THE FINE STRUCTURE OF CERTAIN MEMBERS OF THE ORDER NOSTOCALES

by

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INTRODUCTION

A basic concept in biology is that all living organisms are made up of cells and cell products. As more is learned about the cytology of organisms it is increasingly apparent that two basically different kinds of organisms exist, those with "prokaryotic" cells and those with "eukaryotic" cells (Stanier and Van Niel, 1962). The differences between these kinds of organisms are considered so significant that a number of systematists are proposing that we should revise our views about basic evolutionary affinities and consider merging the animal and plant kingdoms into a single kingdom, the "Eukaryota", with the exception of the bacteria and blue-green algae which would constitute the "Prokaryota". The differences between the cells of prokaryotic and eukaryotic organisms are not as much expressed in cellular function as in differences in morphological organization of the cell machinery. For example, most of the organelles of eukaryotic cells are bounded by membranes which act as selective barriers between these organelles and the rest of the protoplast. Membrane defined nuclei, mitochondria, plastids and dictyosomes are not found in the prokaryotic cell. The genetic material is divided by mitosis in the eukaryotic cell. In the prokaryotic cell the mechanism of division is not understood but is certainly quite different from mitosis.

Until recently, cytologists using the light microscope have been

limited to a resolution of about 0.2 μ. Recently, the electron microscope has increased the limit of resolution to better than 1 mμ. Thus, the electron microscope has a great potential in the study of prokaryotic organisms where most of the species have very small cells.

Recent improvements in fixation techniques (Kellenberger, Ryter and Sechaud, 1958; Luft, 1956; Holt and Hicks, 1961; Pankratz and Bowen, 1963) and embedding techniques (Luft, 1961) have made it practical to use electron microscopy in obtaining a better understanding of the morphology of the blue-green algal cell.

The group of organisms called the blue-green algae or Cyanophyta is a diversified group of relatively "simple" organisms which is considered to be comparable, in certain respects, with bacteria. Even though members of this group have world-wide distribution, relatively little work has been done on them, especially at the electron microscope level.

For this reason, I decided to undertake a comparative morphological study of one of the more common orders in the blue-greens, the Nostocales. Preliminary observations on a wide variety of material immediately pointed out the fact that these "simple" organisms were complex and contained many unusual and unreported inclusions. Because of the absence of typical higher plant and animal cellular organelles a basic understanding of the internal organization of these organisms was considered essential. It is the purpose of this present work, therefore, to

report on the morphological diversity and complexity in the Nostocales and to point out areas for future study. This work may have a bearing on taxonomy, phylogenetic relationships and a more complete understanding of basic biological organization.

LITERATURE REVIEW

General Features of the Order

The Nostocales is an order of filamentous blue-green algae which is characterized by no true branching and by the ability of its members to form short filaments called hormogonia each of which can develop into a new filament (Desikachary, 1959; Fritsch, 1945). Hormogonia form when cell death or other means create local weaknesses in a filament. Vegetative cells as well as hormogonia may be motile moving by a gliding process. The cells of a given species are much alike except that some species form akinetes (spores) and heterocysts (thick-walled cells of unknown function).

Cell Wall

The cell wall in the blue-green algae, following the terminology of Ris and Singh (1961), is composed of two portions: an outer gelatineous element, the sheath, which may be of considerable thickness; and, an inner, relatively rigid, complex layer immediately outside of the plasma membrane.

The sheath may appear either stratified or homogeneous under the light microscope and it can vary in color depending on the pigments present. Yellow or brown colors are due to the pigments fuscorhodin and fuscocholin respectively (Kylin, 1943). When the sheath is red or violet the pigment gleocapsin is present. The chemical nature of these

pigments is uncertain (Kylin, 1943). Calcium and magnesium pectates have also been found in the sheath (Smith, 1950; Desikachary, 1959).

A number of extracellular products are known to be produced by blue-green algae (Fogg, 1962). An auxin-like hormone has been isolated from Anabaena cylindrica and Nostoc punctiforme produces inhibitor and extracellular enzymes. Calothrix brevissima produces large amounts of aspartic acid, glutamic acid and alanine while Anabaena and Microcystis produce toxic compounds which appear to be cylic peptides. Nitrogen fixation and the release of nitrogeneous compounds have been observed mainly in three genera Anabaena, Cylindrospermum and Nostoc. No members of the Oscillatoriaceae have been shown to fix nitrogen. However, one species of the Chroococcales, the unicellular order of blue-green algae, Chloroglea fritschii has this capacity.

Gorham (1962) has intensively investigated toxic compounds of some strains of Microcystis aeruginosa and Anabaena flos-aquae. Microcystis produces a fast-death factor which is a cylic polypeptide. Anabaena produces a very-fast-death factor of unknown structure. Occasionally, blooms of such organisms have killed large numbers of both wild and domestic animals.

In electron microscope studies the sheath may appear layered and contain fibrillar structures as shown by a number of workers (Hopwood and Glauert, 1960; Ris and Singh, 1961; Chapman and Salton, 1962;

Levchenko, 1962; Frank, Lefort and Martin, 1962a; Pankratz and Bowen, 1963). The thickness, number and density of the layers in the sheath appear to vary with the species and environmental conditions (Fritsch, 1945). The denser areas may be composed of long fibers 5 to 10 mμ in thickness (Levchenko, 1962). Frey-Wyssling and Stecher (1954) describe cellulose fibers of 300 mμ diameter and indeterminate length in the sheath of Nostoc sp. Metzner (1955), however, claims there is no good evidence for the existence of cellulose in blue-green algae.

The inner layer of the cell wall contains cellulose according to Kylin (1943) but Metzner (1955) does not support this contention. Regularly arranged pores in the longitudinal walls of Oscillatoria have been observed (Kolkwitz, 189?; Philips, 1904) with the light microscope.

A number of electron microscope studies have described the structure of the inner layer of the cell wall (Drews and Niklowitz, 1956; Niklowitz and Drews, 1956; Shinke and Ueda, 1956; Drews and Niklowitz, 1957; Niklowitz and Drews, 1957; Fuhs, 1958; Hopwood and Glauert, 1960; Hagedorn, 1961; Ris and Singh, 1961; Chapman and Salton, 1962; Frank, Lefort and Martin, 1962a; Hall and Claus, 1962; Levchenko, 1962; Echlin, 1963; Pankratz and Bowen, 1963; Wildon and Mercer, 1963a). Unfortunately a variety of terminologies have been used to describe the wall components. The terminology of Ris and Singh (1961) will be used. The inner layer of the cell wall consists of two components, an outer membrane and an inner

investment immediately outside the plasma membrane. In electron micrographs the inner investment is separated from the plasma membrane by a clear space about 8 to 10 mμ wide and generally ranges from 10 to 20 mμ or more in thickness depending on the species. Shinke and Ueda (1956) and Ris and Singh (1961) found the inner investment of Oscillatoria princeps to be about 0.2 μ thick and to be perforated by pores varying from 30 to 60 mμ in diameter.

The outer membrane has roughly the thickness of a unit membrane. It generally has small undulations which in some species may be very pronounced and project some distance into the sheath (Echlin, 1963).

Pores in the inner investment have been described by early electron microscopists using shadowing techniques (Drawert and Metzner, 1956; Drawert and Metzner, 1958). In Cylindrospermum, Microcoleus and Oscillatoria borneti the pores are between 15 and 19 mμ in diameter and are located in the longitudinal walls over and on either side of the junction of the crosswalls. In Oscillatoria limosa the 13 mμ pores were scattered over the entire wall. Pankratz and Bowen (1963) observed pores in sectioned material of Symploca muscorum which were about 10 mμ in diameter and spaced 18 mμ apart in two rows, one on each side of the intersection of a crosswall with the longitudinal wall.

Some interesting comparisons have been made between the cell wall components of the blue-green algae and bacteria. The cell wall is

interpreted as being the inner layer of the cell wall of the blue-green algae and the cell wall (not the capsular material) of the bacteria. Drews and Meyer (1964) have isolated the components of the cell wall of 2 blue-green algae Anacystis nidulans and Phormidium uncinatum and compared these to the cell wall components of Escherichia coli, a gram-negative bacterium. Compounds found in common were glucosamine, muramic acid, diaminopimelic acid, glutamic acid, alanine, aspartic acid, serine, threonine, leucine, lysine and glycine. Anacystis nidulans contained galactosamine which was not present in Esherichia coli. Similar results were arrived at by Frank, Lefort and Martin (1962b) in a comparison between the cell walls of Phormidium uncinatum and Esherichia coli.

Experiments by Frank, Lefort and Martin (1962a) suggest that the characteristic mucopolymer exists between the plasma membrane and the inner investment in Phormidium uncinatum and that it is removed by lysozyme. Crespi, Mandeville and Katz (1962) produced naked protoplasts from several blue-green algae using lysozyme. These workers found that lysis was accompanied by a release of biliproteins from the cells.

Stanier (1962) has called attention to the fact that muramic acid and diaminopimelic acid have only been found in bacterial and blue-green algal cell walls suggesting taxonomic relationships between the bacteria and the blue-green algae.

Vegetative Cells

Light microscopists have long argued about the organization of the green peripheral protoplasm of the blue-green algal cell. Hegler (1901) believed it contained many small chromatophores but Philips (1904) described one large peripheral chromatophore per cell. Geitler (1958) used high resolution light microscopy to show a peripheral lamellar system in Chroococcus.

The pigments found in the cell are chlorophyll a, several xanthophylls, and at least four different carotenes, one of which, flavicin, is apparently unique to the blue-greens (Fogg, 1956). Either phycoerythrin (red) or phycocyanin (blue) or both occur in a given species and contribute to its characteristic color. They are proteinaceous, water soluble pigments which probably do not occur in other organisms with the exception of phycoerthrin which may be very similar to the red pigment, also called phycoerthrin, found in the Rhodophyceae (Fritsch, 1945). These proteinaceous pigments are destroyed by high light (Desikachary, 1959).

Calvin and Lynch (1952) and Shatkin (1960) have isolated intracellular particles by ultracentrifugation, which contain the chlorophyll and carotenes. Calvin and Lynch considered the particles to be analogous to entire grana of chloroplasts of higher plants while Shatkin considered them to be flattened vesicles resulting from the disruption of

intracellular membranes.

A large number of investigators have used the electron microscope to investigate the cytology of the blue-green algae. One unfortunate result of these works is that a variety of terminologies have been used by different investigators. In an attempt to avoid further confusion in this area the terminology of Pankratz and Bowen (1963) will be adopted.

Blue-green algae cells are enclosed by a plasma membrane of unit membrane appearance and dimensions (ca. 7 mμ thick). The protoplasts contain varying numbers of lamellar elements which Menke (1961) has called thylakoids. The thylakoid consists of a flattened closed sac of varying diameters within the range of several microns. The adjacent unit membranes of a single thylakoid may be appressed to each other to form a myelin-like figure or the sac may be more or less expanded and thus exhibit an intrathylakoidal space (Drawert and Metzner, 1958; Hopwood and Glauert, 1960; Lefort, 1960b; Ris and Singh, 1961). Chapman and Salton (1962) have described membrane elements in the central part of cells of Nostoc muscorum which are darker in appearance than the thylakoids in the peripheral cytoplasm.

The arrangement of the thylakoids in the cells of various species ranges from a regular, concentric pattern in Oscillatoria chalybea to a randomly distributed pattern characteristic of Nostoc muscorum (Menke, 1961).

The thylakoids may originate from the subdividing of a group of thylakoids by an ingrowing crosswall or through an elaboration of the plasma membrane (Bowen and Pankratz, 1963). Echlin (1964) has described an intra-cytoplasmic membranous inclusion in the cells of Anacystis nidulans. It resembles the bacterial mesosome. He coined the term "lamellasome" to describe these inclusions.

A number of different kinds of granular inclusions are found in blue-green algae cells. Cyanophycean granules are generally located in the peripheral part of the cell. They are reported to contain a nitrogenous substance (Fritsch, 1951), which is high in arginine content (Fogg, 1951). Smith (1950) suggests they are probably proteinaceous. Fuhs (1958) obtained a positive reaction for phospholipids in the cyanophycean granules and because the granules stain with Janus green and reduce tetrazolium salts, Drews and Niklowitz (1956, 1957) considered them to be "mitochondrial equivalents". These same workers associated the cyanophycean granules seen in the light microscope with inclusions they called "structured granules" seen by electron microscopy. These are large, dense granules ranging up to 0.5 μ in diameter and show an irregular internal pattern of dense and less dense regions after osmium fixation. They are indistinct and apparently poorly preserved after potassium permanganate fixation (Pankratz and Bowen, 1963). Ris and Singh (1961) considered similar bodies in Oscillatoria to be a type of membrane

differentiation distinct from that of the thylakoids. Pankratz and Bowen (1963) failed to find a membranous component in the structured granules.

Carbohydrate deposits occur in the pigmented part of the cell. They are believed to be between glycogen and starch in chemical composition (Fritsch, 1945). Electron microscope studies, after osmium fixation, reveal elongate granules of medium electron density about 30 mμ in diameter which are most abundant between the thylakoids (Pankratz and Bowen, 1963). These granules are occasionally preserved with potassium permanganate fixation and have been called alpha-granules (α granules) by Pankratz and Bowen (1963). Fuhs (1963) and Giesy (1964) have shown that these structures are removed by diastase digestion and are probably polyglucoside in nature. Giesy (1964) has further shown that in media with a high nitrogen content in which rapid growth occurs few α granules accumulate. With low nitrogen in the culture media these granules accumulated in large numbers.

Menke (1961) reported what appears to be a crystal of fibrillar or tubular array in the cells of Oscillatoria chalybea after potassium permanganate fixation which was located near the crosswall between thylakoids. It appears much like the very elongate α granules in micrographs of Oscillatoria chalybea by Giesy (1964).

Spherical osmiophilic granules ranging in diameter from 30 to 90 mμ are also found between the thylakoids. Pankratz and Bowen (1963) have

called these beta-granules (β granules). Shatkin (1960) suggests that they are storage products. Poorly preserved by potassium permanganate fixation, they are considered to be lipoidal and comparable to the osmiophilic droplets reported by many workers in chloroplasts of higher plants (Pankratz and Bowen, 1963). Fuhs (1958) has suggested that granules of similar appearance in Oscillatoria amoena may be composed of polyphosphates.

Structures which are "vacuole-like" are found in some blue-green algae (Niklowitz and Drews, 1957; Jensen and Bowen, 1961; Hall and Claus, 1962; Pankratz and Bowen, 1963; Wildon and Mercer, 1963a). These structures are generally located in the peripheral cytoplasm. They are up to 2 μ in diameter, frequently contain small dense granules and are not membrane limited. In every case these structures were described in material fixed with potassium permanganate and similar structures have not been described in osmium-fixed material.

Bowen and Pankratz (1963) reported the presence of cylindrical bodies in Symploca muscorum. These non-membranous inclusions are composed of two concentric cylindrical zones of greater density and are found either singly or in clusters near the walls. These bodies range from 100 to 140 mμ in total diameter and up to 1 μ in length. Similar structures have not been reported in any other species.

Polyphosphate bodies have been reported by light microscopists in

many blue-green algae (Spearing, 1937). They occur in all areas of the cell and can be made to increase in size and concentration by incubating the cells in the light in culture media to which dipotassium hydrogen phosphate has been added (Talpasayi, 1963). They are stained with toluidin blue (Keck and Stich, 1957) or lead nitrate-sulphite (Talpasayi, 1963) and can be removed by incubation for 6 hours in cold 10 percent trichloroacetic acid. Tewari and Krishnan (1959) observed that polyphosphates of molecular weight 690 to 1090 gave a weak metachromatic reaction while those of molecular weight 3,600 to 20,000 gave a strong reaction. Polyphosphate particles, isolated from Anabaena cylindrica showed the characteristic red fluorescense of ribonucleic acid under ultraviolet light when stained with buffered acridine-orange (Talpasayi, 1963). Mature spores do not stain for polyphosphates which Talpasayi (1963) suggests could be due to a lower molecular weight of the polyphosphates. Polyphosphate inclusions have been called volutin and metachromatic bodies by various authors (Kuhl, 1962). Electron microscope studies have not demonstrated inclusions that could clearly be identified with polyphosphate bodies.

In Stigonema hexagonal crystals of unknown composition up to 5.8 μ in diameter have been observed in the light microscope (Spearing, 1937). Small crystals have been observed in Dactylococcopsis and gypsin crystals have been observed in Symploca muscorum (Fritsch, 1945). Oscillatoria growing in water rich in sulphides has been reported to

contain droplets of sulfur (Fritsch, 1945).

Over the years much debate has centered around the nuclear area. Chodat (1896) first reported that the blue-green algae lack a true nucleus. A number of workers including Spearing (1937) indicate that certain species such as Oscillatoria sp. have a nuclear membrane. Other workers (Fischer, 1905; Geitler, 1922) claim the central area is not a nucleus but an area filled with reserve material or mitochondria. The presence of chromosomes and a mitotic division process have been claimed by Hegler (1901). Other workers including Wagner (1903) claim cell division is amitotic.

Poljansky and Petruschewsky (1929) first used the Feulgen stain on blue-greens and obtained a positive reaction in the central region of the cell. Some species such as Oscillatoria princeps are Feulgen negative (Shinke and Ueda, 1956). More recent work has shown that Feulgen positive chromatin is present in the central area of the cell. Cassel and Hutchinson (1954) using light microscopy describe three general arrangements of the chromatin: (1) loose, sometimes granular, net-like, (2) rod-like, oriented parallel to the longitudinal axis of the cell in groups, and (3) a very much condensed organization of various shapes. These differences in the distribution are apparently dependent upon the particular species. The presence of a mitotic division process and chromosomes was denied by these workers. Some workers describe

nucleoli-like bodies in the nuclear area (Guilliermond, 1906; Spearing, 1937).

Biswas (1956) isolated the deoxyribonucleic acid from Nostoc muscorum and found two different kinds differing in base composition, metabolic activity and solubility in acid. Biswas (1957) using cytochemical techniques arrived at the conclusion that the deoxyribonucleic acid is bound to a structural protein which is high in tyrosin and arginine. He also subjected cells to digestion with pepsin and trypsin. He found that pepsin, after 20 hours, completely removed the protein from the peripheral cytoplasm but not from the central area. Trypsin in phosphate buffer digested the proteins from the central area. Biswas (1961) analyzed the nucleoproteins of Nostoc muscorum and concluded that they were similar to those found in other organisms.

After osmium tetroxide fixation the nucleoplasm of blue-green algae, in electronmicrographs, appears as small areas of low density containing fibers 2 to 5 mμ wide (Ris and Singh, 1961). In Anabaena cylindrica such an area of low density is located in the central part of the cell and corresponds to the Feulgen positive area observed with the light microscope (Hopwood and Glauert, 1960). No nuclear envelope has been observed and there is no sharp dividing line between nucleoplasm and cytoplasm (Ris and Singh, 1961).

Electron microscope studies reveal polygonal profiles in the central area of the cell which are of medium electron density after osmium tetroxide fixation (Lefort, 1960a, 1960b; Costerton, 1960; Ris and Singh, 1961; Jensen and Bowen, 1961). These "polyhedral bodies" vary in size up to 0.5 μ in diameter and always appear in contact with the fibrous component of the nucleoplasm. In profile they are regularly surrounded by an electron-dense line about 3 mμ wide with a 5 mμ clear space between the line and the body itself (Pankratz and Bowen, 1963). They are preserved with osmium tetroxide, potassium permanganate and formalin-osmium tetroxide fixation (Pankratz and Bowen, 1963). Costerton (1960) suggested they are protein bodies. He observed clear areas in the central region of cells which had been hydrolyzed for 10 minutes at 60°C in 1N hydrochloric acid.

Ribosomes are described by all workers who have used osmium tetroxide fixation. These dense granules are 10 to 15 mμ in diameter and are scattered throughout the cells but they are most numerous in the nucleoplasmic area adjacent to the fibrillar component (Pankratz and Bowen, 1963). They are not preserved by potassium permanganate fixation. Fuhs (1963) has demonstrated that similar granules are removed by ribonuclease in Oscillatoria amoena and Pseudanabaena catenata.

Jensen and Bowen (1961) reported lamellar bodies in the cells of Nostoc pruniforme. They were located in the central area of the cell in

association with the nucleoplasm and the polyhedral bodies. They were about 600 mμ long and about 100 mμ wide. Their profiles showed a very regular periodicity of straight, parallel striations normal to the long axis and spaced about 20 mμ apart.

Plasmodesm-like structures have been observed in the crosswalls of adjacent cells (Pankratz and Bowen, 1963; Wildon and Mercer, 1963a). They are about 17 mμ wide and extend from the plasma membrane of one cell to the plasma membrane of the adjacent cell. Wildon and Mercer (1963a) claim only the outer electron-dense component of the plasma membrane enters into the pore in the crosswall. The inner electron-dense layer of this unit membrane being uninterrupted.

Spearing (1961) attempted to use a number of vital stains to elucidate the nature of the cytoplasmic components of the blue-green cell. He concluded that no reliable information could be obtained with any of his procedures and appropriately included in the title of his long paper, "...Vital staining--a study in the production of artifacts".

A virus which causes the lysis of blue-green algal cells has recently been discovered (Safferman and Morris, 1963; Schneider, Diener and Safferman, 1964; Safferman and Morris, 1964). It was isolated from sewage and tested against 78 different algae of which 11 proved succeptable including species of Lyngbya, Plectonema and Phormidium. This phycovirus resembles a bacteriophage in that it contains deoxyribonucleic acid,

has a polyhederal head about 66 mμ in diameter and a short tail about 16 mμ long.

Akinetes

Spores (akinetes) are formed by all of the Nostocales except the Oscillatoriaceae (Desikachary, 1959). Akinetes are generally large cells with thick walls and have accumulated food reserves mainly in the form of cyanophycean granules (Fritsch, 1945). In mature spores there are no photosythetic pigments, gas vacuoles or polyphosphate granules (Desikachary, 1959). Akinetes frequently develop in close proximity to heterocysts and are assumed to be one means of tiding the algae over unfavorable periods (Fritsch, 1945).

Wildon and Mercer (1963b) have described the ultrastructure of akinetes in Anabaena cylindrica. They are similar to a vegetative cell except that they are much enlarged, have an extra layer of cell envelope and possess a different kind of granule.

Heterocysts

Heterocysts are differentiated cells produced by many members of the Nostocales. These special cells are easily distinguished from other cells by their thick walls and by the generally homogeneous and pale yellowish contents. They may be located in the middle of a filament (intercalary heterocysts) or at the end of a filament (terminal

heterocyst). The cross wall separating a heterocyst from adjacent cells appears to be pierced with a pore when this area is observed under the light microscope (Fritsch, 1945).

The wall of the heterocyst is usually thick and two distinct layers can be seen: (1) an outer broad wall, especially thick near the pore and (2) an inner thin wall. The inner wall contains cellulose according to Geitler (1921) and Bharadwaja (1933). Fritsch (1951), however, does not support this view. A prominent granule, the polar granule, of uncertain composition is frequently observed in the cytoplasm in the pore (Desikachary, 1959).

Heterocysts appear to develop from recently divided vegetative cells (Fritsch, 1951). The characteristic thick outer wall is laid down and the cytoplasm becomes homogeneous in appearance losing its green color. In the Rivulariaceae, however, the heterocysts remain green for long periods of time (Fritsch, 1951). Fogg (1944, 1949) showed that heterocyst formation was inhibited by increasing the nitrogeneous compound or compounds in the culture media. Similarly decreasing nitrogen content of the media increased the number of heterocysts. Fogg (1944) analyzed the combined nitrogen of blue-green algal cells and found an inverse relationship between abundance of heterocysts and the average amount of nitrogen per cell. He suggested that a relationship exists between heterocysts and nitrogen fixation. Other workers consider heterocysts

as providing places where the filament may fragment (Desikachary, 1959). However, Rivularia and a number of other genera possess only terminal heterocysts. In these forms hormogonia are formed by the disintegration of a vegetative cell (Desikachary, 1959).

Fritsch (1951) has proposed that heterocysts produce diffusible enzymes which influence growth and differentiation. A number of workers report they have observed heterocysts germinating and suggest that heterocysts are vestigial reproductive bodies which normally cannot function but on occasion revert and give rise to a new trichome (Geitler, 1921; Desikachary, 1946; Fogg, 1949; Lazaroff and Vishniac, 1963). However, most species which form heterocysts also form akinetes (Fritsch, 1945).

Wildon and Mercer (1963b) have used the electron microscope to investigate the heterocysts of Anabaena sp., Anabaena cylindrica, Fischerella muscicola, Nostoc sp. and Nostoc muscorum. Heterocyst development starts from a vegetative cell with the first indication of development being an increase in cell size. The outer wall is layed down next and the inner wall becomes thicker, especially at the pore region. The pore does not penetrate the crosswall of the adjacent cell. However, plasmodesm-like structures were found in this crosswall. Bodies identified as the polar granules of the light microscopists were occasionally found in the cytoplasm within the pore. These workers

reported that ribosome-like granules became widely scattered and other granular inclusions became fewer and finally disappeared as the heterocyst developed. The lamaellar elements finally branch. In detached heterocysts the cell contents degenerate and such heterocysts lose their cytoplasm. Chapman and Salton (1962) have also described branched lamaellar elements in the heterocysts of Anabaena cylindrica.

Gas Vacuoles

Depending upon the growth conditions the cytoplasm of many species of blue-green algae may contain small irregular structures which resemble air bubbles under the light microscope. These "gas vacuoles" (Kelbahn, 1895) or "pseudo-vacuoles" (Lemmermann, 1910) have been found in species of Microcystis, Aphanizomenon, Anabaena, Calothrix, Gleotrichia, Nostoc, Lyngbya, Anabaenopsis and Oscillatoria (Fogg, 1941). They are of sporatic occurrence in many species but are especially prominent in the planktonic algae causing "water blooms" in lakes and streams and are occasionally found in mud inhabiting forms (Fogg, 1941). Seldom larger than 2 μ, they appear red under high power of the light microscope and do not exhibit birefringence (Molisch, 1903). Gas vacuoles disappear after treatment with boiling water, strong acids, alkalies, detergents, pressure and organic solvents such as chloroform, acetone and phenol (Fogg, 1941). Klebahn (1925) applied pressure to cells by driving a cork into a bottle filled with the algae and water. When the gas vacuoles are

destroyed the algae sink to the bottom of the container. Klebahn (1929) has shown that gas vacuoles contain a gas and after isolating and analyzing it he came to the conclusion that it was nitrogen. Fogg (1941) suggests the gas may be an amine.

Most workers support the theory that gas vacuoles enable the algae to float (Fogg, 1941; Fritsch, 1945). Kolkwitz (1928) suggested that the gas is formed by fermentation under anaerobic conditions and based his hypothesis upon observations of a bacterium, Sarcina ventriculi, which possessed gas vacuoles and had been collected from mud at the bottom of a pond. Lemmermann (1910) suggested the gas vacuoles may screen out strong light.

In electron microscope investigations a variety of structures have been identified as gas vacuoles: electron-transparent regions (Sun, 1961, Wildon and Mercer, 1963a); intrathylakoidal spaces (Hopwood and Glauert, 1961) and structured granules (Costerton, 1960).

METHODS AND MATERIALS

Collections of Algae

Blue-green algae were collected from a number of local sources as well as obtained from permanently maintained culture collections. Some collections made in the field were fixed immediately. Other collections were generally placed in gallon containers, brought to the laboratory and then fixed, cultured or both.

A sample of each collection will be deposited in the herbarium at The Academy of Natural Sciences of Philadelphia. Details about collections made locally can be found on the herbarium sheets.

Following is a list, by families, of the blue-green algae examined and used in this study and their sources.

Family Oscillatoreaceae

Oscillatoria princeps Vaucher ex Gomont. Collected from Lake Ahquabi, Indianola, Iowa, July 6, 1964; Oct. 10, 1964. ISU Jensen 1.

Family Nostocales

Anabaena spiroides var. crassa Lemmermann. Collected from Des Moines River, Sept. 6, 1963; Storm Lake, Iowa, Aug. 26, 1964; Lake Ahquabi, Indianola, Iowa, Oct. 10, 1964. ISU Jensen 2.

Anabaena sp. Collected from Lake Laverne, Ames, Iowa, Sept. 10, 1964. ISU Jensen 3.

Aphanizomenon flos-aquae (L.) Ralfs. Collected from a farm pond near Nevada, Iowa, July 21, 1964; Lake Ahquabi, Indianola, Iowa, Oct. 10, 1964; Lake Okoboji, Milford, Iowa, Oct. 28, 1964. ISU Jensen 4.

Cylindrospermum sp. Collected from Ledges State Park, Boone, Iowa. Sept. 14, 1963. ISU Jensen 5.

Nostoc calcicola Brebisson ex Born. et Flah. Indiana University Culture B382. ISU Jensen 6.

Nostoc carneum Ag. ex Born. et Flah. Collected from Lake Okoboji, Milford, Iowa, 1961. ISU Jensen 7.

Nostoc coeruleum Lyngbye ex Born. et Flah. Collected from a ditch near Council Bluffs, Iowa, July 1, 1964. ISU Jensen 14.

Nostoc commune Vaucher ex Born. et Flah. Indiana University Culture 584. ISU Jensen 8.

Nostoc ellipsosporum (Desm.) Rabenh. ex Born. et Flah. Indiana University Culture B383. ISU Jensen 9.

Nostoc linckia (Roth) Bornet ex Born. et Flah. Collected from Silver Lake, Milford, Iowa, Aug. 1963. ISU Jensen 16.

Nostoc muscorum Ag. ex Born. et Flah. Indiana University Culture 486. ISU Jensen 10.

Nostoc muscorum Ag. ex Born. et Flah. Collected from Big Wall Lake, Dows, Iowa, Aug. 11, 1964. ISU Jensen 15.

Nostoc muscorum Ag. ex Born. et Flah. Franklyn Ott University of Texas Culture 079. ISU Jensen 17.

Nostoc pruniforme Ag. ex Born. et Flah. Collected from Lake Okoboji, Milford, Iowa, June 1960. ISU Jensen 11.

Nostoc pruniforme Ag. ex Born. et Flah. Indiana University Culture Lb756. ISU Jensen 12.

Nostoc punctiforme (Kutz.) Hariot. Indiana University Culture 384. ISU Jensen 13.

Family Scytonemataceae

Plectonema boryanum Gomont. Kaiser Research Foundation M-9.2.1. ISU Jensen 18.

Family Rivulariaceae

Calothrix crustacea Thuret. Kaiser Research Foundation M-13.1.1. ISU Jensen 19.

Calothrix parietina Thuret ex Born. et Flah. Kaiser Research Foundation M-13.1.1. ISU Jensen 22.

Gleotrichia pisum[1] Thuret ex Born. et Flah. Collected from Little Wall Lake, Jewell, Iowa, Sept. 10, 1963; Aug. 26, 1964; Oct. 6, 1964. ISU Jensen 20.

Gleotrichia pisum Thuret ex Born. et Flah. Collected from Clear Lake, Clear Lake, Iowa, Aug. 11, 1964. ISU Jensen 21.

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[1] Gleotrichia echinulata the most common species of Gleotrichia in this area was not studied because another student was working with it.

Culture Technique

Media used were Chu 10 with soil extract with and without 2 percent agar (Chu, 1942), soil extract agar (Starr, 1960), soil water medium (Starr, 1960) and Lazaroff media I and II both with and without 2 percent agar (Lazaroff and Vishniac, 1962). Cultures were generally maintained at 21°C under 500 foot-candles of illumination for 1 to 2 weeks and then transferred to 50-100 foot-candles of illumination. Some cultures were kept at 500 foot-candles of illumination for long periods of time. A 12 hour alternating light and dark period was used.

Pressure Treatment

Algae with gas vacuoles were exposed to 100 lb. p.s.i. in a piston device (Figure 60). The bore was filled with algae suspended in the water in which they were collected and pressure was applied by pushing down suddenly on the piston. A sample was fixed immediately and the remaining algae were allowed to stand at room temperature. These latter samples were checked periodically to observe the reformation of the gas vacuoles. Recovering cells were fixed after 9 hours and 24 hours. Controls were fixed at the beginning of the experiment and from a container containing a similar amount of algae standing at room temperature at each of the above times.

The experiment was repeated twice using Aphanizomenon flos-aquae and once using Anabaena sp.

Nostoc pruniforme (ISU-J11) Inoculation Experiment

Cells of Nostoc pruniforme (ISU-J11) were broken up in a Mickle disintegrator. Material was checked periodically in the light microscope and grinding continued until few whole cells remained. This material was then centrifuged to remove the glass beads and whole cells. The supernatant was then filtered through a 1.3 μ millipore filter. Cultures of Nostoc calcicola, Nostoc muscorum (ISU-J10), Nostoc commune, Nostoc ellipsosporum, Nostoc punctiforme and Nostoc pruniforme (ISU-J12) four days old were inoculated with one drop of the filtrate. The algae were grown at 425 to 450 foot-candles of illumination in Lazaroff I liquid medium except Nostoc pruniforme (ISU-J12) which was grown in soil water medium. All cultures were fixed in osmium tetroxide after 27 days and examined. Untreated controls were fixed for each of the cultures.

Electron Microscopy

Material was fixed in four ways, details of each procedure are given in Appendix A. Most material was fixed in 1 percent osmium tetroxide buffered with veronal acetate as described by Kellenberger, Ryter and Sechaud (1958) and modified by Pankratz and Bowen (1963). Three percent gluteraldehyde (Sabatini, Bensch and Barrnett, 1963) both with and without post osmification (Pankratz and Bowen, 1963) and 4 percent potassium permanganate (Luft, 1956; Mollenhauer, 1959) were also used.

Dehydration was routinely accomplished in increasing concentrations of ethanol (see Appendix A). Epon was used as the embedding medium (Luft, 1961; see Appendix A).

Some of the material of each collection, while soaking in 100 percent Epon at room temperature, was placed on a slide and pressed out under a cover slip. The slide was then incubated in the 60°C oven for 24 hours making the mount permanent and available for future reference.

Sections were cut with a DuPont diamond knife on an LKB ultramicrotome. The sections, 40 to 90 mμ thick, were picked up on clean 400 mesh copper grids. Aqueous uranyl acetate (Watson, 1958a), lead salts (Millonig, 1961; Karnovsky, 1961; Watson, 1958b) and methanol uranyl acetate (Stempak and Ward, 1964) were used to stain the sections.

Specimens were observed using an RCA EMU-3F electron microscope operated at 50 KV using a 30 to 40 μ objective aperature. The micrographs were taken at machine magnifications of 2,000 to 24,000 X on Kodak contrast plates. Development was carried out in Kodak D-19 developer for 3 to 4 minutes. Negatives were routinely enlarged 2.8 to 4.2 times on Kodak F-3, F-4 or F-5 Kodabromide paper.

Enzyme Digestion

After fixation in gluteraldehyde for 1 hour (Appendix A, Schedule II) cells were placed in a number of changes of 0.1M phosphate buffer pH 7.2

over a 15 hour period. This fixation was followed by 3 changes of 5 minutes each in 0.01N hydrochloric acid and incubation at 37°C for 11 hours in a 0.3 percent solution of pepsin (Worthington 2X crystallized) in 0.01N hydrochloric acid. Material was removed after 30 minutes, 1 hour, 2 hours, 4 hours and 11 hours. Control material was placed in 0.01N hydrochloric acid with another control being left in the 0.1M phosphate buffer. When material was removed from the enzyme solution or the 0.01N hydrochloric acid it was rinsed in 3 changes of 5 minutes each with the 0.1M phosphate buffer. The material was stored overnight at 4°C and post-fixed in osmium tetroxide for 3 hours (Appendix A, Schedule I).

Grids bearing sections of cells fixed in 3 percent gluteraldehyde for 12 hours (Appendix A, Schedule II) with no post-osmification were digested in a 0.3 percent solution of pepsin (Worthington 2X crystallized) in 0.01N hydrochloric acid for periods of time up to 24 hours. Controls were placed in 0.01N hydrochloric acid without the enzyme for the same time periods.

Some grids bearing sections of cells fixed in 3 percent gluteraldehyde for 12 hours (Appendix A, Schedule II) with no post-osmification were digested in a 0.1 percent aqueous solution of ribonuclease (Worthington 3X crystallized) at pH 6.8 (Jensen, 1962) for varying periods of time up to 24 hours. Grids with comparable sections were placed in distilled water, in which the pH had been adjusted to 6.8, for the same time periods and served as controls.

OBSERVATIONS AND DISCUSSION

General Observations and Discussion

The usual components of the blue-green algal cell reported in the literature (Lefort, 1960a; Ris and Singh, 1961; Pankratz and Bowen, 1963) were present in all collections studied with only a few exceptions (Figure 1). The normal components include structured granules, α granules, β granules, thylakoids, ribosomes, polyhederal bodies and nucleoplasm (Figures 1, 12, 13, 15 and 18). Observations were made on material fixed for 3 hours with osmium tetroxide unless otherwise indicated.

Cell Wall

In general the wall structure of the blue-green algae studied was in accord with the observations of several other workers (Ris and Singh, 1961; Pankratz and Bowen, 1963). The cell wall is composed of an outer sheath and a bipartite inner layer consisting of an outer membrane and an inner investment immediately outside the plasma membrane (Ris and Singh, 1961).

The sheaths of many of the blue-green algae examined contain fibers 4 to 5 mμ in diameter and of undetermined length (Figures 1, 32, 43, 57 and 73). Structure in the sheath of Nostoc carneum was more apparent in electron micrographs of material fixed with gluteraldehyde-osmium tetroxide (Appendix A, Schedule II) or by osmium tetroxide (Appendix A,

Figure 1. A chart showing the structures present in the blue-green algae examined.

BLUE-GREEN ALGAE	SHEATH STRUCTURE	OUTER MEMBRANE PAPILLAE	INNER INVESTMENT	STRUCTURED GRANULES	ALPHA GRANULES	BETA GRANULES	POLYHEDRAL BODIES	INTRA-THYLAKOIDAL INCLUSIONS	INTRA-THYLAKOIDAL SPACE	OTHER INCLUSIONS
Oscillatoria princeps	0[a]	0	400 mμ	X	X	X	X	0	Small	Pits in inner investment
Anabaena spiroides	0	130 X 40 mμ	9 mμ	X	I[b]	X	X	0	Large	Lamellasomes
Anabaena sp.	0	40 X 60 mμ	10 mμ	X	I	X	X	0	Medium	
Aphanizomenon flos-aquae	0	45 X 150 mμ	14 mμ	X	I	X	X	0	Large	Lamellasomes
Cylindrospermum sp.	0	0	8 mμ	X	I	X	X	0	Small	Filaments; Membrane stacks
Nostoc calcicola	X[c]	0	8 mμ	X	X	X	X	X	Small to medium	
Nostoc carneum	X	0	9 mμ	X	X	X	X	X	Small	Nucleoplasmic crystalloid; Granular areas
Nostoc commune	0	0	8 mμ	X	X	X	X	0	Small to medium	
Nostoc coeruleum	0	0	7 mμ	X	I	X	X	0	Small	Peripheral crystalloid; Lamellasome

[a] 0 indicates absent.

[b] X indicates present.

[c] I indicates insufficient observation.

BLUE-GREEN ALGAE	SHEATH STRUCTURE	OUTER MEMBRANE PAPILLAE	INNER INVESTMENT	STRUCTURED GRANULES	ALPHA GRANULES	BETA GRANULES	POLYHEDRAL BODIES	INTRA-THYLAKOIDAL INCLUSIONS	INTRA-THYLAKOIDAL SPACE	OTHER INCLUSIONS
Nostoc ellipsosporum	0	0	7 mμ	X	X	X	X	X	Small to medium	
Nostoc linckia	0	0	8 mμ	X	X	X	X	X	Small	Peripheral crystalloid; Spheres of membranes
Nostoc muscorum (ISU-J10)	0	0	6 mμ	X	X	X	X	X	Small	
Nostoc muscorum (ISU-J15)	X	0	7 mμ	X	I	X	X	X	Small	Spherical inclusions; Filements; Tubular-like system
Nostoc muscorum (ISU-J17)	0	40 X 50 mμ	11 mμ	X	I	X	X	X	Small to medium	
Nostoc pruniforme (ISU-J11)	0	0	8 mμ	X	X	X	X	X	Small to medium	Nucleoplasmic crystalloid; Tubular-like systems
Nostoc pruniforme (ISU-J12)	0	30 X 30 mμ	8 mμ	X	X	X	X	0	Small to medium	Plate-filament arrangement
Nostoc punctiforme	0	0	8 mμ	X	X	X	X	0	Small	
Plectonema boryanum	X	0	6 mμ	X	X	X	X	0	Small to medium	
Calothrix crustacea	X	0	8 mμ	X	X	X	X	X	Small	
Calothrix parietina	X	0	10 mμ	X	X	X	X	0	Small	

Figure 1 (Continued)

BLUE-GREEN ALGAE	SHEATH STRUCTURE	OUTER MEMBRANE PAPILLAE	INNER INVESTMENT	STRUCTURED GRANULES	ALPHA GRANULES	BETA GRANULES	POLYHEDRAL BODIES	INTRA-THYLAKOIDAL INCLUSIONS	INTRA-THYLAKOIDAL SPACE	OTHER INCLUSIONS
Gleotrichia pisum (ISU-J20)	X	O	14 mμ	X	X	X	X	X	Small to large	Rosettes; Scroll-like-membranous bodies
Gleotrichia pisum (ISU-J21)	X	O	11 mμ	X	X	X	X	O	Large	Filaments; Spherical bodies

Figure 1 (Continued)

Schedule I) when this latter fixation was followed by rapid dehydration consisting of a quick rinse in 50 percent ethanol after fixation and then by immediate immersion in 100 percent ethanol. Both methods revealed fibrillar layers 0.2 to 0.7 μ thick, each layer separated from the next by an osmiophilic layer about 0.1 μ thick (Figures 3 and 4). This organization of the sheath was only occasionally observed in material fixed in osmium tetroxide and dehydrated in the usual manner.

The layering present in the sheath of Nostoc carneum suggests that sheath material is laid down in a cyclic manner. One type of sheath component may be produced for a period followed by the deposition of a new component for a shorter period, resulting in the alternate layering observed. An alternative hypothesis is that the sheath is chemically homogeneous throughout but that the layering represents differential rates of sheath deposition which in turn results in differences in density.

The outer membrane, very close to or touching the inner investment, is from 8 to 14 mμ wide and has the tripartite appearance of a unit membrane (Figures 8, 16 and 25). However, unlike the usual unit membranes it is generally poorly preserved after potassium permanganate fixation (Figures 9 and 14). Previous workers have made similar observations (Ris and Singh, 1961; Pankratz and Bowen, 1963). In most forms the outer membrane exhibits wave-like undulations (Figures 8, 18 and 25). Echlin (1963) has observed that in Anacystis nidulans these undulations

may be quite pronounced and project 50 mμ into the sheath.

In addition to the regular undulations in the outer membrane, scattered long projections were observed in Nostoc pruniforme (ISU-J12), Nostoc muscorum (ISU-J17), Anabaena spiroides, Anabaena sp., and Aphanizomenon flos-aquae. These "papillae" are from 30 to 45 mμ wide and 30 to 150 mμ long (Figures 5, 6, 7, 44, and 61). In Anabaena spiroides the papillae consist of a short stalk and head which appears to have internal structure (Figure 7). The longer, 150 mμ, papillae in Aphanizomenon flos-aquae are circular in profile, about 45 mμ in diameter and contain a dense axial element, about 7 mμ in diameter (Figures 6 and 61).

It is interesting to speculate on the possible function of these papillae. They were present in large numbers on cells of Aphanizomenon flos-aquae. On occasion some cells of Nostoc pruniforme (ISU-J12) possessed them in large number but other cells in the same culture had few or none present. Colonies of Aphanizomenon flos-aquae exhibit movement in which filaments in one part of the colony move in one direction while those in another part of the colony are moving in the opposite direction. After a period of time this movement is reversed. It is possible that these projections have a role in the movement of the trichome within the sheath. The occasional appearance of large numbers of these projections in Nostoc pruniforme (ISU-J12) could be associated with

hormogonia formation. When these projections are observed in large numbers on a particular trichome it may have been in the motile stage when fixed and when few or none of these papillae are observed the trichome may be in the non-motile form in a colony. In Oscillatoria princeps no projections were observed despite the fact that filaments moved in a gliding manner. This was the only species of Oscillatoria investigated and pores were observed while no pores were observed in any of the other blue-greens studied. Dodd (1960) has suggested that movement in the Oscillatoria may be due to the secretion of sheath material which moves the trichome out of the sheath. Pankratz and Bowen (1963) have discussed the possible role of pores in producing the sheath material.

It is therefore possible that at least two methods of producing movement are present in the blue-greens, one involving papillae and another involving pores and the secretion of mucilage.

The inner investment is generally less than 15 mμ thick but actual thickness in mature walls depends on the species (Figure 1). The inner investment is poorly preserved by potassium permanganate fixation and is separated from the plasma membrane by a 30 to 60 mμ electron transparent space in all the species studied (Figures 14, 15, 18, 25 and 61). The wall of Oscillatoria princeps, however, was unusually thick, with an inner investment averaging 400 mμ wide (Figures 8 and 9). In this species there are pits containing cytoplasm projecting almost through the thick

investment. However, a thin layer of inner investment, at least 7 mμ thick, was always observed at the distal end of the pit. The pits are from 80 to 120 mμ in diameter, roughly circular in cross section and range up to 400 mμ deep (Figures 8, 9, 10 and 11). The pits are arranged in rows parallel and perpendicular to the crosswalls with an occasional pit displaced (Figures 10 and 11). The distance between centers of pits in these rows ranges between 200 and 280 mμ. The electron-density of the inner investment is lowest in the immediate vicinity of the pits (Figure 10).

Shinke and Ueda (1956) and Ris and Singh (1961) described the wall structure of Oscillatoria princeps and both groups interpreted the pits in the inner investment as pores. Examination of their published micrographs reveals much plasmolysis and consequent withdrawal of protoplasm from the interior of the pits. The plasmolysis and the fact that the majority of their sections were made in planes more or less oblique to the longitudinal cell walls may account for their observations.

Rows of fine pores were observed adjacent to the crosswalls in the inner investment of Oscillatoria princeps but not in any of the other blue-greens studied. The rows are double, each pore is about 15 mμ in diameter and the pores are spaced about 14 to 28 mμ apart (Figures 8 and 11). Previous reports of pores have been made by Drawert and Metzner (1956, 1958) in Cylindrospermum, Microcoleus and Oscillatoria and by

Pankratz and Bowen (1963) in Symploca muscorum.

The cell walls of gram-negative bacteria appear to have the same morphological organization as the cell walls of the blue-green algae. In these bacteria the cell wall consists of an outer membrane and a mucopolymer layer immediately outside of the plasma membrane (Claus and Roth, 1964). The mucopolymer is a macromolecule composed of muramic acid, glucoseamine, alanine, glutamic acid and diaminopimelic acid (Stent, 1963). Lysozyme removes this mucopolymer layer in bacteria producing a spheroplast in the case of gram-negative bacteria (Stent, 1963). The mucopolymer layer in blue-green algae can also be removed by lysozyme (Crespi, Mandivelle and Katz, 1962). Based on the observation that the mucopolymer layer of the gram-negative bacterial wall is poorly preserved by osmium fixation (for a recent discussion of this problem see Claus and Roth, 1964) Frank, Lefort and Martin (1962a) concluded that this component of the blue-green algal cell would also be poorly preserved. However, it is entirely possible that the inner investment of blue-green algal wall may contain the mucopolymer and that its relatively good preservation after osmium fixation is due to other components not present in the mucopolymer layer of the bacterial wall. The production of spheroplasts by lysozyme in blue-green algae would seem unlikely if the relatively thick inner investment remained intact.

Thylakoids, Membranes and Membrane-like Inclusions

The plasma membrane of all the species examined is, as previously reported (Lefort, 1960b), a unit membrane 8 mμ thick and consists of two outer electron-dense layers and a middle electron transparent layer (Figure 19). An infolding of the plasma membrane was occasionally observed in some cells of Nostoc calcicola, Nostoc commune and Nostoc punctiforme, particularly in older cultures (Figures 12 and 13). These usually appeared as continuities between the plasma membrane and the thylakoidal membranes as described in Symploca muscorum by Pankratz and Bowen (1963). Similar but much more elaborate membrane complexes were observed in cells of Nostoc coeruleum, Apahnizomenon flos-aquae and Anabaena spiroides (Figures 7 and 37). Complexes of this sort ranging up to 200 mμ in diameter were especially frequent in Nostoc coeruleum. Although actual connection to the plasma membrane was not demonstrable in every section, the fact that these structures were always located near the plasma membrane and that several sets of serial sections showed such connection, strongly suggests that these are elaborations of the plasma membrane.

These structures resemble the lamellasomes described by Echlin (1964) in Anacystis nidulans and as he pointed out they are similar to the bacterial mesosomes which are found primarily in the gram-positive bacteria. Bacterial mesosomes were considered reductive sites by Woutera and Leene

(1964). Cole (1964) has proposed that mesosomes may have a role in the partition of the replicated DNA during bacterial cell division. The fact that they are regularly found in only a few species of blue-green algae argues against their having the latter function in this group.

Photosynthetic lamellar units, the thylakoids, topologically consisting of separate closed membranous sacs, were found in all cells studied. Depending both upon the species and the conditions of culture, the thylakoids exhibited considerable variation in morphology (Figure 1). One variation involved the degree of flattening of the individual thylakoids. At one extreme, flattening is so complete that the appressed unit membranes formed a "myelin-like" configuration where in cross-section the adjacent electron-dense layers of the two unit membranes appear to fuse into a single wider electron-dense zone (Figures 19, 20 and 31). More commonly, the thylakoidal membranes are not tightly appressed. Although these elements are very flattened, a small electron-transparent intrathylakoidal zone is visible (Figures 12, 18 and 31). In some of the cells of certain species this zone in some thylakoids may be more or less expanded forming fairly large intrathylakoidal vesicles as described by Pankratz and Bowen (1963)(Figures 5 and 44). Examples of this whole range of thylakoidal flattening may occasionally be found in the same cell (Figure 44). It seems likely that these variations are dependent on the physiological state of the cells.

Thylakoids in cells of Aphanizomenon flos-aquae are characteristically tremendously expanded. In this species one half or more of the cell volume regularly consists of transparent intrathylakoidal space, giving the cells a highly vacuolate appearance (Figures 61 and 62). Flattened thylakoids were rarely observed. Similar but less extreme thylakoidal expansion was observed in some cells of Gleotrichia pisum (ISU-J20), Gleotrichia pisum (ISU-J21) and Anabaena spiroides (Figures 50, 56 and 47) but other cells in the same colonies had flattened thylakoids. It seems likely that this extreme dialation of thylakoids may be related to the planktonic habit. However, Oscillatoria princeps (Figure 8) and Microcystis sp. (Bowen[1]) may be planktonic and do not show such thylakoidal expansion.

In all species studied thylakoids tended to be independent of each other unlike other algae and higher plants. An exception to this was noted in Calothrix parietina where in several cells a stacked arrangement was observed (Figure 20). Although this is considered to be an abnormal situation it does show that unlike the lamellar grana of higher plants a distinct interthylakoidal space of about 3 mμ persists. This observation suggests a basic difference in the outer and inner protein layers of

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[1]Bowen, C. C. Department of Botany, Iowa State University, Ames, Iowa. Cytology of blue-green algae. Personal Communication. 1965.

the thylakoid membranes, perhaps due to particles of some sort on the outer surface of the membrane which physically prevent them from coming into direct contact with each other. One observes the consequent myelin-like organization of adjacent thylakoids.

In old cultures of Nostoc commune and Nostoc calcicola the thylakoids became contorted and fold out producing a branched appearance which can be very regular (Figures 14 and 19). This arrangement has been observed in Nostoc spp. by Menke (1961) and Wildon and Mercer (1963a), as well as Symploca muscorum recovering from high-light-induced chlorosis (Bowen and Pankratz, 1963).

Thylakoids assume elaborate patterns in some stages of heterocyst differentiation in Anabaena spiroides and Nostoc linckia (Figures 21, 22, 23 and 24). Studies of heterocysts carried out by Chapman and Salton (1962) in Anabaena cylindrica and by Wildon and Mercer (1963b) in Anabaena sp., Anabaena cylindrica, Fischerella muscicola, Nostoc sp. and Nostoc muscoum failed to show such patterns. However, it is possible that heterocyst development proceeds differently in different species. Hundreds of heterocysts were observed in the several species of Nostoc examined but the unusual thylakoidal morphology was observed only in heterocysts of Nostoc linckia.

Centrally located inclusions presenting a roughly circular profile varying from 200 to 400 mμ in diameter and surrounded by a unit membrane

and containing moderately osmiophilic material were observed in a number of the blue-greens examined (Figures 13, 14 and 15). Similar structures in Microcystis sp. are formed by the expansion of the end of a thylakoid and have been called intrathylakoidal granules (Bowen[1]). The inclusions observed in this study have not been observed in connection to the thylakoids, but are considered to be identical. This kind of inclusion, although not described by the authors, can be seen in published micrographs of Nostoc muscorum by Chapman and Salton (1962).

The intrathylakoidal granule is an electron-dense crystalline material in Nostoc muscorum (ISU-J17), Nostoc ellipsosporum and Nostoc muscorum (ISU-J10) (Figures 16 and 17). The crystals are needle-shaped and vary in dimension from 5 to 15 mμ wide and up to 40 mμ long. Unlike the intrathylakoidal granules which tend to be centrally located these crystalline inclusions generally are located peripherally. The number of intrathylakoidal inclusions per cell was quite variable but a few profiles of these were observed in every cell.

Unusual stacks of "membrane-like" structures were observed in Cylindrospermum sp. They ranged from 0.3 to 0.7 μ in their longest dimension and each consist of about 20 "membranes" lying parallel to one

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[1]Bowen, C. C. Department of Botany, Iowa State University, Ames, Iowa. Cytology of blue-green algae. Personal Communication. 1965.

another but separated by a space of about 6 mμ (Figure 25). Each individual "membrane" was about 8.5 mμ thick and had the tripartite appearance of a unit membrane. Only four of these previously unreported inclusions were observed in the hundreds of cells studied.

While these "membranes" resemble those of the thylakoids they are not as electron-dense. Unlike thylakoids each individual "membrane" in these unusual inclusions does not form a more or less flattened closed vesicle but rather forms a simple disc with the "membrane" apparently terminating at the disc's edge. These were not observed in potassium permanganate fixed Cylindrospermum sp. cells from the same collection. The low frequency of their occurrence, however, could account for their not being seen.

Unusual "spherical membrane" systems were observed in all vegetative cells and heterocysts of Nostoc linckia with up to 30 such structures observed in a section of a single cell (Figures 26 and 27). They are from 0.2 to 0.6 μ in diameter, and must consist of at least 3 long but relatively narrow membranous sheets each about 9 mμ thick and resembling a unit membrane. Topologically these long thin "membranes" must be wound in three dimensions around a common center to result in a spherical inclusion. The "membranes" appear to fuse to one another in many instances but the individual "membranes" are about 10 mμ apart and appear to branch (Figure 27). No "membranes" extend into the central

area of the sphere which is about 0.1 to 0.3 μ in diameter. However, α granules, β granules and ribosomes are found in this zone. In a number of cases the spherical membrane systems appear to be fused to each other in which case the membranes of the outer partial spheres appear to end at the surface of the inner sphere (Figure 27).

Unusual "scroll-like membrane systems" were observed in heterocysts of Gleotrichia pisum (ISU-J20) (Figures 28 and 29). These were 0.4 to 0.9 μ in diameter and up to 2 μ in length. In cross section these bodies were seen to be composed of an unbranched tripartite membrane 11.5 mμ thick arranged in a spiral with 5 to 13 turns. The membranes are about 150 mμ apart and like the previously described spherical membrane systems do not extend into the central zone, about 0.3 μ in diameter, where α granules, β granules, ribosomes and even occasional thylakoids occur. When these inclusions are sectioned longitudinally (Figure 29), it is apparent that the general shape is cylindrical with one membrane per inclusion arranged as in a scroll. The individual membranes are striking in appearance and quite different from membranes previously described because of the prominent and precisely defined central electron-transparent zone 5.5 mμ wide. The adjacent electron-dense zones or layers of the membrane are asymmetrical with the zone oriented to the outside of the inclusion 3.5 mμ in width and the inner zone 2.5 mμ wide. At the membrane "edges" at the ends of the cylinder and at the inner

and outer ends of the "scroll" the membranes simply terminate and there is no continuity of connection between the two electron dense zones of a single membrane. Ribosome-like particles, 16 mμ in diameter, are regularly arranged between the membranes and appear to be in close contact with the innermost electron dense zone of the membrane (Figure 28). A number of these bodies are located in each heterocyst and up to 8 have been observed in a single section of one heterocyst. Observation of these heterocysts with the light microscope reveals light elongate areas scattered throughout the still green heterocysts. Perhaps these areas correspond to scroll-like membranous bodies because similar areas were not observed in vegetative cells.

These unusual "membrane" systems are unique in that biological membranes, in general, have no ends or edges. The blue-green algae are a very ancient group of organisms and it is possible that these variations in cytology have evolved slowly and with great diversity. It is equally possible that some of these unusual membranes are evidences of infections by a virus, a symbiont or a commensal. There is really no evidence one way or the other and work involving cross infection techniques, enzymatic digestion and speration and chemical analysis of these components needs to be carried out.

Crystalloids

Regions showing a high order of periodic structure with several different patterns of organization were observed with varying regularity in some species of blue-green algae (Figure 1). They were located in the central part of the cell in some species and in the peripheral cytoplasm in others but were always interthylakoidal. Those found in the central part of the cell were generally elongate in profile and were associated with the nucleoplasm and polyhederal bodies. Crystalloids found in the peripheral cytoplasm tended to be rectangular in profile. In general all crystalloids stained densely with uranyl acetate after osmium fixation. Preservation ranged from indifferent to poor with potassium permanganate fixation.

The crystalloids termed "lamellar bodies" by Jensen and Bowen (1961) were again found in Nostoc pruniforme (ISU-J11). This was the same material studied previously and had been maintained in culture at Iowa State University for over 4 years. Very similar structures were found in Nostoc carneum but not in any other Nostoc spp. including Nostoc pruniforme (ISU-J12). They were present in about 1 out of every 10 cells which were sectioned medially.

The crystalloids in Nostoc pruniforme (ISU-J11) were from 0.1 to 1 μ long and about 130 mμ wide (Jensen and Bowen, 1961). The electron-dense striations, at right angles to the longitudinal axis were 4 mμ across

and spaced 15 to 30 mμ apart (Figures 30 and 33). In some material finer lines of the inclusion parallel to the long axis were observed between the striations (Figure 30). These lines were 2.8 mμ wide and 5.6 mμ apart. The crystalloids were observed quite regularly with their ends in contact with polyhederal bodies (Figures 30 and 33).

In Nostoc carneum the crystalloids were from 0.5 to 1 μ long and were 90 to 180 mμ wide (Figures 31 and 34). Like the crystalloids of Nostoc pruniforme (ISU-J11) one or both ends of the crystalloids were often seen to be in contact with polyhederal bodies. These crystalloids though similar in appearance to those in Nostoc pruniforme (ISU-J11) had striations about 4 mμ wide at right angles to the long axis, which were spaced from 10 to 15 mμ apart (Figure 31). Finer lines, between the striations and parallel to the long axis, were observed in some cases (Figure 31). These lines 2 mμ wide were spaced about 3 mμ apart.

Variation in spacing in these crystalloids depends on how close the section plane is to the crystalloid axis. However, the difference in spacing between Nostoc pruniforme (ISU-J11) and Nostoc carneum appears to be a valid difference.

In Nostoc pruniforme (ISU-J11) poorly defined organized areas, about 1 μ across and roughly square in profile, were occasionally observed in the central part of the cell (Figure 35). Striations about 3 mμ wide and spaced about 20 mμ apart with similar striations crossing these at an

angle were observed within the organized area.

Crystalloids in the peripheral cytoplasm were observed in Nostoc linckia, Nostoc coeruleum and Gleotrichia pisum (ISU-J20).

In Nostoc linckia the crystalloids, roughly square in profile, varied from 70 to 800 mμ in their longest dimension although one was observed as large as 1 by 2 μ. They were seen in about 1 out of every 10 sections of cells examined which suggests that they were present in most cells. The crystalloids in profile exhibited electron-dense parallel striations 4 mμ wide which were spaced 6 mμ on centers (Figures 27 and 36). In some cases the crystalloid appeared compound and seemed to consist of several smaller crystalloids each with its parallel striations at a different angle (Figure 36).

Crystalloids in Nostoc coeruleum were quite variable in size with the smallest being about 400 by 400 mμ in profile and the largest 700 by 800 mμ in profile. They were observed in about 1 out of every 10 sections of cells. Although somewhat variable in appearance, in profile these always exhibited dense striations 4 mμ wide spaced about 9 mμ on centers. In some of these crystalloids the only striations were parallel, in others one set of parallel striations was crossed by a similar set of striations (Figures 37, 38, 39, 67 and 68).

In Gleotrichia pisum (ISU-J20) crystalloids of varied appearance were observed. Roughly square profiles with dense parallel striations,

4 mμ wide, spaced about 6 mμ apart on centers were and most frequently observed (Figure 40). In some similar profiles striations were not clearly evident. Profiles about 500 by 700 mμ were observed twice with a crosshatched appearance consisting of two sets of 7 mμ electron dense parallel lines spaced 10 mμ apart on centers which intersect with each other at right angles (Figure 41).

In general these crystalloids in Gleotrichia pisum (ISU-J20) were difficult to identify in the electron microscope because on the fluorescent screen they were about the same size and had the same general density as polyhedral bodies. It was necessary to take micrographs to ascertain if one were observing a polyhedral body or a crystalloid. Because polyhedral bodies are found in large numbers in cells it is certain that crystalloids in this species were of more frequent occurrence than the 1 cell in 20 in which they were actually detected in the sections.

It is of interest to compare the crystalloids observed in these blue-green algae with similar structures seen in other cells (Figure 2). The crystals described by Schnepf (1964) and Gezelius (1959) are similar in appearance and have about the same lattice spacing as the peripheral crystals reported here in Nostoc linckia and Nostoc coeruleum. The protein crystals described by Roth and Porter (1964) are also similar in appearance and lattice spacing to the compound crystalloids observed in Nostoc coeruleum except that in the latter there is no surrounding

Figure 2. A chart showing the location, composition and periodic spacing of some biological crystals.

AUTHOR	ORGANISM OR TISSUE	LOCATION	COMPOSITION	SPACING
Gezelius (1959)	Dictyostelium	Cytoplasm	Unknown	9 mμ
Schnepf (1964)	Lathraea	Cytoplasm of leaf parenchyma cells	Unknown	7 mμ
Tandler and Shipkey (1964)	Warthin's tumor	Vesicle in cytoplasm	Unknown	6 to 9 mμ
Roth and Porter (1964)	Aedes aegypti L.	Vesicle in cytoplasm of oocyte	Protein	11 mμ
Thornton and Thimann (1964)	Avena sativa	Vesicles in cytoplasm of coleoptile cells	Unknown	12 to 16 mμ
	Acer rubrum	Vesicles in cytoplasm of phloem parenchyma cells	Unknown	20 mμ
	Eucalyptus camaldulensis	Vesicles in cytoplasm of cultured cells	Unknown	16.6 mμ
Cronshaw (1964)	Avena sativa	Vesicles in cytoplasm of coleoptile cells	Unknown	15 mμ
Fujita (1964)	Rat	Mitochondria in thyroid follicular cells	Unknown	10 mμ
Crawley (1964)	Acetabularia	Vacuole	Polymerized 3-sub. derivative of indole	20 to 40 mμ
Stuart, Fogh and Plager (1960)	FL cells infected with virus	Cytoplasm	Virus	17 mμ dense granules
Dales and Franklin (1962)	L cells infected with virus	Cytoplasm	Virus	16 to 25 mμ dense granules
Strunk (1959)	Midge	Golgi of midgut gland cells	Protein	9 mμ dense granules

AUTHOR	ORGANISM OR TISSUE	LOCATION	COMPOSITION	SPACING
Engelbrecht and Esau (1963)	Beet infected with beet yellows virus	Chloroplast	Virus?	7 mμ
Hannay and Fitz-James (1955)	Bacillus thurengiensis	Cytoplasm	Protein	35 mμ
Schwartz and Zinder (1963)	Escherichia coli	Cytoplasm of cells infected with bacteriophage f2	Virus	17 mμ dense granules

Figure 2 (Continued)

membrane. The crosshatched crystals observed in Gleotrichia pisum (ISU-J20) are identical in appearance and lattice spacing to those observed by Tandler and Shipkey (1964) except the latter are membrane limited.

The crystalloids in Gleotrichia pisum (ISU-J20) with parallel striations, although not membrane limited, are similar in appearance but with a different spacing than crystals observed in vesicles by Cronshaw (1964) and Thornton and Thimann (1964). Thornton and Thimann (1964) suggest the crystals they observed may be involved in the phototropic response while Cronshaw (1964) postulates that such membrane limited crystals are storage organelles, lysosomes, and contain hydrolytic enzymes since lysosomes of rat liver cells contained similar crystals (DeDuve, 1960).

Viruses are known to form intracellular crystalline aggregates, however these generally appear in profile as a series of dense granules each about 10 percent smaller than the virus particle itself. In most cases the spacing is about 20 mμ on centers (Stuart, Fogh and Plager, 1960; Dales and Franklin, 1962). All crystals which appear granular, however, are not viruses. Strunk (1959) reported a protein crystal rich in iron from the Golgi of the midgut gland of a midge which was composed of 6 mμ granules spaced 9 mμ apart. The eyespot of Euglena is also composed of 100 to 300 mμ granules which are tightly packed in a hexagonal

pattern giving the appearance of a crystal (Wolken, 1956). Engelbrecht and Esau (1963) observed a crosshatched crystal with a 7 mμ spacing in chloroplasts of beets infected with beet yellows virus. These resemble the crystalloids observed in Nostoc coeruleum as well as the crosshatched crystalloid in Gleotrichia pisum (ISU-J20).

Sections of cells of Escherichia coli infected with the bacteriophage T2 do not exhibit crystalloid arrays but infected cells show an increase in the number of 2 mμ fibers observed in the nucleoplasm of uninfected cells. The phage heads only become visible later (Kellenberger, Ryter and Sechaud, 1958). However, more recently Schwartz and Zinder (1963) have described a crystalline array composed of dense granules spaced 17 mμ apart in Escherichia coli cells 50 minutes after infection with the RNA containing bacteriophage f2.

Preliminary efforts to infect cultures of Nostoc calcicola, Nostoc muscorum (ISU-J10), Nostoc commune, Nostoc ellipsosporum, Nostoc punctiforme and Nostoc pruniforme (ISU-J12) in which crystalloids were not observed with the crystalloid found in Nostoc pruniforme (ISU-J11) were unsuccessful.

The composition and function of these inclusions will have to be solved using separation techniques followed by chemical analysis or by new methods in cytochemical analysis at the electron microscope level.

Filaments

Five different types of unusual filamentous elements were observed in the cytoplasm of Nostoc pruniforme (ISU-J12), Nostoc pruniforme (ISU-J11), Cylindrospermum sp., Nostoc muscorum (ISU-J15) and Gleotrichia pisum (ISU-J21) respectively.

In Nostoc muscorum (ISU-J15) the filaments were in packets roughly parallel to one another and profiles of such packets ranged up to 0.4 μ across (Figure 43). These filaments were about 0.3 μ long and 7 mμ in diameter and were located in the interthylakoidal spaces of the cell, generally near the cell wall. Observed in about 1 out of every 20 sections of cells examined, these seemed to be more common in older cultures.

Filaments about 5 to 7 mμ in diameter and of undetermined length were observed in bundles in Cylindrospermum sp. (Figures 48 and 49). Profiles of some filament-bundles covered areas as large as 2 by 0.4 μ but most were smaller.

In Nostoc pruniforme (ISU-J12) an unusual arrangement of filaments in association with a plate-like membranous structure was regularly observed (Figures 44, 45, 46 and 47). The plates were always located close to the crosswall and usually parallel to it. Roughly circular, about 1.2 μ in diameter and 19 mμ thick, these plates in cross section appeared to consist of 3 layers, 2 outer electron-dense layers about 7 mμ thick and

an inner more electron-transparent layer about 5 mμ thick (Figure 44). Filaments about 14 mμ in diameter and from 240 to 340 mμ in length project out from the plate at right angles. In cross section these filaments are spaced 7 mμ apart in a repeating hexagonal pattern with an occasional filament missing (Figures 45 and 47). Each filamentous element appears to have an electron-transparent inner core about 7 mμ in diameter which is surrounded by an electron-dense layer 3.5 mμ thick. With potassium permanganate fixation only the plate is preserved (Figure 46). The plate and filament arrangement was observed in almost every cell when the section was cut in a median longitudinal plane.

Unusual arrays of filaments in Gleotrichia pisum (ISU-J21) more or less completely fill certain interthylakoidal regions which range in size to over 1 μ in length (Figures 50, 51 and 52). In longitudinal section groups of parallel filaments 140 to 260 mμ across are seen within each large complex. The filament groups are separated by a thin layer of ribosome containing cytoplasm about 20 mμ across (Figures 50 and 51). Five to 13 groups of filaments are seen in each profile of a large inclusion. The individual filaments, are arranged roughly in rows in each group, were 8.4 mμ in diameter and consist of a dense outer layer 2.8 mμ across and a lighter 2.8 mμ central portion (Figure 52). A central dark strand 1.5 mμ in diameter is observed in the center of filaments cut in cross section (Figure 52). These inclusions were

observed in about 1 out of every 10 sections of cells examined. Even more regularly spaced filaments of quite different appearance are frequently found in association with the former filaments. These 15 mμ diameter filaments are quite electron dense, spaced about 14 mμ apart on centers and arranged in a repeating hexagonal pattern (Figures 50 and 52). Such aggregates typically occupy areas about 0.1 μ to 0.5 μ across in profile.

Occasionally sections of Nostoc muscorum (ISU-J15) and Nostoc pruniforme (ISU-J11) show roughly circular profiles of tubular-like systems up to 0.4 μ in diameter (Figures 53, 54 and 55). They consist of electron-transparent elongate elements 7 mμ in diameter and are separated from one another by common 7 mμ electron dense walls. The individual elements are arranged in 3 dimensional whorled and convoluted patterns. Recently Gunning (1965) has described similar structures in the chloroplasts of Avena sativa. He calls them "stromacenters" and believes they are aggregates of fibers 8.5 mμ in diameter and of undetermined length.

No filamentous components have been previously described in prokaryotic cells except for the bacterial flagella. However, in eukaryotic cells microtubular filaments 14 to 25 mμ in diameter have been observed in connection with the mitotic apparatus and in the cytoplasm between divisions (Ledbetter and Porter, 1963; De-The, 1964).

A variety of functions have been suggested for these filaments some of which are chromosome movement, involvement in cytoplasmic streaming and cell movement, deposition of cell wall materials and maintenance of cell shape (Ledbetter and Porter, 1963; De-The, 1964). Filamentous material with a diameter of about 7 mμ has been reported in the cytoplasm of mononuclear phagocytes (DePetris, Karlsbad and Pernis, 1962).

The function of filaments seen in blue-green algae which similarly range from 5 to 15 mμ in diameter is not clear. How fundamental are the differences both between the varied filamentous structures seen in blue-green algae and between these and similar structures in higher cells? If the filaments in blue-green algae functioned in the equational division of the nucleoplasm, movement, wall deposition or maintenance of cell shape one would expect them to be generally present in all cells of blue-green algae. The position of the unusual plate-filament arrangement in Nostoc pruniforme (ISU-J12) at the crosswalls of adjacent cells suggests that they may have a function in cell division. However, other species do not possess such structures. Nostoc pruniforme (ISU-J12) grows fair in soil water media and grows poorly or not at all in other media tried. It is possible that the plate-filament arrangement is some sort of intracellular parasite.

Other Inclusions

In 1 out of every 100 cells of Nostoc muscorum (ISU-J15) examined, the intrathylakoidal spaces contained a cluster of 10 or more spheres (Figure 42). Each sphere is about 30 mμ in diameter with an outer 3 mμ dense layer.

Rosette-like inclusions 85 to 90 mμ in diameter were observed in Gleotrichia pisum (ISU-J20) (Figure 56). In sections through the centers of these inclusions they appear in profile to be composed of a round central 30 mμ element surrounded by 5 round less electron-dense 30 mμ elements (Figure 56). These structures are interpreted as being aggregates of eight, probably similar, spherical elements, one being in the center and seven packed around it. The individual spheres closely resemble the spherical inclusions just described in Nostoc muscorum (ISU-J15). The relative electron-dense appearance of the center sphere may simply be due to the fact that superimposed on it are profiles of the spheres above and below it in the section. These rosette-like elements appear to be present in all cells with at least a few being observed in every intrathylakoidal space. Careful study of cells of Gleotrichia pisum (ISU-J20) in a collection made in August, 1964, from Little Wall Lake, did not reveal these inclusions. However, in collections made in September, 1963, and October, 1964, from the same area these inclusions were observed in large numbers.

In Nostoc carneum granular areas of medium electron-density and with a suggestion of about a 17 mμ periodicity were observed in the peripheral cytoplasm between the thylakoids (Figure 32). In profile these areas covered areas up to 0.5 by 1 μ.

Spherical bodies from 0.7 to 1 μ in diameter with at least two different components were regularly observed in cells of Gleotrichia pisum (ISU-J21) (Figure 57). In profiles of these medium electron-dense, slightly granular, inclusions less dense circular areas up to 280 mμ in diameter can be seen. These lighter regions appear to be filled with highly convoluted filamentous elements, about 15 mμ in diameter. These interthylakoidal bodies are present in about 1 out of every 5 sections of cells examined.

It is possible that the rosettes in Gleotrichia pisum (ISU-J20) and the spherical structures in Nostoc muscorum (ISU-J15) represent viral inclusions. Rosettes are in the size range reported for intracellular bacteriophage heads (50 x 70 mμ) (Kellenberger, Ryter and Sechaud, 1958) while the spherical inclusions in Nostoc muscorum (ISU-J15) are somewhat smaller.

The frequency of occurrence of the granular areas in Nostoc carneum and the spherical inclusion in Gleotrichia pisum (ISU-J21) suggests they may be storage products. The large granules observed in Gleotrichia pisum (ISU-J21) should serve as a reminder to cytologists that one cannot

assume that the largest granules, visible in blue-green algae cells with the light microscope, always represent the cyanophycean granules.

Gas Vacuoles

In control cells of Aphanizomenon flos-aquae, gas vacuoles are visible in the light microscope (Figure 58). In electron micrographs of fixed material, ordered parallel arrays of electron-transparent cylindrical vesicles are visible in interthylakoidal regions of the cells (Figure 61). Individual vesicles are from 0.1 to 1 μ in length and about 75 mμ in diameter, have conical ends, and are bounded by a membrane which appears in profile as a single electron-dense line 2 mμ wide (Figures 61 and 63).

Cells of Aphanizomenon flos-aquae that are examined shortly after being subjected to pressure have a 28 percent smaller volume than they have prior to treatment (Figure 59). In these cells no gas vacuoles are visible under the light microscope (Figure 59). After pressure was applied the colonies sank to the bottom of the container and changed from light green to dark green. The arrays of electron-transparent vesicles seen in electron micrographs of control material are conspicuously absent in cells fixed immediately after pressure treatment (Figures 62 and 64). Instead, many short membranous elements, each 6 mμ wide and 200 mμ or more in length and resembling a unit membrane, are seen in the regions of the treated cells where vesicles appeared in control cells (Figures

62 and 64).

Gas vacuoles are visible in the light microscope after 9 hours of recovery and are extensive after 24 hours of recovery from pressure treatment. Electron micrographs of cells allowed to recover for 9 hours show some scattered electron-transparent vesicles in the locality of the 6 mμ elements. Cells allowed to recover for 24 hours show fairly extensive arrays of such vesicles (Figure 65). Control material from each time period possess the electron-transparent vesicles.

Cells of Nostoc coeruleum and Nostoc pruniforme (ISU-J11) which have gas vacuoles present when examined in the light microscope also have the scattered electron-transparent vesicles when examined with the electron microscope (Figures 39, 67 and 68). Cells of these two species which do not have gas vacuoles in the light microscope also do not possess the electron-transparent areas when examined with the electron microscope (Figure 15). Unlike usual membranes the membranes of the gas vacuoles are not preserved by potassium permanganate fixation (Figures 38 and 66).

From these observations it is concluded that the aggregates of electron-transparent vesicles observed in electron micrographs represent the gas vacuoles observed by light microscopists (Bowen and Jensen, 1965). It is further concluded that each vesicle, a "gas vesicle", can somehow collect a gas and prevent its diffusion into the cytoplasm and that these gas vesicles can reversibly collapse and expand. The membranes making up

the gas vesicles (about 2 mμ thick) have a smaller thickness than any membrane reported in biological material to date.

Recently, Stanier[1] has shown that similar vesicles are present in Pelodictyon (a green bacterium) and Thiodictyon elegans (a purple sulfur bacterium). These organisms also possess gas vacuoles when examined with the light microscope.

Enzyme Digestion

In preliminary efforts to characterize some of the components of Nostoc pruniforme (ISU-J11) cells were treated with several enzymes. In material that was fixed in gluteraldehyde (Appendix A, Schedule II), sectioned and digested with pepsin for 24 hours the only cell components showing significant change were the structured granules (Figure 69). In the absence of osmium tetroxide fixation the centers of structured granules in control cells appear poorly preserved and suggest poor infiltration of the embedment, although they show some relatively electron-dense peripheral regions (Figure 70). The structured appearance of these granules after osmium tetroxide fixation was not evident after gluteraldehyde fixation. In sections treated with pepsin structured

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[1] Stanier, Germaine. Department of Bacteriology, University of California, Berkeley, California. Discussions on the cytology of bacteria. Personal Communication. 1965.

granules did not show the electron-density observed in controls. It is difficult to know how much weight must be given to the fact that other structures in the cell such as polyhedral bodies were not affected by this pepsin treatment. The protein components of most of the cell may well have been protected from enzyme attack by the surrounding epoxy embedment. The structured granules on the other hand, appear poorly infiltrated and hence exposed to enzyme attack. This may account for the fact that preliminary attempts to treat these cells with ribonuclease did not result in removal of ribosomes. Fuhs (1963) reported that material fixed in formaldehyde, embedded in epoxy resin, sectioned and then exposed to enzymes was not digested. However, Giesy (1964) succeeded in removing the α granules in material which was fixed in osmium tetroxide and embedded in an Araldite-Epon mixture. He treated sections with hydrogen peroxide, hydrochloric acid and pepsin which resulted in removal of the α granules.

In material fixed for 1 hour in gluteraldehyde and then digested in pepsin before embedding the structured granules were essentially removed after 4 hours of digestion except for small, relatively electron-transparent remnants (Figure 72). In control material, treated with 0.01N hydrochloric acid but without the enzyme, the structured granules were also much affected. Some relatively electron-dense fragments and a fibrous residue can still be seen (Figure 71). Tissue which remained in 0.1M

phosphate buffer at pH 7.2 for 4 hours showed relatively well preserved structured granules (Figure 73).

These results suggest that structured granules probably contain a proteinaceous component as proposed by other workers (Smith, 1950; Fritsch, 1951; Fogg, 1951). Interpretation of results of proteolytic digestion of cells before embedment is equivocal; however, it seems worthy of note that the crystalloids and tubular systems were apparently unaffected by either the dilute hydrochloric acid or pepsin (Figure 72), suggesting that these structures are not primarily proteinaceous.

General Discussion

On the basis of results presented in this study it is of interest to speculate on the relationship between bacteria and the blue-green algae. The blue-greens and bacteria both have a prokaryotic cellular organization. The typical organelles of higher plants are absent and the nuclear material is not set off from the rest of the cytoplasm by membranes. Gas vacuoles found in both groups are aggregates of similarly organized gas vesicles. Diaminopimelic acid is present in the cell wall of both groups. The morphology of the cell wall in the gram-negative bacteria and blue-greens is also similar. One major difference is the fact that the photosythetic pigments of bacteria are bacteriochlorophyll whereas the blue-green algae have chlorophyll a as do higher plants.

In all studies of blue-green algae to date in which cellular organization is clearly discernible the protoplasm has been shown to contain polyhedral bodies associated with the nucleoplasm. No similar structures have been observed in any bacteria. These bodies appear to be a consistent inclusion within the cells of filamentous forms while their presence has been shown in coccoid forms so far investigated. It would therefore be of utmost significance to know whether polyhedral bodies exist in the remaining groups of blue-green algae not yet studied with the electron microscope.

A variety of previously unreported inclusions of unknown function have been observed, many unique to a single species or collection. What is their significance? It has been suggested that some of the complex membranous, filamentous and crystalline inclusions may represent viral commensals. If this is the case they are quite different from previously described viruses or commensals. Unfortunately crude attempts to infect related blue-greens with such elements were unsuccessful, but this should be pursued further. On the other hand, the blue-green algae species studied are members of a very ancient group. In eons of time each species may have slowly evolved along a different line to the extent that these inclusions may represent real "organelles", some of which may no longer be functional.

The range of organisms containing microtubules has been extended to the blue-green algae. The presence of these filamentous structures in the cytoplasm of blue-green algae is of considerable interest. Although they are of the same general dimensions as the bacterial flagellum, comparable structures have not been observed in the cytoplasm of bacteria.

Several of the "membrane" systems observed here should be of great interest to those who have attempted to make models of biological membrane organization. The membrane of the gas vesicle (2 mμ) is much thinner than any previously reported membrane and, unlike other biological subcellular membranes, does not have a tripartite appearance. Is this membrane composed of a single layer of lipoidal molecules? When the gas vesicle is collapsed the two appressed membranes have the appearance and dimensions of a single unit membrane. Perhaps further investigation of these membrane systems will suggest modifications of the classical Danielli generalized model of the unit membrane. What configuration of molecules results in the impermeability of these thin membranes to a gas and the consequent retention of this gas? What is the gas contained in the gas vesicles and how is it evolved? How do these membrane systems originate and are they initially derived from other membrane systems of the cell? These are some of the intriguing questions opened up by the study of the gas vacuoles.

In several cases branchings and endings of membrane profiles were observed. Fusion and subsequent breakage of membranes must be directly connected to the phenomena of pinocytosis, formation of vesicles from Golgi, discharge of materials through the cell membrane and the development of thylakoids in chloroplasts. However, these phenomena have never been observed probably because they occur very rapidly much like the fusion and breakage of soap films. It is possible that the membrane systems in the blue-green algae, where branching and ends of membranes are seen, are very different than the typical unit membrane. Certainly no branching or breaks have been observed in the membranes of the thylakoids or the plasma membrane in this group of organisms. Perhaps the most interesting and potentially informative observation made here has been the wide range and variation of membrane organization in the blue-green algal cell. In general, the membrane systems of prokaryotic cells have been considered to be very simple and membrane-limited organelles almost non-existent.

The fact that two collections were quite different under the electron microscope yet each keyed out to "Nostoc pruniforme" is just one of several observations suggesting the potential value of electron microscopy in taxonomic studies of the blue-green algae.

SUMMARY

1. A number of species of blue-green algae in the order Nostocales were studied by means of electron microscopy. Most of the collections were fixed in osmium tetroxide and embedded in Epon. The sections were stained with lead salts or uranyl acetate.

2. The cell wall consists of a sheath and an inner complex layer consisting of an outer membrane and an inner investment located immediately outside the plasma membrane. The outer membrane forms papillae in Aphanizomenon flos-aquae, Anabaena spiroides, Anabaena sp., Nostoc pruniforme (ISU-J12) and Nostoc muscorum (ISU-J17). The possible function of these structures in producing movement is discussed.

3. The inner investment of Oscillatoria princeps was unusually thick, 0.4 μ, with pits of cytoplasm projecting into the layer. Pores at an angle through the inner investment over the crosswalls were also present. The possibility that the inner investment of blue-green algae is equivalent to the mucoplymer layer in the gram-negative bacteria is discussed.

4. The plasma membrane, a unit membrane, was observed to form invaginations in Nostoc calcicola, Nostoc commune and Nostoc punctiforme. Lamellasome-like structures were observed in Nostoc coeruleum, Aphanizomenon flos-aquae and Anabaena spiroides.

5. The cells examined contained the usual inclusions reported

for the blue-green algae. These inclusions are thylakoids, α granules, β granules, structured granules, ribosomes, polyhedral bodies and nucleoplasm. However, a number of new inclusions are described.

6. Variation in thylakoid morphology is described. In some species the end of a thylakoid expands to form an intrathylakoidal granule which may be composed of a material which is of medium electron-density or needle-shaped crystals.

7. Stacks of "membrane-like" structures were observed in Cylindrospermum sp. They consist of about 20 "membranes" lying parallel to one another but separated by a space of about 60 mμ.

8. Unusual "spherical membrane" systems were observed in vegetative cells and heterocysts of Nostoc linckia. They consist of at least 3 long but relatively narrow membranous sheets each about 9 mμ thick and resembling a unit membrane. These long thin membranes are wound in three dimensions around a common center resulting in a spherical inclusion.

9. Unusual "scroll-like membrane systems" were observed in heterocysts of Gleotrichia pisum (ISU-J20). These bodies, about 0.4 to 0.9 μ in diameter and 2 μ in length, are composed of a tripartite membrane 11.5 mμ thick arranged as in a scroll with 5 to 13 turns.

10. Elongate crystalloids, in profile, associated with the nucleoplasm were observed in Nostoc carneum and Nostoc pruniforme (ISU-J11). Although they had the same overall appearance the striations

at right angles to the long axis were spaced 10 to 15 mμ apart in Nostoc carneum and 15 to 30 mμ apart in Nostoc pruniforme (ISU-J11).

11. Crystalloids which are roughly square in profile were observed in Nostoc linckia, Nostoc coeruleum and Gleotrichia pisum (ISU-J20). These crystalloids are compared to similar crystals observed in other cells. The possibility that some of the crystalloids are viruses is discussed.

12. Five different types of unusual filamentous elements were observed in Nostoc pruniforme (ISU-J12), Clyndrospermum sp., Nostoc muscorum (ISU-J15), Gleotrichia pisum (ISU-J21) and Nostoc pruniforme (ISU-J12) respectively. Comparison of these 5 to 15 mμ filaments to filaments found in other cells is made.

13. In Nostoc muscorum (ISU-J15) clusters of small spherical inclusions about 30 mμ in diameter were observed in vacuole-like intrathylakoidal spaces. Rosette-like elements were observed in intrathylakoidal spaces of Gleotrichia pisum (ISU-J20). They consist of a central 30 mμ sphere which is probably surrounded by 7 similar spheres.

14. Granular areas of medium electron density with a suggestion of 17 mμ periodicity were observed in the peripherial cytoplasm between the thylakoids in Nostoc carneum.

15. Spherical bodies from 0.7 to 1 μ in diameter with at least two different components were regularly observed in cells of Gleotrichia

pisum (ISU-J21). These bodies are of medium electron-density and contain less dense spherical inclusions filled with highly convoluted filamentous elements.

16. From experiments using pressure to destroy the gas vacuoles it is concluded that the gas vacuole observed in the light microscope is an aggregate of gas vesicles which can collect a gas and prevent its diffusion into the cytoplasm. The gas vesicles can reversibly collapse and expand. The biological significance of these membranes and other membrane systems observed in this study is discussed.

17. Preliminary enzyme digestion experiments indicate that structured granules have a proteinaceous component.

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APPENDIX A: FIXATION, DEHYDRATION, AND EMBEDDING SCHEDULES

Schedule I. Osmium fixation.

A. Three hours at room temperature in one percent osmium tetroxide in Michaelis buffer modified from Kellenberger, Ryter and Sechaud (1958) and Pankratz and Bowen (1963) as follows:

1. 5 cc of Michaelis buffer (a. below)

   7 cc of 0.1N hydrochloric acid

   13 cc of distilled water

   0.25 cc of a 1M calcium chloride solution

   pH = 6.1 to 6.2

2. Dilute (1) above 1:1 with 2 percent osmium tetroxide

3. Add 0.1 ml of 1 percent Bacto-tryptone solution (b. below) per ml of fixative

   a. Michaelis buffer

      1.94 grams of sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)

      2.94 grams of sodium veronal (Barbital)

      3.4 grams of sodium chloride

      add water to 100 cc and store in refrigerator

   b. Bacto-tryptone solution

      1 gram of Bacto-tryptone

      0.5 grams of sodium chloride

      add water to 100 cc

B. Dehydration and embedding modified from Luft (1961) as follows:

1. 5 minutes each in 50, 70 and 95 percent ethanol at room temperature
2. 3 changes of 5 minutes each in 100 percent ethanol at room temperature
3. 3 changes of 5 minutes each in propylene oxide at room temperature
4. 15, 30 and 45 minutes in 1:3, 1:1 and 3:1 mixtures of Epon (with accelerator) to proplyene oxide respectively at room temperature
5. 100 percent Epon (with accelerator) 12 hours at room temperature in specimen vials and swirl on a slow rotating mixer
6. Place in shallow open aluminum boats
7. Polymerize by placing specimens at 35°C for 12 hours, 45°C for 12 hours and 60°C for 3 to 5 days

C. Epon mixture (Luft, 1961)

1. Mixture A

   62 cc of Epon 812

   100 cc of Dodecenyl succinic anhydride (DDSA)

2. Mixture B

   100 cc of Epon 812

89 cc of Nadic Methyl Anhydride (NMA)

3. Add 3 parts of A to 2 parts of B

4. Add 0.2 ml of DMP-30 per 10 ml of Epon mixture and mix thoroughly

Schedule II. Gluteraldehyde fixation with and without post-fixation in osmium tetroxide

A. One to 12 hours at 4°C in phosphate buffered gluteraldehyde (Sabatini, Bensch and Barrnett, 1963) as follows:

1. Phosphate buffer

   0.1M KH_2PO_4 use 13 ml

   0.1M Na_2HPO_4 use 37 ml

   pH = 7.2 to 7.4

2. Dilute buffer (1.) with 25 percent gluteraldehyde to obtain fixing solution of 3 percent gluteraldehyde

3. After fixation rinse 3 x 5 minutes in phosphate buffer (1.) if no post fix is used dehydrate and embed according to Schedule I-B

4. Rinse 5 minutes in Michaelis buffer (Schedule I-A-1) and then post fix in osmium tetroxide for 3 hours at room temperature (Schedule I-A)

5. Dehydrate and embed according to Schedule I-B

Schedule III. Potassium permanganate

A. Five minutes at room temperature in unbuffered potassium permanganate (Luft, 1956; Mollenhauer, 1959) as follows:

1. Fix in 4 percent aqueous potassium permanganate
2. Rinse thoroughly in several changes of 30 percent ethanol until solution is no longer colored
3. Continue dehydration and embed according to Schedule I-B

APPENDIX B: EXPLANATION OF FIGURES

Key to all Labels

A - α granules

B - β granules

C - Crystalloid

CW - Crosswall

F - Filaments

GV - Gas vesicle

I - Inner investment

IT - Intrathylakoidal space

IG - Intrathylakoidal granule

L - Lamellasome

LW - Longitudinal wall

N - Nucleoplasm

OM - Outer membrane

P - Papillae

PB - Polyhedral bodies

PG - Polar granule

PI - Pits

PL - Plate

PM - Plasma membrane

PO - Pores

R - Ribosomes

RO - Rosettes

S - Sheath

SB - Spherical bodies

SI - Spherical inclusions

SG - Structured granules

T - Tubular systems

TH - Thylakoids

Figure 3. Sheath (S) structure in Nostoc carneum after osmium tetroxide fixation followed by rapid dehydration. Fibrillar layers are separated by osmiophilic layers (arrow). Stained with aqueous uranyl acetate. Approximately X36,000.

Figure 4. Sheath (S) structure in Nostoc carneum after gluteraldehyde-osmium fixation. Fibrillar layers are separated by osmiophilic layers (arrows). Stained with aqueous uranyl acetate. Approximately X14,000.

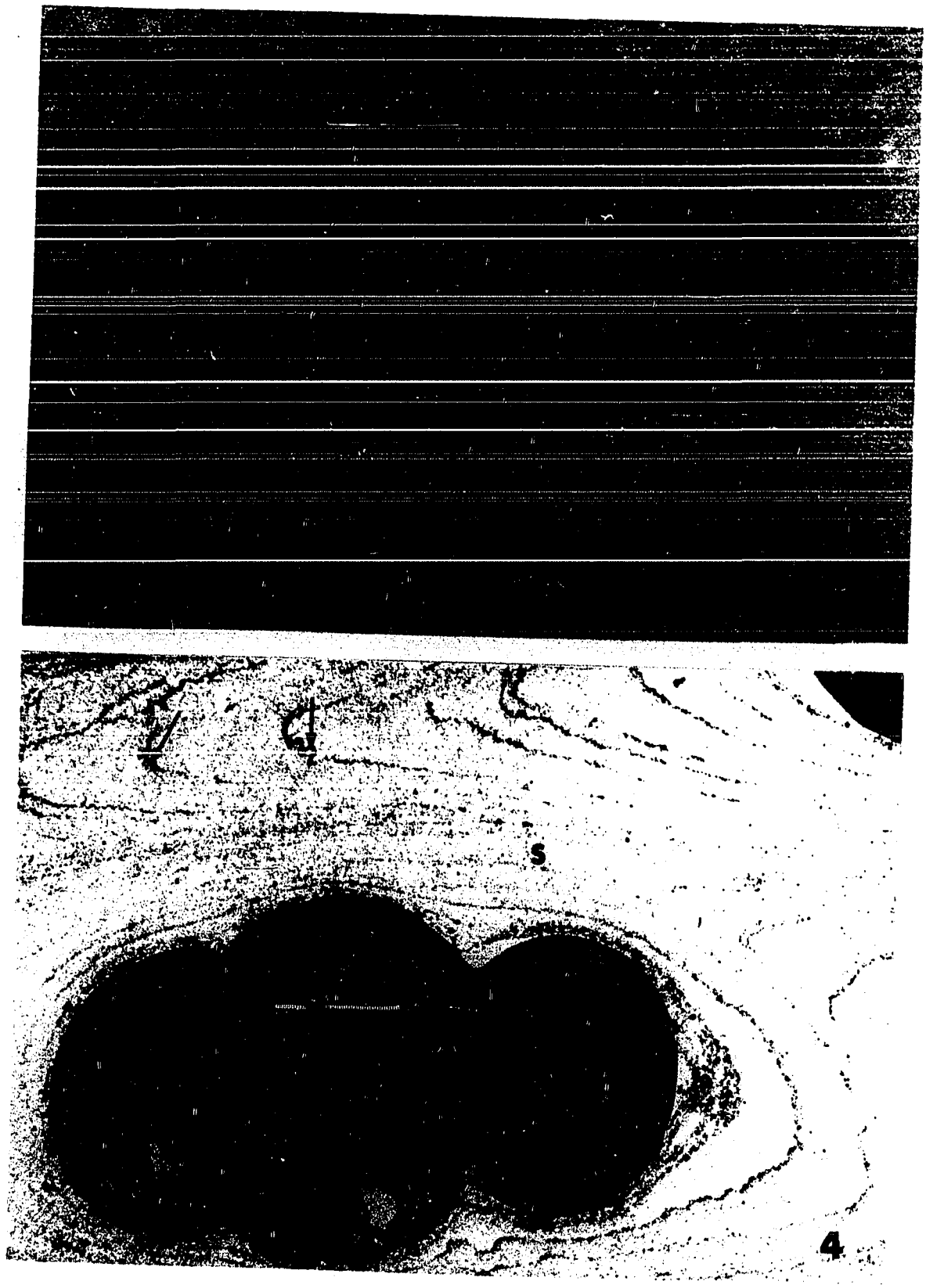
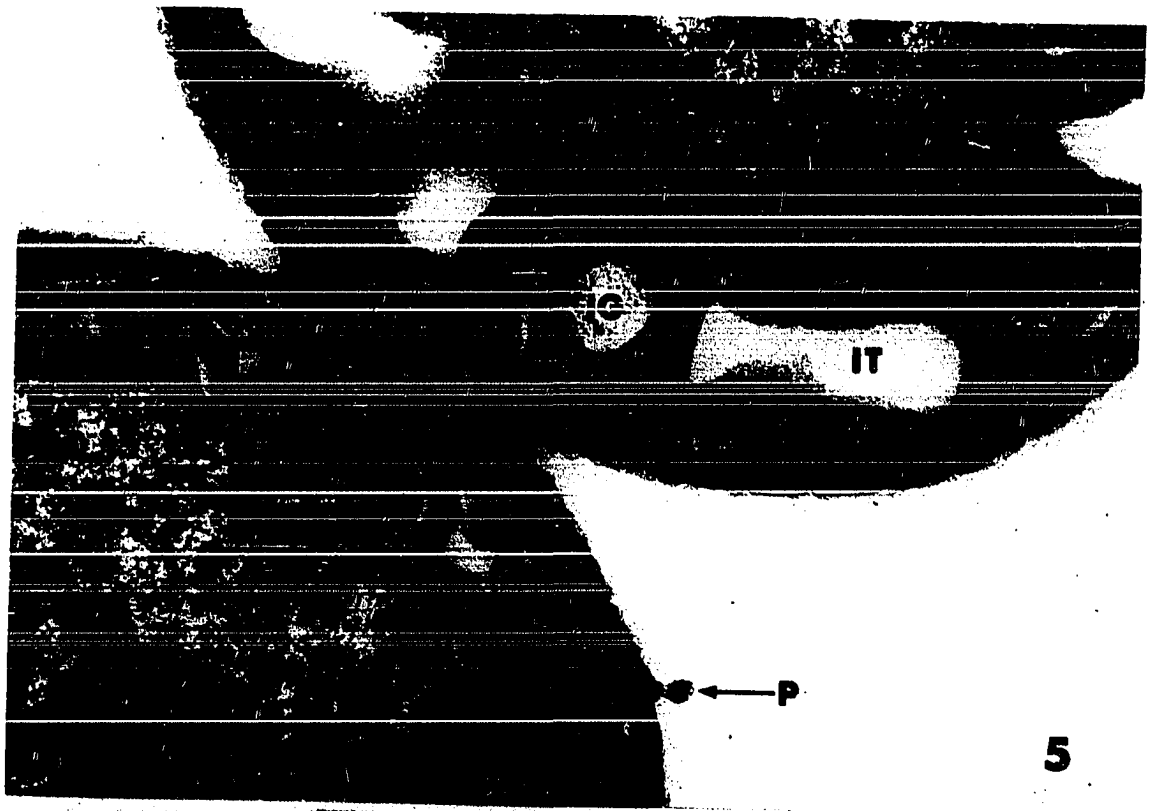

S
4

Figure 5. Portions of 2 cells of Nostoc muscorum (ISU-J17) showing a papilla (P), an intrathylakoidal granule (IG) and a large intrathylakoidal space (IT). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

Figure 6. Enlargement of a papilla on Aphanizomenon flos-aquae showing the outer membrane (OM) forming the outer boundary of the papilla and a dense axial element (arrow). Figure 6a is a similar papilla cut in cross section which also shows the surrounding outer membrane (OM) and the dense central axial element (arrow). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X120,000.

Figure 7. Papilla (P) on Anabaena spiroides showing an internal organization. A lamellasome (L) and a β granule (B) are also shown. Osmium tetroxide fixation and stained with lead hydroxide. Approximately X100,000.



5

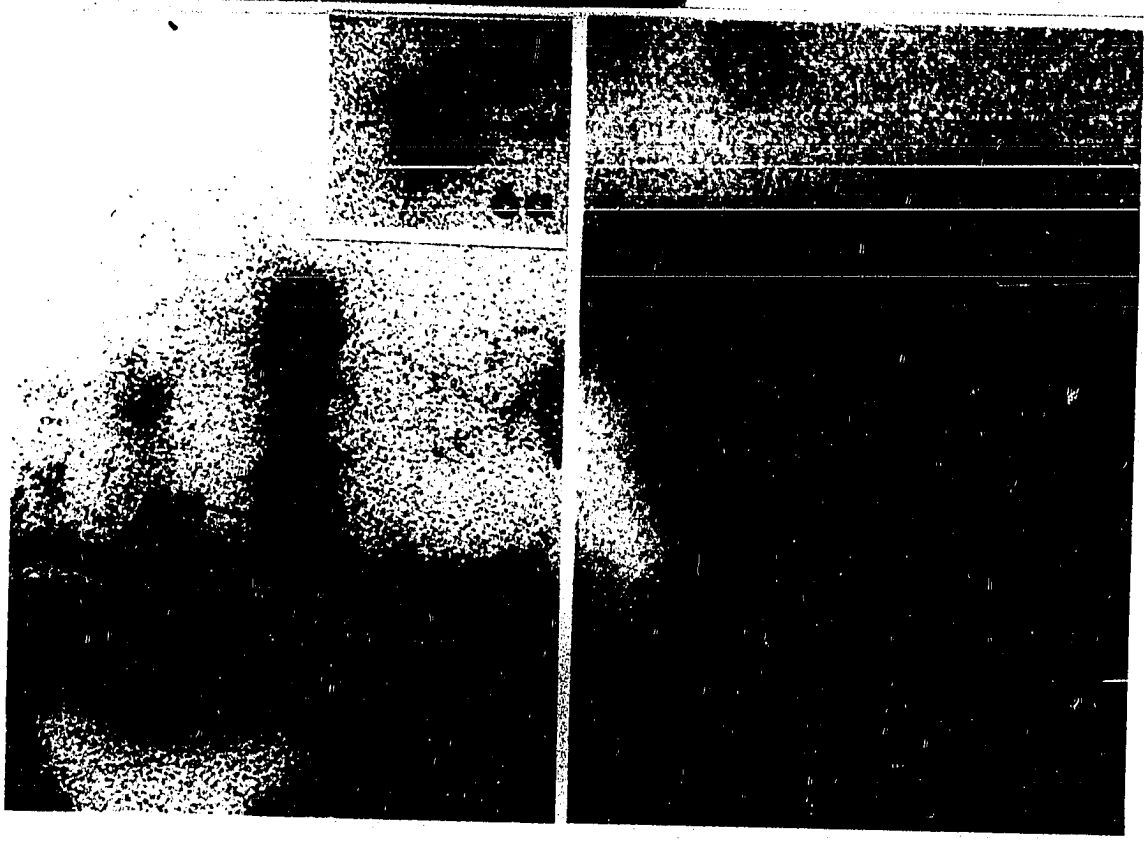


Figure 8. Cell wall of Oscillatoria princeps showing pits (PI) projecting into the inner investment (I), the outer membrane (OM) with undulations and pores (PO) through the inner investment over the crosswall (CW). The thylakoids (TH) in this species are partially expanded. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 9. Cell wall of Oscillatoria princeps showing poor fixation of inner investment (I) and outer membrane (OM) after potassium permanganate fixation. Pits (PI) containing cytoplasm are shown projecting into the inner investment. Approximately X72,000.

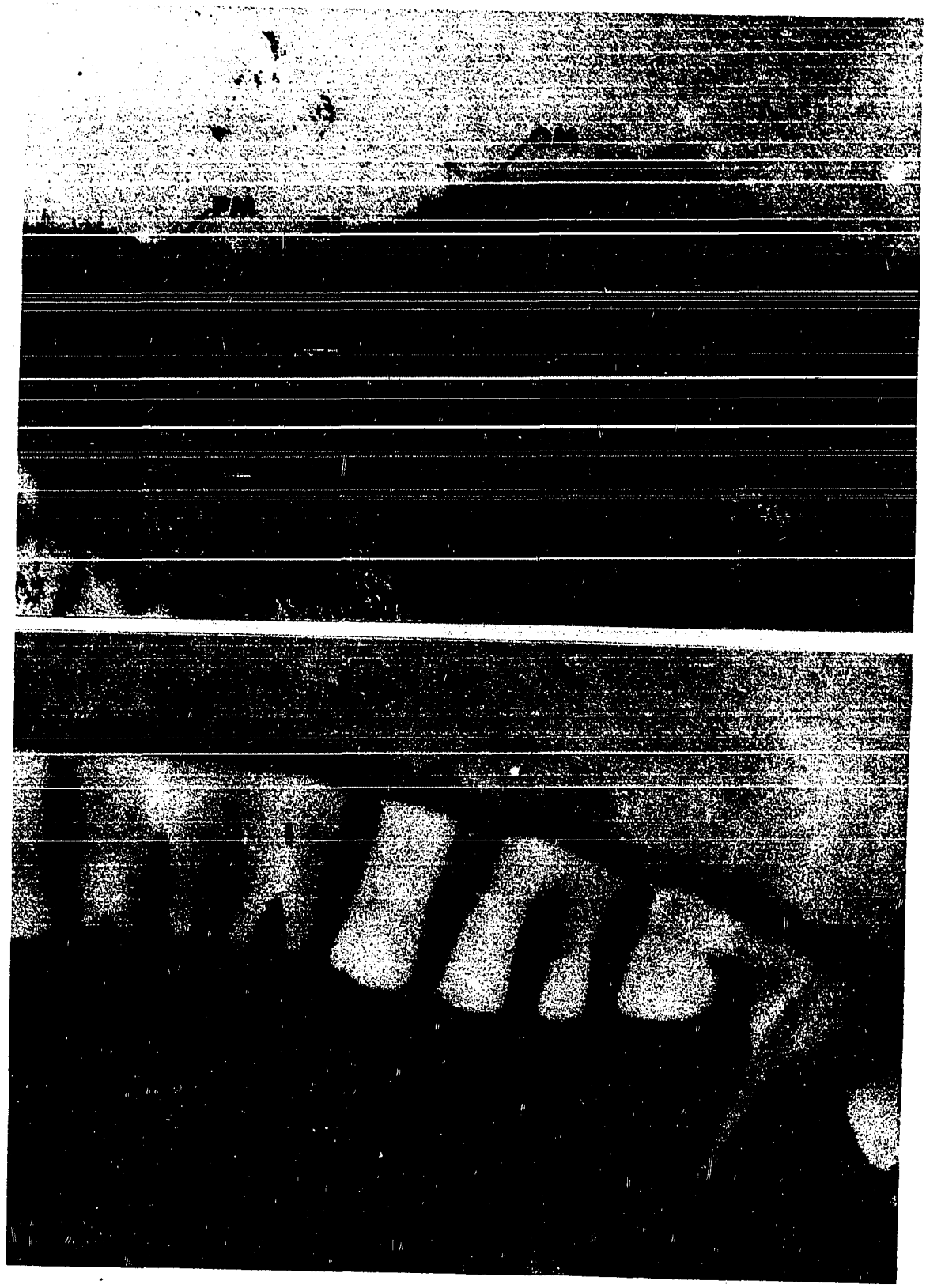
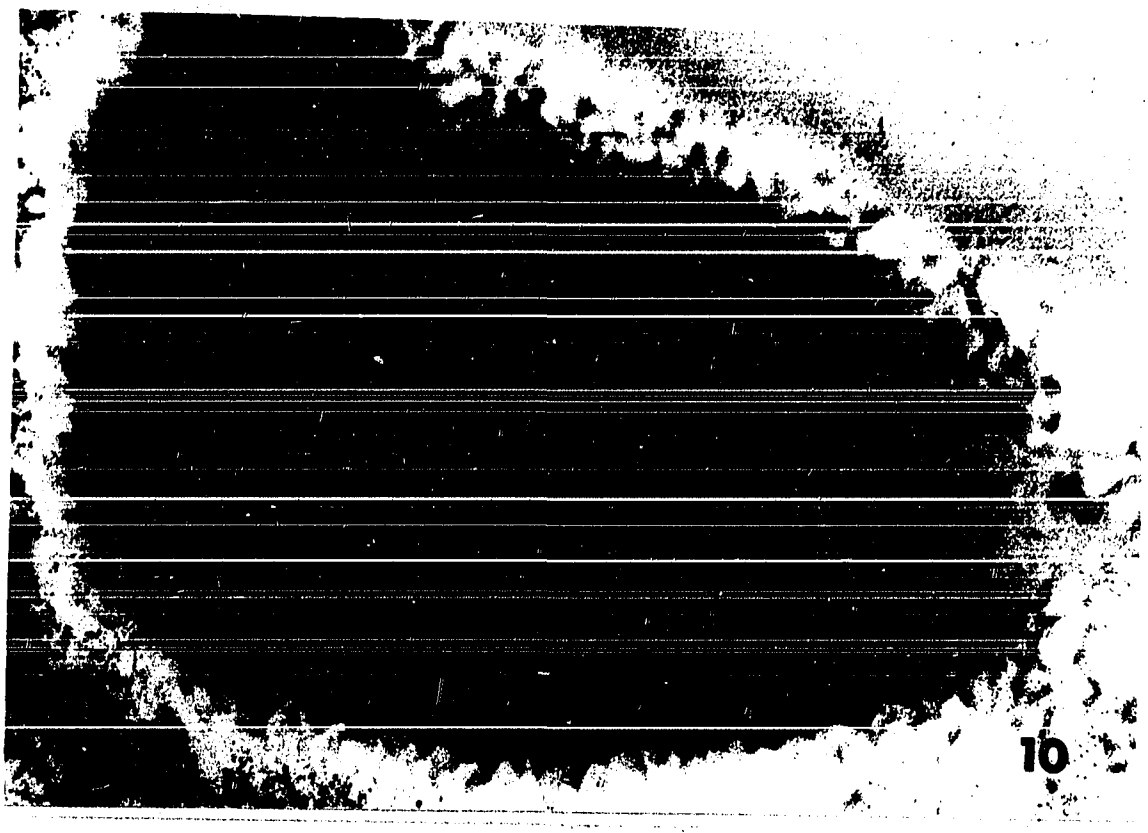


Figure 10. Tangential section through the inner investment (I) of Oscillatoria princeps showing rows of pits (PI) in cross section. The inner investment (I) immediately surrounding the pits is of lower electron density (arrow) than the rest of the inner investment. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 11. Section cut at an angle through the inner investment (I) of Oscillatoria princeps showing rows of pores (PO) over a crosswall and a row of pits (PI). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.


10

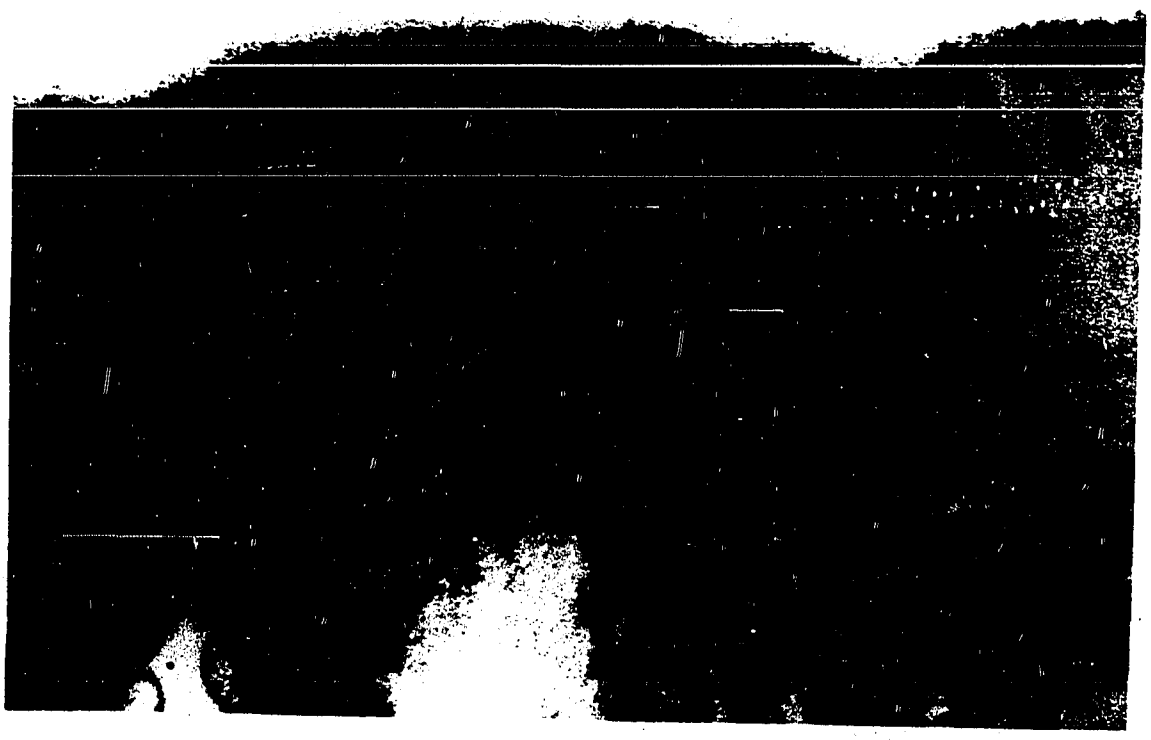


Figure 12. Cell of Nostoc commune showing structured granule (SG), β granules (B), polyhedral body (PB), thylakoids (TH) which are slightly expanded and an infolding of the plasma membrane (arrow). Potassium permanganate fixation. Approximately X51,000.


SG

Figure 13. Cell of Nostoc calcicola from an old culture showing polyhedral body (PB), intrathylakoidal granules (IG), thylakoids (TH) which appear to be "branched" and an infolding of the plasma membrane (arrow). Potassium permanganate fixation. Approximately X50,000.

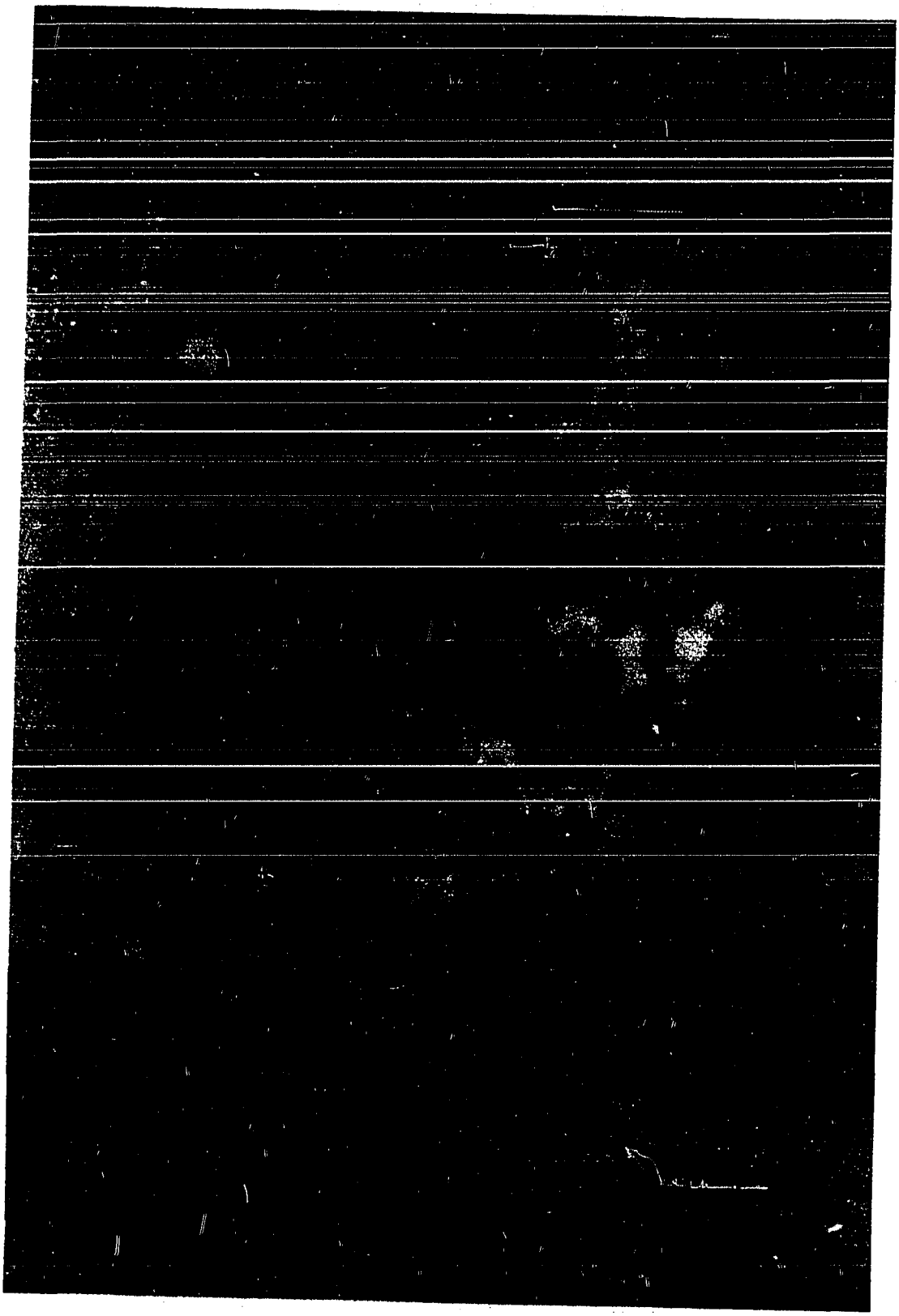


Figure 14. Portion of a cell of Nostoc calcicola showing poor fixation of the outer membrane and inner investment by potassium permanganate (arrow). An intrathylakoidal granule (IG) and a thylakoid (TH) which has folded out are also shown. Approximately X72,000.

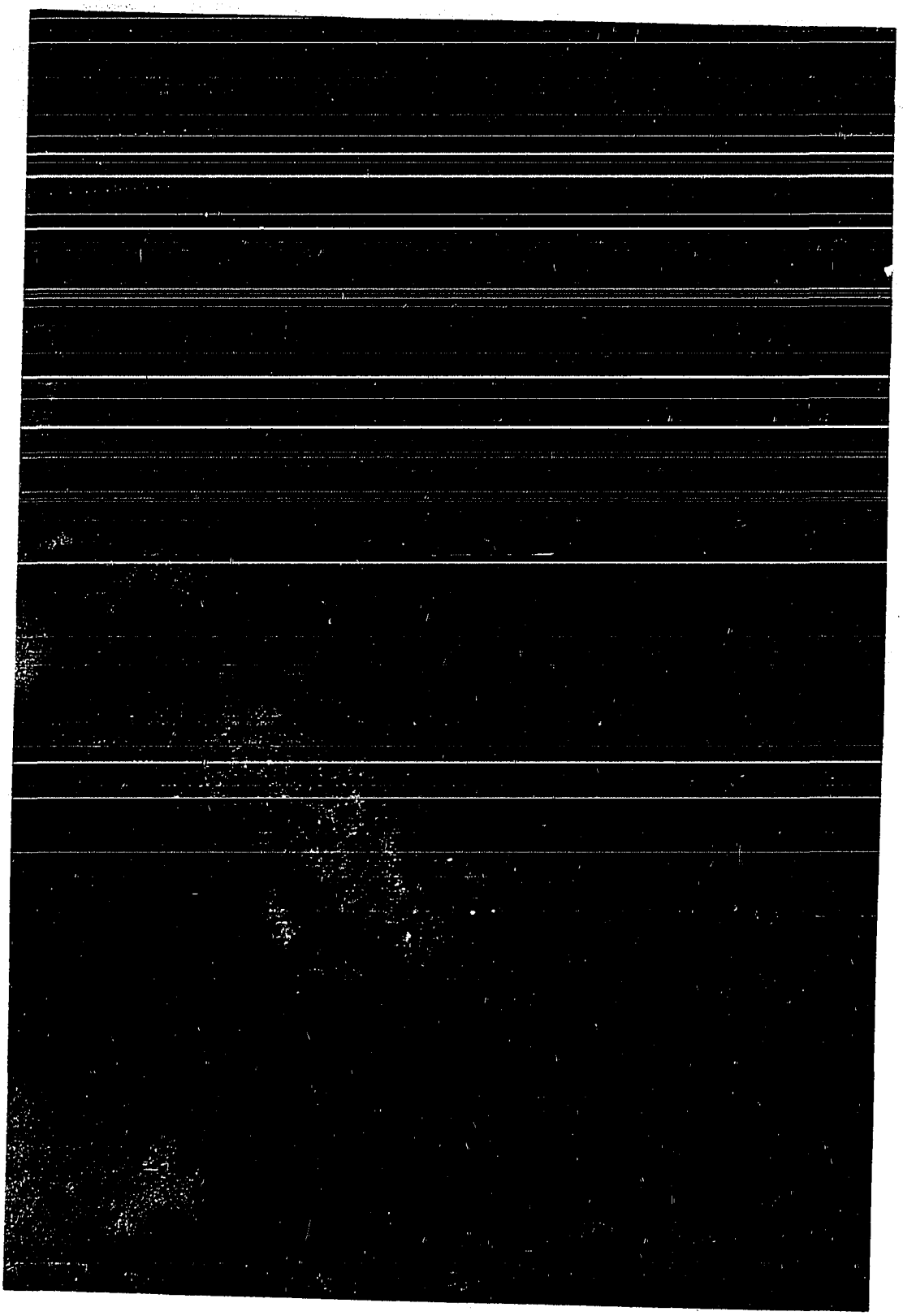


Figure 15. Cell of Nostoc pruniforme (ISU-J11) showing outer membrane (OM), inner investment (I), structured granule (SG), β granules (B), thylakoids (TH) which are somewhat expanded, intrathylakoidal granules (IG), numerous ribosomes (R) and polyhedral bodies (PB) associated with the nucleoplasm (N). Osmium tetroxide fixation and stained with lead hydroxide. Approximately X72,000.


TH
OM
I
15

Figure 16. Portion of a cell of Nostoc muscorum (ISU-J17) in which the intrathylakoidal granule (IG) is a crystalline material. The unit membrane nature of the outer membrane is also apparent (OM). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

Figure 17. Portion of a cell of Nostoc ellipsosporum in which the intrathylakoidal granule (IG) is a crystalline material. Osmium tetroxide fixation and stained with lead hydroxide. Approximately X72,000.


OM

Figure 18. Cell of Nostoc punctiforme in the process of division showing ingrowing crosswall (CW), outer membrane (OM) with wave like undulations, inner investment (I), structured granules (SG), β granules (B), thylakoids (TH) with a small intrathylakoidal space and a polyhedral body (PB) associated with the nucleoplasm (N). Osmium tetroxide fixation and stained with lead hydroxide. Approximately X50,000.

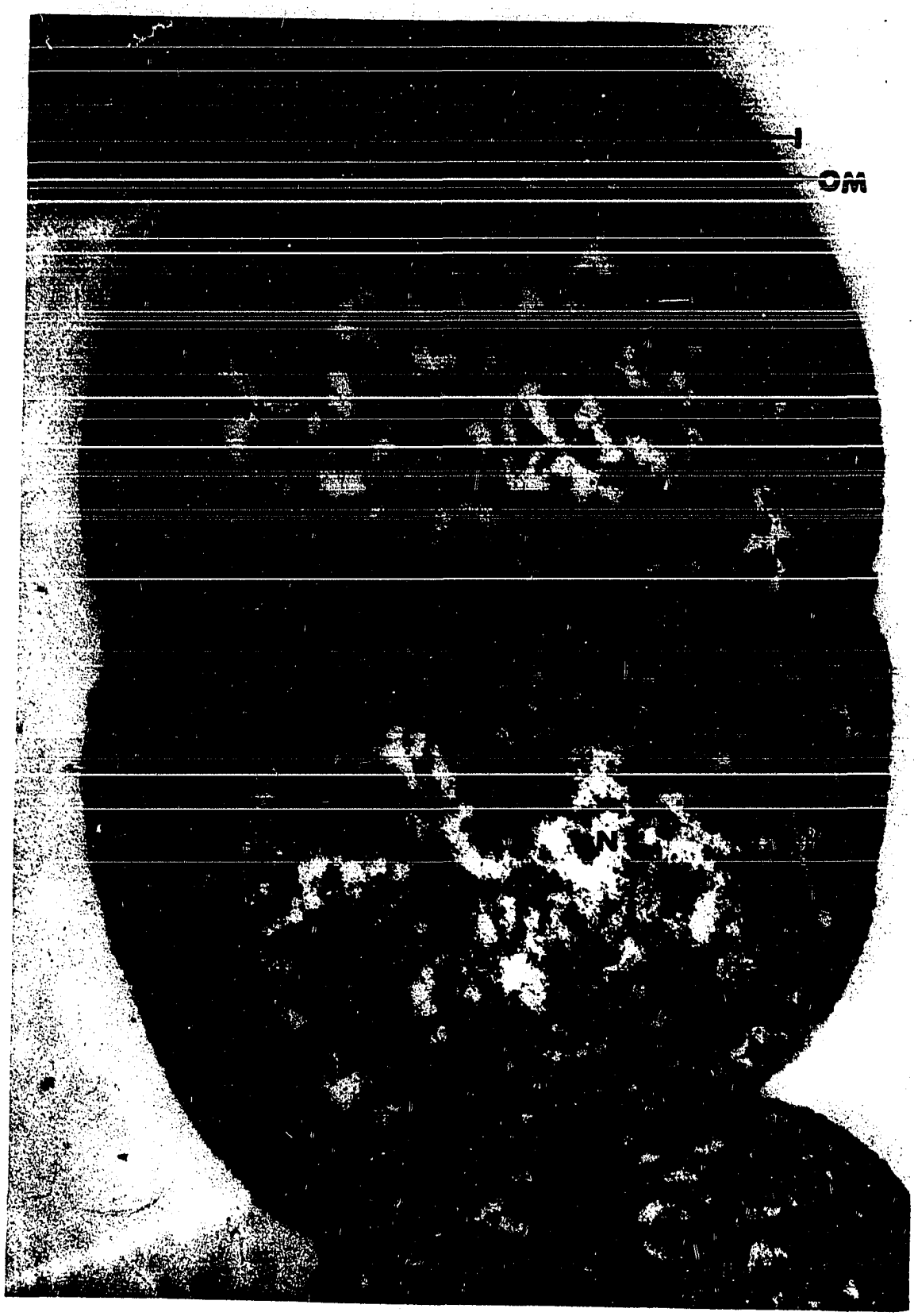

OM
N

Figure 19. Portion of a cell of Nostoc calcicola showing unit membrane nature of the plasma membrane (PM). Some thylakoids (TH) exhibit a myelin configuration and some have "branched" in a very regular manner. Potassium permanganate fixation. Approximately X72,000.

Figure 20. Portion of a cell of Calothrix parietina in which the thylakoids (TH) are arranged in stacks. The inner protein layers of the thylakoid membrane are in contact producing a myelin configuration but the outer protein layers remain separated by a small space (arrow). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

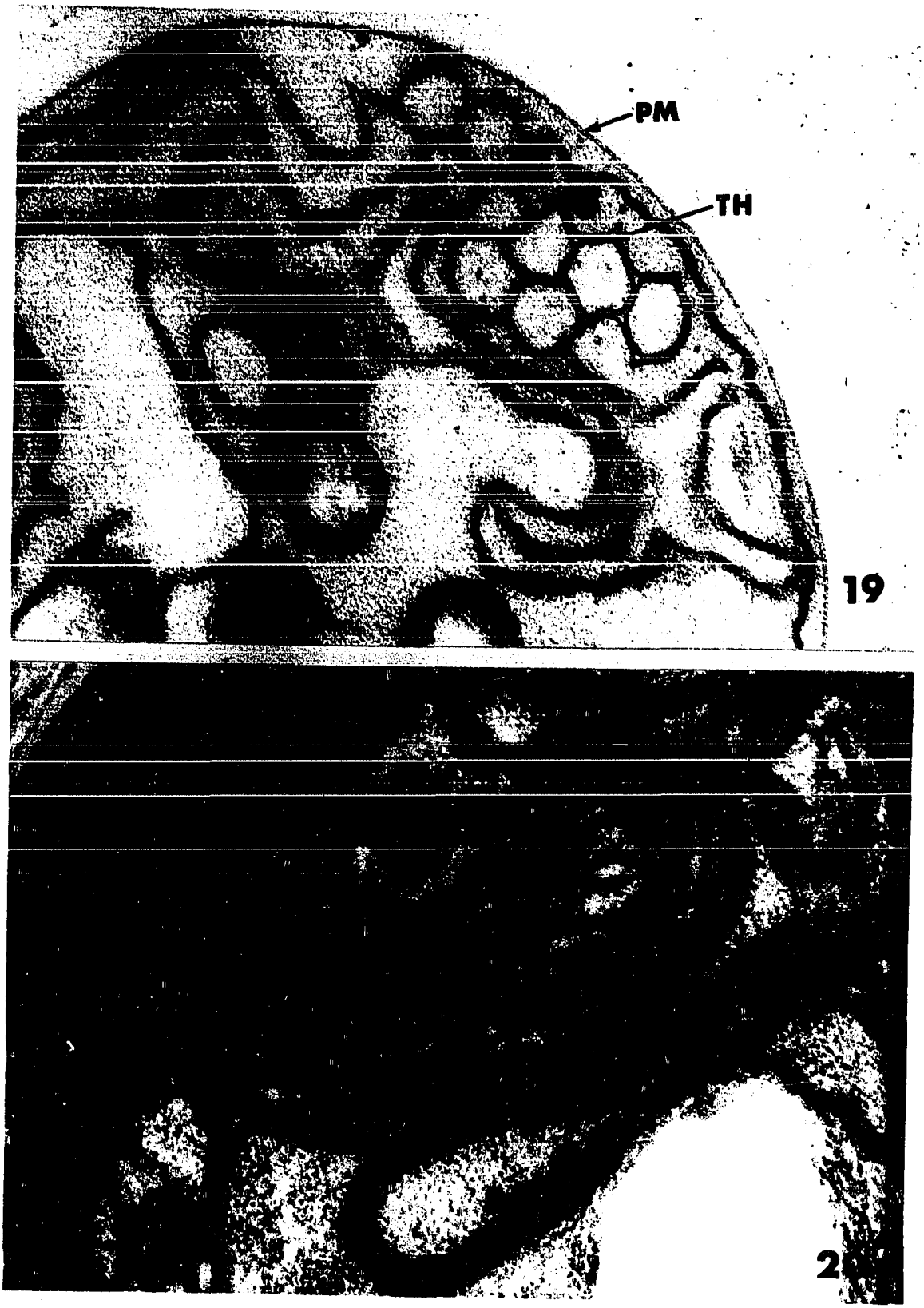

PM
TH
19
2

Figure 21. Heterocyst of Anabaena spiroides containing polar granule (PG) and showing elaborate thylakoid (TH) pattern. Osmium tetroxide fixation and stained with lead hydroxide. Approximately X28,000.

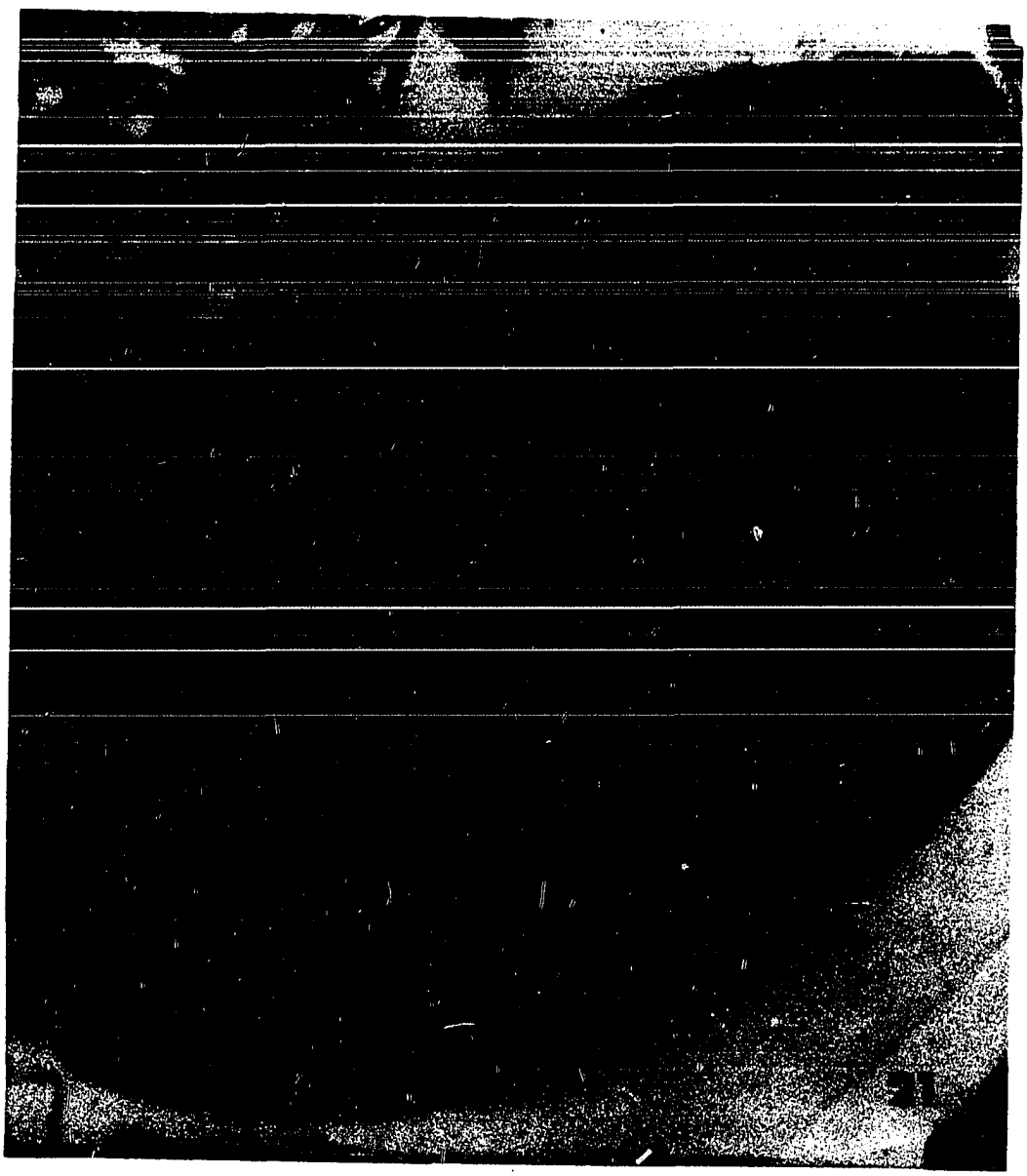


Figure 22. Cross section through elaborate thylakoid (TH) pattern in the pore region of a heterocyst of Anabaena spiroides. Osmium tetroxide fixation and post stained with aqueous uranyl acetate. Approximately X50,000.

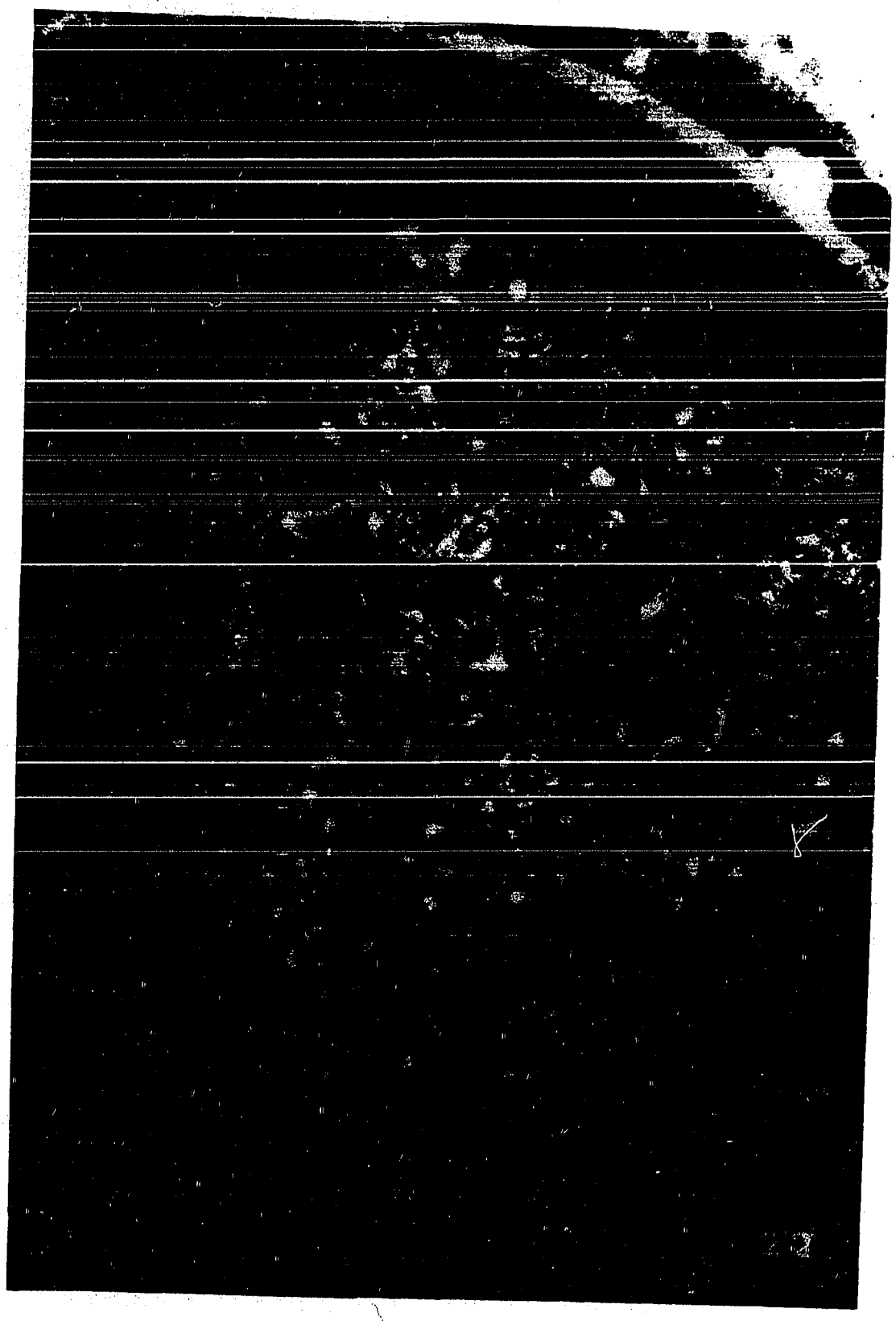


Figure 23. Section through the pore region of a heterocyst of Anabaena spiroides showing a polar granule (PG) and an elaborate thylakoid (TH) arrangement. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

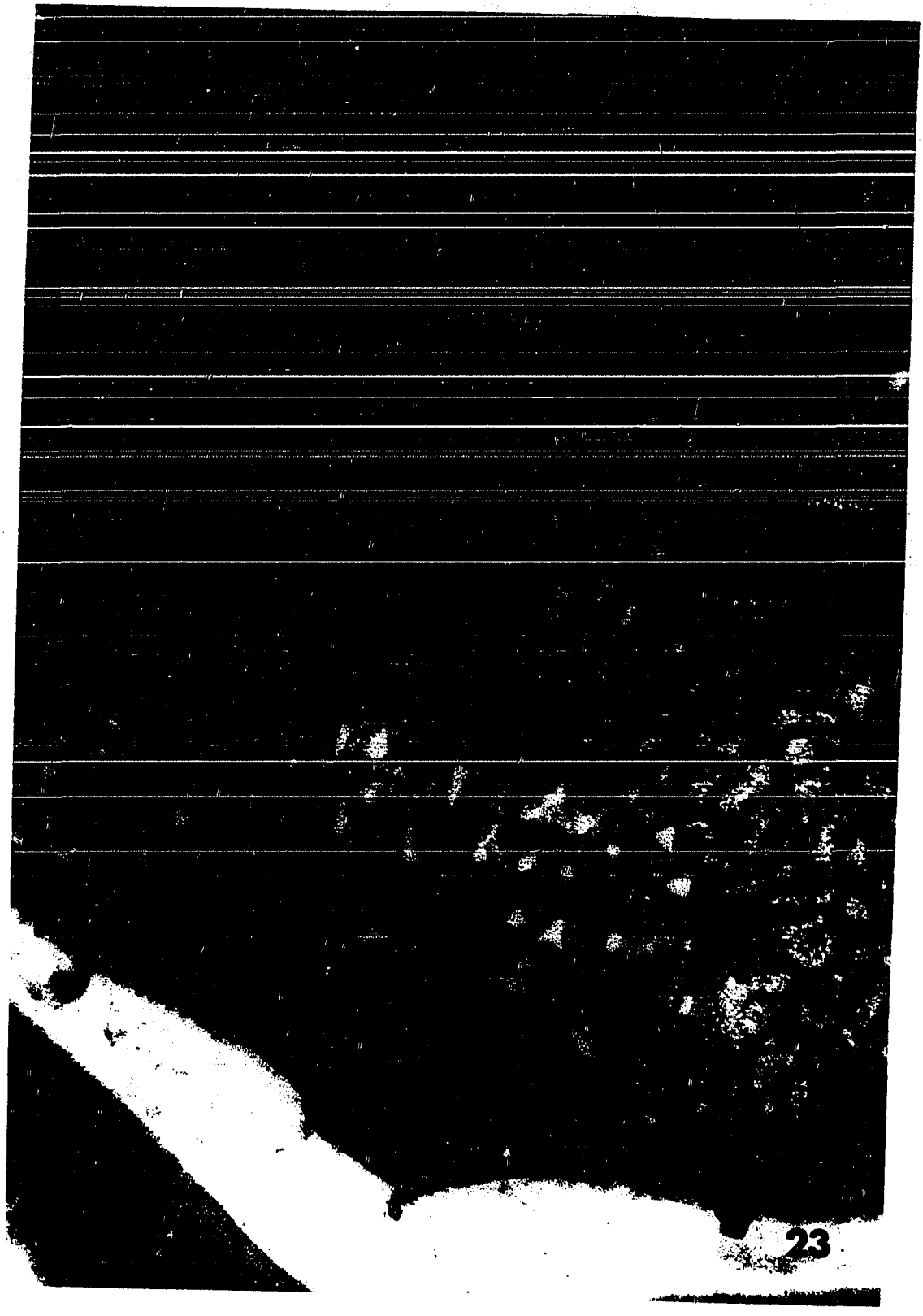
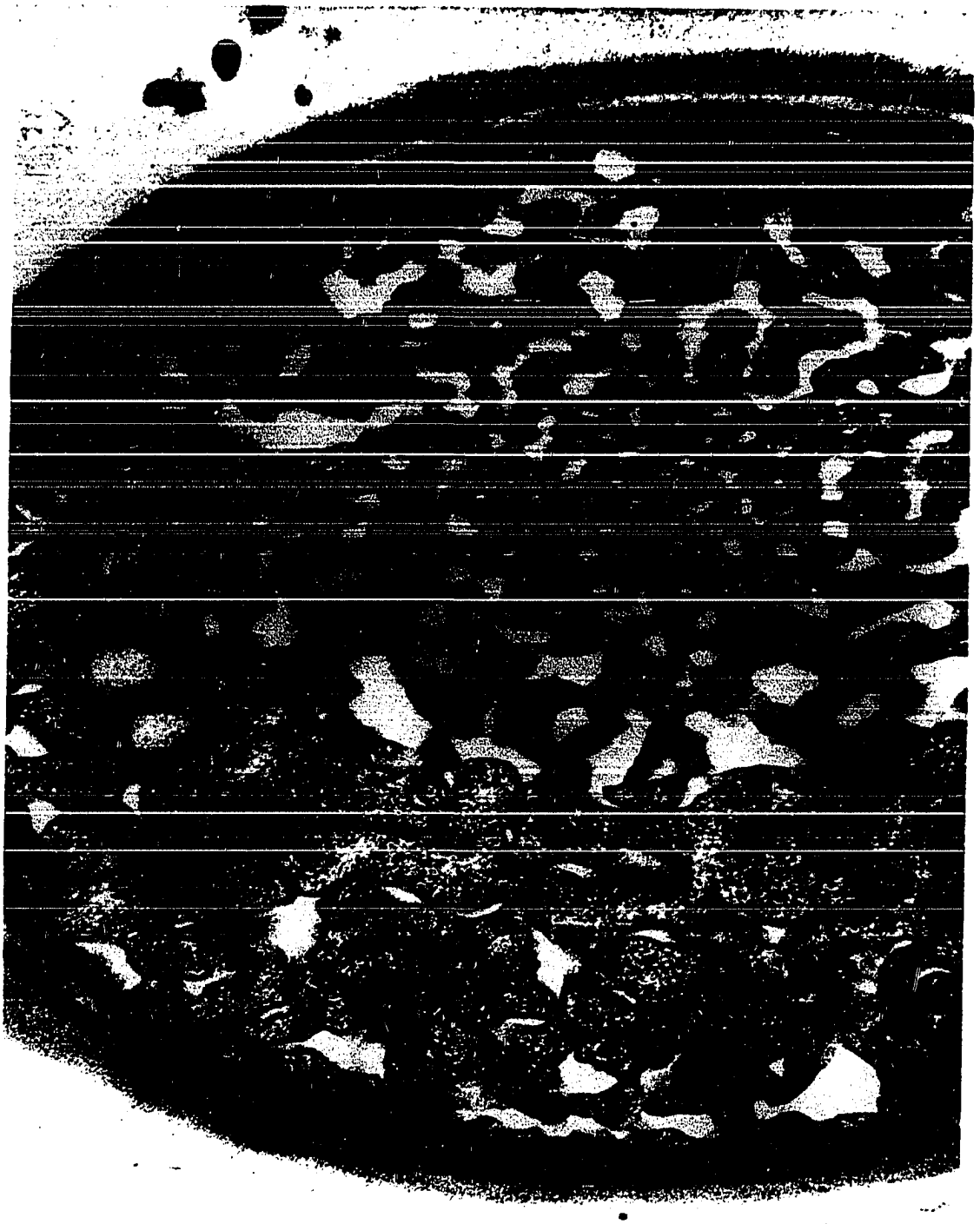

23

Figure 24. Cross section through pore region of a heterocyst of Nostoc linckia showing elaborate thylakoid (TH) arrangement. Osmium tetroxide fixation and stained with lead hydroxide. Approximately X50,000.



24

Figure 25. Portion of a cell of Cylindrospermum sp. containing a stack of membrane-like structures (arrow). The outer membrane (OM) appears as a unit membrane with wave like undulations. The inner investment (I) is immediately outside the plasma membrane (PM). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X70,000.

Figure 26. Portion of a cell of Nostoc linckia containing spherical membrane systems. Three ends of the membranes (arrows) of one inclusion are shown. Within the inclusion α granules (A) are located. Osmium tetroxide fixation and stained with lead hydroxide. Approximately X120,000.

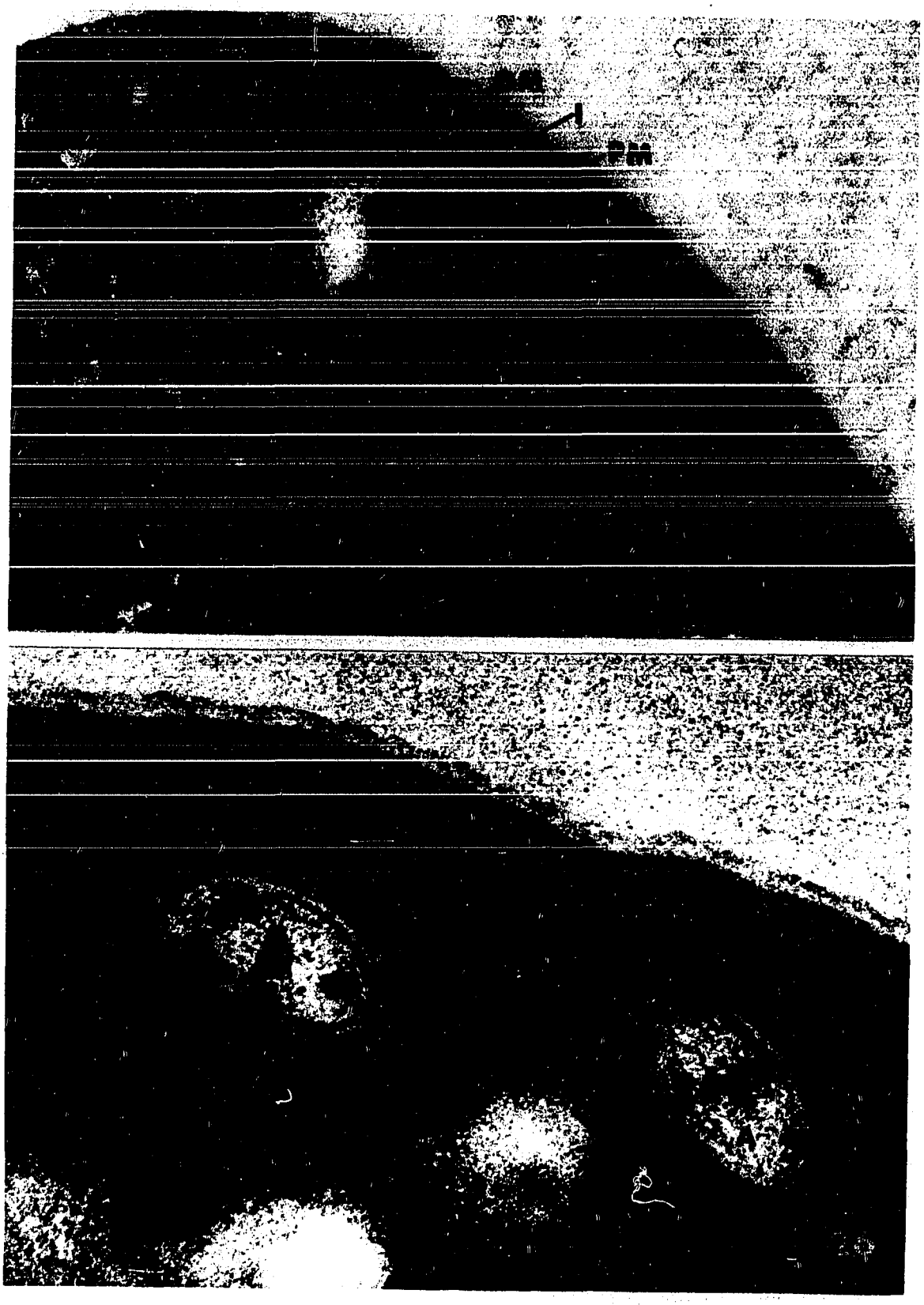

1 µm

Figure 27. Portion of a cell of Nostoc linckia containing spherical membrane systems and a crystalloid (C), The "membranes" of the spherical membrane systems appear to branch and fuse (arrows). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

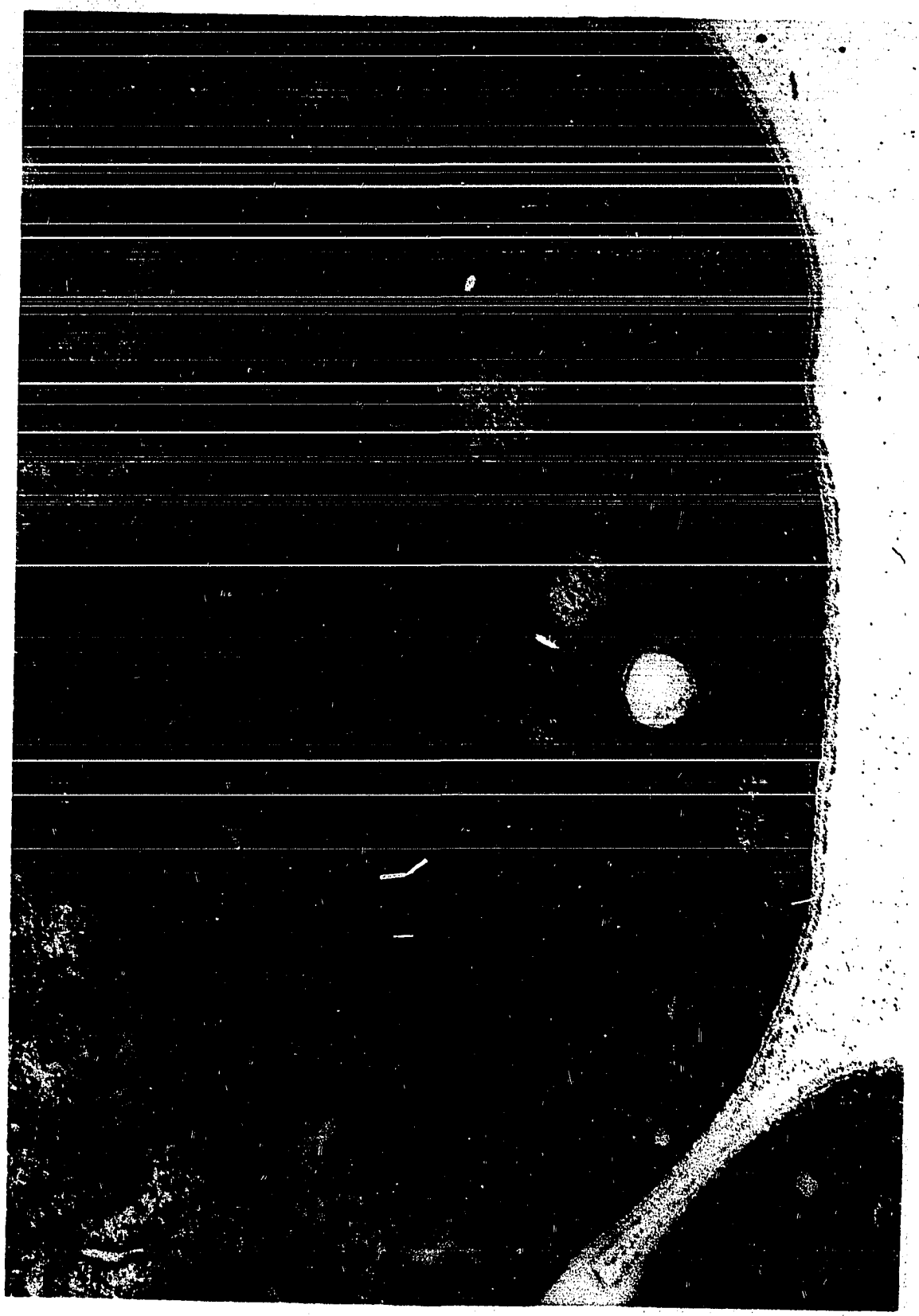


Figure 28. Portion of a heterocyst of Gleotrichia pisum (ISU-J20) containing scroll-like membrane systems cut in cross section. The membrane ends (arrows) at the inner and outer zone of the scroll are evident and ribosome-like particles (R) can be seen between the membranes. Within the scroll-like membrane system a β granule (B), a thylakoid (TH) and numerous ribosomes (R) can be seen. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X73,000.

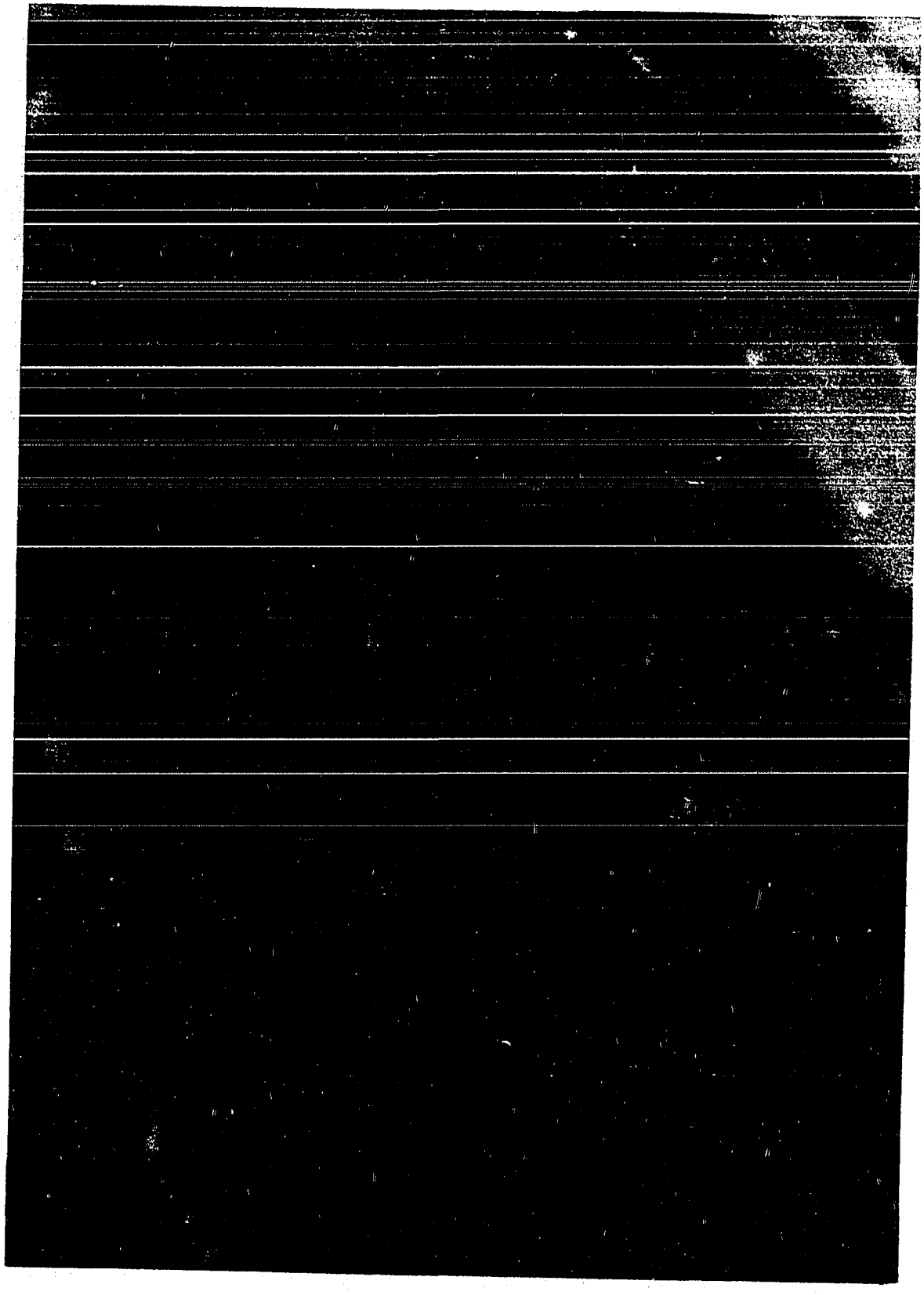


Figure 29. Portion of a heterocyst of Gleotrichia pisum (ISU-J20) containing scroll-like membrane systems sectioned longitudinally. The edges of the membrane at the end of the cylinder are evident (arrows). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X73,000.

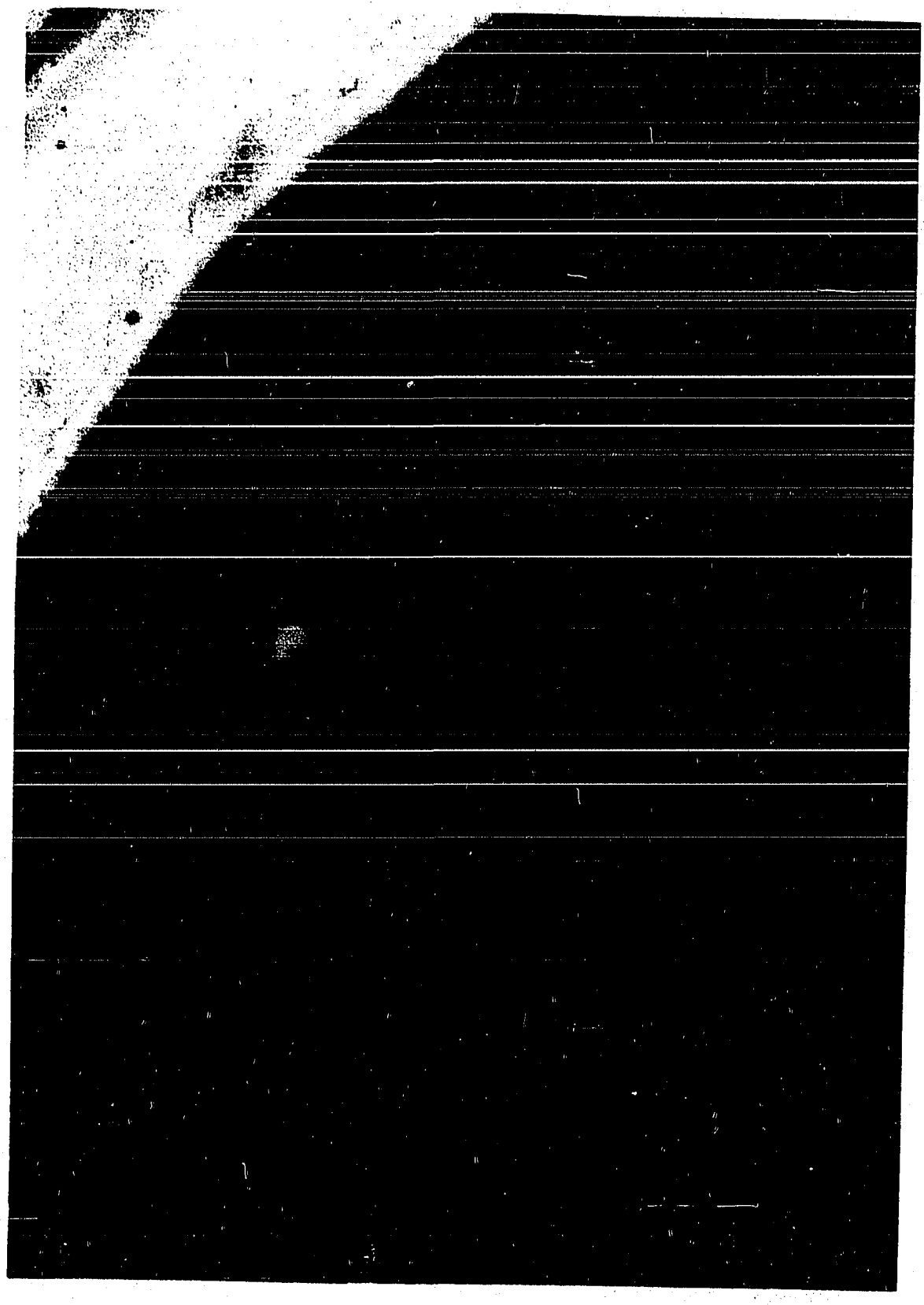


Figure 30. Portion of a cell of Nostoc pruniforme (ISU-J11) containing a crystalloid (C) associated with two polyhedral bodies (PB). Figure 30a is an enlargement of the crystalloid (C) and shows the osmiophilic striations at right angles to the longitudinal axis and finer lines parallel to the long axis (arrows). Osmium tetroxide fixation and stained with lead hydroxide. Figure 30 approximately X72,000. Figure 30a approximately X120,000.

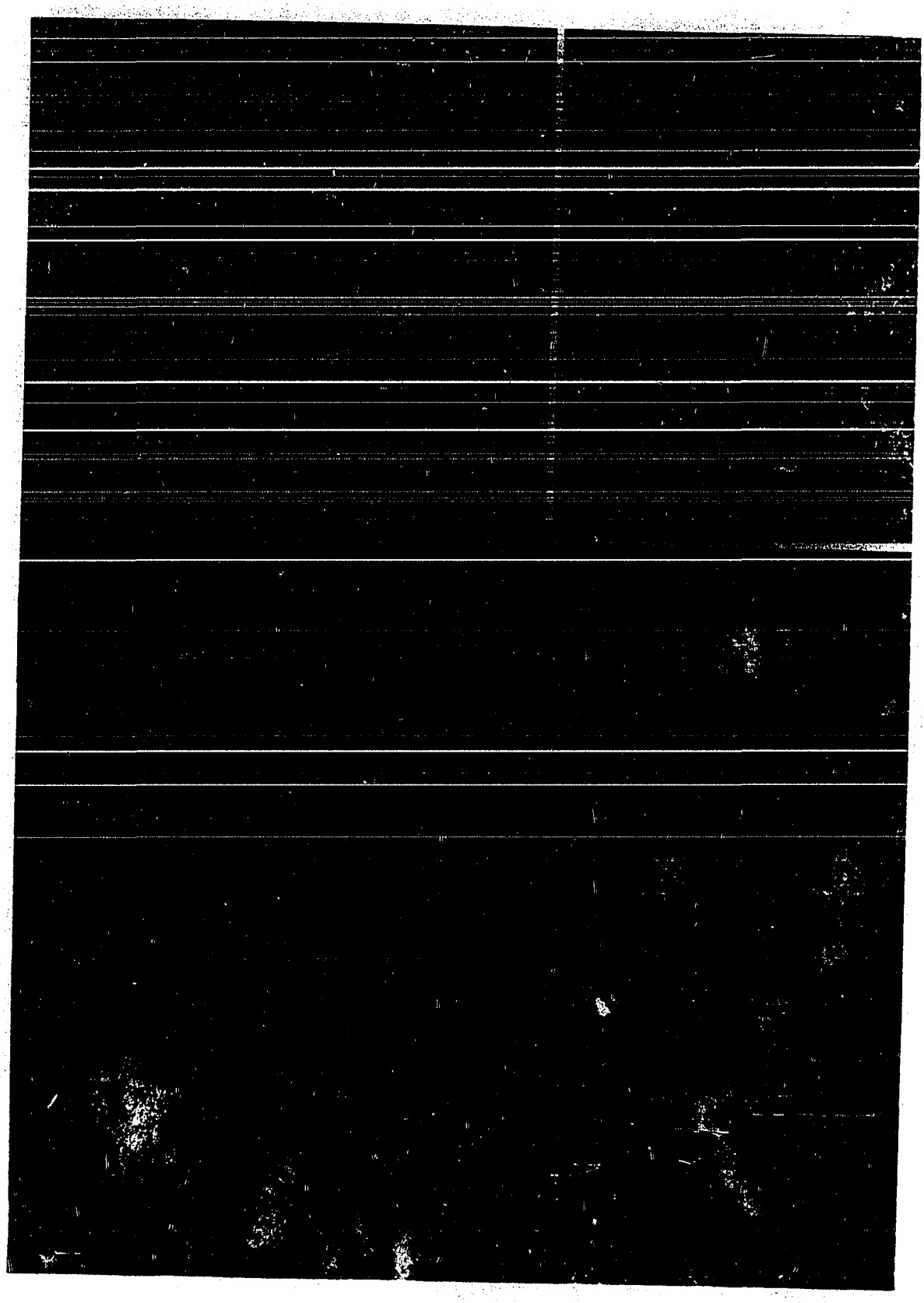


Figure 31. Portion of a cell of Nostoc carneum containing a crystalloid (C). Numerous polyhedral bodies (PB) can also be seen in association with the nucleoplasm (N). The thylakoids (TH) are observed in a myelin-like configuration in places. Figure 31a is an enlargement of the crystalloid and shows the osmiophilic striations at right angles to the longitudinal axis and finer lines parallel to the long axis (arrows). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Figure 31 approximately X72,000. Figure 31a approximately X120,000.

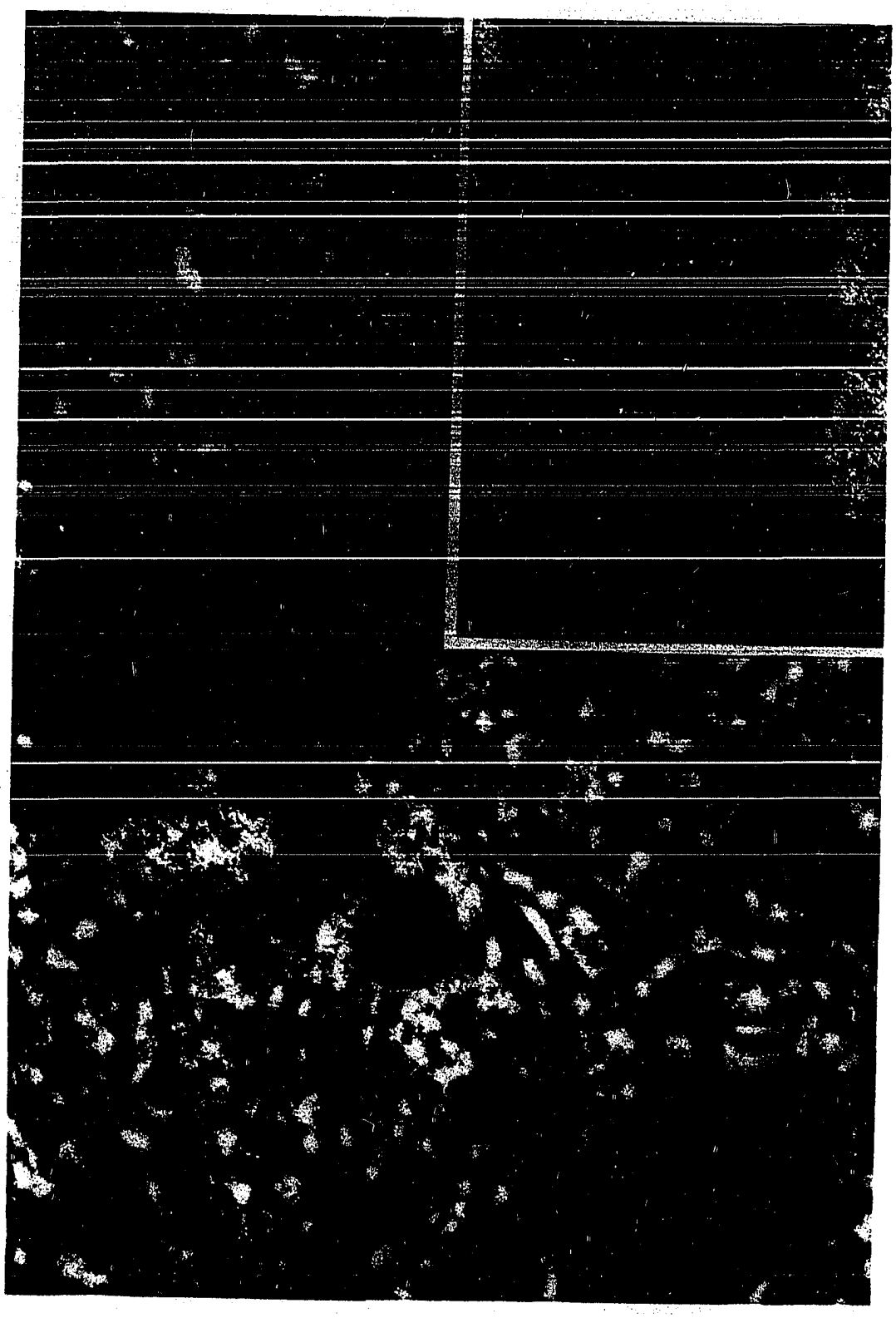


Figure 32. Portion of a cell of Nostoc carneum containing a granular area with an indication of periodicity (arrows). Osmiophilic fibers are evident in the sheath (S). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 33. Portion of a cell of Nostoc pruniforme (ISU-J11) containing a crystalloid (C) associated with a polyhedral body (PB). Osmium tetroxide fixation and stained with lead hydroxide. Approximately X72,000.

Figure 34. Portion of a cell of Nostoc carneum containing a crystalloid (C), a polyhedral body (PB) and a structured granule (SG). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

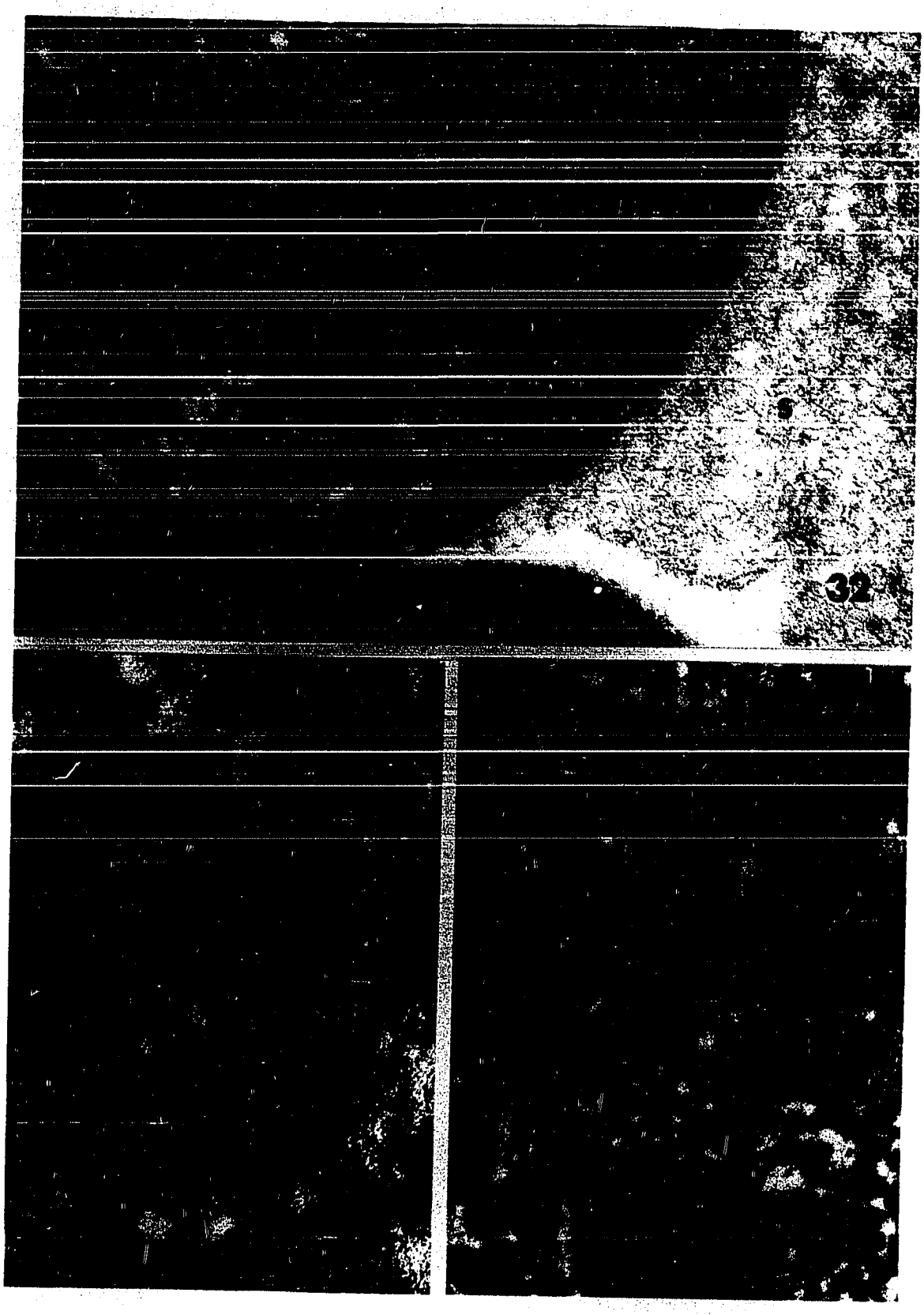
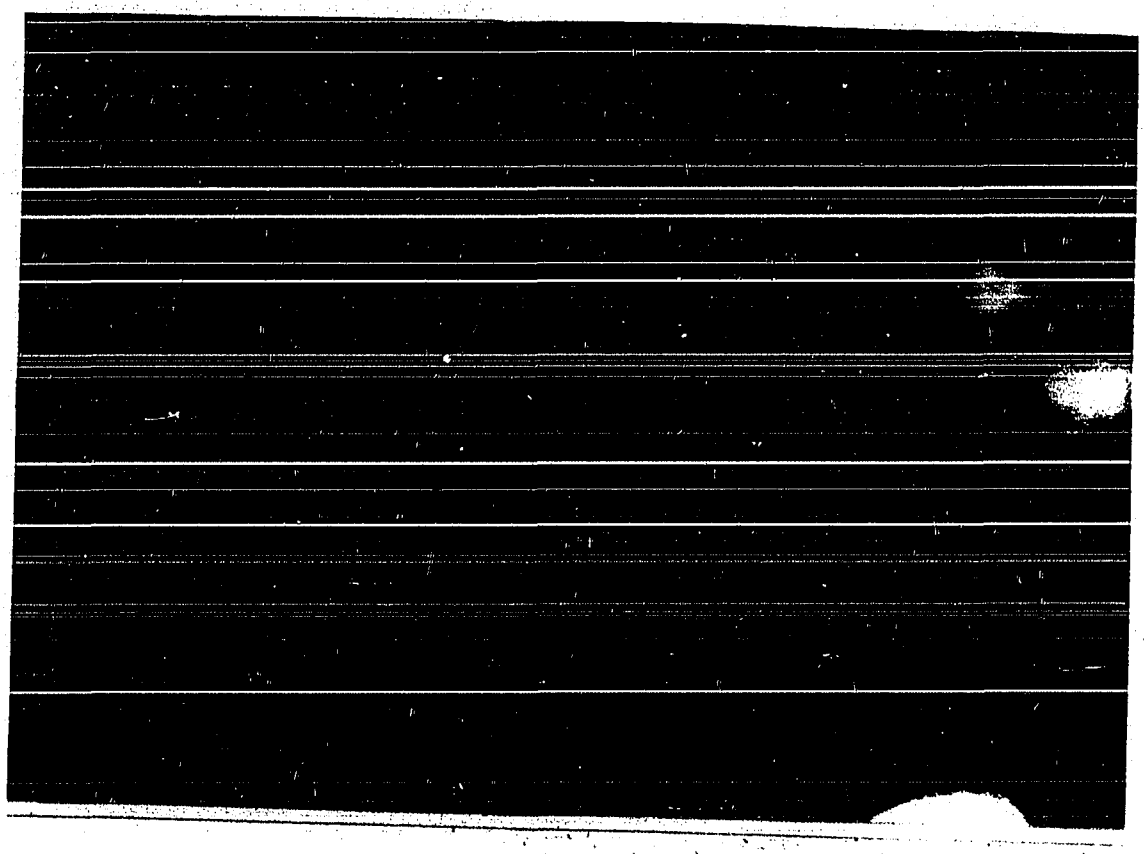

32

Figure 35. Portion of the central part of a cell of Nostoc pruniforme (ISU-J11) containing a poorly defined organized area with crosshatched striations (arrows). Numerous polyhedral bodies (PB) surround the area. Osmium tetroxide fixation and stained with lead hydroxide. Approximately X72,000.

Figure 36. Portion of a cell of Nostoc linckia containing a compound crystalloid which is apparently composed of several smaller crystalloids (C). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.



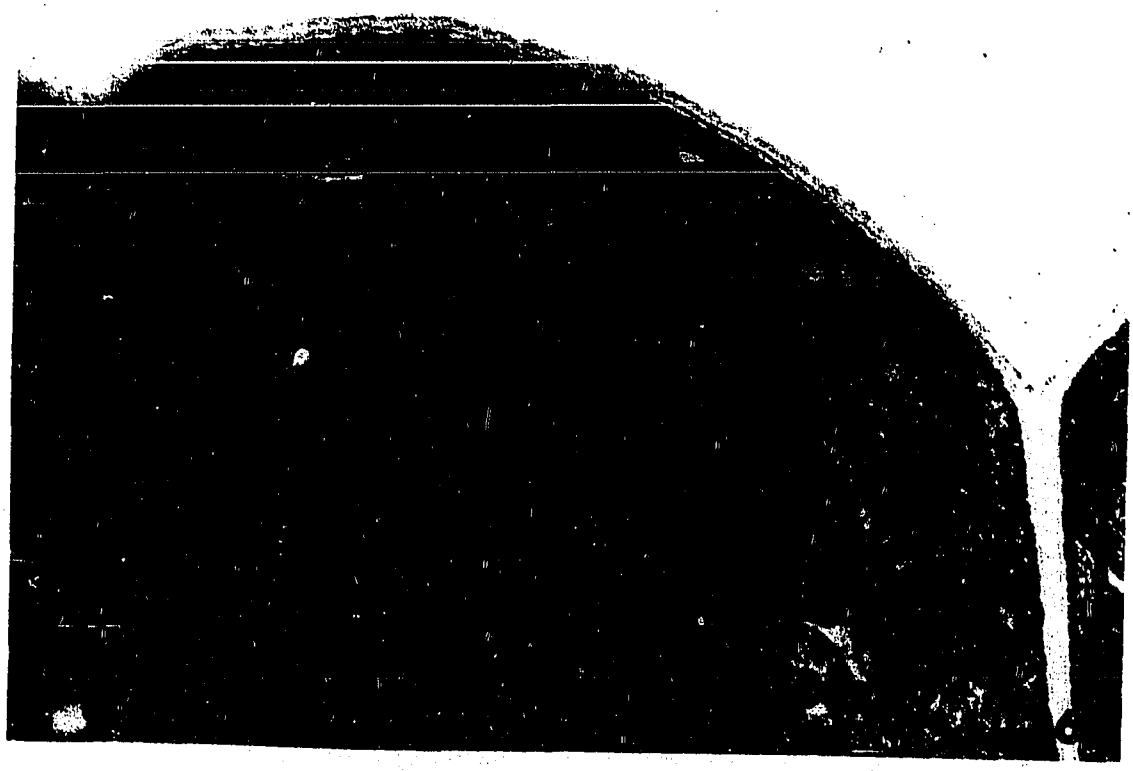
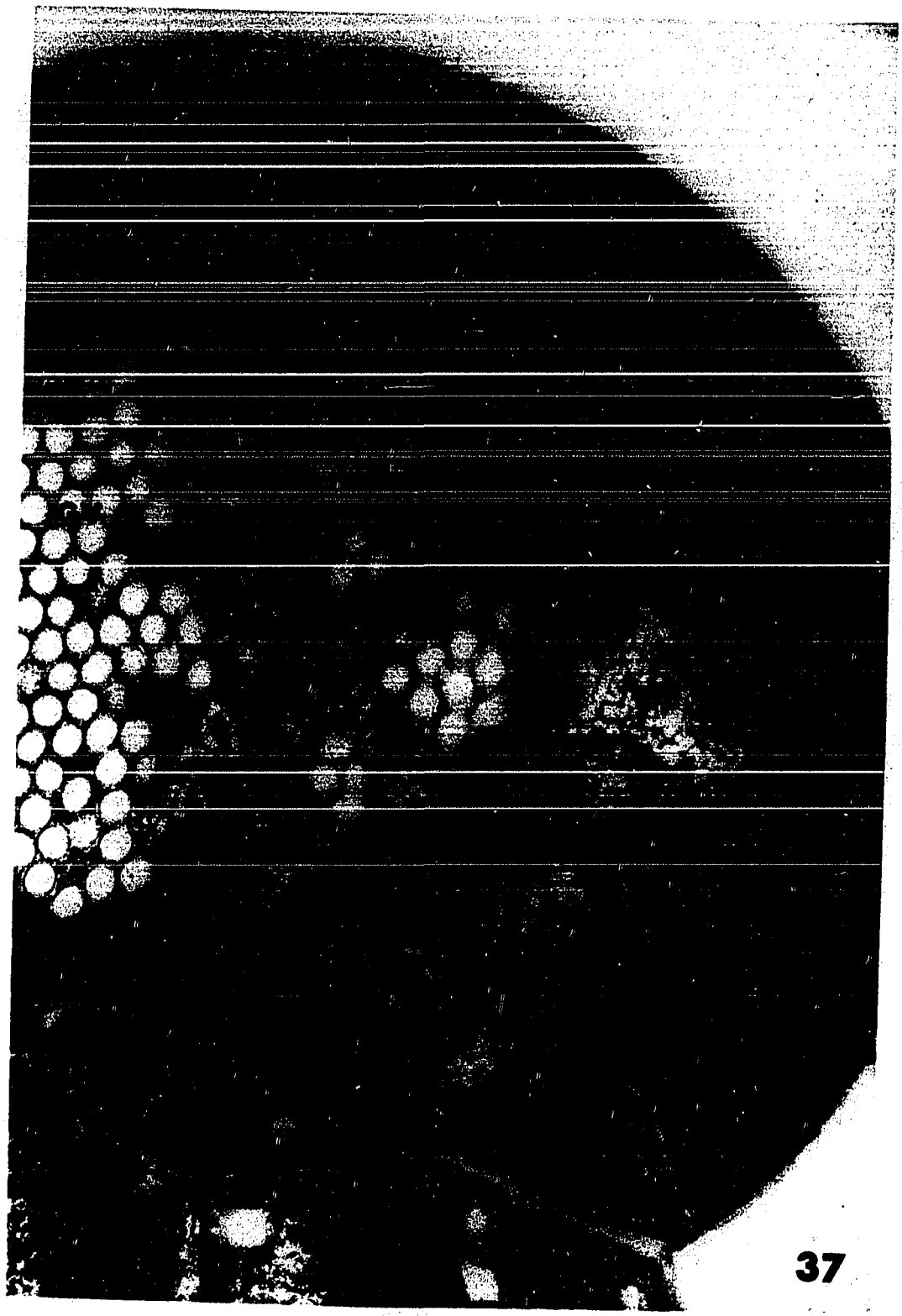


Figure 37. Cell of Nostoc coeruleum containing a crystalloid (C) with crosshatched striation, 2 lamellasomes (L) and numerous gas vesicles (GV) cut in cross section. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.



37

Figure 38. Portion of a cell of Nostoc coeruleum showing poor preservation of a crystalloid (C) and the gas vesicles (GV) after potassium permanganate fixation. Approximately X72,000.

Figure 39. Portion of a cell of Nostoc coeruleum containing a crystalloid (C) with parallel striations and numerous gas vesicles (GV). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

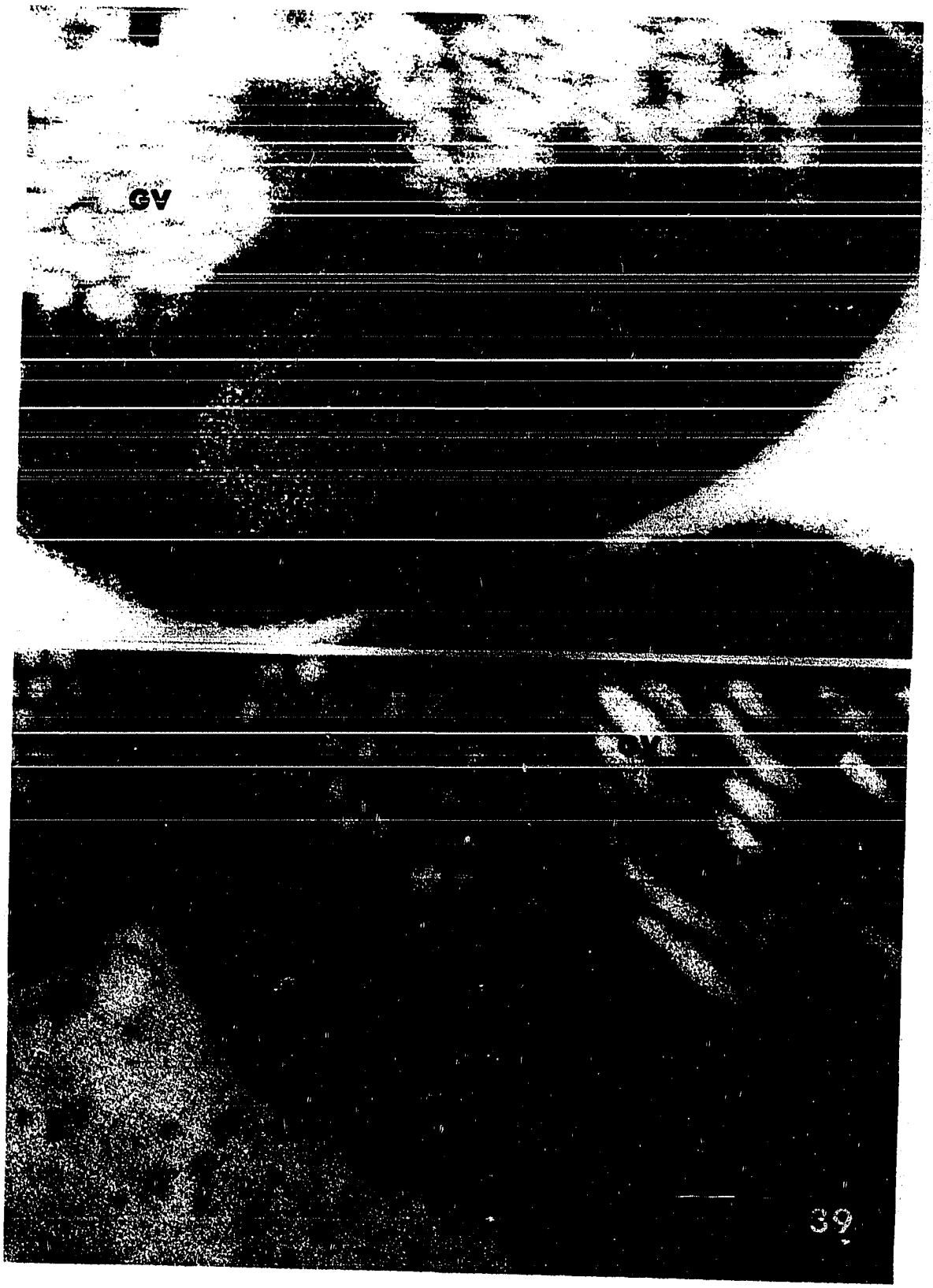

GV
GV
39

Figure 40. Portion of a cell of Gleotrichia pisum (ISU-J20) containing a crystalloid (C) with parallel striations. A large intrathylakoidal space (IT) is also evident. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 41. Portion of a cell of Gleotrichia pisum (ISU-J20) containing a crystalloid (C) with crosshatched striations. A structured granule (SG), a β granule (B) and large intrathylakoidal spaces (IT) are also evident. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

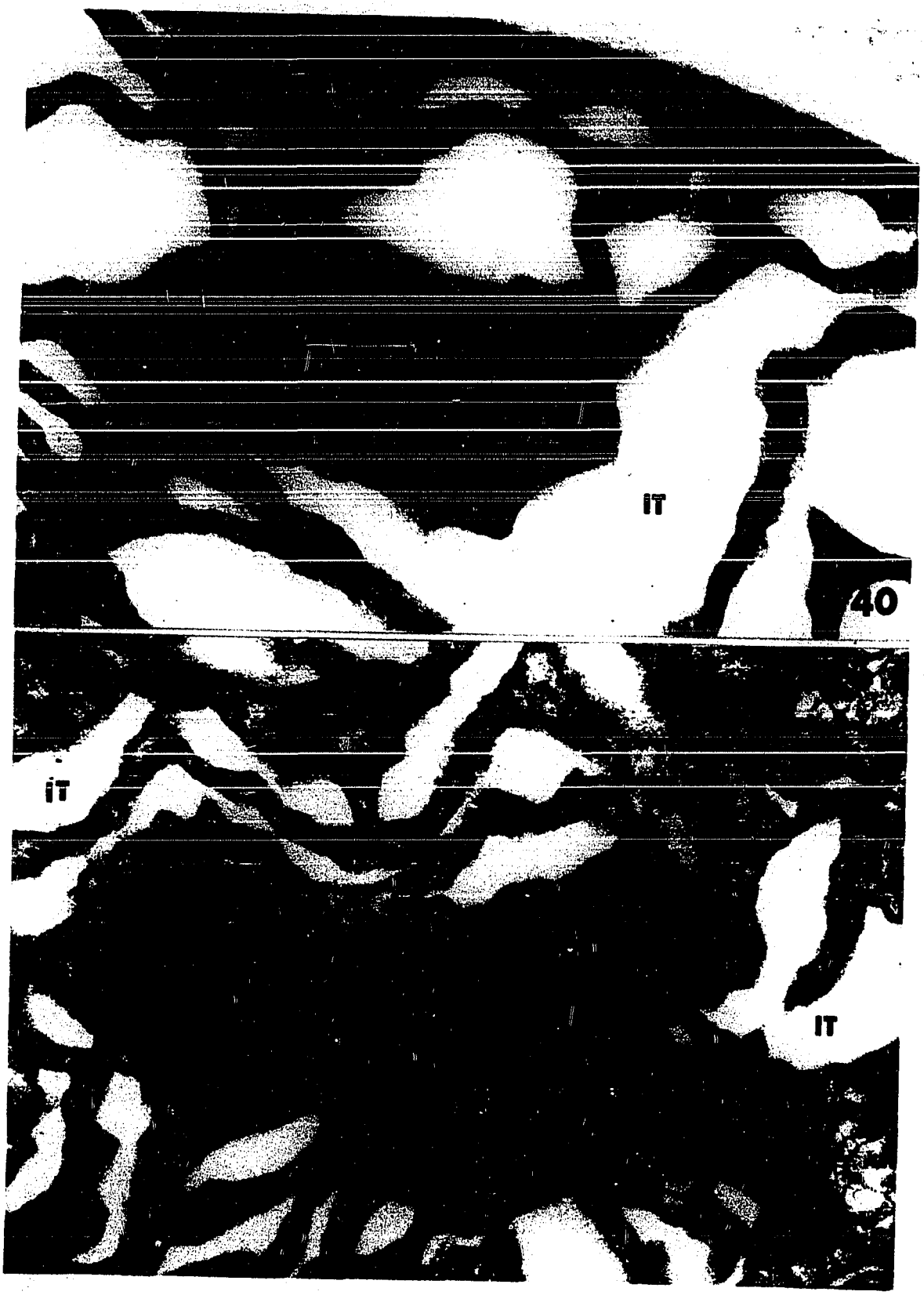

IT
40
IT
IT

Figure 42. Portion of a cell of Nostoc muscorum (ISU-J15) containing spherical inclusions (arrow), a structured granule (SG) and β granules (B). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

Figure 43. Portion of a cell of Nostoc muscorum (ISU-J15) containing a packet of filaments (F), large structured granules (SG), and β granules (B). Osmiophilic fibers in the sheath (S) are also evident. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

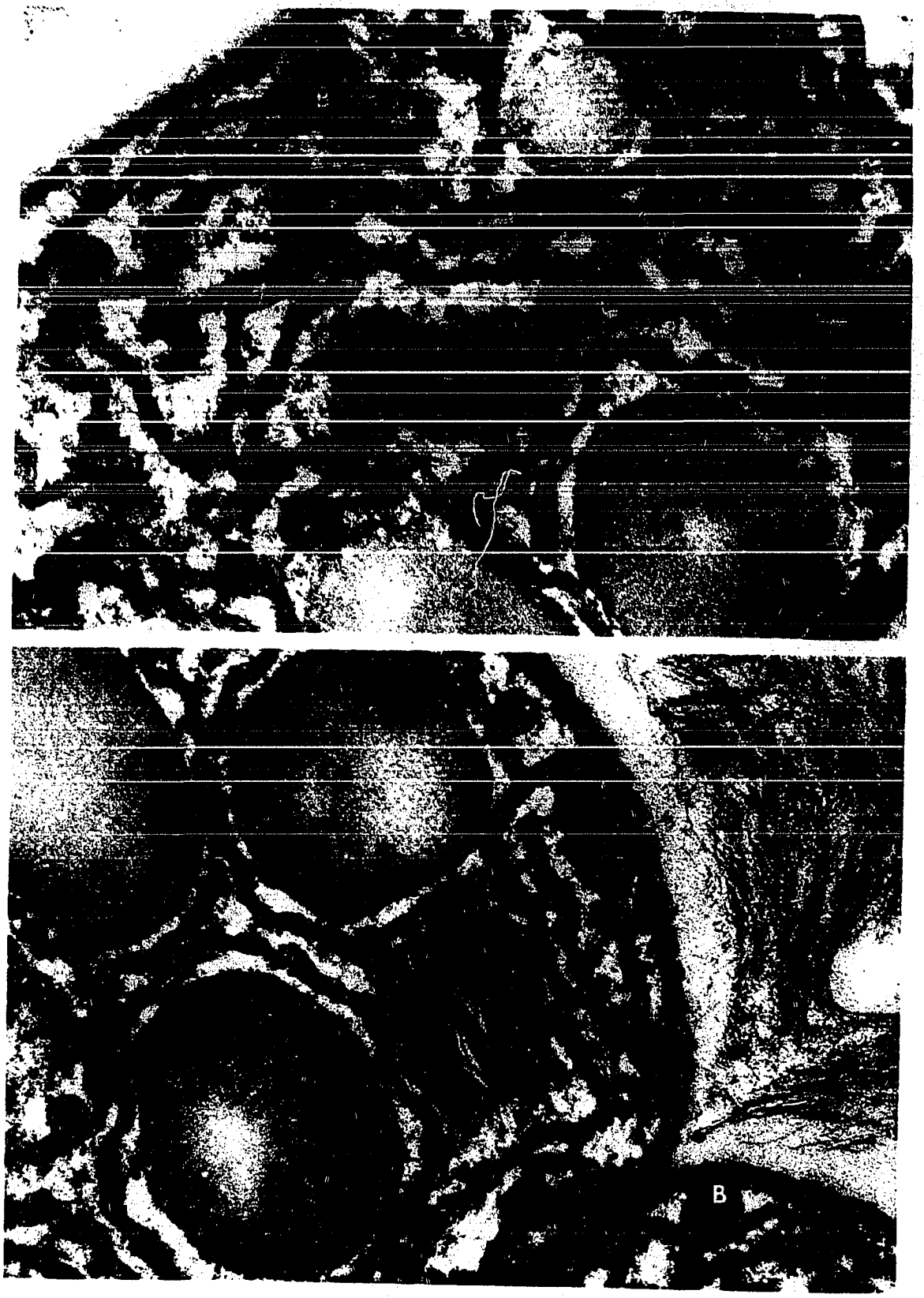

B

Figure 44. A section of part of two cells of Nostoc pruniforme (ISU-J12) containing a plate (P) and filament (F) arrangement. The thylakoids (TH) can be seen in various degrees of expansion. A papilla (P) is also evident. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 45. A section which is cut in a plane near the long axis of the plate (P) of the plate and filament (F) arrangement in Nostoc pruniforme (ISU-J12). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 46. A section through the narrow axis of the plate of the plate-filament arrangement in Nostoc pruniforme (ISU-J12) showing the plate (P) and the lack of fixation of the filaments (arrow) after potassium permanganate fixation. Approximately X72,000.


IT
46

Figure 47. Cross section of the filaments (F) of the plate-filament arrangement in Nostoc pruniforme (ISU-J12). Most of the filaments are arranged in a repeating hexagonal pattern. The outer electron-dense layer and the inner electron transparent zone is apparent in some of the filaments (arrows). Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X92,000.

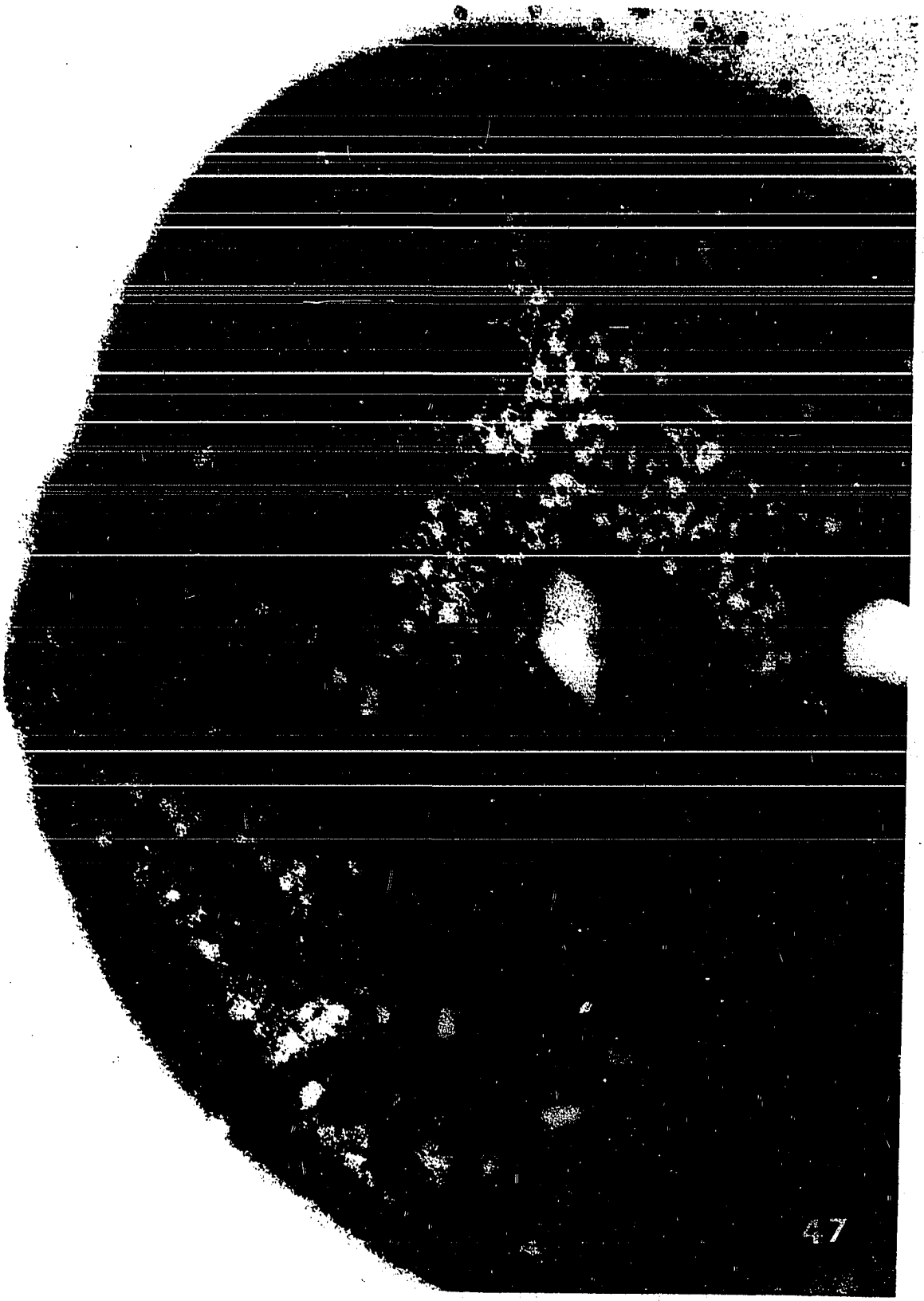

47

Figure 48. Portion of a cell of Cylindrospermum sp. containing a bundle of filaments (F) cut in longitudinal section. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

Figure 49. Portion of a cell of Cylindrospermum sp. containing a bundle of filaments (F) cut in cross section. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.

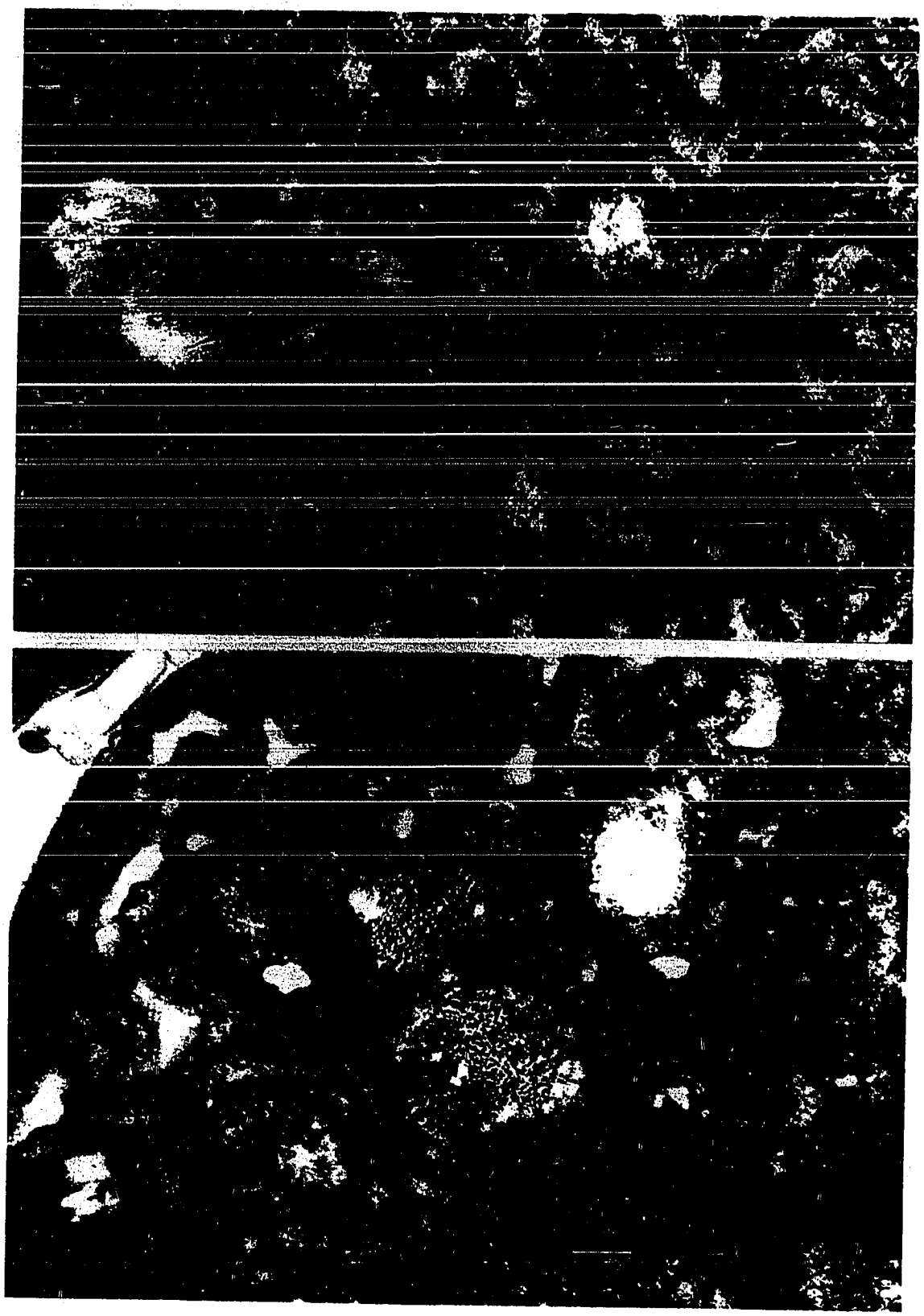


Figure 50. Portion of a cell of Gleotrichia pisum (ISU-J21) containing filaments (F) cut longitudinally. The lighter staining groups of filaments are separated by a layer of ribosome containing cytoplasm (arrow). Darker staining more regularly spaced filaments (F) are evident above the large group of filaments. Large intrathylakoidal spaces (IT) are also evident. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

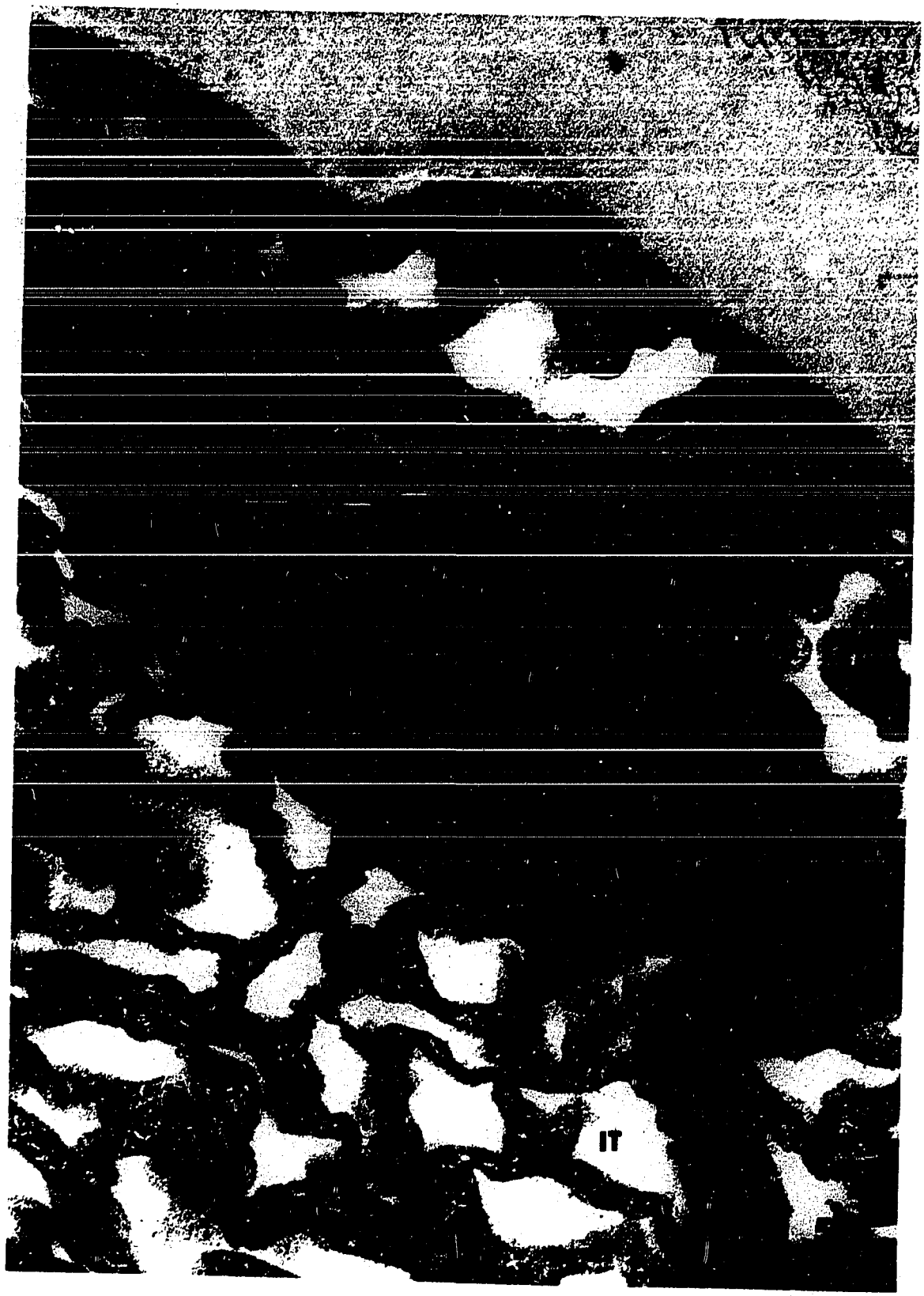

IT

Figure 51. Portion of a cell of Gleotrichia pisum (ISU-J21) containing groups of filaments (F) cut in longitudinal section which are separated by a band of ribosome containing cytoplasm (arrow). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

Figure 52. Portion of a cell of Gelotrichia pisum (ISU-J21) containing filaments (F) cut in cross section. The more regular arrangement of the more osmiophilic filaments (F) on the right is evident. Figure 52a is an enlargement of the less dense filaments. The outer osmiophilic layer and a central dark strand can be seen in several of these filaments (arrows). Osmium tetroxide fixation and stained with methanol uranyl acetate. Figure 52 approximately X72,000. Figure 52a approximately X120,000.

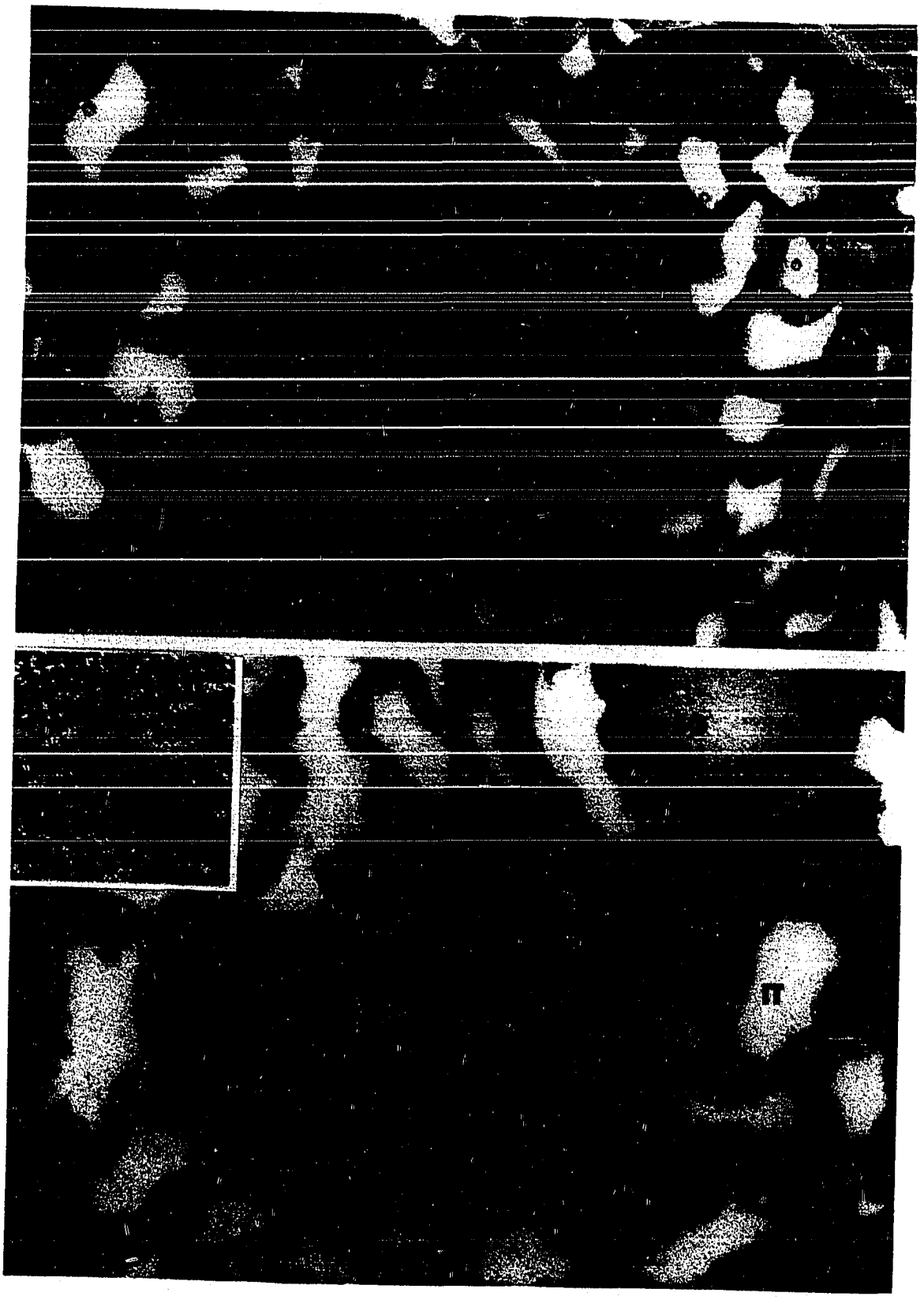


Figure 53. Portion of a cell of Nostoc pruniforme (ISU-J11) containing tubular-like system (T). Osmium tetroxide fixation and stained with lead hydroxide. Approximately X72,000.

Figure 54. Portion of a cell of Nostoc muscorum (ISU-J15) containing tubular-like system (T). The tubes have been cut longitudinally. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

Figure 55. Portion of a cell of Nostoc muscorum (ISU-J15) containing tubular-like system (T) sectioned in cross and longitudinal section. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

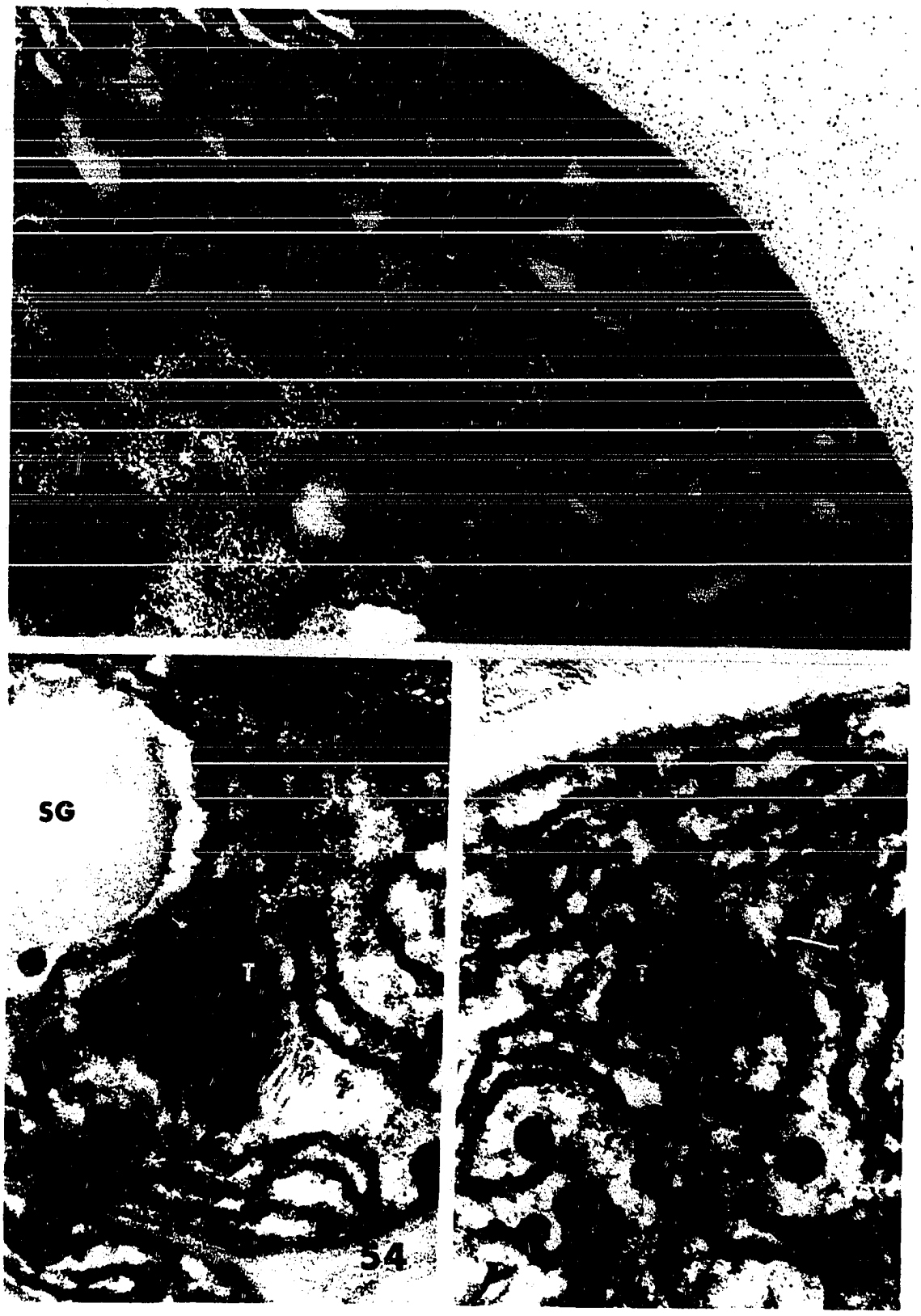

SG
T
54
T

Figure 56. Portion of a cell of Gleotrichia pisum (ISU-J20) containing rosette-like inclusions (RS) within the large intrathylakoidal spaces (IT). Figure 56a is an enlargement of one of the rosette-like inclusions showing a central sphere which is surrounded by 5 similar spheres. Osmium tetroxide fixation and stained with methanol uranyl acetate. Figure 56 approximately X72,000. Figure 56a approximately X120,000.

Figure 57. Portion of a cell of Gleotrichia pisum (ISU-J21) containing large spherical bodies (SB) with less electron dense circular areas (arrow) which contain highly convoluted filamentous elements. A structured granule (SG), large intrathylakoidal spaces (IT) and osmiophilic fibers in the sheath are also evident. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

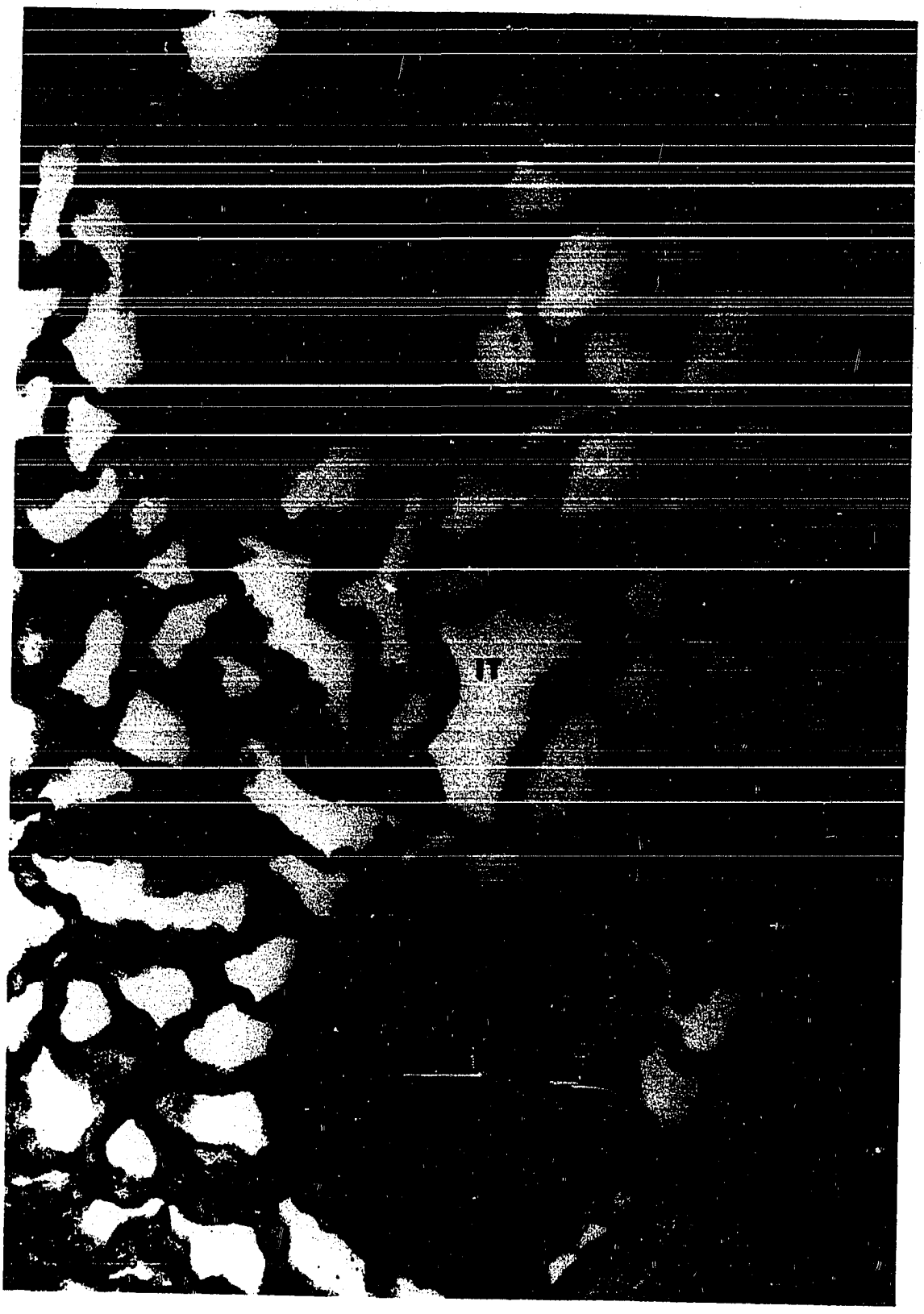


Figure 58. Photomicrograph of normal living filaments of Aphanizomenon flos-aquae. The highly refractile areas within the cells are the gas vacuoles (arrows). Approximately X2,500.

Figure 59. Photomicrograph of living filaments of Aphanizomenon flos-aquae after the gas vacuoles have been destroyed by pressure. Note the absence of the highly refractile areas and the decrease in cell size. Approximately X2,500.

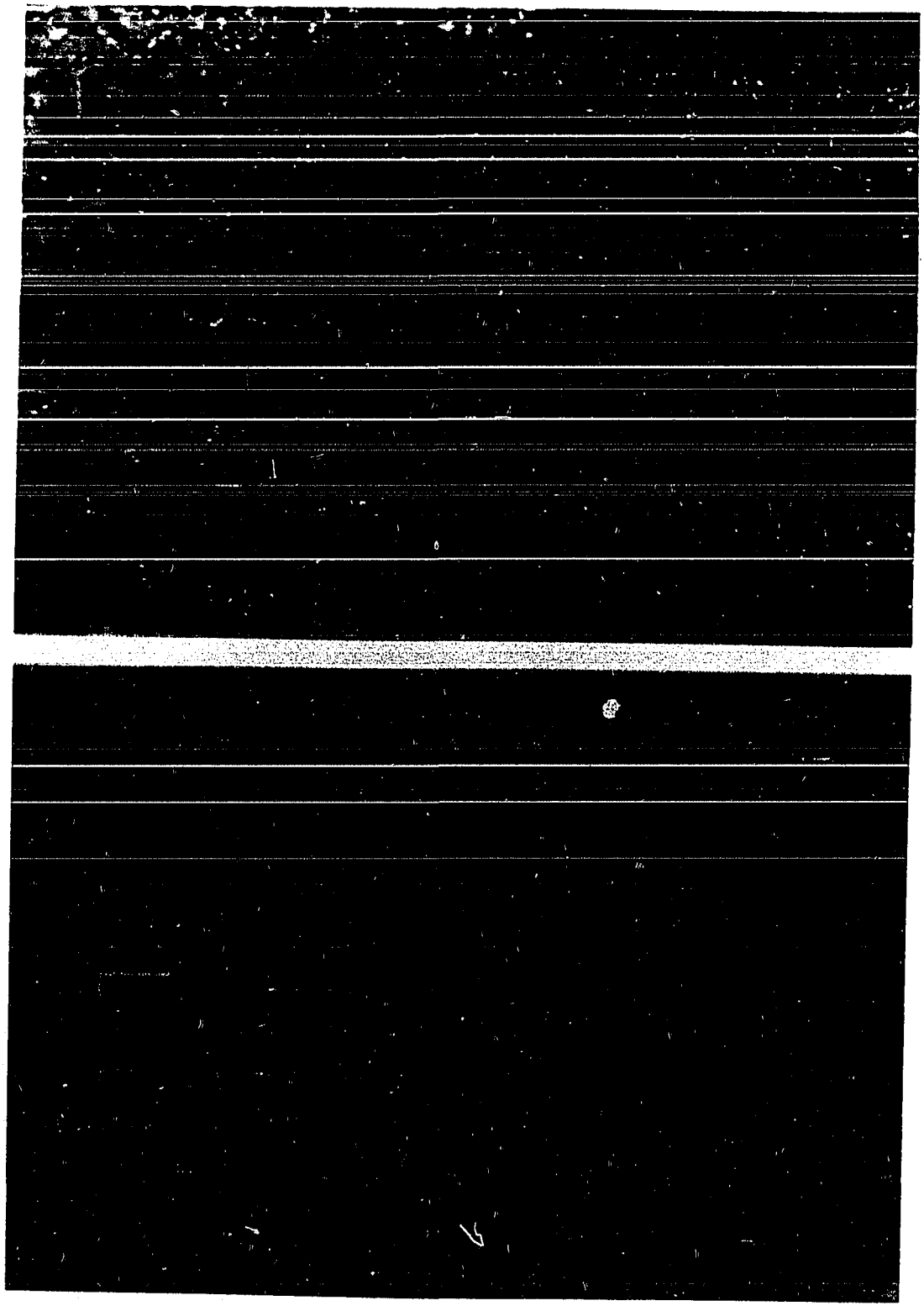


Figure 60. Photograph of the brass-piston-O-ring and cylinder device used to apply pressure to destroy the gas vacuoles.

Figure 61. Portion of two cell of Aphanizomenon flos-aquae containing aggregates of gas vesicles (GV) which make up a gas vacuole. The gas vesicles are shown in cross and longitudinal section. Large intrathylakoidal spaces characteristic of this species are evident (IT). A long papilla formed by the outer membrane is also evident (P). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.


GV
P
IT
G
60
61

Figure 62. Portion of a cell of Aphanizomenon flos-aquae fixed shortly after pressure treatment, showing collapsed gas vesicles (GV). The large intrathylakoidal spaces (IT) are also evident. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

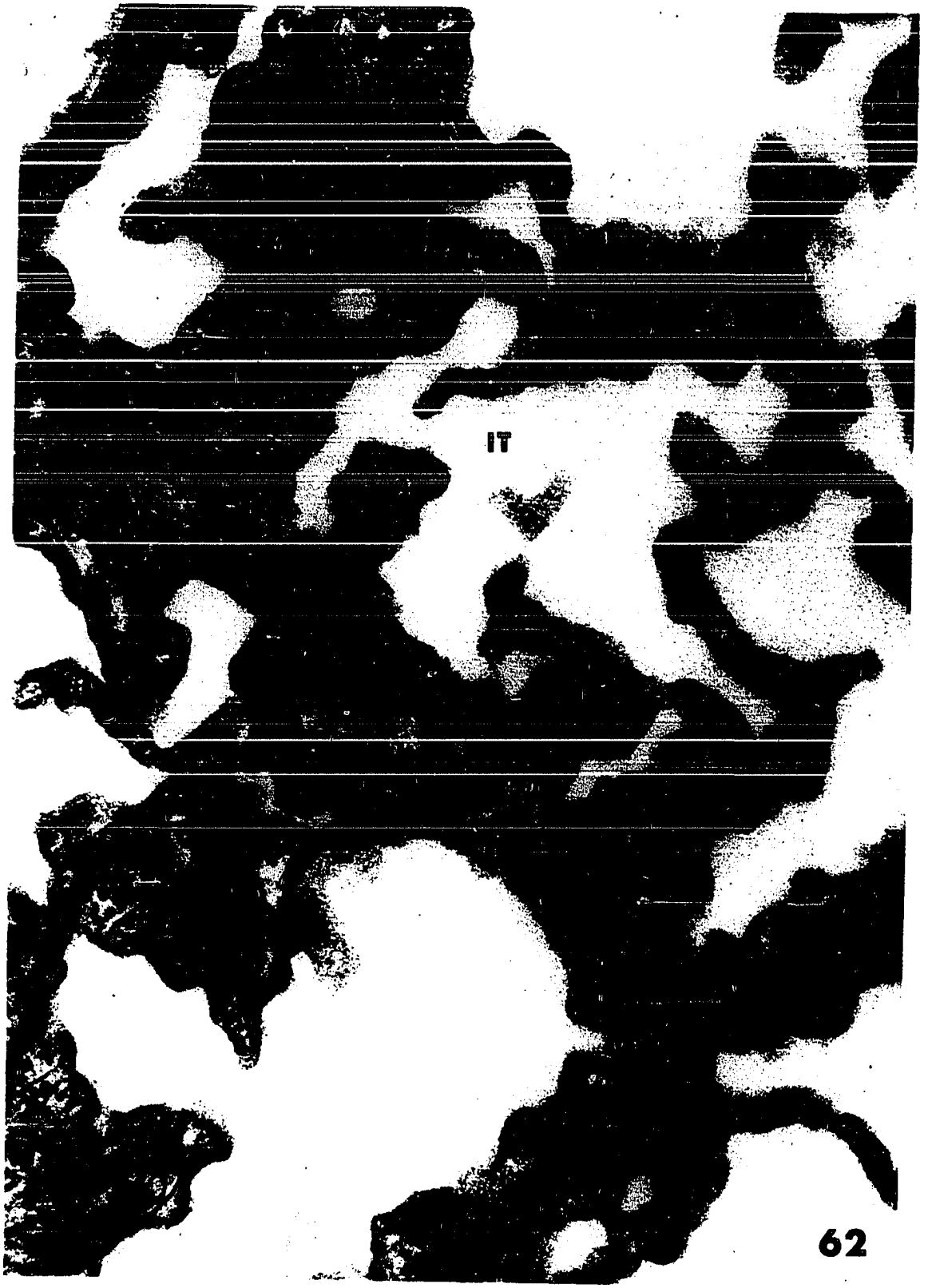
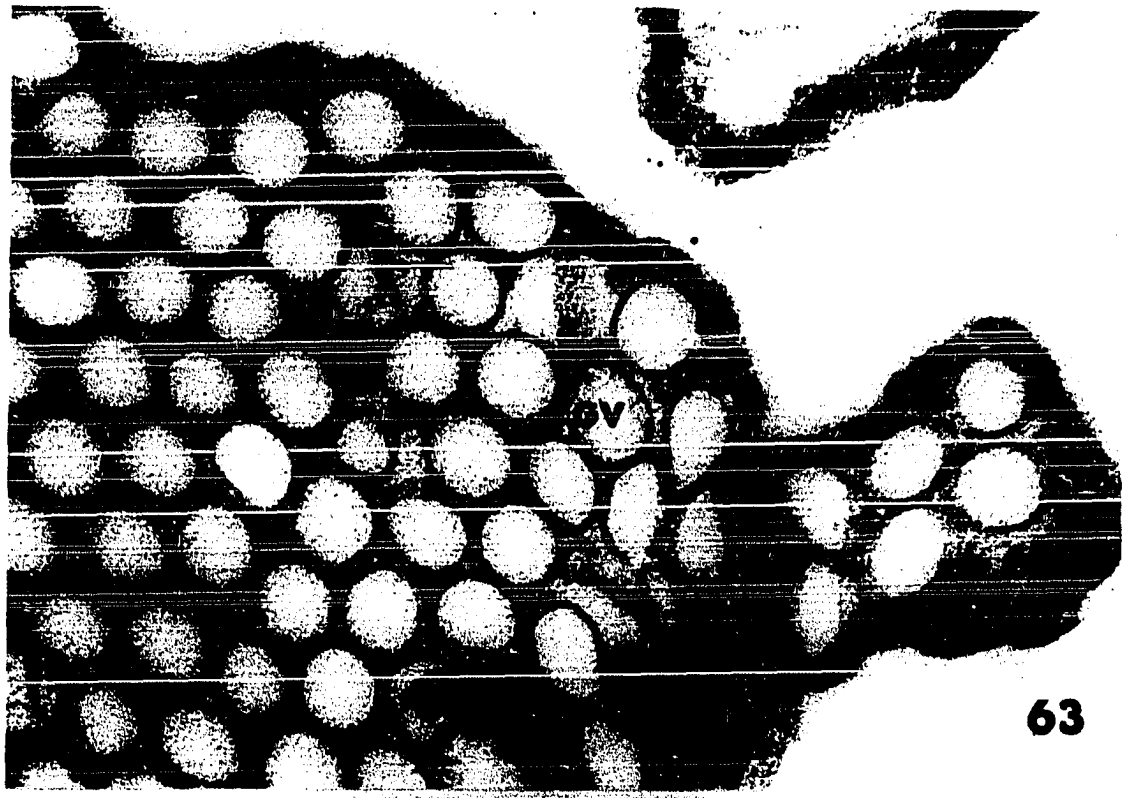

IT
62

Figure 63. Portion of a cell of Aphanizomenon flos-aquae showing gas vesicles (GV) cut in cross section. A partially collapsed gas vesicle can also be seen (arrow). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X144,000.

Figure 64. Portion of a cell of Aphanizomenon flos-aquae showing unit membrane nature of collapsed gas vesicles (GV) and the unit membrane nature of the thylakoid membrane (TH). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X144,000.


63

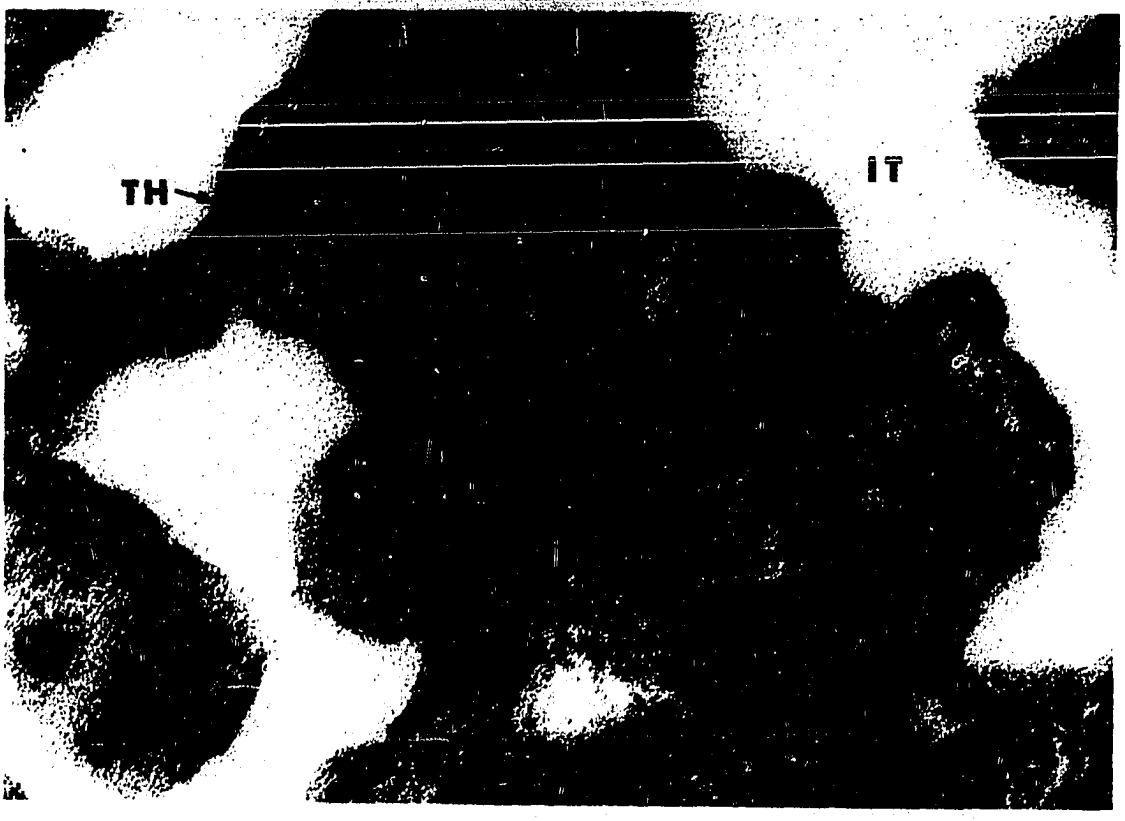

TH
IT

Figure 65. Cell of Aphanizomenon flos-aquae fixed 24 hours after pressure treatment showing reexpanded and collapsed gas vesicles (GV). Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.


GV

Figure 66. Portion of 2 cells of Aphanizomenon flos-aquae showing lack of fixation of gas vesicles (GV) with potassium permanganate fixation. However, thylakoid membranes are preserved (TH). Approximately X72,000.

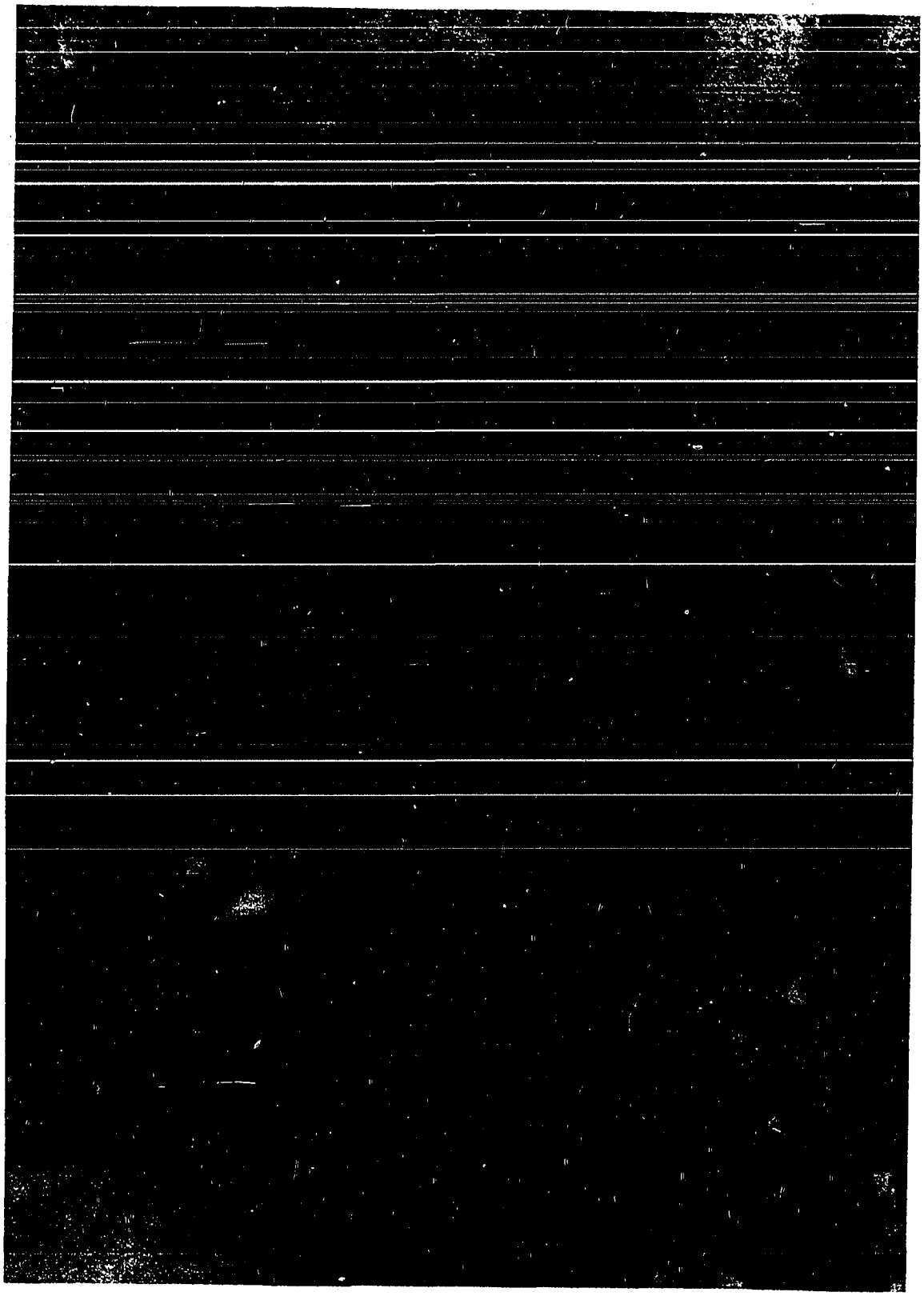
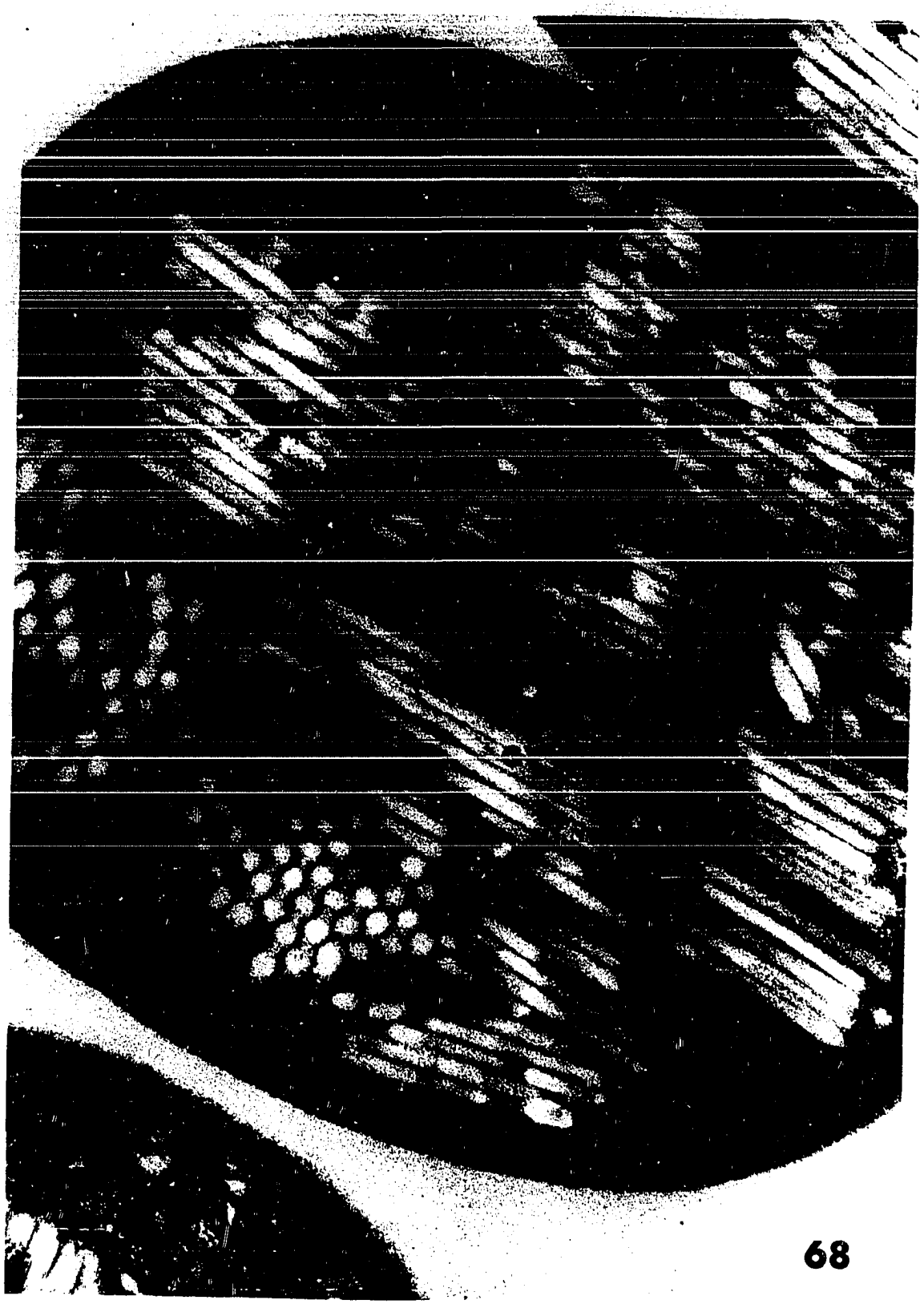


Figure 67. Cell of Nostoc coeruleum showing gas vesicles (GV) cut in cross section. The gas vesicles are arranged in a repeating hexagonal pattern. The entire aggregate of gas vesicles comprises a gas vacuole. A large crystalloid (C) is also present in the section. Osmium tetroxide fixation and stained with methanol uranyl acetate. Approximately X72,000.

67

Figure 68. Section of a cell of Nostoc coeruleum showing gas vesicles (GV) of the gas vacuole cut mainly in longitudinal section. A crystalloid (C) with crosshatched striations is also present. Osmium tetroxide fixation and stained with aqueous uranyl acetate. Approximately X72,000.



68

Figure 69. Portion of a cell of Nostoc pruniforme (ISU-J11) fixed in gluteraldehyde, embedded in Epon, sectioned and digested 24 hours with pepsin. The structured granule (SG) is less dense. The polyhedral bodies (PB) and a crystalloid (C) are apparently unaffected. Stained with methanol uranyl acetate. Approximately X72,000.

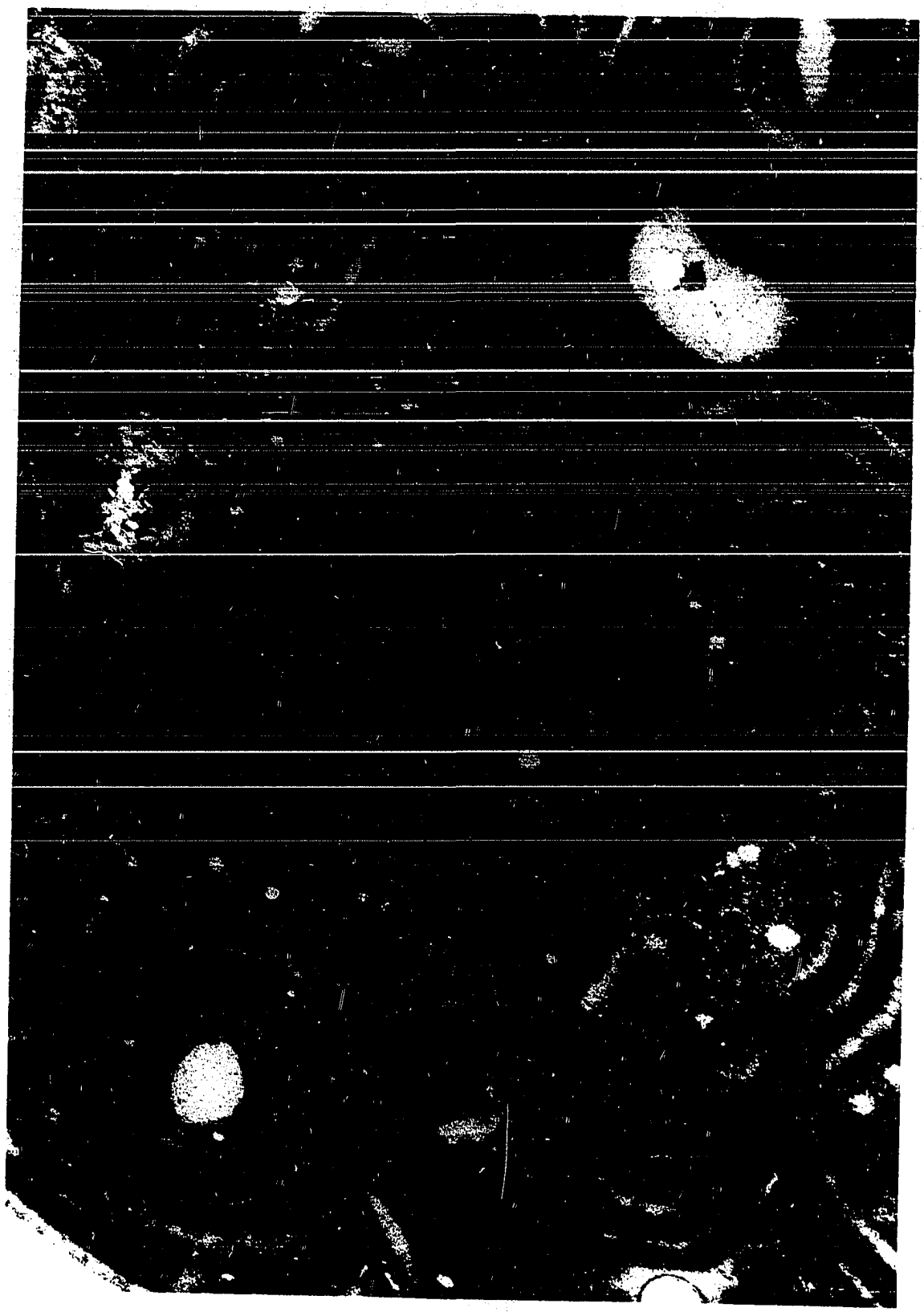


Figure 70. Cell of Nostoc pruniforme (ISU-J11) fixed in gluteraldehyde, embedded in Epon, sectioned and placed in 0.01N hydrochloric acid for 24 hours. The structured granules (SG) contain a dense component. Stained with methanol uranyl acetate. Approximately X52,000.

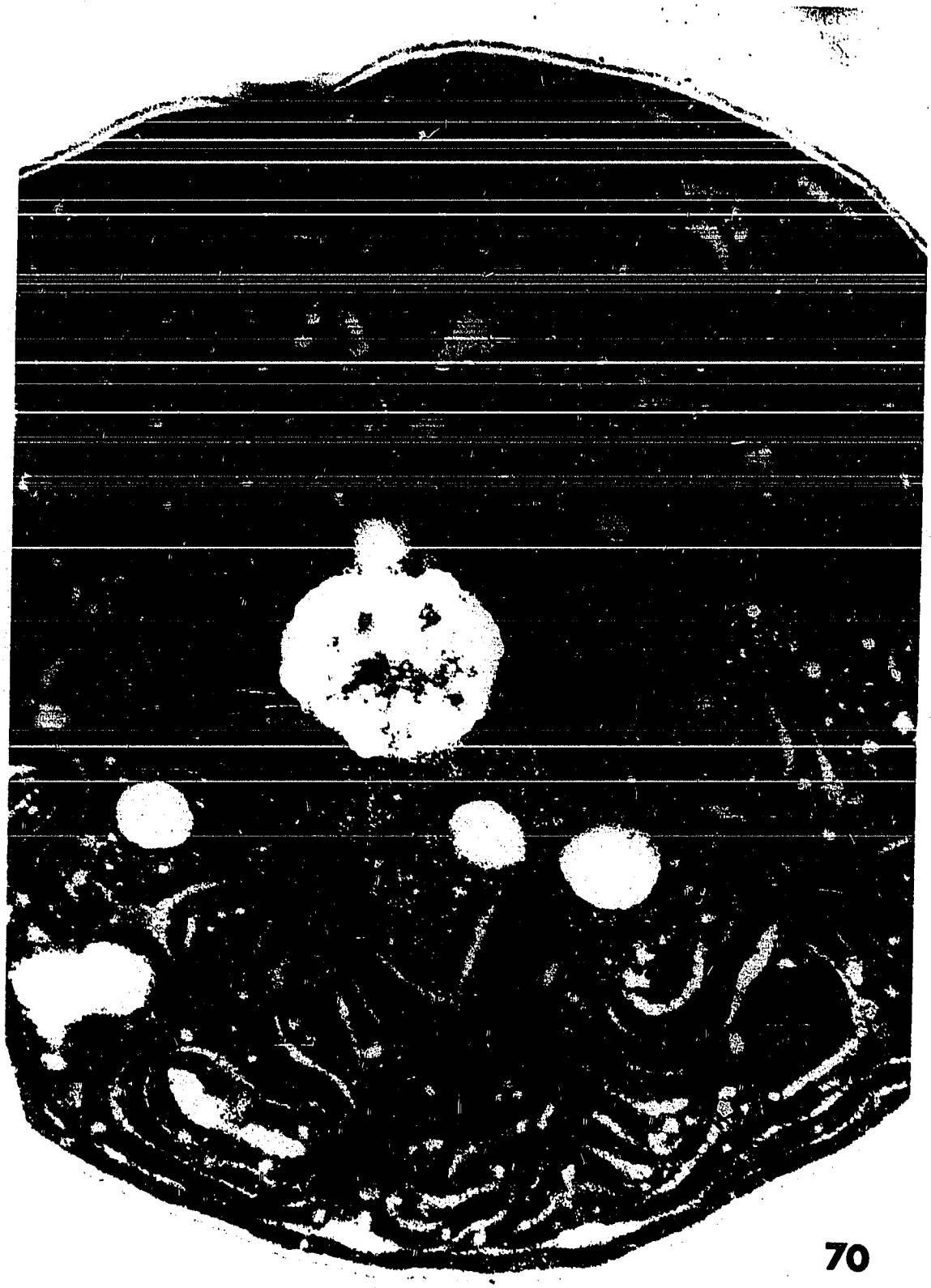


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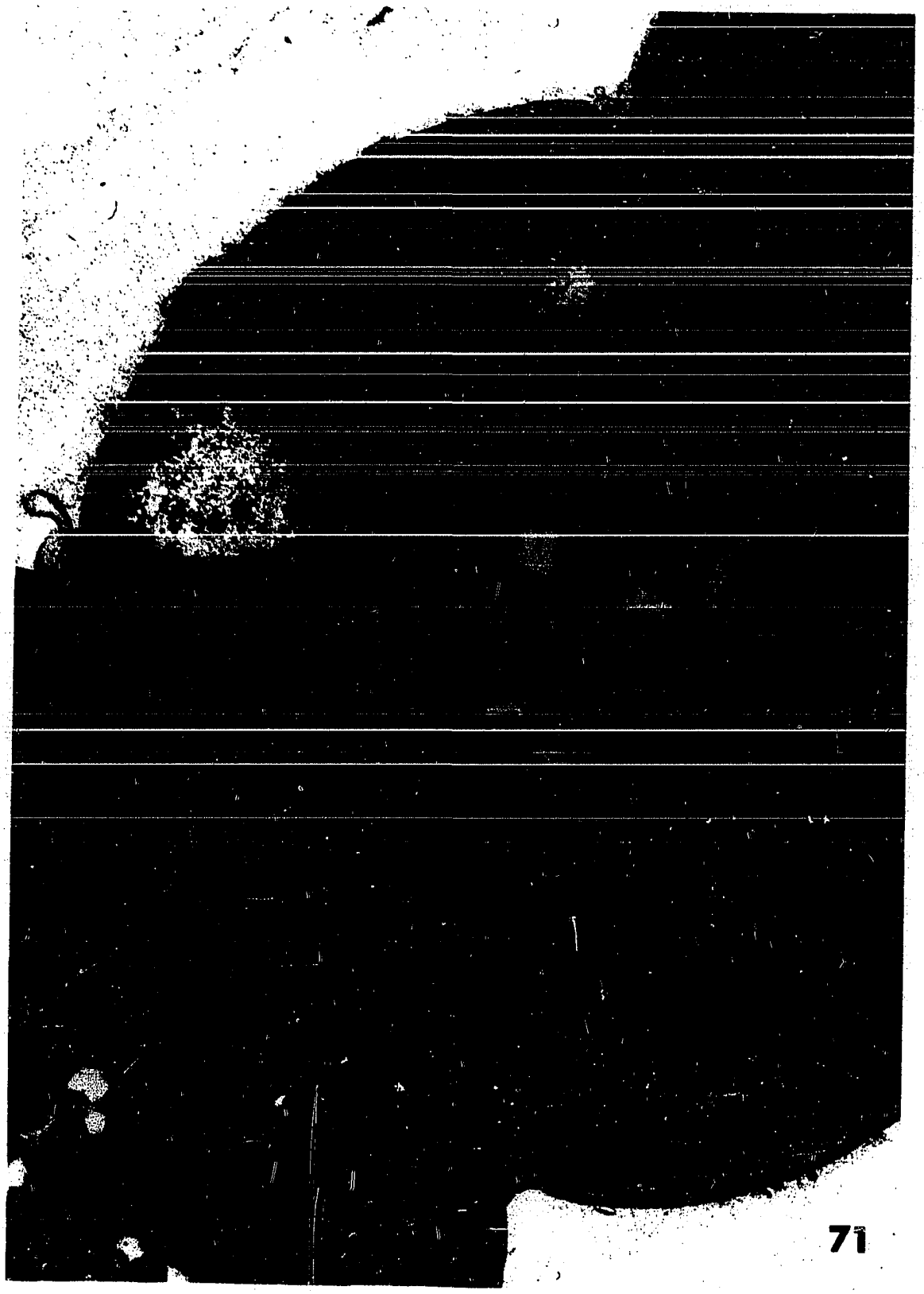
Figure 71. Cell of Nostoc pruniforme (ISU-J11) fixed in gluteraldehyde, placed in 0.01N hydrochloric acid 4 hours, post-fixed in osmium tetroxide and then embedded in Epon. Electron-dense fragments and a fiberous component of the structured granule (SG) can be seen. A crystalloid (C) is also present. Stained with methanol uranyl acetate. Approximately X52,000.



71

Figure 72. Portion of a cell of Nostoc pruniforme (ISU-J11) fixed in gluteraldehyde, digested with pepsin for 4 hours, post-fixed in osmium tetroxide and then embedded in Epon. Structured granule (SG) is essentially removed except for a small remnant. A crystalloid (C) and a tubular-like system (T) are apparently unaffected. Stained with methanol uranyl acetate. Approximately X72,000.

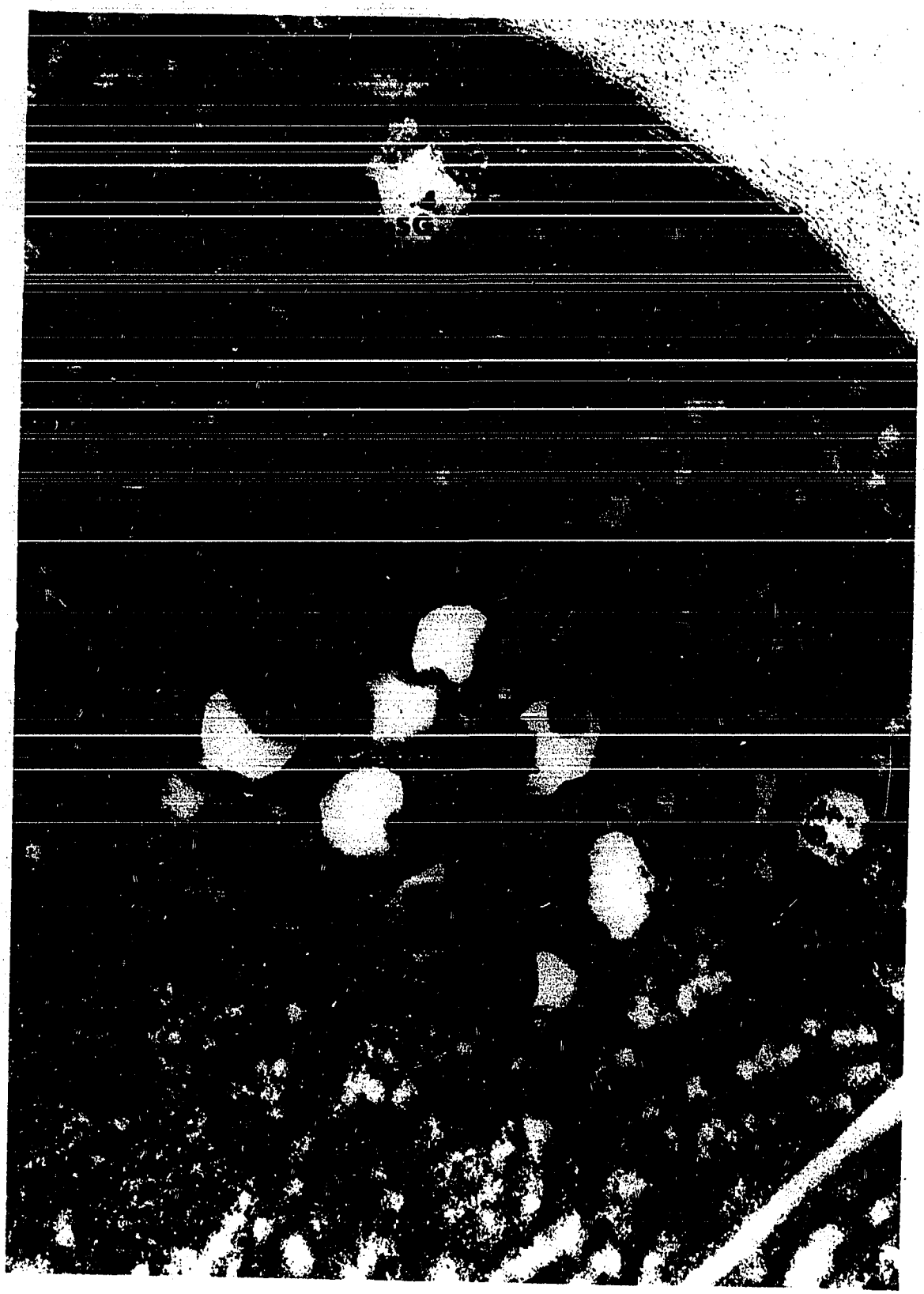
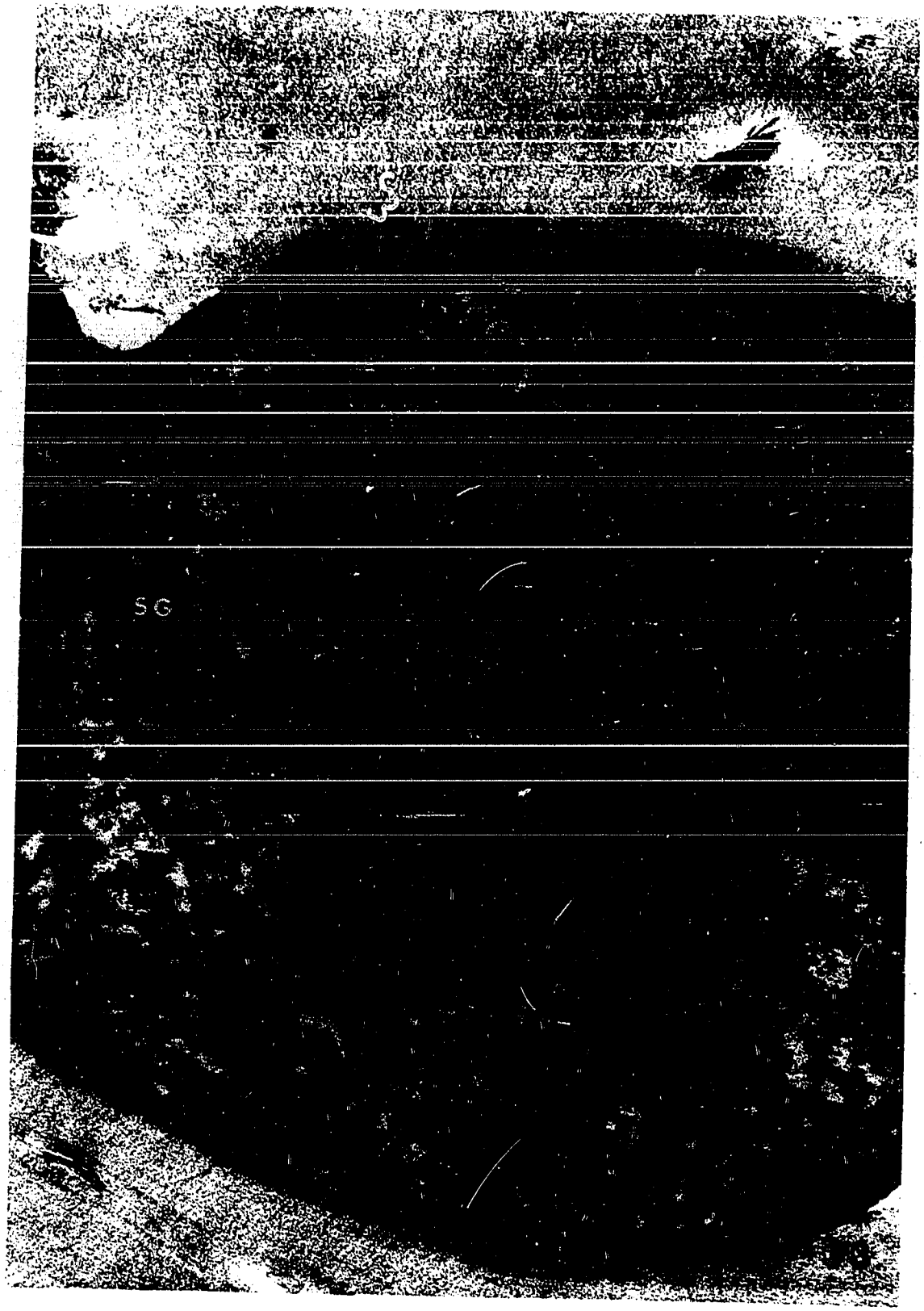


Figure 73. Cell of Nostoc pruniforme (ISU-J11) fixed in gluteraldehyde, left in phosphate buffer during the enzyme digestion experiment, post-fixed in osmium tetroxide and embedded in Epon. Structured granules (SG), a crystalloid (C) and a tubular-like system (T) are evident. Sheath structure (S) is also more evident after this fixation. Stained with methanol uranyl acetate. Approximately X52,000.


SG