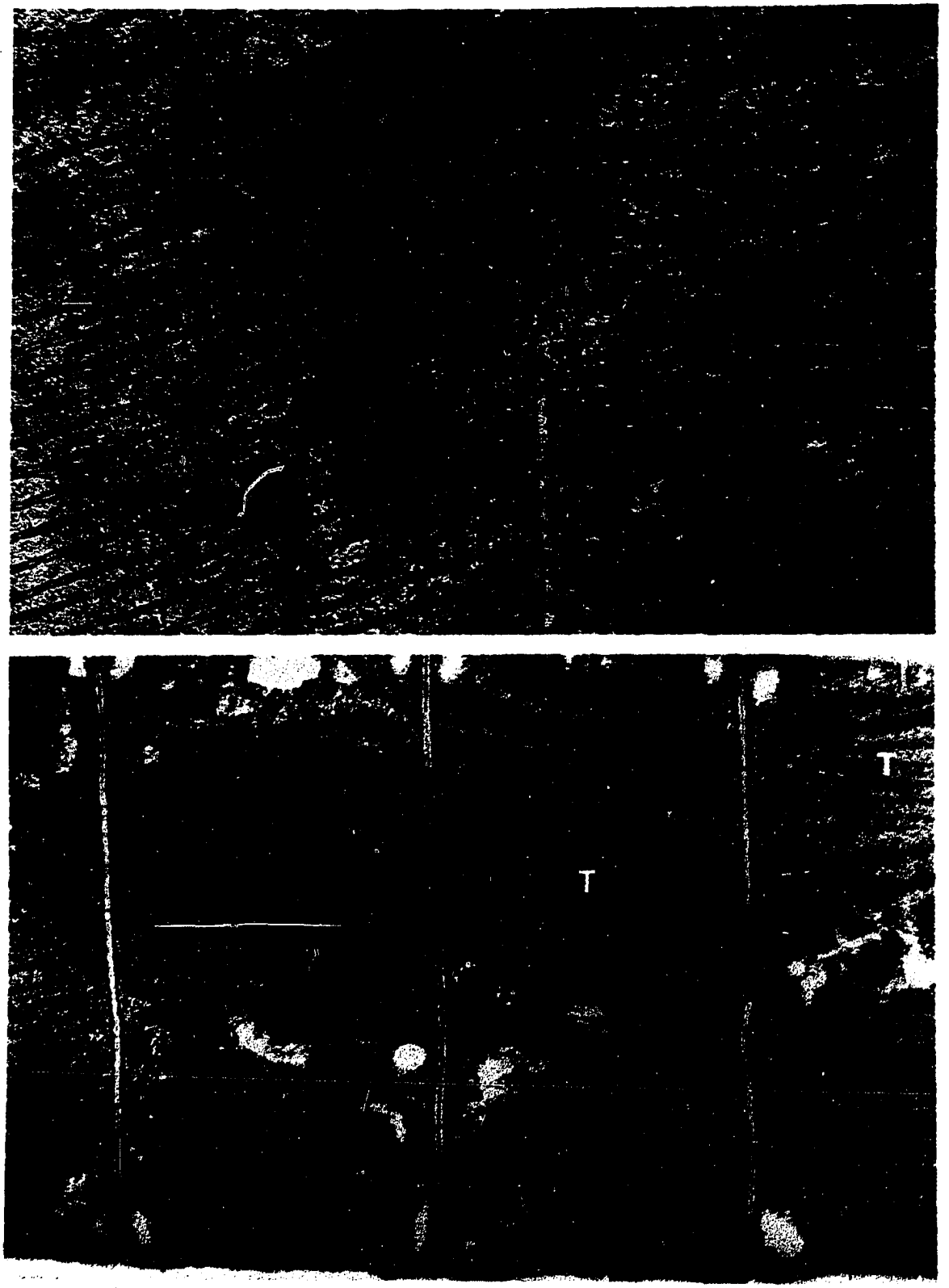

T
T

Figure 80. Cell of Microcoleus vaginatus (Type A), fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate. Thylakoids fill much of the cell and can be seen in cross-section and tangential section. 36,000 X.

Figure 81. Cell of Microcoleus vaginatus (Type B), prepared as in Figure 80. Sheath material (S) is loosely associated with the cell and usually separated during fixation for electron microscopy. 36,000 X.


81

Figures 82 and 83. Thylakoids of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Apparent sub-unit structure of the thylakoid membrane is seen as dark beads or dark rings with light cores (arrows). Figure 82, 752,000 X; Figure 83, 560,000 X.

Figure 84. Thylakoids of Microcoleus vaginatus (Type A), fixed and stained as in Figures 82 and 83. The image of the membrane gives the impression of rows of dense sub-units separated by an electron-transparent zone. 320,000 X.

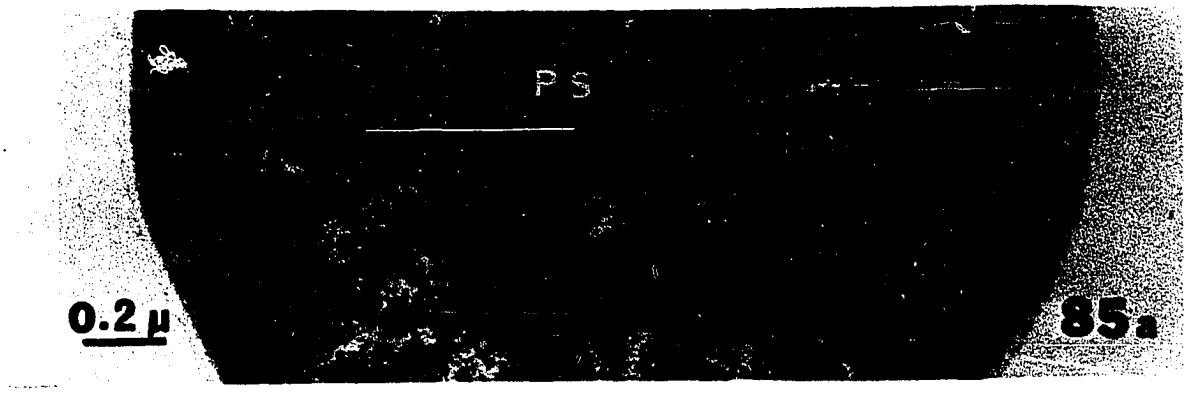

0.02 μ

Figure 85a. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate. Arrays of phycobilisomes (PS) are evident. 55,500 X.

Figure 85b. Schematic representation of a possible model for a phycobilisome in blue-green algae containing phycocyanin.

1. A phycobilisome represented as a discoid arrangement of phycocyanin hexamers. One phycocyanin sub-unit is shaded. Increases in phycocyanin concentration might involve the addition of layers of hexameric sub-units.

2. Face view of three possible forms of association between a phycobilisome and the thylakoid membrane (T). Each form would display a periodicity of six on rotational reinforcement.


PS
0.2 µ
85a

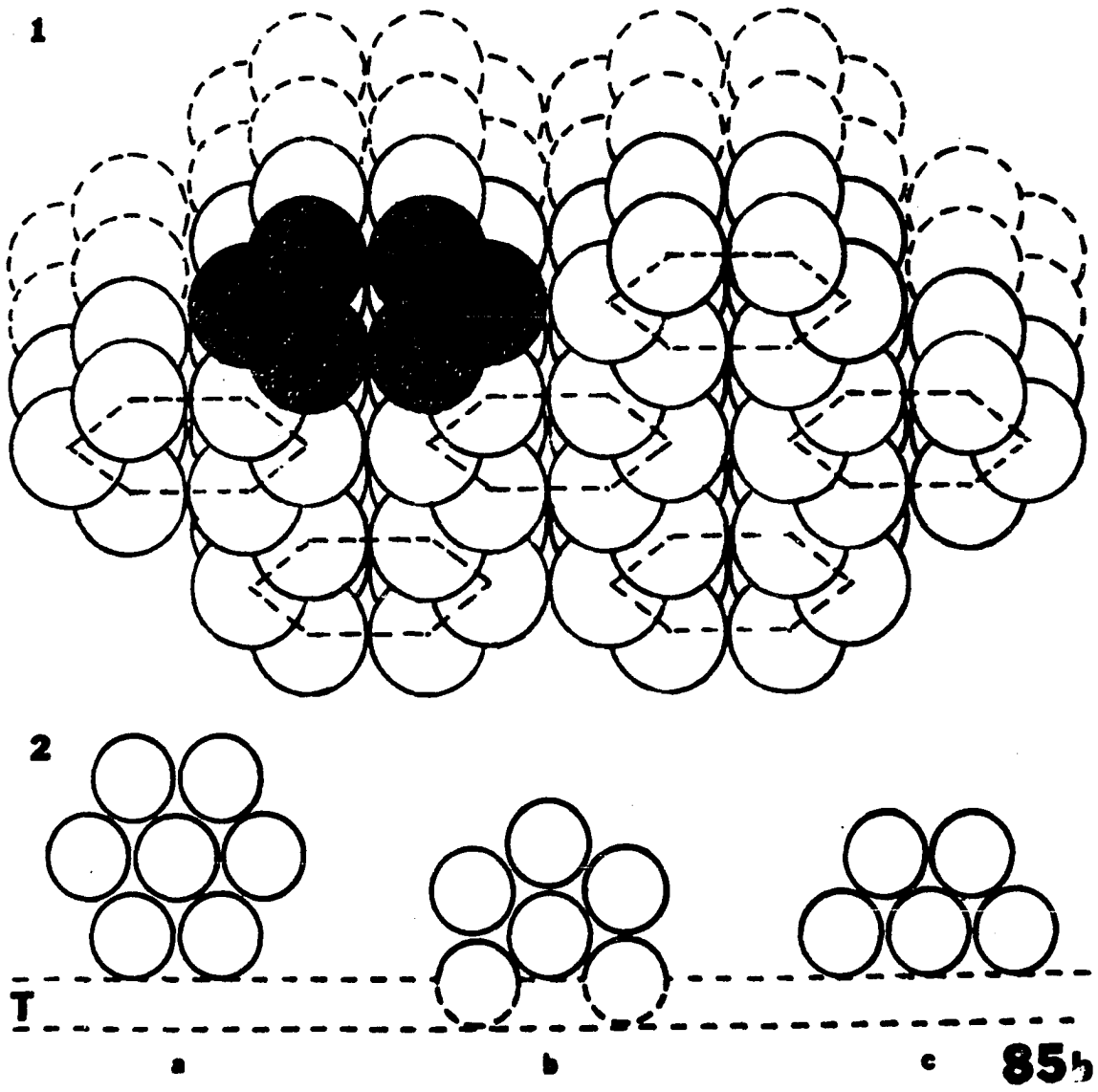

1
2
T
a
b
c
85b

Figure 86. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Phycobilisomes appear on thylakoids throughout the cell. 75,500 X.

Figure 87. Portion of a cell of Nostoc muscorum, fixed in glutaraldehyde-picric acid and osmium tetroxide, and stained with uranyl acetate and lead citrate. Phycobilisomes also are evident with this fixation. 75,500 X.

Figure 88. Portion of a cell of Symploca muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. 75,500 X.

Figure 89. Portion of a cell of Arthrospira Jenneri, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Phycobilisomes are outlined against an electron-transparent gas vacuole. 75,500 X.

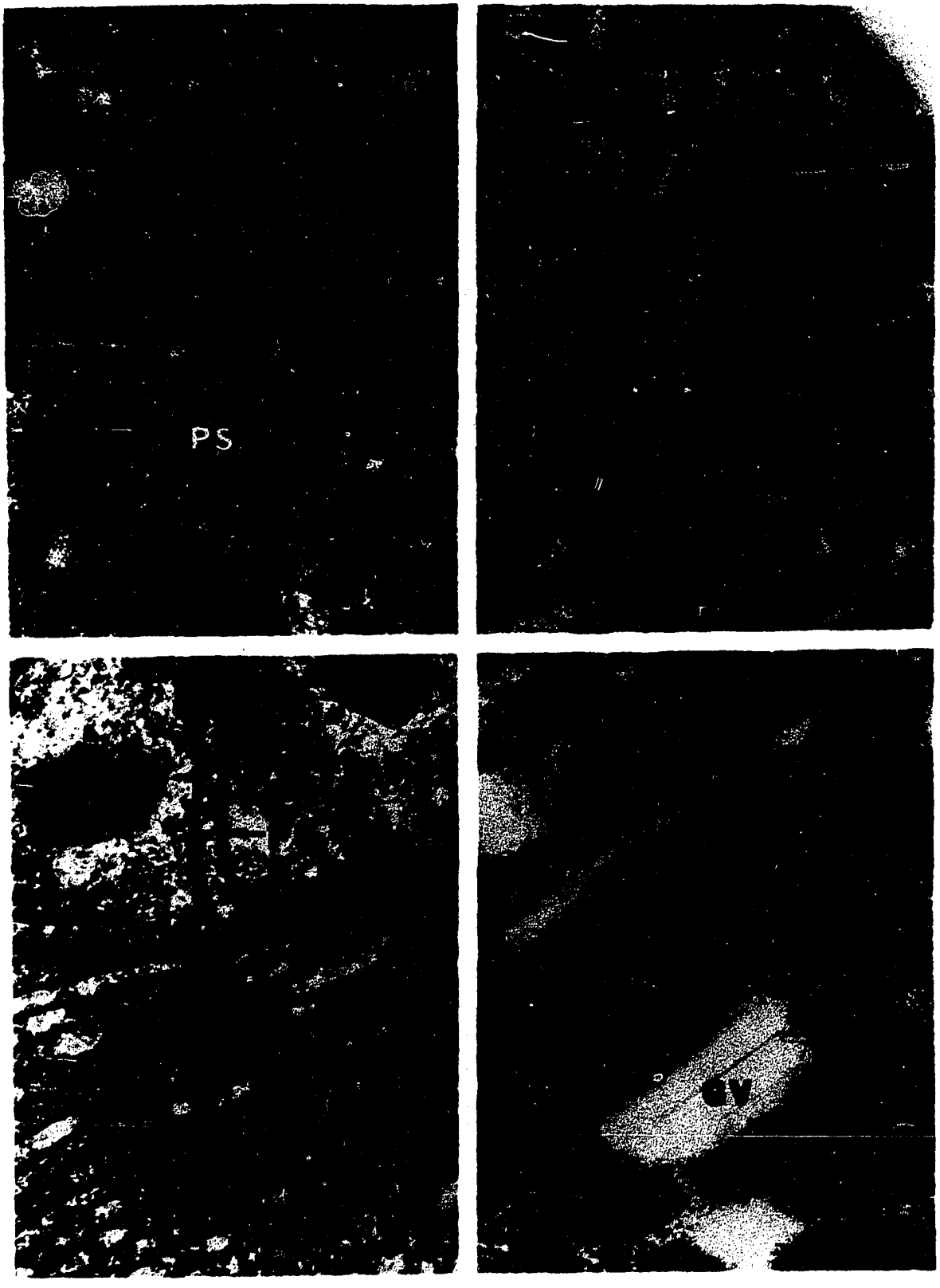
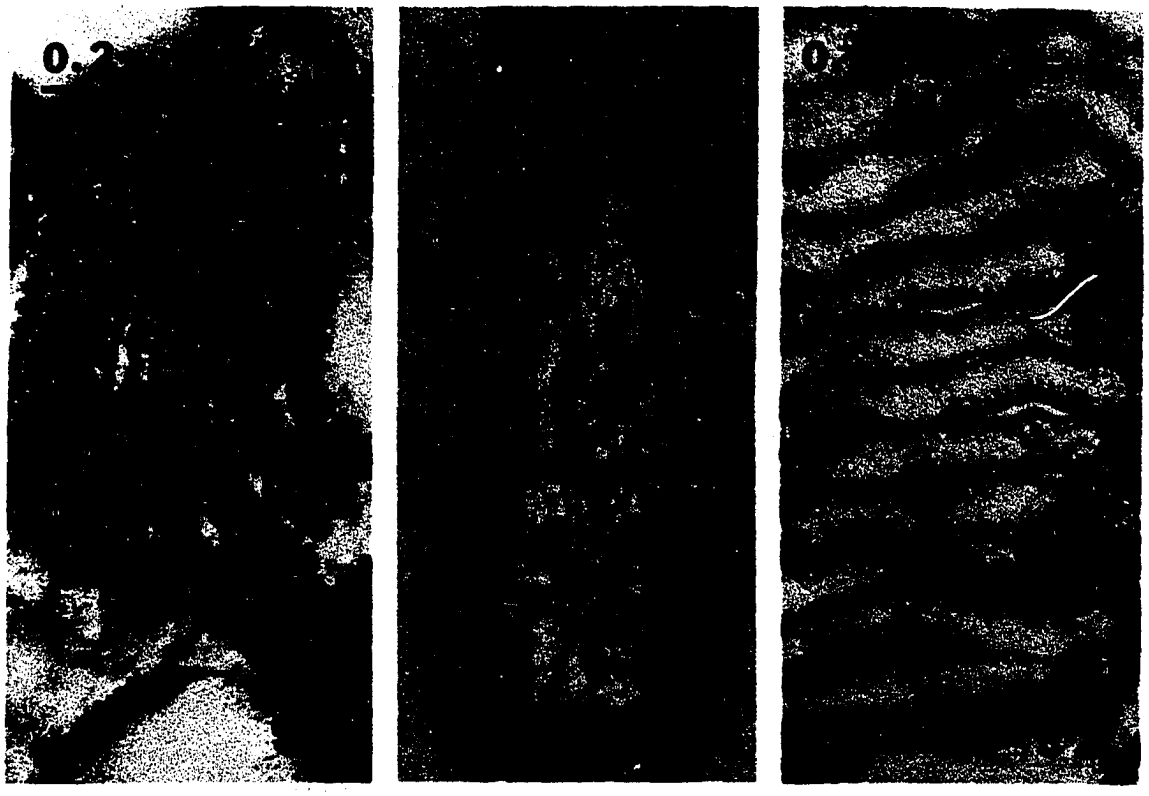

PS

Figure 90. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate. The plane of sectioning cuts through a profusion of interdigitating phycobilisomes on adjacent thylakoids. 55,500 X.

Figure 91. Portion of a cell of Anacystis nidulans, fixed in glutaraldehyde-osmium tetroxide and stained with uranyl acetate. Phycobilisomes are seen on thylakoid surfaces except the most interior surface facing the nucleoplasm. 55,500 X.

Figure 92. Portion of a cell of Arthrospira Jenneri, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Regular arrays of phycobilisomes in side view line the exterior surfaces of the thylakoids. 96,500 X.

Figure 93. Portion of a cell of Nostoc muscorum, fixed in glutaraldehyde-picric acid and osmium tetroxide and stained with uranyl acetate and lead citrate. Phycobilisomes are seen in face and side view. 96,500 X.



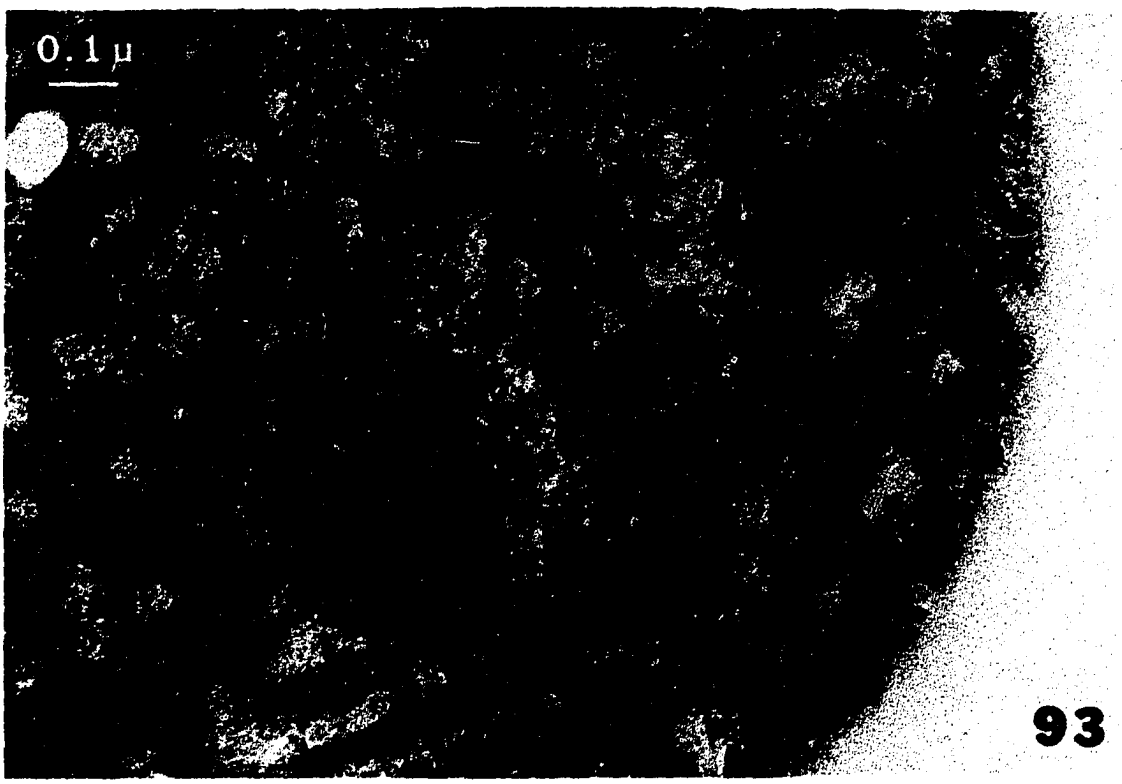

0.1 μ
93

Figure 94. Portion of a cell of Microcoleus vaginatus (Type A), fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. A phycobilisome (arrow) appears to have sub-unit structure. 290,000 X.

Figure 95. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. At high magnification, phycobilisomes appear to exhibit internal structure (arrow). 580,000 X.

Figure 96. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Phycobilisomes appear to have sub-unit structure (arrow). 55,500 X.

Figure 97. An enlargement of Figure 96. 365,000 X.

Figure 98. Rotational reinforcement of a phycobilisome in Figure 97 at periodicities of 5, 6, 7, and 12. Reinforcement appears strongest at n = 6.

Figure 99. Portion of a cell of Nostoc muscorum, fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Phycobilisomes show internal structure which rotational reinforcement suggests may involve six sub-units. 140,000 X.

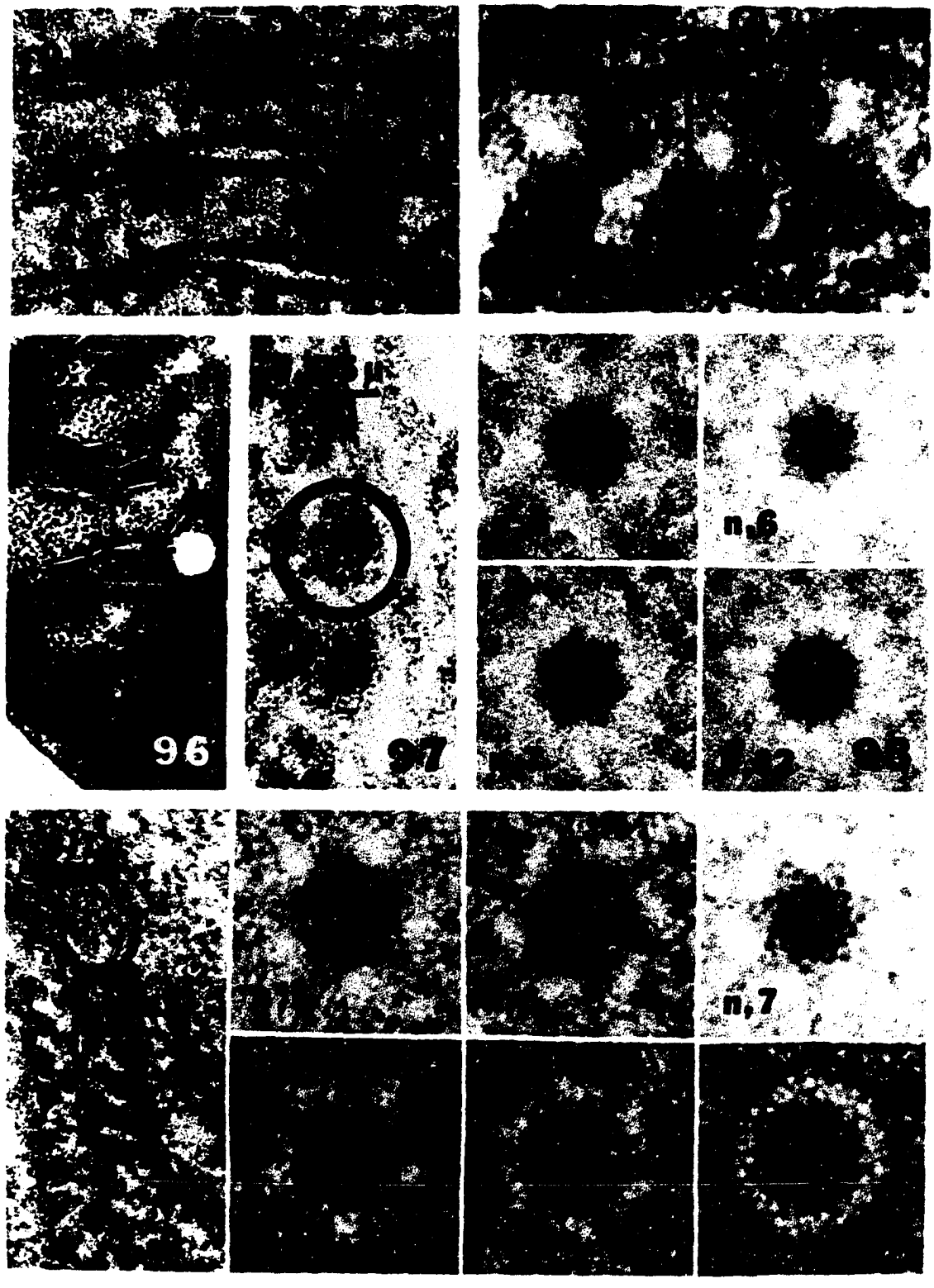
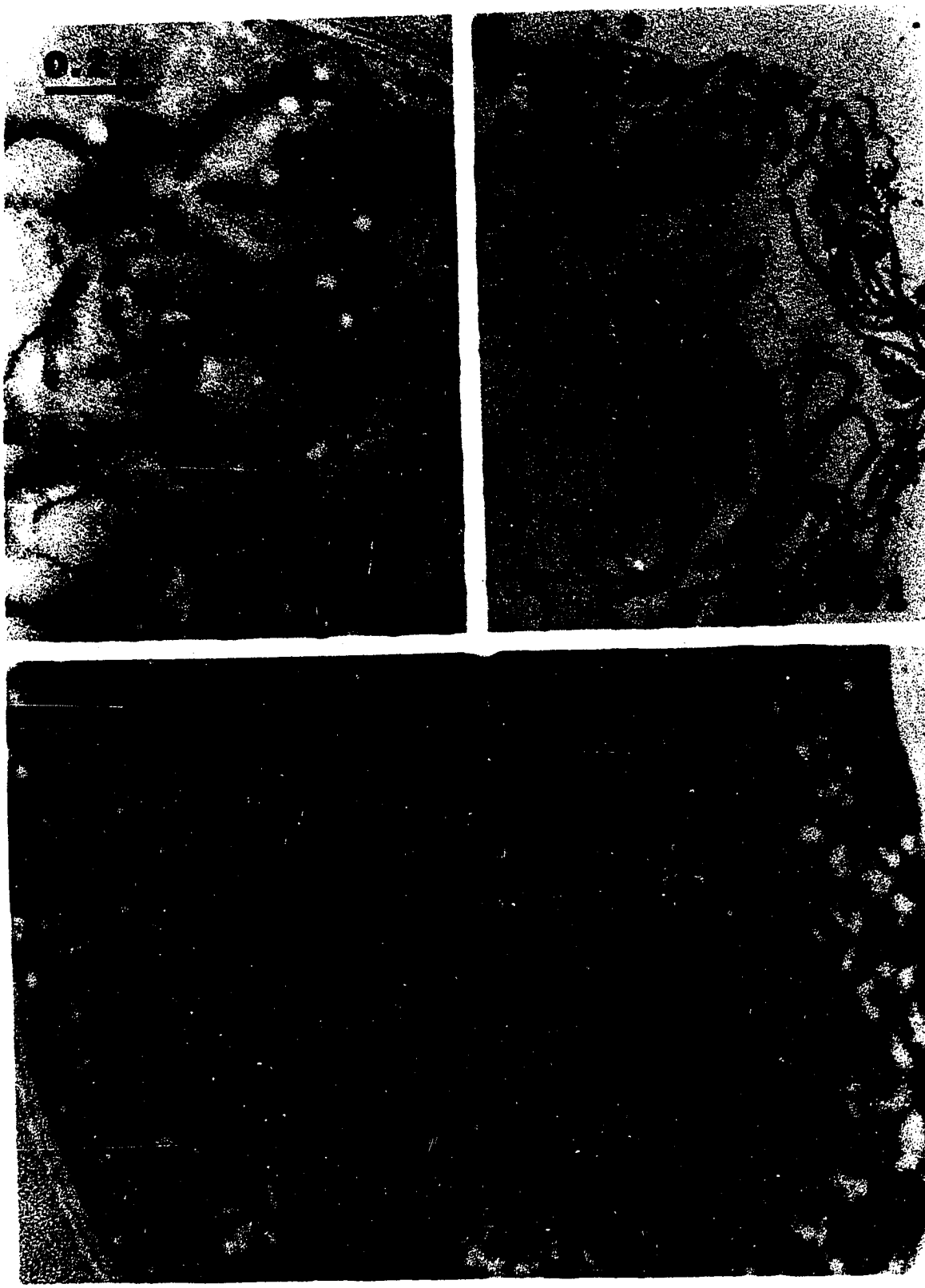

96
97
n,6
n,7

Figure 100. Portion of a cell of Nostoc muscorum after extrusion from a French press, treated with deoxycholate, fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate and lead citrate. The phycobilisomes have been removed from thylakoid surfaces. 75,500 X.

Figure 101. Portion of a cell of Nostoc muscorum, treated as in Figure 100 but without deoxycholate. Phycobilisome remnants can be seen clinging to thylakoids. 46,000 X.

Figure 102. Portion of a cell of Nostoc muscorum, extracted with 80% methanol-20% acetone, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Thylakoids have suffered the loss of chlorophyll and carotenoids, but phycobilisomes remain in position. 75,500 X.



Figures 103 and 104. Portions of cells of unfixed Nostoc muscorum, infiltrated with glycerol and subjected to freeze-etching. Thylakoids appear to be covered with globular units (arrows). 104,000 X.

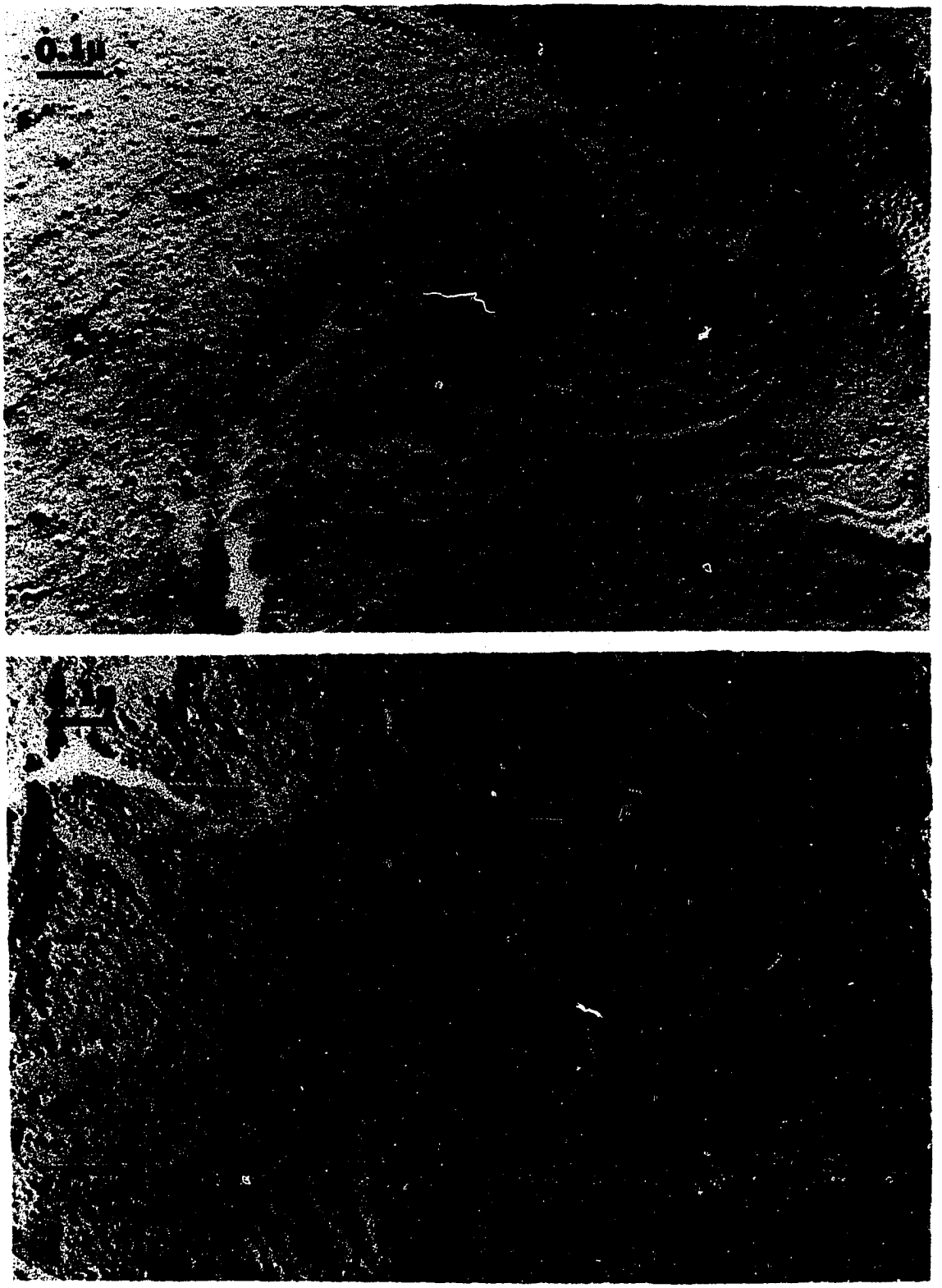


Figure 105. *In vivo* absorption spectrum of *Anacystis nidulans*.

Figure 106. *In vivo* absorption spectrum of *Arthrospira Jenneri*.

Figure 107. *In vivo* absorption spectrum of *Microcoleus vaginatus* (Type A).

Figure 108. *In vivo* absorption spectrum of *Microcoleus vaginatus* (Type B).

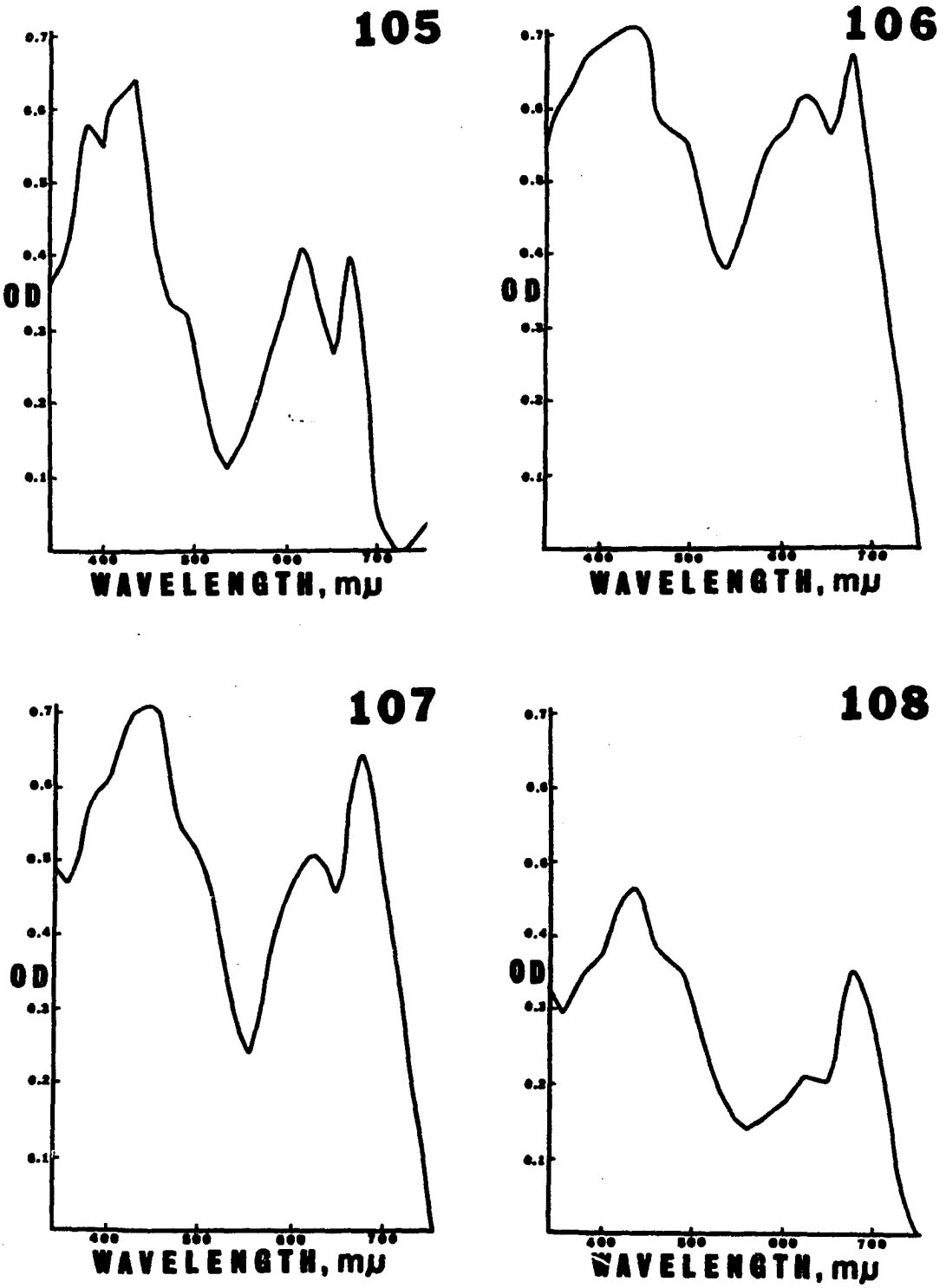

105
106
107
108
OD
WAVELENGTH, mμ

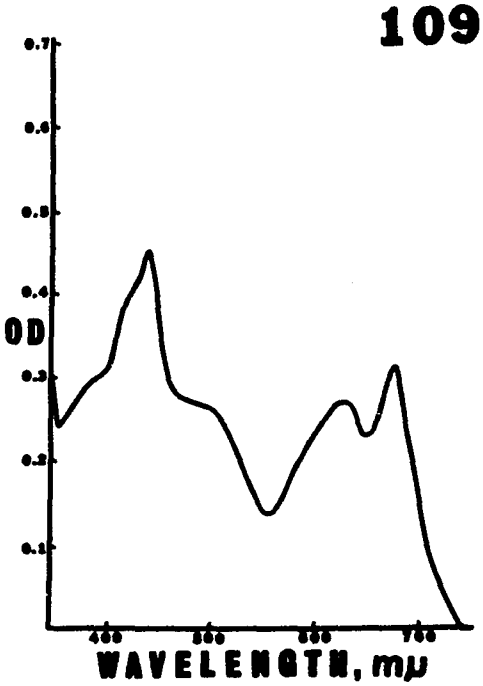
Figure 109. *In vivo* absorption spectrum of *Nostoc muscorum*.

Figure 110. *In vivo* absorption spectrum of *Spirulina major*.

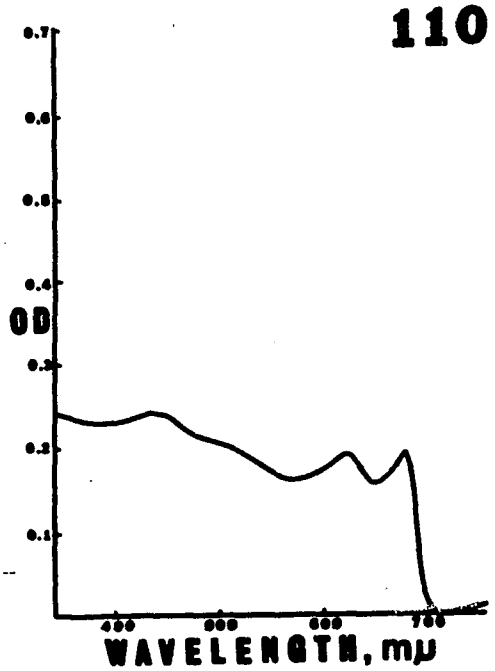
Figure 111. *In vivo* absorption spectrum of *Symploca muscorum*.

Figure 112. *In vivo* absorption spectrum of *Tolypothrix distorta*.

109



110



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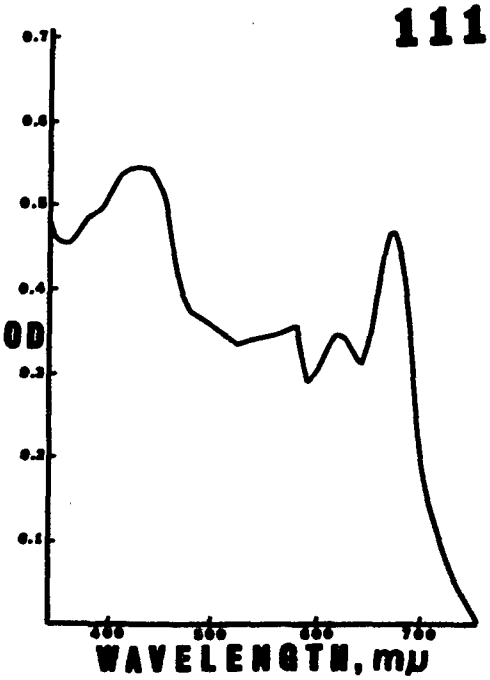


112

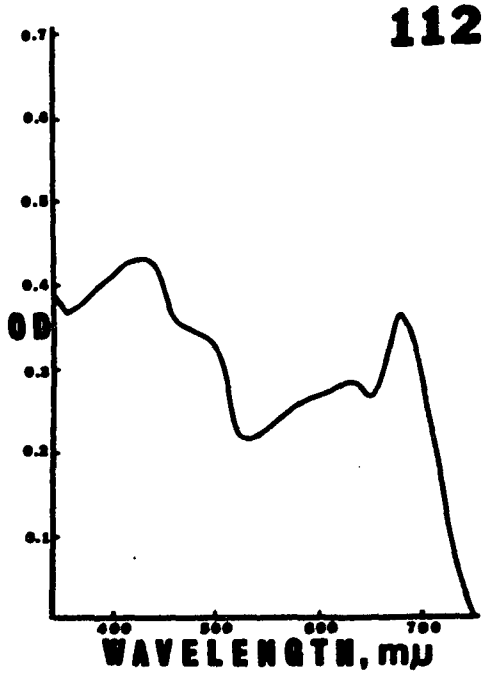
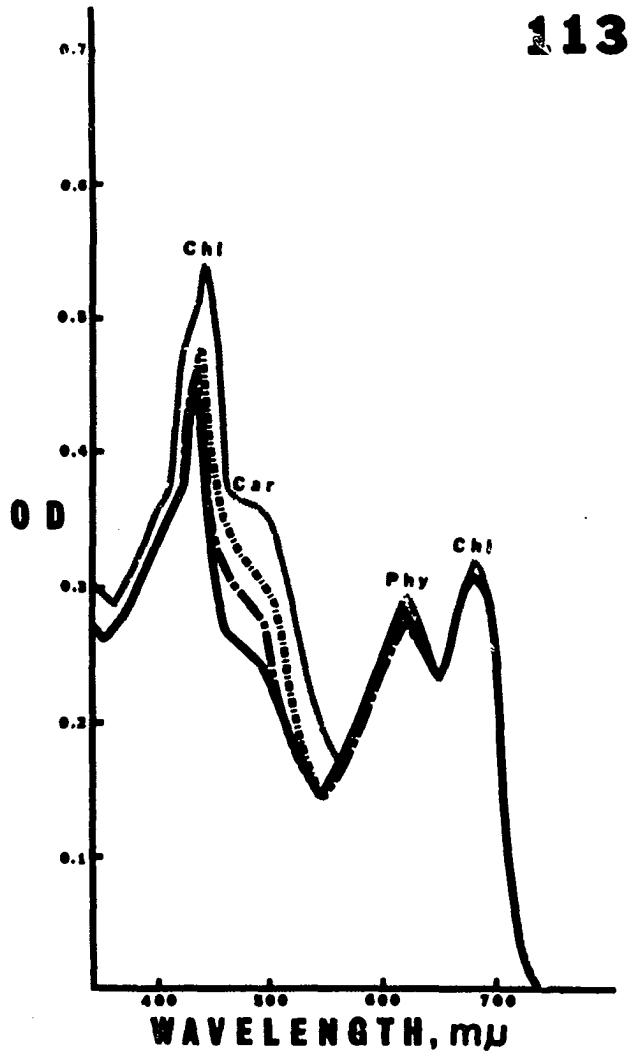


Figure 113a. *In vivo* absorption spectra of *Nostoc muscorum* grown at different light intensities for three weeks. ▬▬▬▬, 20 ft-c; ▬▬ ▬ ▬, 100 ft-c; ▪▬▪▬▪▪, 200 ft-c; ▪▪▪▪▪▪▪▪▪▪▪▪▪,900-1,000 ft-c. Chl, chlorophyll; Phy, phycocyanin; Car, carotenoids.

Figure 113b. *In vivo* absorption spectra of *Nostoc muscorum* grown at different wavelengths for three weeks. ▬▬▬▬, red light; ▪▪▪▪▪▪▪▪▪▪▪▪▪, orange light.


113a
Chl
Car
Phy
Chl
O D
WAVELENGTH, mμ

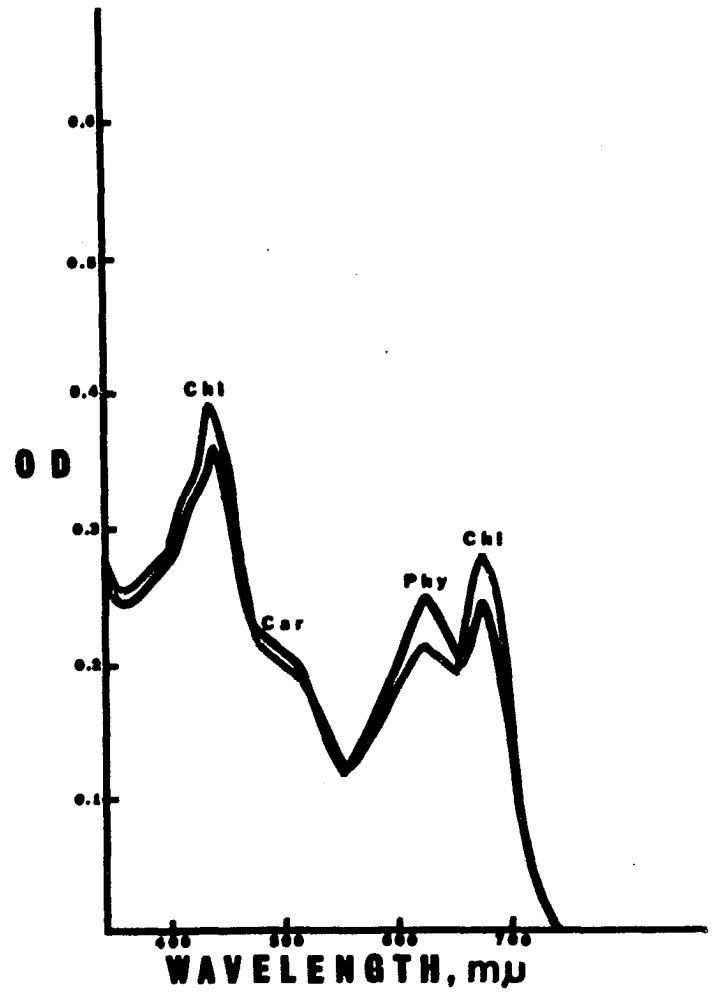
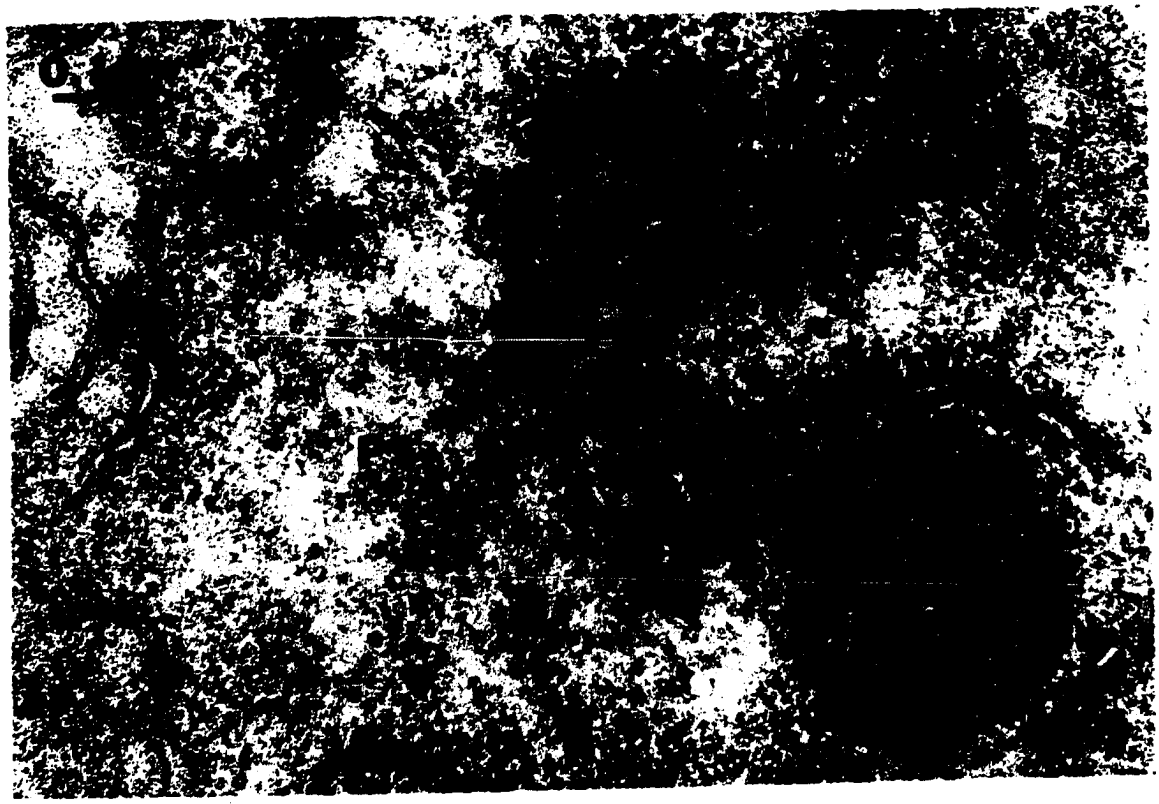

113b
Chl
Car
Phy
Chl
O D
WAVELENGTH, mμ

Figure 114. Cell of Nostoc muscorum grown for three weeks at 200 ft-c, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Phycobilisomes are apparent. Thylakoids are largely peripheral. 42,500 X.

Figure 115. Portion of a cell of Nostoc muscorum, grown for three weeks at 100 ft-c, fixed in osmium tetroxide, and stained with uranyl acetate. Phycobilisomes are evident. 116,000 X.


0.5μ
T
114



Figures 116 and 117. Portions of cells of Nostoc muscorum, grown for three weeks at 900-1,000 ft-c, fixed in osmium tetroxide, and stained with uranyl acetate. Structured granules are numerous and large. Phycobilisomes are less prominent than at lower light intensities. 55,500 X.


0.2 μ

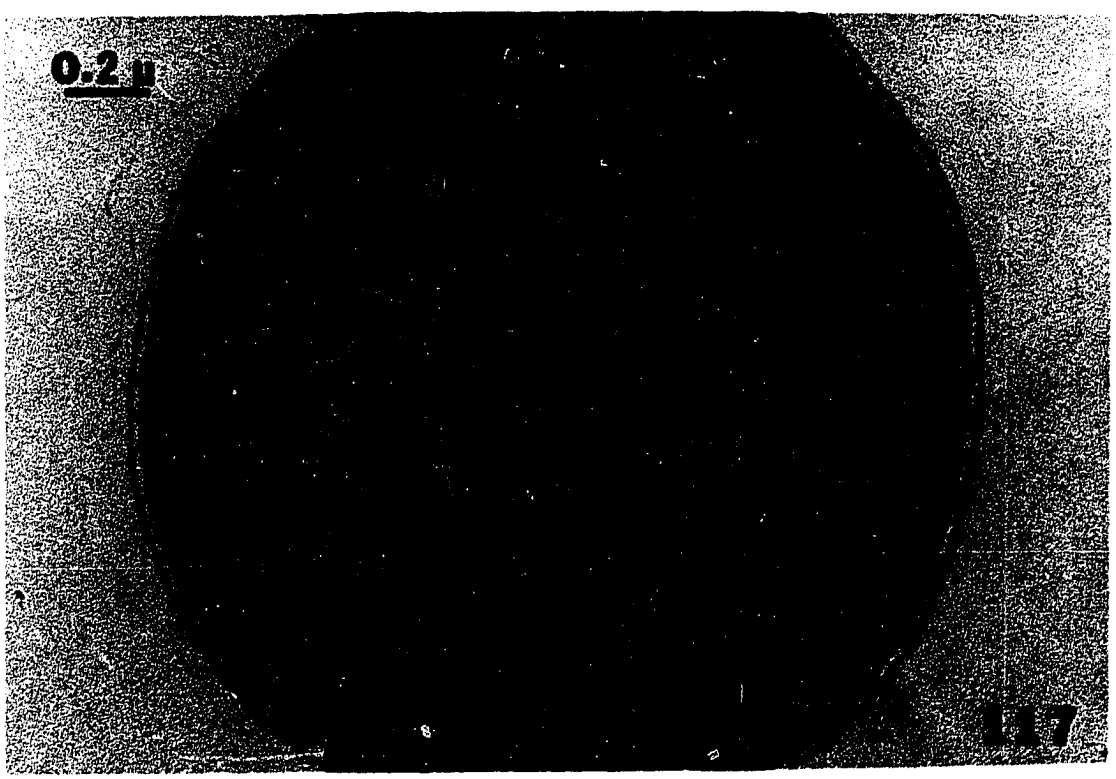
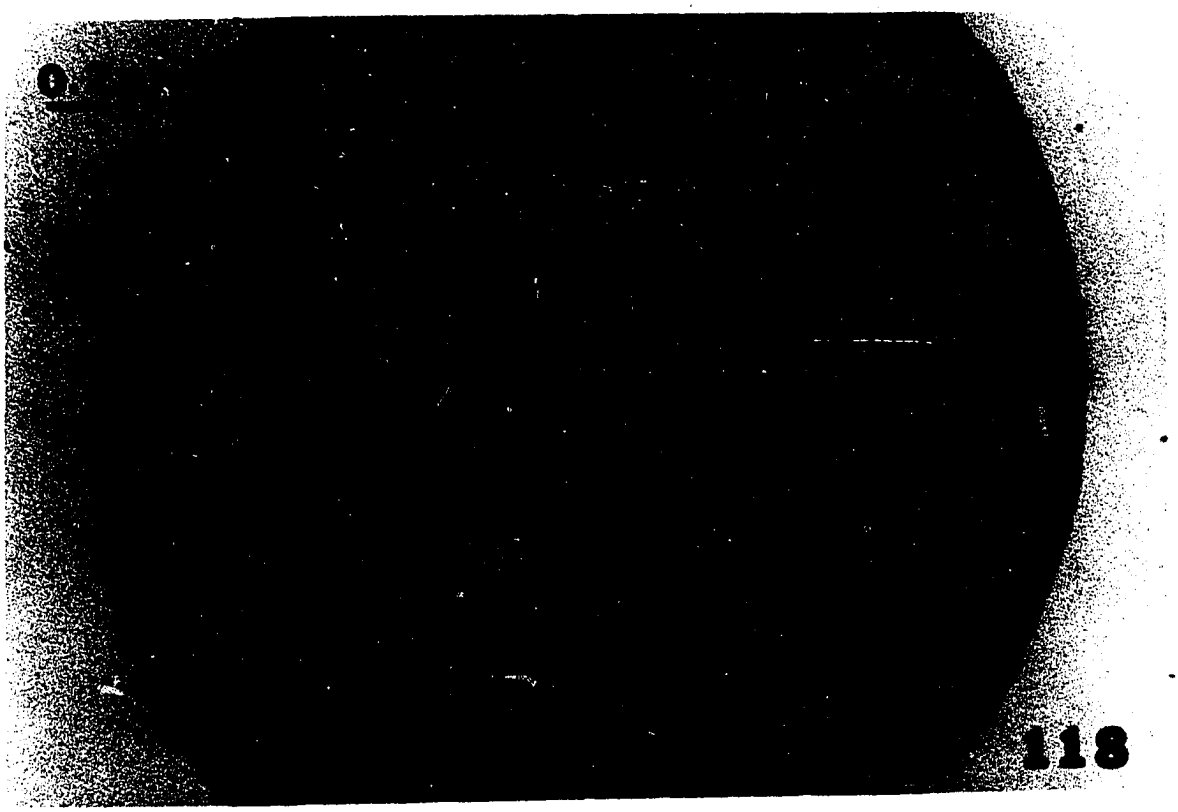

0.2 μ

Figure 118. Cell of Nostoc muscorum, grown for three weeks at 20 ft-c, fixed in osmium tetroxide, and stained with uranyl acetate. Expanded intrathylakoidal spaces are seen throughout the cell. 55,500 X.

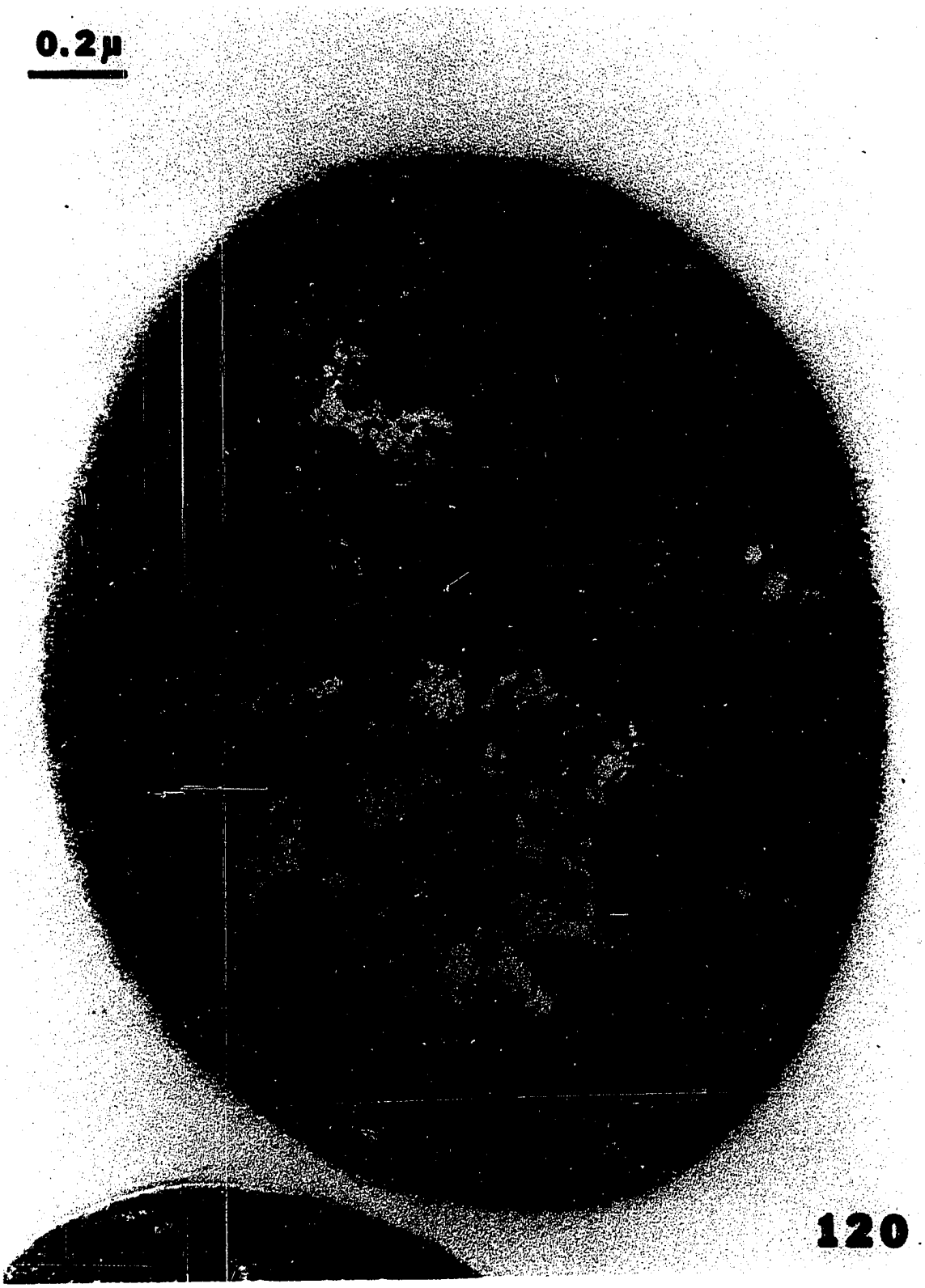
Figure 119. Cell of Nostoc muscorum, grown for three weeks in orange light, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. The circular pattern of thylakoids is characteristic. Phycobilisomes are not prominent. 42,500 X.



118

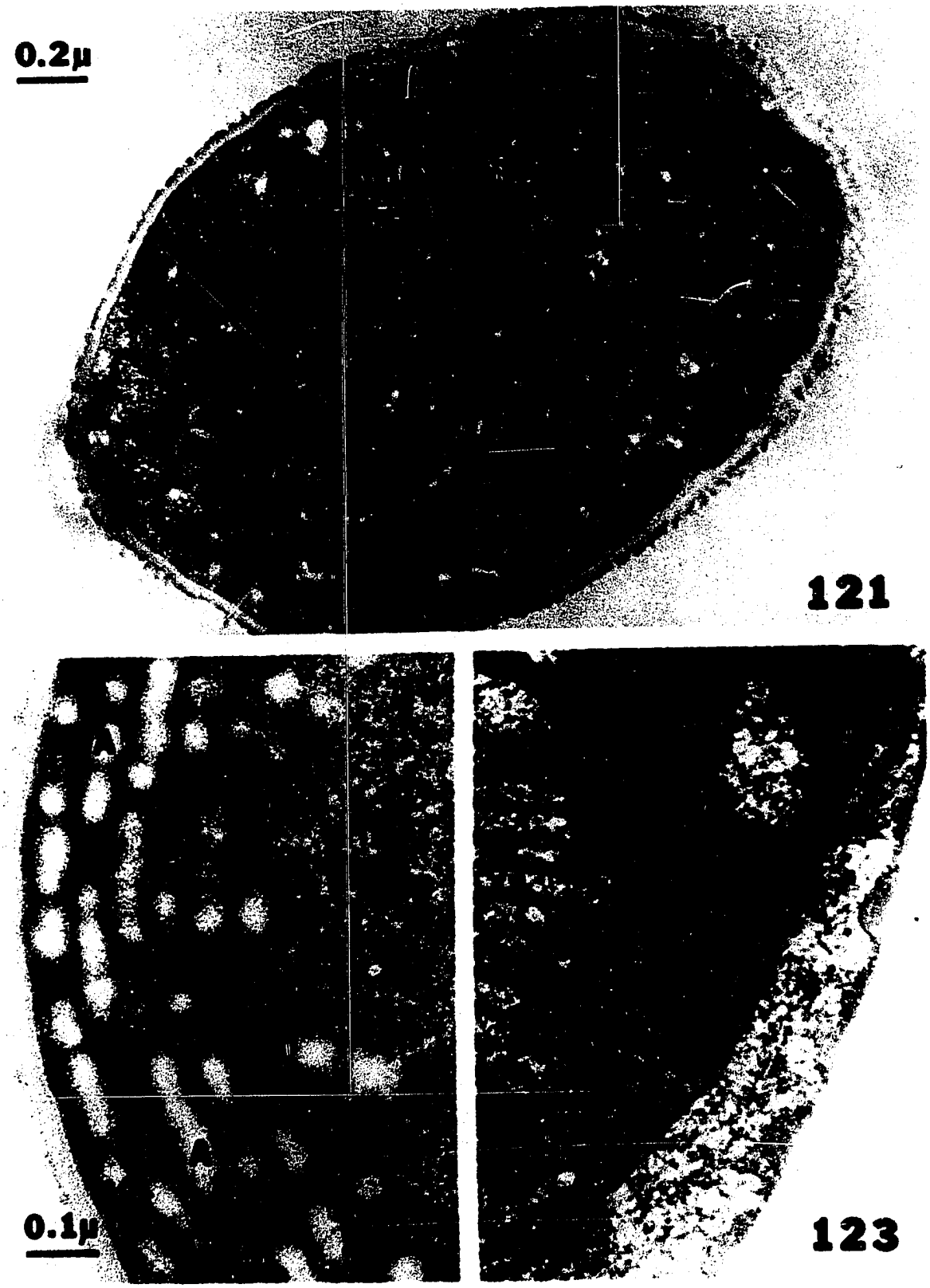
119

Figure 120. Cell of Nostoc muscorum, grown for three weeks in red light, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Phycobilisomes are enlarged and numerous. 75,500 X.


0.2μ
120

Figures 121 and 122. Portions of cells of Nostoc muscorum, grown in the dark for five weeks with 1% sucrose added to the medium, fixed in glutaraldehyde-osmium tetroxide, and stained with uranyl acetate and lead citrate. The intrathylakoidal spaces or appressed inner membranes stain intensely. There is evidence of an accumulation of α-granules. Figure 121, 55,500 X; Figure 122, 116,000 X.

Figure 123. Portion of a cell of Microcoleus vaginatus (Type A), grown in the dark for five weeks with 1% glucose added to the medium, fixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Intrathylakoidal spaces or appressed inner membranes stain intensely, and the thylakoids frequently appear to be stacked. 75,500 X.


0.2μ
121
0.1μ
123