Purification and Properties of Some Fresh Water and Marine Cyanobacteria
Belonging to the Orders Chamaesiphonales and Pleurocapsales

By

John Battin Waterbury

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Abstract

A total of 34 strains of cyanobacteria (blue-green algae) representative of the orders Chamaesiphonales and Pleurocapsales were isolated in axenic culture from marine and fresh water sources. Two strains isolated from fresh water, both assignable to the genus Chamaesiphon, are unicellular cyanobacteria which reproduce by budding; the buds being formed in succession at one pole of the oval cell. These buds are the structures currently termed "exospores" by algologists.

The other 32 strains, some marine and some fresh water, reproduce by the formation of small spherical spores produced through the multiple fission of a vegetative cell. In these strains the peptidoglycan and lipopolysaccharide-containing cell wall layers common to all cyanobacteria are enclosed by a third, fibrous layer which increases in thickness during vegetative cell enlargement: spores are liberated by rupture of this wall layer of the parental cell. In some strains, the fibrous wall layer is synthesized during multiple fission, and the spores, each enclosed by this layer, are immotile after release. In others, the synthesis of the fibrous wall layer is arrested during multiple fission, and the spores, initially lacking this layer, show transient gliding motility after release.

Among the spore-forming strains, six major strain clusters could be distinguished in terms of their developmental patterns and each strain cluster could be identified with an existing genus. These included strictly unicellular forms (Dermocarpa, Xenococcus), forms which undergo only one or two vegetative divisions (Dermocarpella), and forms where extensive vegetative growth normally precedes spore formation (Myxosarcina, Chroococcidiopsis, Pleurocapsa). Revised definitions of the orders Chamaesiphonales and Pleurocapsales and of some of their constituent genera are proposed in the light of these findings.

The potential taxonomic utility of certain physiological and chemical properties was also examined. These included: ionic requirements including sodium, chloride, calcium and magnesium; vitamin requirements; nitrogen sources; temperature relationships; heterotrophy; pigment composition; and mean DNA base composition. There were no clear-cut correlations between genera defined in terms of developmental patterns and groups defined in terms of any of the physiological and chemical properties examined. However, many of the latter properties will be useful in distinguishing species within each genus. Strains of marine origin can be distinguished from fresh water strains in culture by their elevated requirements for sodium, halide, calcium and magnesium.

This work has revealed the intrinsically unsatisfactory nature of the existing classification of cyanobacteria based almost exclusively on the description of field materials. Many of the characters previously used to differentiate species (and even genera) are not valid.

It is therefore proposed that all future taxonomic descriptions should be based on the detailed characterisation of axenic cultures, and that cultures should replace herbarium specimens as reference materials.

R. Y. Stanier, Chairman
Dissertation Cttee

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I. INTRODUCTION

With respect to cell structure and development, the cyanobacteria (blue-green algae) are more diverse than any other major prokaryotic group. The primary taxonomic sub-divisions among the cyanobacteria which have been proposed by phycologists are based on the structural and developmental differences within the groups. One sub-group, comprising the order Chroococcales, consists of unicellular organisms that reproduce exclusively by binary fission. A second major sub-group consists of filamentous organisms, which reproduce by fragmentation of the filament or trichome. These filamentous cyanobacteria are assigned by most algal taxonomists to at least two orders, the Stigonematales and Nostocales, distinguished by the structure of the trichome, branched or unbranched, respectively.

The third major sub-group of cyanobacteria is characterized by the ability to reproduce through the formation of spores, either endogenous ("endospores") or exogenous ("exospores"). All these organisms are sessile and grow attached to solid substrates. The exospore-formers (*Chamaesiphon*) and some of the endospore-formers (e.g., *Dermocarpa*) are unicellular, and have been placed on the basis of their unicellularity in a single order, the Chamaesiphonales (or Dermocarpales). However, many endospore-formers can undergo extensive vegetative growth prior to endospore formation, thus producing cellular aggregates consisting of irregular masses or chains of cells. These organisms are commonly assigned to a separate order, the Pleurocapsales.

Spore-forming cyanobacteria are at present a poorly characterized group. The existing descriptions are based almost exclusively on field observations which have rarely permitted an elucidation of the details of their relatively complex cellular life cycles. Very few of these organisms have so far been cultivated, even in the unialgal state. At the time this study was initiated, only five strains (one *Dermocarpa*, one *Xenococcus*, two *Chroococcidiopsis* and one *Chamaesiphon*) (see Tables I, II and IV) had been isolated in pure culture.

Nearly all the groups of endospore-formers are well represented in the microflora of the marine littoral zone, where they occur as epiphytes on other algae and as epiliths or endoliths on calcareous rocks and shells. The initial goal of this study was to isolate and purify representatives of this marine endospore-forming cyanobacterial population, in order to gain a better insight into the properties of the group. Subsequently a number of fresh water strains, including both endospore and exospore formers, were purified and added to the collection. This has enabled us to make a comparative study of the life cycles and physiological properties of a series of cyanobacteria reasonably representative of the two orders Chamaesiphonales and Pleurocapsales. Since the taxonomic conclusions which have emerged from this study are radically different from those of all previous workers, a critical analysis of the past literature, primarily taxonomic in content, is best deferred until it can be presented in the context of the present findings.

In conclusion, we shall note previous reports on the cultivation of members of these two orders. Several authors have used unialgal cultures for observations on the structure and development of

Chroococcidiopsis spp. (24, 39, 40, 41), *Pleurocapsa fuliginosa* (4) and *Hyella* spp. (47, 56). Castenholz (11) has defined the conditions for the laboratory culture of a thermophilic endospore-former, which he identified as *Pleurocapsa minor*. Two strains, identified in the present study as a *Chamaesiphon* sp. and a *Chroococcidiopsis* sp., had been previously studied in pure culture by Stanier *et al.* (70) who identified them as a *Synechococcus* sp. and a *Chlorogloea* sp. respectively. In all the above cases, taxonomic identifications were probably made according to the system of Geitler (27, 29), although this fact is sometimes not explicitly stated. Where relevant, the data provided in these reports will be discussed in the context of the present findings.

II. METHODS AND MATERIALS

A. Biological Material

The sources and histories of the 40 axenic strains used in this study are summarized in Tables I, II, III and IV. In these tables the strains are listed, in order of accession, within four groups: I. strains of marine origin capable of reproducing by multiple fission; II. strains of non-marine origin capable of reproducing by multiple fission; III. unicellular strains of marine origin reproducing only by binary fission and IV. unicellular strains of non-marine origin reproducing solely by budding. The symbols A (axenic) and U (unialgal) indicate the state of strains at their time of accession from other investigators and from culture collections. Strains which were unialgal upon receipt were subsequently purified by the author.

T A B L E I

STRAINS OF MARINE ORIGIN, CAPABLE OF REPRODUCING BY MULTIPLE FISSION

Strain Number	Name (if any) Previously applied	Source and Date collected	Previous History (if any)	Ref.
7301	<i>Dermocarpa violacea</i> (1)	marine aquarium, Scripps Institute of Oceanography, La Jolla, California Dec. 1964	isolated by R. Lewin (strain 1416/1 ---A---> CCCAP 1965 ---A---> IPCC 1972	
7302		sea water tank, Arizona Marine Station, Puerto Penasco, Mexico Mar. 1971		
7303		sea water tank, Arizona Marine Station, Puerto Penasco, Mexico Mar. 1971		
7304		epiphyte on <i>Rhodochorton</i> sp. high intertidal zone, Bodega Marine Laboratory, California Jan. 1970		
7305	<i>Dermocarpa</i> sp. (1)	marine aquarium, Scripps Institute of Oceanography, La Jolla, California	isolated by R. Lewin ---A---> IPCC 1971	
7306		rock chip, high intertidal zone, Pillar Point, California Feb. 1970		
7307		rock chip, high intertidal zone, Horseshoe Cove, Bodega Marine Laboratory, California Jan. 1970		
7310		snail shell, intertidal zone, Arizona Marine Station, Puerto Penasco, Mexico Mar. 1970		
7312		"		

T A B L E I (Continued)

Strain Number	Name (if any) Previously applied	Source and Date collected	Previous History (if any)	Ref.
7314		Snail shell, intertidal zone, Arizona Marine Station, Puerto Penasco, Mexico Mar. 1971		
7317		"		
7319		"		
7320		"		
7321		"		
7322		"		
7324		"		
7325		"		
7326		"		
7440		rock chip, high intertidal zone, Deuville, Normandy, France Mar. 1974		
7506		rock chip, collected by T. LeCampion- Alsumard, Marseille, France Sept. 1974		
7516	<i>Hyella</i> <i>caespitosa</i> (2)	rock chip, Station B, l'île Riou, Calanque des Contrebandiers, Marseille, France 1972	isolated by T. LeCampion- Alsumard U → IPCC - Apr. 1975	(46,47)

(1) identified by R. Lewin

(2) identified by T. LeCampion-Alsumard

IPCC: Institut Pasteur Culture Collection

CCCAP: Cambridge Culture Center for Algae and Protozoa

U: Unialgal

A: Axenic

T A B L E II
STRAINS OF MARINE ORIGIN, CAPABLE OF REPRODUCING BY MULTIPLE FISSION

Strain Number	Name (if any) Previously applied	Source and Date collected	Previous History (if any)	Ref.
6712	<i>Chlorogloea</i> sp.	water sample, reservoir, Marin County, California 1967	isolated by R. Kunisawa	70
7203	<i>Myxosarcina</i> (1) <i>chroococcoides</i> <i>Chroococcidiopsis</i> <i>thermalis</i> (2)	soil sample, near Greifswald East Germany 1962	EMAU (Strain A149) 1962 U → CCCAP (Strain 1451/1) 1965 A → IPCC 1972	39,41
7327	<i>Pleurocapsa</i> <i>minor</i>	Hunter's Spring, Oregon 1969	isolated by R. Castenholz (Strain OH-69-PM) U → IPCC 1973	11
7431	<i>Chroococcidiopsis</i> <i>thermalis</i> (1)	mineral spring, near San Diego, Cuba 1964	isolated by Komarek (Strain 1964/48) U → IPCC 1974	39,41
7432	<i>C. thermalis</i> (1)	mineral spring, near Santa Fe, Cuba 1965	isolated by Hindak (Strain 1965/21) U → IPCC 1974	39,41
7433	<i>C. thermalis</i> (1)	soil, dried pool, Pinar del Rio, Cuba 1966	isolated by Hindak (Strain 1966/27) U → IPCC 1974	39,41

T A B L E II (Continued)

Strain Number	Name (if any) Previously applied	Source and Date collected	Previous History (if any)	Ref.
7434	<i>Chroococcidiopsis cubana</i> (1)	pool botanical garden, Havana, Cuba, 1965	isolated by Hindak (Strain 1965/19) ---U---> IPCC 1974	39,41
7436	<i>C. cubana</i> (1)	drainage ditch, Nueva Gerona, Cuba, 1965	isolated by Hindak (Strain 1965/108) ---U---> IPCC 1974	39,41
7437	<i>C. cyanosphaera</i> (1)	pool, botanical garden, Havana, Cuba, 1965	isolated by Hindak (Strain 1965/25) ---U---> IPCC 1974	39,40
7438	<i>C. cyanosphaera</i> (1)	mineral spring, near Santa Fe, Cuba, 1965	Isolated by Hindak (Strain 1965/26) ---U---> IPCC 1974	39,40
7439	<i>C. doonensis</i> (1)	sand, sea beach, near Mamaia, Romania, 1968	isolated by Hindak (Strain 1968/64) ---U---> IPCC 1974	39,41

EMAU: Ernst - Moritz - Arndt - University

(1) identified by Dr. Komarek

(2) subsequent identification by Dr. Komarek

TABLE III

UNICELLULAR STRAINS OF MARINE ORIGIN, WHICH REPRODUCE EXCLUSIVELY BY BINARY FISSION

Strain Number	Name (if any) Previously Applies	Source and Date of Collection	Previous History (if any)	Ref.
7001	<i>Anacystis marina</i> (1)	City Island, New York	Isolated by C. Van Baalen (Strain n° 6)	70,74
	<i>Synechococcus</i> sp. (2)		A ----> IPCC 1970	
7002	<i>Agmenellum quadruplicatum</i> (1)	Magueyes Island, La Parguera, Puerto Rico	Isolated by C. Van Baalen (Strain RR-6)	70,74
	<i>Synechococcus</i> sp. (2)		A ----> IPCC 1970	
7335		Snail shell, intertidal zone, Arizona Marine Station, Puerto Pensasco Mexico Mar. 1971		
7336		Sea water tank, Berkeley, California 1971		
7338		Tidal pool, Bodega Head, California 1970		
7339		Tidal pool, Bodega Head, California 1970		

(1) Identified by Dr. Francis Drouet

(2) Identified by Stanier et al. (70)

TABLE IV

UNICELLULAR STRAINS OF NON-MARINE ORIGIN, WHICH REPRODUCE EXCLUSIVELY BY BUDDING

Strain Number	Name (if any) Previously Applied	Source and Date of Collection	Previous History (if any)	Ref.
6605	<i>Synechococcus</i> sp. Typological Group IA	Water from Strawberry Canyon, Berkeley, California 1966	Isolated by M. M. Allen A ----> IPCC 1966	70
7430	<i>Chroococcidiopsis</i> sp. (1)	Periphyton, creek, Sarka-valley near Praha, Czechoslovakia 1963	Isolated by Hindak (Strain 1963/133) U ----> IPCC 1974	39,41

(1) Subsequently identified as *Chamaesiphon* sp. by
Dr. Komarek (personal communication).

B. Media and Conditions of Cultivation

1. Principal Media and Growth Conditions

Three basic media, the compositions of which are shown in Table V were used routinely during the course of this study. Medium BG-11 (70) was used as the standard medium for strains of non-marine origin. When it was used without a source of combined nitrogen or with nitrogen sources other than sodium nitrate, the sodium concentration of medium BG-11 was maintained by the addition of sodium chloride at a final concentration of 1.0 gm/l (w/v). Medium MN, used as the standard medium for marine strains, has a sea water base and is fortified with the minerals of medium BG-11. Medium ASN-III is a strictly synthetic medium suitable for the growth of marine strains. It is superior to medium MN for the cultivation of strains 7302, 7303, 7304, 7305, 7306 and 7310, and was used for their maintenance. Merck analytical grade chemicals and deionized water, passed through a Millipore Super-Q Water Purifier, were used for the preparation of all media. Solid media contained Difco Bacto agar at a final concentration of 1.2% (w/v). The mineral base and the agar solutions were separately autoclaved (2) at the required concentration and mixed just prior to pouring the plates.

Three strains (7301, 7317, 7002) have an obligate requirement for vitamin B₁₂. For the culture of these strains, vitamin B₁₂ was added to media after autoclaving at a final concentration of 4 µg/l.

The media described above are poorly buffered. For some experiments, buffering capacity was increased by addition of Tris [tris(hydroxymethyl)amino methane]-HCl buffer (pH 7.5), at a final concentration of 0.1% (w/v), to media after autoclaving. However, at this concentration several strains, notably 7301, 7302, 7303, 7304, 7305 and 7306 were

Table V

COMPOSITION OF STANDARD MINERAL MEDIA

Ingredient	Amount per liter			
	BG - 11	MN	ASN - III	
Deionized water	1000 ml	250 ml	1000 ml	
Sea water	-	750 ml	-	
NaCl	-	-	25.0 gm	
MgSO ₄ - 7H ₂ O	0.075 gm	0.04 gm	3.5 gm	
MgCL ₂ - 6H ₂ O	-	-	2.0 gm	
KCl	-	-	0.5 gm	
CaCl ₂ - 2H ₂ O	0.036 gm	0.02 gm	0.5 gm	
NaNO ₃	1.5 gm	0.75 gm	0.75 gm	
K ₂ HPO ₄ - 3H ₂ O	0.04 gm	0.02 gm	0.02 gm	
Citric acid	0.006 gm	0.003 gm	0.003 gm	
Ferric ammonium citrate	0.006 gm	0.003 gm	0.003 gm	
EDTA (disodium potassium salt)	0.001 gm	0.0005 gm	0.0005 gm	
Na ₂ CO ₃	0.02 gm	0.02 gm	0.02 gm	
Trace metals A-5	1.0 ml	1.0 ml	1.0 ml	
pH after autoclaving	7.1	8.5	7.3	
Osmolarity (in milliosmoles) ⁽¹⁾		690	825	

A-5 Trace Metal Mix

Compound	Amount (gm/l)
H ₃ BO ₃	2.86
MnCl ₂ - 4H ₂ O	1.81
ZnSO ₄ - 7H ₂ O	0.222
Na ₂ MoO ₄ - 2H ₂ O	0.039
CuSO ₄ - 5H ₂ O	0.079
Co(NO ₃) ₂ - 6H ₂ O	0.0494

(1) Determined by freezing point depression

inhibited by Tris buffer.

Illumination of cultures was continuous, and was provided by fluorescent light sources. Osram Interna fluorescent lamps were used to illuminate liquid cultures of small volume and plate cultures. Osram white universal circular fluorescent lamps, suspended above the culture vessels, were used to provide a reasonably even illumination of liquid cultures grown in Fernbach flasks of 1500 ml capacity. Most of the organisms studied do not tolerate high light intensities. Stock cultures and plate cultures were generally exposed to light intensities between 200 and 400 Lux measured at the surface of the cultures. Fernbach flasks were exposed to initial intensities of 200 Lux, subsequently increased to 500 Lux as the cultures developed.

No attempt was made to determine temperature optima for the strains examined. With one exception all strains grew well at 25 to 28°C, which was routinely used for their cultivation. One strain of thermophilic origin (7327) failed to grow at this temperature and was routinely cultivated at 37°C.

Stock cultures were maintained in 100 ml Erlenmeyer flasks containing 50 ml of liquid media, and incubated without agitation or aeration. Cultures of larger volume (≥ 1500 ml) were gassed continuously with air containing approximately 1% CO_2 (v/v), and the sodium carbonate concentration was increased to 0.2 gm/l in order to maintain the pH in a favorable range. These cultures were slowly agitated by magnetic stirrers, the gassing mixture being introduced above the level of the liquid through a Pasteur pipette at a rate of approximately 10 cc/min.

2. Test Media and Determination of Growth Requirements

The members of the Pleurocapsales all develop in liquid media in the form of clumps of cells, which often adhere to the glass walls of the culture vessel. As a result, it is not possible to measure quantitatively either growth rates or growth yields. Consequently, all experiments to determine the influence of nutrients or temperature on growth were qualitative, only the presence or absence of growth under a given set of conditions was recorded.

a. Ionic requirements

Heavy cell suspensions from young liquid stock cultures were pipetted onto well dried agar plates containing all nutrients except the ion to be tested. After the liquid of the inoculating suspension had been absorbed by the agar, cells were transferred from these plates with a platinum spade to tubes of liquid medium (7.0 ml) containing a graded series of concentrations of a given ion. A transfer to fresh medium of the same composition was made from tubes where growth had occurred at an ionic concentration close to the limiting value. Only if growth took place on subculture was the result recorded as positive.

The minimum sodium concentration which would support growth was determined with the basal medium shown in Table VI. The chloride concentration in this medium was maintained at a minimum value of 0.2 M. The maximum level of contamination by sodium in the medium, with no added sodium chloride, was calculated to be less than 1.5×10^{-5} M.

The minimum chloride concentration which would support growth was determined using the basal medium shown in Table VII. The sodium concentration was held constant at 0.1 M for the non-marine strains and at 0.5 M for the marine strains by the addition of sodium sulfate. The

Table VI

BASAL MEDIUM FOR EXAMINATION OF SODIUM REQUIREMENTS

Compound	Amount per liter
NaCl	Variable
MgCl ₂ - 6H ₂ O	17.0 gm
KCl	1.0 gm
CaCl ₂ - 2H ₂ O	1.5 gm
MgSO ₄ - 7H ₂ O	0.08 gm
KNO ₃	0.89 gm
K ₂ H PO ₄ - 3H ₂ O	0.02 gm
Citric acid	0.003 gm
Ferric ammonium citrate	0.003 gm
EDTA (disodium potassium salt)	0.0005 gm
K ₂ CO ₃	0.026 gm
Trace metals A-5	1.0 ml

pH after autoclaving ~ 7.5

Table VII

BASAL MEDIUM FOR EXAMINATION OF CHLORIDE REQUIREMENTS

Compound	Amount per liter
NaCl	variable
Na_2SO_4	variable
$\text{Na}_2\text{SO}_4 - 7\text{H}_2\text{O}$	7.0 gm
K_2SO_4	0.58 gm
$\text{Ca}(\text{NO}_3)_2 - 4\text{H}_2\text{O}$	0.8 gm
NaNO_3	0.75 gm
$\text{K}_2\text{HPO}_4 - 3\text{H}_2\text{O}$	0.02 gm
Citric acid	0.003 gm
Ferric ammonium citrate	0.0005 gm
EDTA (disodium potassium salt)	0.02 gm
Na_2CO_3	1.0 ml
Trace metals A-5 (modified) (1)	

pH after autoclaving ≈ 7.5

(1) $\text{MnSO}_4 - \text{H}_2\text{O}$ was substituted for $\text{MnCl}_2 - 4\text{H}_2\text{O}$ in equimolar amounts.

maximum level of contamination by chloride in the medium with no added sodium chloride was calculated to be less than 3.4×10^{-6} M.

Experiments on the effects of magnesium and calcium concentrations on the growth of marine strains were conducted using medium ASN-III, in which the levels of magnesium sulfate and calcium chloride were varied.

Experiments attempting to substitute potassium for sodium were conducted using medium ASN-III in which sodium chloride was replaced by equimolar concentrations of potassium chloride. The effect of potassium on minimum sodium concentrations supporting growth was determined using medium ASN-III in which the concentration of sodium chloride was varied while the chloride level was maintained at 0.4 M by the addition of potassium chloride. The halotolerance of marine strains was examined by the use of medium ASN-III containing increased concentrations of sodium chloride.

b. Vitamin requirements

Strains were screened for vitamin requirements in ASN-III (marine strains) or BG-11 (non-marine strains). The media were prepared with water from a Millipore Super-Q Water Purifier and all glassware was carefully washed with concentrated nitric acid. Initial experiments were conducted with a filter-sterilized vitamin mixture containing vitamins B₁₂, biotin and thiamine at final concentrations of 4 µg/l, 2 µg/l and 10 µg/l, respectively. Subsequent experiments were conducted solely with vitamin B₁₂ at a final concentration of 4 µg/l. Liquid cultures containing 50 ml of medium, with and without vitamins, were inoculated from plate cultures to minimize carry-over from the previous growth media. At least four successive transfers in vitamin-free medium were made in order to demonstrate the lack of an obligate

vitamin requirement. Strain 7002, which has an obligate vitamin requirement for vitamin B₁₂ (74) was used as a control. It invariably failed to grow after one transfer in a medium not containing vitamin B₁₂.

c. Nitrogen sources

All strains were screened for their ability to use ammonia, nitrate and urea as sources of combined nitrogen. All three compounds were used at a final concentration of 0.125 gram atoms of nitrogen per liter in liquid media. All strains were also tested for their ability to grow aerobically in tubes of liquid media lacking a source of combined nitrogen.

The screening of strains for the ability to synthesize nitrogenase anaerobically was performed by a method recently developed by Rose Marie Rippka in Dr. R. Y. Stanier's laboratory at the Pasteur Institute, Paris. Samples (approximately 10 ml) of young stock cultures were twice washed aseptically by centrifugation to eliminate nitrate. The cells were resuspended in 10 ml of an appropriate nitrogen-free medium, buffered with Tris-HCl (pH 7.5) and placed in sterile 75 ml flasks, sealed with serum stoppers. CO₂ (1% v/v) was added to each flask with a sterile syringe. The flasks were incubated at room temperature on a rotary shaker, and illuminated from above with a circular fluorescent lamp at an intensity of 1500 Lux. After 36 hours, the nitrogen starved cells had synthesized large amounts of glycogen. At this time DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] was added to each flask at a final concentration of 2×10^{-5} M, sufficient to inhibit completely photosynthetic oxygen production (6). After the addition of DCMU, each flask was gassed with argon for 15 minutes to remove any oxygen present, and acetylene (10% v/v) was added with a sterile syringe.

The gas phase of the flask was periodically sampled, and the presence of ethylene was determined by gas chromatography (17, 65).

d. Heterotrophy

All strains were tested for their capacity to grow both photo-heterotrophically and chemoheterotrophically with glucose, fructose and sucrose at a final concentration of 0.3% (w/v). The tests for photo-heterotrophy were conducted in tubes containing 7.0 ml of mineral medium supplemented with 10^{-5} M DCMU and the sugar to be tested (63). The ability to grow chemoheterotrophically was tested in 100 ml flasks containing 50 ml of mineral medium supplemented with the appropriate sugar, and with Tris-HCl buffer (when tolerated by the strain in question). These flasks were incubated in the dark at either 25, 30, or 35°C, depending on the temperature characteristics of growth of the particular strains examined.

e. Maximum temperature permitting growth

Temperature maxima for growth were determined for all strains in tube cultures. These cultures were incubated in 7.0 ml mineral medium at a series of different temperatures in water baths and illuminated from above with white universal circular fluorescent lamps at an intensity of approximately 300 Lux.

f. Effects of chromatic illumination on pigment synthesis

The effects of chromatic illumination were examined on liquid cultures grown in 100 ml flasks containing 50 ml of mineral medium. The flasks were incubated in opaque plastic boxes equipped with loosely fitting clear plastic lids, through which the flasks were illuminated with either red or green light, produced by Roscolene No. 821 (red) and Roscolene No. 871 (green) gelatin filters (Rosco Laboratories, Inc.,

Figure 1

Absorption spectra and percent transmittance of
Roscolene No. 821 (.....) and Roscolene No. 871
(-----) gelatin filters.

Port Chester, New York). The absorption spectra of these filters are shown in Figure 1. White universal fluorescent lamps were used as light sources. The photosynthetic effectiveness of the light transmitted by the two filters is markedly different. In order to obtain roughly equal growth rates in red and green light, white light intensities at the level of the filters were adjusted to 1800 Lux and 4000 Lux for the cultures grown in red and in green light, respectively.

3. Other Culture Methods

a. Slide culture techniques

Many of the cyanobacteria included in this study reproduce very slowly: weeks may be required for completion of their growth cycles. Thus, it was necessary to find a method of slide culture that would sustain growth and permit semi-continuous observations of development over long periods of time. It was found that Falcon No. 3009 Cooper culture dishes, which were originally designed for the examination of liquid tissue cultures with an inverted microscope, could be used as growth chambers for this purpose. Prior to inoculation 8.0 ml of agar medium was added to the culture dish which just filled the space between the upper and lower halves. After the medium had hardened, the cover was rotated and detached from the underlying agar, the surface of which was inoculated with a suitable dilution of freshly released spores. The cover was then replaced, and the dish was incubated at 25°C in a small clear plastic box which contained tissue paper moistened with a copper sulfate solution to retard evaporation and prevent fungal growth. These cultures were illuminated from above with light at intensities between 200 and 300 Lux. At intervals, the Cooper dishes were placed on a microscope stage and examined. Photographs were made

with a Zeiss Universal microscope equipped with a long focal length condenser and a 16X neofluar objective, using bright field illumination.

The above system has optical limitations imposed both by the thickness of the agar layer and the relatively low power of the objective. However, it permits good growth over long periods (up to four weeks) without risk of contamination and has proved extremely useful for determining the developmental cycles of the cyanobacteria examined.

Two of the strains studied (6605, 7430) had such small cells that their developmental cycles could not be satisfactorily determined by the Cooper dish culture method described above. Instead, traditional slide culture techniques were employed. Glass slides and coverslips were carefully washed, rinsed and sterilized in glass petri dishes. A hot solution (4.0 ml) of medium BG-11 + 1% agar, prepared and sterilized as previously described, was pipetted into a sterile glass petri dish, so that a uniformly thin layer of medium was formed at the bottom of the dish. Squares, about one-third the size of the cover slips used, were cut out of this layer and transferred aseptically to the surface of a sterile microscope slide. The upper surface of the agar was inoculated with a suitable dilution of cells and then covered with a sterile cover slip, subsequently sealed into place with paraffin (two parts paraffin to one part mineral oil w/w). These preparations were incubated, illuminated and photographed as described above, except that a 40X neofluar objective was used for their examination.

It should be noted that no attempt was made to incubate Cooper dish and slide cultures under conditions which permitted the growth of each strain at maximal rates. For example, light intensities were

always kept very low because experience showed that freshly liberated spores are often very sensitive to light. For this reason, the relative growth rates revealed by time lapse photographs of developmental sequences are not strictly comparable with one another, and in many cases are far below the growth rates obtainable at optimal light intensities and temperatures.

b. Cultures for electron microscopy

Several methods were used to grow cells for fixation and subsequent electron microscopic observation. Most fixations were done on cells grown in liquid cultures or on the surface of agar plates, using the standard mineral media, in some cases supplemented with combinations of vitamin B₁₂, Tris-HCl buffer and sucrose (0.3% w/v). In order to make preparations of undisturbed colonies the organisms were grown in 0.5% agar overlays on plates of solid media. In order to observe cell attachment cultures were grown on the surface of small squares of dialysis membrane which were placed on the surface of agar plates.

c. Growth on oyster shells

Oyster shells were carefully washed, broken into small chips and sterilized by autoclaving. Initial experiments were conducted in small glass petri dishes (approx. 50 mm in diameter) containing several oyster shell chips and partially filled (10 ml) with medium MN. These dishes were inoculated with axenic cultures of cyanobacteria. Subsequent experiments were conducted in 50 ml cotton-stoppered flasks, containing oyster shell chips and 15 ml of medium MN. These flasks were inoculated either with an axenic culture of a cyanobacterium or with both the cyanobacterium and an acid producing chemoheterotrophic bacterium. All cultures were incubated at 25°C (the Petri dishes in a large sterile

glass container), and illuminated from above with fluorescent lamps (Interna) at an intensity of approximately 200 Lux. The medium was changed at intervals of 3 to 4 weeks throughout the experiments.

d. Spore motility

Spore motility was observed on standard agar media, in Petri dishes or in Cooper dishes. Plates were inoculated with cells from stationary phase liquid cultures and incubated at 25°C in clear plastic boxes illuminated laterally with unidirectional light at intensities varying from 50 to 200 Lux. In some strains, spore motility proved difficult to demonstrate. Our experience has shown that it may be necessary to use several different light intensities and conditions of culture in order to determine unambiguously the presence or absence of this property.

e. Spore counts

In order to count the number of spores released by single parental cells of *Dermocarpa* and *Dermocarpella*, Cooper dish cultures were inoculated with well dispersed cells from stationary phase liquid cultures and incubated at 25°C in clear plastic boxes. These were illuminated laterally with unidirectional light at intensities varying from 50 to 200 Lux. Following multiple fission and spore release, motile spores move away from the remains of the parental cell wall. When the released spores were sufficiently dispersed, photographs were taken, and spore counts determined on prints.

C. Isolation and Purification of Strains

1. Cyanobacteria

The nutritional requirements of the members of the Pleurocapsales do not appear to differ from those of other cyanobacteria that occur in the same natural habitats and their rates of growth are, in general,

lower than those of other cyanobacteria. Consequently, the establishment of cultures is best achieved by direct isolation from favorable source materials collected in the field. Rock chips, mollusk shells and macroalgae (e.g., *Rhodochorton*) from the intertidal zone are good natural sources of the marine representatives, and all isolates discussed in this thesis were obtained from these sources. Samples were kept moist during transport to the laboratory by placing them in closed bottles or tubes which contained a piece of damp filter paper. Samples should not be covered with water, since this often leads to rapid overgrowth by non-photosynthetic bacteria.

As soon as possible after collection, the samples were examined microscopically in order to determine the most appropriate isolation procedure. Material was scraped off the natural substrate and suspended in sterile liquid medium, tough aggregates were dispersed in a tissue homogenizer or by emulsification between two flame-sterilized microscope slides. When microscopic examination revealed the presence of a large number of contaminating organisms, the suspension was washed several times in sterile medium by repeated low-speed centrifugations. This treatment reduced substantially the population of contaminants.

After such preliminary treatment, the sample was streaked on several plates, and also inoculated into a tube of liquid medium. Since well-isolated colonies are relatively rare on primary plates, the probability of a successful isolation is increased if several plates are prepared from each sample. Members of the Pleurocapsales develop on plates as small, compact colonies, which are often tough and adhere to the agar surface. Early detection and transfer of such colonies is essential, in order to minimize the possibility of overgrowth by other

organisms. Consequently, plate cultures were examined daily with a dissecting microscope, and microcolonies were transferred as soon as they could be recognized. If such colonies could be easily dispersed, they were suspended in sterile medium and restreaked. However, in many cases it was preferable to pick the entire colony off the plate with a platinum spade under the dissecting microscope. Such colonies were transferred into tubes of liquid medium, from which additional plate cultures were prepared after sufficient growth had occurred. When primary plates did not yield any well-isolated colonies, a second set of plates was prepared from the primary liquid culture. Plating was continued until pure cultures were obtained.

With the precautions described above, the primary isolation and subsequent purification of members of the Pleurocapsales, as well as other cyanobacteria that form discrete colonies, can be readily achieved by standard bacteriological procedures. All strains isolated directly from natural samples were of marine origin. However, the same procedures were used successfully to obtain axenic cultures of fresh water strains which were received from other investigators in the unialgal state, many of which were heavily contaminated with non-photosynthetic bacteria and fungi at the time of their receipt.

Cultures were presumed to be axenic when plates showed only one type of colony. This was confirmed by microscopic examination of liquid cultures and by the absence of growth after transfer to mineral medium supplemented with glucose (0.3% w/v) and yeast extract (0.05% w/v) and incubated in the dark.

2. Acid Producing Chemoheterotrophic Bacteria

Acid producing chemoheterotrophic bacteria present in a uni-algal culture of strain 7506 were isolated and purified on plates of medium MN, supplemented with 0.5% glucose, 0.05% Difco nutrient broth, 0.1% ammonium chloride, 0.01% phenol red and 1.2% Difco Bacto agar. Cultures were incubated at room temperature in the dark. Acid producing colonies were purified by successive streaking on the same medium.

D. Microscopic Techniques

All light micrographs, except where expressly noted, were taken with bright field illumination using a Zeiss Universal microscope equipped with Neofluar objectives.

The actual fixation, dehydration and embedding procedures for electron microscopy have been previously described by Rippka *et al.* (1974) (64). In general, cells were prefixed (0.5%) and fixed (3.5%) in glutaraldehyde, with subsequent post-fixation in osmium (1.0%). During fixation, marine strains were buffered in 0.143 M veronal acetate buffer at pH 7.2 supplemented with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (1.0 gm/l), sucrose (34.0 gm/l) and NaCl (10.0 gm/l). Non-marine strains were buffered in 0.143 M veronal acetate buffer at pH 7.2 supplemented with $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (1.0 gm/l).

The initial steps of the fixation procedures varied with the growth conditions used. Cells grown in liquid media were centrifuged at low speed prior to prefixation, while cells grown on solid media could be scraped off the agar surface and suspended in prefixative directly. In both cases the cells were enrobed in agar following fixation in glutaraldehyde. Entire undisturbed colonies, grown in agar

overlays, were removed by cutting small blocks out of the overlay and dropping them directly into prefixative which made it unnecessary to enrobe them subsequently in agar. Small squares of dialysis membrane with cells attached to their upper surface were removed from the surface of agar plates and dropped directly into prefixative. The cells remained attached to the membrane throughout the entire fixation, dehydration and embedding procedure. With the exception of the squares of dialysis membrane, which required flat embedding, all preparations were embedded in Beem capsules.

E. Chemical Methods

1. Isolation of DNA

Cells were suspended in lysis mixture (9) and broken in a French pressure cell at 20,000 PSI. Following deproteinization in chloroform-isoamyl alcohol, 24/1 v/v), the (deoxyribonucleic acid) DNA was isolated by selective adsorption onto DNA-grade hydroxyapatite (8) in the presence of 8 M urea in 0.24 M sodium phosphate buffer pH 6.9 (MUP). The hydroxyapatite was washed repeatedly, first with MUP to remove protein and RNA (ribonucleic acid) and subsequently with 0.014 M sodium phosphate buffer (pH 6.9) to remove the urea. The purified DNA was eluted with 0.4 M sodium phosphate buffer (pH 6.9) and dialyzed against 0.1X standard saline citrate (54). Melting curves were determined in 0.1X standard saline citrate (0.0195 M with respect to Na^+) using a Gilford recording spectrophotometer. DNA base compositions were calculated as described by Mandel and Marmur (1968) (54).

2. Other Procedures

a. Fatty acid composition

Fatty acid analyses were performed by methods previously described

by Kenyon (1972) (38).

b. Pigment analysis

Pigment analyses were performed by methods previously described by Rippka *et al.* (1974) (64).

c. Protein determination

Protein was determined by the method of Lowry *et al.* (1951) (49).

III. RESULTS

A. Circumscription of the Orders Chamaesiphonales and Pleurocapsales

In most recent treatments of the taxonomy of cyanobacteria (7, 16, 25, 27), all members which reproduce regularly by the formation of spores are placed in two orders: the Chamaesiphonales (or Dermocarpales) and the Pleurocapsales. The distinguishing property of the former order is the unicellular nature of its representatives, which reproduce either by exospore or by endospore formation. The Pleurocapsales, on the other hand, is composed of colonial or filamentous cyanobacteria which reproduce only by the formation of endospores.

Observations made during the course of this study on the structure and development of representatives of these two orders have revealed that the processes of cell division underlying exospore and endospore formation are fundamentally different. *Chamaesiphon*, the classical representative of an exospore former, is in fact a unicellular cyanobacterium which reproduces by budding, i.e., by a binary fission which yields daughter cells of unequal size. This distinctive mode of cell division also occurs in other groups of prokaryotes, for example certain purple bacteria (*Rhodopseudomonas* sp.) (59, p. 29) and certain nitrifying

bacteria (*Nitrobacter* sp.) (77, p. 451). The structures described in the phycological literature as "exospores" of *Chamaesiphon* are in reality the smaller daughter cells produced by this special variant of binary fission.

The formation of endospores, both in the Pleurocapsales and in the Chamaesiphonales, occurs through the multiple fission of a parental cell. This leads to the production of at least four and often many more smaller daughter cells, which are then liberated by rupture of the wall of the parental cell. This mode of reproduction has not been reported in any other group of prokaryotes. Another character shared by all endospore-formers, but absent from *Chamaesiphon*, is the presence of an outer cell wall layer with a distinctive structure and mode of growth.

For these reasons, we propose to make an ordinal separation between the endospore-forming and budding cyanobacteria. The order Chamaesiphonales will be redefined as comprising those cyanobacteria which reproduce by budding; thus excluding the unicellular endospore-formers. The latter organisms will be reassigned to the order Pleurocapsales, now defined as including all cyanobacteria which reproduce by multiple fission.

B. Structure and Development in the Pleurocapsales

1. General Features

a. Growth and reproduction

The members of the Pleurocapsales are distinguished from all other cyanobacteria by their mode of reproduction, which occurs through the formation and liberation of small spherical cells known in the algological literature as endospores. Endospores are produced by multiple fission: i.e., by a series of rapid, successive cleavages of a vegetative cell :

into at least four (and usually many more) daughter cells, all enclosed by the outer wall layer of the parental cell. No increase in cell mass occurs during the cleavage process and consequently the volume of each daughter cell is considerably less than half that of the parent cell. The spores are subsequently released through rupture of the outer wall layer of the parental cell.

In some members of the Pleurocapsales (*Dermocarpa* and *Xenococcus*), multiple fission is the sole mode of cell division: the spore increases greatly in cell mass to produce a large spherical vegetative cell which undergoes multiple fission followed by spore release. In other members of the group, however, spore enlargement is followed by one or more binary fissions, to produce a cell aggregate. It is composed of cells that are much larger than endospores and are held together by the common outer wall layer. The gross form of such aggregates is determined by the planes of successive binary fissions. Regular binary fission in three planes at right angles to one another produces cubical packets of cells, characteristic of *Myxosarcina* and *Chroococcidiopsis*. In the *Pleurocapsa-Hyella* group, the planes of successive binary fissions are less regular and the aggregate consists of a mass of cells, sometimes bearing extensions in the form of cell chains, either branched or unbranched. Some or all of the cells that compose such aggregates eventually undergo multiple fission and release endospores.

Most of the existing reports on multiple fission in the Pleurocapsales indicate that it takes place by successive binary fissions (16, p. 41; 25, p. 811; 30, p. 52); however, Setchell and Gardner (66, p. 20 and 26) claimed the occurrence of simultaneous cleavage in *Dermocarpa* sp. This specific claim of Setchell and Gardner could not

be verified in the present investigation. As will be described in the following pages, multiple fission in the Pleurocapsales always takes place by rapid, successive binary fissions. However, in some members of the group (including *Dermocarpa*) this fact cannot be ascertained by light microscopy, and can be established only through the examination by electron microscopy of thin sections of dividing cells (Figures 7, 8, 27 and 28).

The designation of the reproductive cells produced by members of the Pleurocapsales as *endospores* is undesirable, since this term has long been applied by bacteriologists to a cell of an entirely different type. The bacterial endospore is a highly specialized resting cell that arises through an unequal binary fission, becomes engulfed by its larger sister cell, and thereafter undergoes an elaborate differentiation prior to its eventual release through lysis of the enclosing sister cell. The endospore of the Pleurocapsales is always formed through multiple fission and is not a resting cell, since it initiates vegetative growth immediately after its release. We propose to abandon the designation of *endospore* for the reproductive cell characteristic of the Pleurocapsales, and shall refer to it hereafter as a *spore*.

b. Wall structure and its role in growth and development

i. Cell division

As first clearly shown by Frank *et al.* (22, 23), the cell wall of cyanobacteria is homologous in structure and chemical composition with the wall of other Gram negative prokaryotes. It is composed of at least two distinct layers: an inner layer, which appears homogeneous and electron-dense in thin sections, and an outer layer, with the structure of a unit membrane when viewed in thin sections. The inner layer is

destroyed by treatment with the enzyme lysozyme, and its synthesis by growing cells is inhibited by penicillin (14, 18, 23, 26, 33, 34, 55). Both these treatments result in lysis of the enclosed cells. The sensitivity of this wall layer to lysozyme and to penicillin indicates that it is composed largely or entirely of peptidoglycan. The chemical analyses of the purified cell walls of several cyanobacteria (18, 22, 23, 34) have shown the presence of the characteristic molecular constituents of peptidoglycans: muramic acid, glucosamine, meso-diaminopimelic acid, D-alanine and D-glutamic acid. We shall term this layer the PG layer (Figure 9). Recent analyses of cell wall preparations from cyanobacteria (78), have also shown the presence of lipopolysaccharides, which are known to be characteristic components of the outer wall layer in most Gram negative prokaryotes (57). It is therefore probable that the outer wall layer in cyanobacteria is homologous both in structure and in chemical composition with the corresponding wall layer in other Gram negative groups. We shall term this the LP layer (Figure 9).

The PG and LP wall layers of cyanobacteria are often enclosed by an additional layer, readily distinguishable in electron micrographs of thin sections by its location, thickness and fine structure. In members of the group where such a layer is sufficiently distinct to be detectable by light microscopy, it has been designated by algologists as a *sheath* or *enclosing membrane*. Electron microscopic observations (21, p. 74-77; 44) have shown that the structure of this so-called sheath layer may vary widely among the cyanobacteria. In filamentous forms such as *Lyngbya*, this layer consists of a tubular sheath which surrounds the multicellular trichome, each constituent cell of which is completely enclosed only by the PG and LP wall layers (43).

Our electron microscopic observations have shown that in all members of the Pleurocapsales the PG and LP layers of the wall are completely enclosed by a fibrous outer layer (Figure 9) which is closely appressed to the surface of the LP layer. This layer, which will be termed the F layer, cannot as a rule be resolved by light microscopy.

In the Pleurocapsales, as in other Gram negative prokaryotes, the PG and LP layers of the wall maintain a constant and characteristic thickness during the growth of the cell, except at the sites of transverse wall synthesis where the PG layer often shows a localized and transient thickening, coincident with the change in the polarity of its extension. In the Pleurocapsales, the PG layer is approximately 6.0 nm wide, and the LP layer approximately 7.5 nm wide. These dimensions are very similar to those reported in other groups of Gram negative prokaryotes and in other cyanobacteria. Consequently, during the enlargement of the enclosed cell, the PG and LP layers increase in area, but not in thickness. However, the F layer increases in thickness as well as in area during the growth of the enclosed cell, sometimes attaining a thickness of 1000 nm (Figure 9).

Transverse wall formation accompanying binary fission in the Pleurocapsales is initiated by centripetal ingrowth of the PG and LP layers (Figure 39). Shortly after a transverse wall, composed of these two layers, has been completed, each daughter cell starts to synthesize the material of the F layer evenly over its entire surface. Daughter cells, each completely surrounded by the PG and LP layers, are thus pushed apart by the synthesis of a new F layer, which thickens progressively until the onset of the following division. However, they remain enclosed by the F layers formed during previous generations (Figure 58).

Particularly if binary fission is extensive, electron micrographs of sections passing through transverse wall layers reveal clearly the existence of a series of F layers, laid down in the course of successive cell divisions (Figure 58). The outermost, older portions of the F layer tend to become less dense and irregular in structure, probably because they are stretched to accommodate the increasing volume of the common mass of cells which they enclose. The F layer thus serves as a semi-elastic integument, which holds together small groups of cells formed by a few successive binary fissions (Figure 58). Eventually, enlargement of the cell mass will lead to the rupture of the outermost enclosing F layers. Nevertheless, very large cell aggregates in such genera as *Myxosarcina*, *Chroococcidiopsis*, and *Pleurocapsa* remain coherent, probably as a result of continued adhesion between the F layers of adjacent cells even after the destruction of the common outer F layer (Figure 39).

The synthesis of the wall layers during the course of multiple fission does not occur in the same manner in all representatives of the Pleurocapsales. In *Xenococcus* and *Chroococcidiopsis*, multiple fission is accompanied by the synthesis of a new F layer, enclosing each cleavage product. The resulting spores are consequently each enclosed by a PG layer, an LP layer and a thin F layer at the time of their release. The much thicker F layer of the parental cell remains as a common enclosing structure until the spores are liberated (Figures 20 and 38b). In all other members of the Pleurocapsales, synthesis of the F layer is arrested during the course of multiple fission, and as a consequence the spores are individually enclosed only by PG and LP wall layers at the time of their release from the common F layer of the parent cell. Once

the vegetative development of the spores is initiated, each spore starts to synthesize a new F layer (Figures 7, 8, 27, and 28).

ii. Gliding motility

Gliding movement, which is the sole mode of cellular locomotion in the cyanobacteria, occurs in many filamentous groups and in several unicellular forms (7, p. 296; 25, p. 800-804). With the exception of two reports that the spores of *Pleurocapsa fuliginosa* (4) and *Endonema* sp. (58) are capable of jerking movements, motility has not been described in any members of the Pleurocapsales. During vegetative growth, these organisms are invariably immotile. However, in members of the group which produce spores initially devoid of an F layer, the spores are transiently motile following their release, becoming immotile between 6 and 48 hours later, concomitant with the initiation of the synthesis of a new F layer.

Spore motility is most evident if the newly released spores are placed in a light gradient, where they show a pronounced phototactic response. Phototaxis may be either positive or negative, depending upon the light intensity. Migration of spores from a single parental cell of strain 7302 in a light gradient is shown in Figure 4. Comparison of successive fields shows that immediately after liberation all (or nearly all) of the spores are motile; thereafter, the capacity for movement is lost progressively by the members of the population. Some spores become immotile after six hours or less, while others remain motile as long as 48 hours. The rate of movement by the spores of this strain in a light gradient is approximately 120 μm per hour at 22°C.

In strains where the spores are surrounded by an F layer at the time of their release, spore motility has never been observed. Even in a

light gradient, the spores remain closely clustered around the parental cell (Figure 15).

From the foregoing discussion, it follows that in most cyanobacteria which are capable of undergoing multiple fission, the cells produced by this division process are readily distinguishable from the cells produced by binary fission not only by their small size, but also by a difference in wall structure correlated with a capacity for gliding movement. These differences in wall structure and motility between spores and vegetative cells do not exist in the genera *Xenococcus* and *Chroococcidiopsis*, where the distinction rests solely on a difference in cell size. In *Xenococcus*, which divides exclusively by multiple fission, the parental cell is usually so much larger than the spore that size alone is an adequate character for the recognition of a spore. In *Chroococcidiopsis*, which divides both by binary and by multiple fission, the similarity in size of the spores and the vegetative cells makes discrimination between them, and hence discrimination between binary and multiple fission, extremely difficult.

iii. Spore release

In the algological literature, spore release in the Pleurocapsales has been attributed to the rupture or gelatinization of the enclosing "membrane" of the parent cell following multiple fission (7, p. 295; 16, p. 171; 25, p. 812; 30, p. 56). Light micrographs of the F layer of parental cells following spore release in strains of *Dermocarpa* suggest that this layer is torn open at the time of spore release (Figure 10). Probably the spores begin to enlarge following multiple fission and their increase in cell volume causes the rupture of the common parental F layer in a manner analogous to its rupture following repeated binary

fissions, as described in the previous section.

iv. Attachment to solid substrates

In nature, members of the Pleurocapsales are typically found attached to solid substrates. In the laboratory, when grown in unagitated liquid media, they adhere to the glass surface of the culture vessel. Several observations suggest that only freshly released spores are capable of becoming attached to glass, although subsequent growth of the F layer may increase the strength of attachment. When a stationary phase culture is inoculated into a fresh liquid medium, almost all the cells undergo multiple fission and release spores shortly after transfer. The spores attach firmly to the surface of the glass culture vessel. If the cells are subsequently detached by vigorous shaking, they do not reattach, remaining as suspended clumps in the liquid medium until the next round of multiple fission takes place. Moreover, when cultures are grown on solid media, the spores stick to the agar surface and to each other shortly after their release, making it difficult to remove them from the surface of the plate.

Spores do not appear to possess specialized organelles for attachment, analogous to the holdfasts of *Caulobacter* (60). However, thin sections of strain 7326, growing on the surface of a dialysis membrane which had been placed on the surface of an agar plate, show that the F layer flares out around the base of the cell, increasing the surface area in contact with the substrate, and thus probably increasing the effectiveness of attachment (Figures 26c and 29).

c. Ultrastructure

The cellular fine structure of the Pleurocapsales is, in general, similar to that of other cyanobacteria (44); no features which could be

considered distinctive of this group have been observed. The structure and arrangement of the various layers of the cell wall have been discussed in a previous section. The cytoplasmic membrane appears in thin sections as a unit membrane, approximately 7.0 nm in width (Figure 9). The photosynthetic thylakoids are structurally similar to those described for other groups of cyanobacteria (13, 45). The individual thylakoids are 17.5 nm in width, and composed of two closely appressed unit membranes (Figure 9). In the Pleurocapsales, the thylakoids are dispersed throughout the cytoplasm in somewhat irregular parallel arrays. In each array, the individual thylakoids are normally separated by a distance of 35 to 45 nm; the intervening space is in part occupied by a regular array of spherical phycobilisomes attached to the outer surfaces of each thylakoid. Individual phycobilisomes range in diameter from 25 to 30 nm (Figure 23a).

In certain strains of this group (e.g., 7304, 7307, 7310, 7312, 7314) a second membrane configuration is evident in many cells. It consists of stacks of closely appressed unit membranes which do not appear to bear phycobilisomes on their surfaces (Figures 7a and 19a).

The structure of the nucleoplasm is similar to that of other prokaryotic organisms, appearing in thin sections as relatively electron-transparent areas filled with strands of DNA (Figures 7a, 23a, 39 and 40c). In strains of *Dermocarpa* (7301, 7304) and *Xenococcus* (7306), where large numbers of spores are produced, the DNA appears to segregate into many well-defined nucleoids just prior to the onset of multiple fission (Figure 7a).

Most of the cytoplasmic inclusions described in other groups of cyanobacteria are evident in the cells of the Pleurocapsales. These

include carboxysomes (67, 68, 69) (Figures 39 and 40c), often associated with the nucleoplasm; polyphosphate granules (Figure 23a); and glycogen (Figure 9).

Freshly released spores are very similar, if not identical, in fine structure to mature vegetative cells; the only obvious difference concerns the presence or absence and (if present) the thickness of the F layer of the cell wall.

Thin sections of strain 7305 reveal that a very small minority of the cells contain electron-dense cylindrical inclusions with conically shaped ends measuring 50 nm in diameter and 110 nm in length, that are located in the nucleoplasm (Figure 23a). Negatively-stained preparations of the supernatants of stationary phase cultures of this strain reveal the presence of virus-like particles, whose head dimensions correspond to those of the inclusions seen in thin sections and whose tails are 6 nm in diameter and 400 nm in length (Figure 23b). It is thus likely that the inclusions present in the thin sections are cyanophage heads.

2. Specific Developmental Patterns

Three primary properties influence the life cycles of the members of the Pleurocapsales. These are the relative importance of binary and multiple fissions; the planes of successive binary fissions; and the presence or absence of spore motility. In terms of these criteria, the strains examined can be divided into six groups, each of which corresponds to a previously described genus, as shown in Table VIII. The assignment of strains to these genera is shown in Table IX.

For each of the six groups, the developmental cycle has been established on the basis of three modes of examination: the gross appearance

of mass cultures^{*}; semi-continuous observations with the light microscope on the growth of colonies from single spores in Cooper dishes[†]; and electron microscopic examination of thin sections prepared from material at various stages of growth. In the ensuing accounts, the evidence obtained from these modes of examination will be presented, followed by a schematic reconstruction of the life cycle characteristic for each of the six genera.

*Examination of mass cultures is best done on material which has been grown on a solid substrate (i.e., agar). In groups which produce large aggregates of vegetative cells with a distinctive arrangement (*Myxosarcina*, *Chroococcidiopsis*, and *Pleurocapsa*) the characteristic cell arrangement is often much less evident or absent in liquid cultures.

†In the numerous plates which illustrate development in Cooper dish and slide cultures (Figures 5, 6, 16, 17, 18, 25, 32, 36, 37, 43, 44, 45, 47, 48, 50, 51, 52, 55, 56, 57, 62, 65, 66) the times (in hours) following the first observation are indicated by the number on each individual photomicrograph.

Table VIII

KEY TO SUBGROUPS OF THE PLEUROCAPSALES

- I - Cell division solely by multiple fission.
- A Spores motile *Dermocarpa*
- B Spores immotile *Xenococcus*
- II - Cell division by a combination of binary and multiple fission.
- A Spore development leads to formation of a vegetative cell which undergoes one or two binary fissions, to produce a single apical cell which divides by multiple fission and releases spores; the basal cell(s) subsequently enlarges and repeats the cycle
. *Dermocarpella*
- B Spore development followed by repeated binary fissions in three planes at right angles to one another, producing a cubical aggregate of cells, all of which normally undergo multiple fission.
1. Spores motile *Myxosarcina*
2. Spores immotile *Chroococcidiopsis*
- C Spore development followed by repeated binary fissions in many different planes, to produce irregular, sometimes filamentous aggregates of cells. Some or all of the cells in the aggregate undergo multiple fission.
. *Pleurocapsa*

Table IX
GENERIC ASSIGNMENT OF STRAINS

I.	<u>Dermocarpa</u>
A	Strains of marine origin
	7301
	7302
	7303
	7304
B	Strains of non-marine origin
	7437
	7438
II.	<u>Xenococcus</u>
	Strains of marine origin
	7305
	7306
	7307
III.	<u>Dermocarpella</u>
	One strain of marine origin
	7326
IV.	<u>Myzosarcina</u>
	Strains of marine origin
	7312
	7325
V.	<u>Chroococcidiopsis</u>
	Strains of non-marine origin
	6712
	7203
	7431
	7432
	7433
	7434
	7436
	7439

Table IX (Continued)

VI. Pleurocapsa

A Strains of marine origin

7310
7314
7317
7319
7320
7321
7322
7324
7440
7506
7516

B One strain of non-marine origin

7327

a. Dermocarpa

The genus *Dermocarpa*, which is represented in our collection by six strains (7301, 7302, 7303, 7304, 7437, 7438), includes unicellular forms which divide exclusively by multiple fission and produce motile spores. The examination of mass cultures reveals populations of spherical cells which vary greatly in size. The contents of most of these cells appear homogeneous. However, some of the larger cells have clearly undergone cleavage, and are filled with many small spores (Figures 2 and 3). Spore size is uniform in each strain: some strains have spores 1.5 to 2.0 μm in diameter, others have spores 3 to 4 μm in diameter (see Table X). At the time of their release, the spores display gliding movement, as revealed by their phototactic response to unidirectional illumination (Figure 4). Following the cessation of movement, the spores begin to enlarge symmetrically and increase in volume until multiple fission occurs. The development of the six strains as observed in Cooper dish cultures is very similar, and is illustrated by sequences for strains 7302 (Figure 5) and 7437 (Figure 6). Until cleavage has been completed, the contents of the developing cells appear homogeneous. In each strain, the size of the parental cell at the time of multiple fission varies widely: maximum observed diameters for the cells of each strain are listed in Table X.

The developmental process has also been studied by electron microscopic examination of thin sections (Figures 7 and 8). Figure 7a shows the typical appearance of a cell just prior to multiple fission. It contains numerous discrete nuclear bodies (arrows), and the three wall layers are still in close contact with one another. The F layer, as a

TABLE X

STRUCTURAL PROPERTIES OF *Dermocarpa* STRAINS

Strain	Diameter of spore in μm	Greatest diameter observed for parental cell in μm
7301	3 - 4	30
7302	1.5 - 2	30
7303	1.5 - 2	30
7304	1.5 - 2	20
7437	3 - 4 (2 - 3)*	30 (25)*
7438	3 - 4 (2 - 2.8)*	20 (20)*
* From Komarek (39).		

result of continuous thickening during vegetative growth, has attained a width ranging from 500 to 1000 nm; details of wall structure in a cell just prior to the onset of division are shown in Figure 9. Figure 7b shows a cell in the course of multiple fission; arrows point to three dividing cells. At this stage of development, the cleavage products, each enclosed by the PG and LP wall layers, have retracted from the surrounding F layer of the parental cell, and are also separated from one another. A cell which has completed multiple fission is shown in Figure 8a. Division stages are no longer present, and the final cleavage products, of roughly equal size, are surrounded only by the PG and LP wall layers. A rarely observed developmental abnormality is shown in Figure 8b. In this cell, a large portion of the cytoplasm has failed to undergo cleavage.

Old cultures contain many empty F layers of parental cells. Their appearance in phase contrast photomicrographs (Figures 11a and 11b) suggests that spore release has occurred by rupture of the F layer at a single point.

The developmental cycle of *Dermocarpa* is shown schematically in Figure 11.

As shown in Table X, spore size is a constant strain character. The number of spores produced by single parental cells is therefore a function of the size of the parental cell at the onset of multiple fission, which is in turn affected by environmental factors. When a culture of *Dermocarpa* in the stationary phase of growth is transferred to fresh medium, nearly all the cells undergo multiple cleavage and release spores within 12 to 24 hours. Hence, the number of spores

TABLE XI

The Number of Spores Released by Single Parental Cells of *Dermocarpa* Strains

Strains	Total Number of Determinations	Numbers of Spores Released	
		Range	Individual Values
7301	11	16-293	16,16,21,21,23,23,30,32,32,281,293
7302	9	70-1048	70,71,78,80,95,155,206,340,1048
7303	9	181-868	181,458,540,594,595,716,845,853,868
7304	6	67-199	67,163,171,174,180,199
7437	16	116-267	116,120,130,137,142,152,158,160, 163,192,213,243,247,263,267

produced from single parental cells of each strain varies widely as shown in Table XI.

Since multiple fission occurs through a rapid series of successive binary fissions, the number of spores formed from a single parental cell should, in principle, conform to the series $2^2, 2^3, 2^4 \dots 2^n$. In fact, nearly all spore counts on the progeny of single parental cells deviate from this series; indeed, some of the determined values (e.g., 21, 23, 95, 181, 206, 340) are roughly intermediate between two successive numbers of the series. This suggests that the numerous successive nuclear divisions which accompany vegetative growth, do not always occur in synchrony. Relatively minor deviations from the successive numbers of the series such as 30 ($2^5 = 32$) or 70 ($2^6 = 64$) might also be explained by irregular cell divisions, resulting either in the formation of anucleate spores (number of spores greater than expected) or in the formation of multinucleate undivided segments (number of spores less than expected). In one instance of spore release from a parental cell of strain 7301, microscopic observations on the liberated spores were continued until vegetative enlargement was well underway. Of the 281 spores released, only 256 (2^8) actually developed, showing that about 10 percent of the spore progeny were non-viable. These non-viable spores may have failed to receive nuclei. Some of the electron micrographs of thin sections of strain 7304 (Figure 8b) reveal sporangia that contain, together with numerous single spores of equal size a large segment of cytoplasm which has failed to cleave completely. Such undivided segments have also been seen with the light microscope. They have never been observed to develop following release.

When examined in the light microscope, a very small fraction of the vegetative cells of strain 7437 contain a well-defined equatorial band or plate, suggestive of the occurrence of a binary fission (Figure 12a). However, electron micrographs of thin sections do not confirm this interpretation. Instead, rare cells show a localized thickening and invagination of the fibrous outer layer, which never penetrates deeply into the cytoplasm (Figure 12b). This localized thickening of the outer wall layer appears to correspond to the equatorial band seen by light microscopy. The phenomenon is confined to strain 7437.

Although all strains of *Dermocarpa* produce spherical cells when growing dispersed in liquid cultures, the cell form is modified when the spores develop in dense masses attached to a solid substrate (e.g., the glass surface of a culture flask). Under these circumstances, the cells become pyriform (Figure 13), as a result of mutual compression during their enlargement. The apparent polarity of such cells is imposed by the growth conditions and thus is not a genetically determined property, as is the polarity of the cell in *Dermocarpella* as described in a later section.

Figure 2

Photomicrographs of mass cultures of *Dermocarpa* grown on agar plates of medium MN. The spherical cells of varying size appear either homogeneous or filled with spores.

a. and b. Strain 7301, X1000; c. Strain 7302, X1000;
d. and e. Strain 7303, X1000.

Figure 3

Photomicrographs of mass cultures of *Dermocarpa* grown on agar plates. a. Strain 7304 grown on medium MN, X1000; b. Strain 7437 grown on medium BG-11, X1000; c. and d. Strain 7438 grown on medium BG-11, X1000. In Figure 3c, the field includes many parental F layers left behind following multiple fission and spore release.

Figure 4

Photomicrographs of *Dermocarpa* strain 7302 grown in a Cooper dish culture on medium MN and illuminated laterally with unidirectional light. The three pictures show the phototactic response of freshly released motile spores when placed in a light gradient. The numbers in the upper left hand corners of the pictures indicate the time (in hours) after the preparation was made. The arrows indicate the direction of illumination. Note the remnants of the parental F layer in the upper right hand corner of each picture X200.

Figure 5

Development of *Dermocarpa* strain 7302 on medium MN in a Cooper dish culture. Successive photomicrographs of a single field, showing spore development over a period of 288 hours. X 500 .

Figure 6

Development of a Cooper dish culture of *Dermocarpa* strain 7437 on medium BG-11, over a period of 238 hours. Arrows on the photograph taken at 238 hours point to remnants of the parental F layer after spore release. X 500 .

Figure 7

Electron micrographs of thin sections of *Dermocarpa* strain 7304 grown in liquid medium MN. a. A cell just prior to the onset of multiple fission. It contains many separate nucleoids (N), frequently associated with carboxysomes (C). In one area (arrow) the thylakoids occur in a closely appressed stack. X14,000. b. A cell in the course of multiple fission. The cleavage products have retracted from the parental F layer. Dividing cells are indicated by arrows. X9250.

Figure 8

Electron micrographs of thin sections of *Dermocarpa* strain 7304 grown in liquid medium MN. a. A cell which has completed multiple fission and is filled with spores surrounded only by the PG and LP wall layers. X 11,500. b. A cell undergoing multiple fission which contains a large, uncleaved segment of cytoplasm. X 12,250.

Figure 9

Electron micrograph of a thin section of *Dermocarpa* strain 7302 grown on an agar plate of medium MN. Note the fibrous outer cell wall (F) which is 750 nm thick and of variable density, the lipopolysaccharide layer of the Gram-negative cell wall (LP), the peptidoglycan layer of the Gram-negative cell wall (PG), the cytoplasmic membrane (CM), the photosynthetic thylakoids (T) composed of two closely appressed unit membranes, and glycogen granules (arrows). X83,000.

Figure 10

The F layers of parental cells, emptied of their spore contents, in old cultures of *Dermocarpa* strain 7304 (Figure 10a) and strain 7437 (Figure 10b). Phase contrast, X 1000.

Figure 11

Schematic Developmental Cycle of Dermocarpa

- A Symmetric spore enlargement
- B Multiple cleavage leading to spore formation
- C Spore release
- D Period of spore motility

Figure 12

a. Photomicrograph of *Dermocarpa* strain 7437 grown on medium BG-11. One cell (arrow) has a well-defined equatorial band or plate, suggestive of the occurrence of binary fission. X1000. b. Electron micrograph of a thin section of *Dermocarpa* strain 7437 showing a localized thickening of the F layer (arrows) that appears to correspond to the equatorial band observable by light microscopy (compare with Figure 12a). X7000.

Figure 13

Photomicrographs of *Dermocarpa* strains 7302 (a. and b.) and 7304 (c. and d.) grown in unagitated flasks of medium MN. In such cultures many cells develop in dense masses attached to the wall of the flask. Arrows point to pyriform cells produced through mutual compression of adjacent cells which have developed under crowded conditions attached to a substrate. X1000.

b. *Xenococcus*

The developmental cycle of *Xenococcus*, represented in our collection by strains 7305, 7306 and 7307, closely parallels that of *Dermocarpa*; division occurs solely by multiple fission. The only difference between the two groups is that in *Xenococcus* synthesis of the F layer accompanies multiple fission. Hence, the spores are completely enclosed by a thin F layer at the time of release, and are permanently immotile.

The examination of mass cultures of *Xenococcus* by light microscopy (Figure 14) reveals uncleaved spherical cells of widely varying size, larger cells that have cleaved into spores and many dividing cells which could be construed as being in the course of binary fission. This picture can be adequately interpreted only by following the development of Cooper dish cultures and by the electron microscopic examination of thin sections. The dividing cells are in fact engaged in multiple fission, the division planes being visible (unlike the corresponding stages of *Dermocarpa*) because the transverse walls are relatively thick and refractile as a result of the presence of the F layer.

Freshly released spores are 2-3 μm in diameter in all strains (Table XII). In contrast to *Dermocarpa* spores, they are never motile following their release and hence remain clustered around the F wall layer of the parental cell, even when exposed to unidirectional illumination (Figure 15a and 15b). As in *Dermocarpa*, the size of the parental cell at the onset of multiple fission may vary considerably. The greatest diameters observed for parental cells were approximately 15 μm in strains 7305 and 7307, and 25 μm in strain 7306 (Table XII).

TABLE XII

STRUCTURAL PROPERTIES OF *Xenococcus* STRAINS

Strain	Diameter of spore in μm	Greatest diameter observed for parental cell in μm
7305	2 - 3	15
7306	2 - 3	25
7307	2 - 3	15

The developmental cycles of the three strains as observed in Cooper dish cultures are shown in Figures 16-18. The resolution is not sufficient to permit detection of the successive cleavages, readily evident in light micrographs taken under more favorable optical conditions (Figure 14): hence only cell enlargement and subsequent spore release are observable in the Cooper dish cultures. The development of strains 7305 (Figure 16) and 7306 (Figure 17) is readily interpretable; both clearly reproduce by multiple fission, their growth cycles being comparable to that of *Dermocarpa* (Figures 5 and 6). However, the development of strain 7307 in Cooper dish cultures (Figure 18) is somewhat more difficult to interpret. At the time of spore release, some larger cells also emerge from the F layer of the parental wall. The most probable explanation for this fact, supported by electron microscopic observations to be described below, is that spores are not released immediately following their formation, but proceed to develop within the F layer of the parental cell wall, and eventually again undergo multiple fission. Spore release then occurs; but since maturation of the enclosed cells is not synchronous, some have not yet formed spores at the time of release. These constitute the larger cells, indicated by arrows in the later photomicrographs of Figure 18.

Electron micrographs of thin sections of cells of strain 7307 at various stages of division are shown in Figures 19-21. Figure 19a shows a cell just prior to the onset of multiple fission. It is enclosed by a wall of which the three layers are closely appressed, the F layer being approximately 500 nm thick. Several widely separated areas containing DNA fibrils are present, although the segregation of nucleoids is not as evident as in the comparable stage of *Dermocarpa* (see Figure 7a). Figures

19b, 20a, and 20b show successive cleavage stages. At the two-celled stage (Figure 19b), the retraction of the PG and LP layers from the surrounding parental F layer, characteristic of multiple fission as already described for *Dermocarpa*, has occurred. At the two- and four-celled stages (Figures 19b and 20a), the cleavage products are surrounded by a loose network of fibers, which probably represent newly synthesized F layer material. At later stages of multiple fission (exemplified by Figure 20b), each cleavage product is surrounded by a well-defined F layer of uniform thickness, that is much thinner than the parental F layer. These figures can, accordingly, be interpreted without ambiguity as stages of a primary multiple fission; except for the process of a newly formed F layer enclosing the cleavage products, they correspond very closely to comparable stages in *Dermocarpa* (Figures 7a, 7b, and 8a). Figure 21 shows a section of a group of cells, all enclosed by a common F layer; several of the enclosed cells have started to undergo cleavage. This probably represents an aggregate in which spores that were not released following their formation have undergone growth and subsequent multiple fission within the F layer of the original parental cell.

During multiple fission, in strains 7305 and 7307, the successive cleavages occur in alternating planes, although not necessarily in three regular planes at right angles to one another. However, during the early stages of multiple fission in strain 7306 some cells undergo two or even three successive divisions in the same plane (Figure 22).

Multiple fission and spore release can be induced in *Xenococcus*, as in *Dermocarpa*, by the transfer of cultures to fresh media. Under these conditions, the number of spores released depends on the size of the

cells at the time of transfer, and can vary from as few as four spores (Figure 14c) to 100 or more.

A diagrammatic representation of the developmental cycle of *Xenococcus* is shown in Figure 24.

Figure 14

Photomicrographs of mass cultures of *Xenococcus* grown on agar plates of medium MN. a., b. and c. Strain 7305, X1000; d. and e. Strain 7306, X1000; f. and g. Strain 7307, X1000. Arrows point to dividing cells.

Figure 15

An illustration of the absence of a phototactic response by spores of *Xenococcus* strain 7305 (Figure 15a and 15b) and *Chroococcidiopsis* strain 6712 (Figure 15c). Each photomicrograph shows a group of freshly released spores in Cooper dish cultures subjected to unidirectional illumination. The spores remain closely and evenly clustered around the remains of the parental cells.

a. and b., X 500; c. X 300. Compare with the behavior of the motile spores of *Dermocarpa* (Figure 4).

Figure 16

The development of *Xenococcus* strain 7305 on medium MN in a Cooper dish culture over a total period of 347 hours. The cell had completed spore release after 131 hours. After 347 hours, spore release from cells of the second generation had begun. The successive divisions which lead to spore formation (see Figure 14) are not visible, as a result of the relatively poor resolution under these conditions of examination. X 500.

Figure 17

The development of one spore of *Xenococcus* strain 7306 on medium MN in a Cooper dish culture over a total period of 123 hours. X 500. The developmental pattern is very similar to that of strain 7305 (see Figure 16).

Figure 18

The development of two spores of *Xenococcus* strain 7307 on medium MN in a Cooper dish culture over a total period of 334 hours. In this strain, multiple fission is often followed by either delayed or incomplete spore release. Spore enlargement may therefore occur within the F layer of the parental cell. The arrows in the photographs taken at 264 and 286 hours point to large cells which have developed in this manner. X 500.

Figure 19

Electron micrographs of thin sections of *Xenococcus* strain 7307 grown in an agar overlay on medium MN. a. A cell prior to the onset of multiple fission. The PG, LP and F wall layers are closely appressed to one another. The cell contains two internal membrane configurations: the photosynthetic thylakoids and a stack of closely appressed unit membranes (arrow). X12,000. b. The two cell stage of multiple fission. Note that the daughter cells have retracted from the F layer of the parental cell wall. X15,000.

Figure 20

Electron micrographs of thin sections of *Xenococcus* strain 7307 from the same preparation as the cells illustrated in Figure 19. a. The four cell stage of multiple fission. X14,500. b. A cell which has cleaved into numerous spores, each surrounded by a distinct F layer. X11,250.

Figure 21

An electron micrograph of a thin section of *Xenococcus* strain 7307 grown on a plate of medium MN. The cells in the aggregate have begun to develop while remaining enclosed in the F layer of the previous generation. X13,000.

Figure 22

a. and b. Photomicrographs of *Xenococcus* strain 7306, grown on a plate of medium MN, showing cells that have cleaved repeatedly in one plane (arrows). X1000. c. An electron micrograph of a thin section of a cell of strain 7306, which has cleaved several times in one plane during the early stages of multiple fission.

X8000.

Figure 23

- a. An electron micrograph of a thin section of strain 7305 grown in liquid medium ASN-III. It shows an unreleased spore surrounded by a thin F layer. Note the phycobilisomes (arrows) which are evenly spaced on the thylakoids, the burnt-out polyphosphate inclusions (P) and the very electron-dense cylindrical bodies. X 34,000.
- b. and c. Electron micrographs of negatively stained preparations of the culture medium from very old (two months) flask cultures of strain 7305. These micrographs reveal the presence of a cyanophage which probably corresponds to the electron-dense cylindrical bodies seen in thin sections. This preparation was stained with 0.5% sodium molybdate. X 215,500.

Figure 24

Schematic Developmental Cycle of *Xenococcus*

A Symmetric spore enlargement

B Multiple cleavage leading to spore formation

C Release of immotile spores

c. *Dermocarpella*

The developmental cycle of *Dermocarpella*, represented by only one strain (7326), is similar to that of *Dermocarpa* except that multiple fission is always preceded by at least one binary fission, and occurs in only one of the daughter cells produced through binary fission. In fact, the special mode of development which is characteristic of *Dermocarpella* reveals with particular clarity the distinctions between binary and multiple fission as these processes occur in the Pleurocapsales.

In Cooper dish cultures (Figure 25) the freshly released spores, each approximately 2 μ m in diameter, undergo a brief period of motility (not illustrated). They then begin to enlarge asymmetrically to produce an ovoid cell which measures approximately 10 x 13 μ m at the time of division. A transverse binary fission yields a small cell (the basal cell) and a large cell (the apical cell). The basal cell may undergo one further division in a plane at right angles to the first, to produce a pair of basal cells of equal size. The apical cell, on the other hand, invariably undergoes multiple fission, followed by spore release (photomicrograph taken at 193 hours in Figure 25). The details of cell division cannot be resolved in Cooper dish cultures; but the various division stages which precede spore release are clearly distinguishable in mass cultures examined under more favorable optical conditions (Figures 26a and 26b).

Electron micrographs of thin sections of dividing cells (Figures 27a, 27b, 28a and 28b) reveal further details of the division process. The cell in Figure 27a has just completed the first binary fission which separates the large apical cell from the small basal cell. The three

wall layers of the basal cell remain closely appressed, whereas the PG and LP wall layers of the apical cell have begun to retract from the F layer. Figure 27b shows an individual in which multiple fission of the apical cell has begun, while the basal cell has undergone a second binary fission. The two basal cells have already started to synthesize a new F layer, and remain closely appressed to the F wall layer of the parental cell. Figure 28a shows a later stage of multiple cleavage, and Figure 28b an individual in which multiple cleavage is complete. The F layer enclosing the basal cells has now become relatively thick; no synthesis of a new F layer has occurred around the spores delimited in the apical cell.

After spore release has taken place through rupture of the F wall layer of the apical cell, the basal cell (or cells) can undergo enlargement and repeat the division cycle. Each such repetition causes a thickening of the F layer which underlies the basal cells, and which consists of an F layer that has just been synthesized, together with one or more F layers synthesized during earlier generations. However, the F layer which surrounds the newly developing apical cell consists largely of newly synthesized material, together with adherent remnants of the F layer from the immediately previous generation. The F layer in the region of the apical cell is therefore frequently thinner and more irregular than that underlying the basal cell. This feature of wall structure, distinctive of *Dermocarpella*, is particularly evident in Figures 27a and 28a.

As in all members of the Pleurocapsales, the freshly released spores of *Dermocarpella* can attach to solid substrates. The possible influence of attachment on the polarity of ensuing development was studied by

preparing thin sections of individuals which had been grown on the surface of pieces of dialysis membrane. As shown in Figures 29a and 29b, the polarity of asymmetric spore growth and subsequent division are completely unrelated to the site of attachment. The enlarging ovoid cell shown in Figure 29a (compare with Figure 27a) is attached to the substrate laterally, by a region which will subsequently become part of the apical cell. The mature individual in Figure 29b is attached to the substrate by the pole of the apical cell. Hence, it is evident that the polarity of the growing spore is intrinsic, and is not determined by the point at which it happens to adhere to a substrate.

The number of spores released by single individuals of *Dermocarpella* (Table XIII) is much less variable than in *Dermocarpa* and *Xenococcus*. This is probably attributable to the fact that the onset of binary fission occurs after the cell has attained a fixed size, so that the volume of the apical cell (and hence the number of spores that it produces) is relatively constant.

The developmental cycle of *Dermocarpella* is shown schematically in Figure 30.

TABLE XIII

The Number of Spores Released by Single Parental
Cells of *Dermocarpella* Strain 7326

Strain	Total Number of Determinations	<u>Numbers of Spores Released</u>	
		Range	Individual Values
7326	7	60-120	60, 76, 80, 94, 101, 107, 120

Figure 25

Successive photomicrographs of the development of a single spore of *Dermocarpella* strain 7326 on medium MN in a Cooper dish culture over a period of 216 hours. Asymmetric enlargement of the initially spherical spore leads, after 169 hours, to the formation of a large ovoid cell, which has probably already undergone binary fission, although the transverse wall is not resolved in this figure. After 193 hours spore release from the apical cell has occurred. The remaining basal cell is visible in the photograph at hour 216. X 500.

Figure 26

a. and b. Photomicrographs of a liquid culture in medium MN of *Dermocarpella* strain 7326. These figures show division stages which cannot be resolved in Cooper dish cultures. They include: 1, an individual which has just undergone the first division, a binary transverse fission yielding a small basal and a large apical cell; 2, an individual containing a single basal cell, and an apical cell which has completed multiple fission; 3, individuals containing a pair of basal cells, and an apical cell which has completed multiple fission; 4, individuals in which spore release from the apical cell has occurred, revealing the outline of the parental F wall layer, which also encloses the basal cell or cells. X1000. c. An electron micrograph of a thin section of a *Dermocarpella* cell, at an early stage of growth, on a piece of dialysis membrane placed on the surface of a plate of medium MN. Arrows indicate the surface of the dialysis membrane. The F layer of the cell wall flares out to produce a broad basal attachment of the cell to the membrane. X57,000.

Figure 27

Electron micrographs of thin sections of *Dermocarpella*

strain 7326 grown on plates of medium MN. a. An in-

dividual which has just completed the binary fission which separates the apical cell from the basal cell.

The PG, LP and F wall layers of the basal cell remain closely appressed to one another, whereas the PG and LP

layers of the apical cell have pulled away from the F layer. Note the variation in thickness of the F layer

(arrows). X14,500. b. An individual which has under-

gone a second binary fission to form two basal cells.

The apical cell has begun to undergo the successive divisions of multiple fission producing cleavage products which are initially not of equal size. The basal cells

have begun to synthesize new F layer material over their entire surfaces, but no new synthesis of F layer material has occurred around the dividing cells of the apical portion.

X15,000.

Figure 28

Electron micrographs of thin sections of *Dermocarpella* strain 7326. a. An individual nearing the completion of multiple fission. The F layer surrounding the basal cells is markedly thicker than in the earlier developmental stage shown in Figure 27b. No F layer material surrounds the cleavage products in the apical cell. Note the variation in thickness of the enclosing F layer (arrows).

. X13,500. b. An individual which has completed multiple fission and is filled with spores each surrounded only by the PG and LP wall layers. X10,500.

Figure 29

Electron micrographs of thin sections of *Dermocarpella* strain 7326 grown on pieces of dialysis membrane placed on plates of medium MN. a. A developing ovoid cell which is laterally attached to the dialysis membrane. X41,000. b. An individual attached to the dialysis membrane by its apical cell. X14,600.

Figure 30

Schematic Developmental Cycle of Dermocarpella

- A Asymmetric spore enlargement
- B Binary fission giving rise to a small basal cell (b) and a larger apical cell (a)
- C Multiple cleavage of the apical cell leading to spore formation
- D Spore release
- E Period of spore motility
- F Enlargement of basal cell

d. *Myxosarcina*

In *Myxosarcina* spore enlargement is followed by repeated binary fissions in three regular planes, to produce cubical packets of cells which then undergo multiple fission and release motile spores. This mode of development is shown by two strains, 7312 and 7325. Both the spores and the vegetative cells of strain 7325 are somewhat larger than those of strain 7312 (Table XIV). In large cell aggregates of strain 7325, the arrangement of the cells tends to become somewhat irregular (Figure 31d), whereas the cells in large aggregates of strain 7312 remain in a regular cubical array (Figure 31b).

The developmental cycles of the two strains in Cooper dish cultures are closely similar, and are illustrated by a series of photomicrographs of strain 7312 (Figure 32). The spore enlarges symmetrically until it reaches a predetermined size, characteristic of the strain (Table XIV). Following enlargement the spore begins to divide by repeated binary fissions in three regular planes leading to the formation of a cubical packet of cells. Almost all the cells in the aggregate undergo multiple fission simultaneously, which is followed by rupture of the surrounding F layers and release of spores. Following a brief period of motility (not illustrated) the spores begin to enlarge, and the cycle is repeated.

Both strains of *Myxosarcina* produce a relatively small (and probably constant) number of spores per parental cell (Table XIV), regardless of the culture conditions. However, culture conditions do affect the number of binary fissions that precede the onset of multiple fission: the more favorable the conditions of growth, the earlier is the onset of multiple fission.

TABLE XIV

STRUCTURAL PROPERTIES OF *Myrosarcina* STRAINS

Strain	Diameter of spore in μm	Diameter of cell at the time of first binary fission in μm	Number of spores produced per cell
7312	2	5	probably 4
7325	3	8 - 10	greater than 4 but not known

The developmental cycle of *Myxosarcina* is represented schematically in Figure 33.

Figure 31

Photomicrographs of *Myxosarcina* strains 7312 and 7325 growing on plates of medium MN. a. - e., X1000, f., X500. a. Young aggregates of strain 7312, showing the uniformity of cell size and the regularity of the planes of successive divisions. b. Older aggregates of strain 7312, showing the maintenance of fairly regular cubical packets of cells. c. Young aggregates of strain 7325, showing the uniformity of cell size and the initial regularity of the successive divisions. d. Older aggregates, in which the regularity of cell arrangement has been largely lost. e. Cell aggregates of strain 7325 in which many packets of cells have completed multiple fission, and are filled with spores. f. Cell aggregates from an old liquid culture of strain 7325, in which the common enclosing F layer of the cell wall is clearly visible (arrows).

Figure 32

Successive photomicrographs of the development of a single spore of *Myxosarcina* strain 7312 on medium MN in a Cooper dish culture over a total period of 554 hours. The spore enlarges symmetrically until it reaches a size specific for each strain. It then begins to divide by binary fissions in three regular planes, to produce a cubical packet of cells. Almost all the cells in the packet undergo multiple fission simultaneously. X 500.

Figure 33

Schematic Developmental Cycle of Myxosarcina

e. *Chroococcidiopsis*

The genus *Chroococcidiopsis* is not distinguishable from *Myxosarcina* in mass cultures. In such cultures, it develops as cubical or somewhat irregular cell masses (Figure 34) which, when crushed, break up into smaller cell clusters, typically containing from 2 to 8 cells, most of which are of similar size (Figure 35). As will be explained below, these cell clusters consist mostly of vegetative cells in the course of binary fission. The slightly smaller endospores are rarely observed in mass cultures, although a few groups of endospores (arrows) are present in Figure 35a.

Among the eight strains of *Chroococcidiopsis* in our collection, seven (7203, 7431, 7432, 7433, 7434, 7436 and 7439) are structurally indistinguishable and, as will be described in a later section, also indistinguishable in physiological and chemical respects. Strain 6712 differs from the remaining strains in the size of the vegetative cells and spores (Table XV) and also in physiological and chemical respects.

The developmental cycle characteristic of *Chroococcidiopsis* is difficult, if not impossible, to determine by the examination of mass cultures, but is revealed by semi-continuous observations of growth in Cooper dish cultures. Figure 36 shows the development over a period of 302 hours of a single spore of strain 6712. The spore underwent an initial enlargement, and started to divide after 72 hours. The first two divisions occurred at right angles to one another, producing after 120 hours a tetrad of vegetative cells, each considerably larger than the initial spore. Thereafter, the cell aggregate continued to grow until 229 hours, although the arrangement and size of its constituent cells could no longer be resolved. The release of small spores started at

TABLE XV

STRUCTURAL PROPERTIES OF *Chroococcidiopsis* STRAINS

Strain	Diameter of spore in μm	Diameter of cell at the time of first binary fission in μm	Probable number of spores produced per cell
6712	4	6.3	4
7203	3 (2.5 - 3.5) [*]	5	4
7431	3 (2.5 - 3.5) [*]	5	4
7432	3 (2.5 - 3.5) [*]	5	4
7433	3 (3 - 4) [*]	5	4
7434	3 (3 - 4) [*]	5	4
7436	3 (2.5 - 3.5) [*]	5	4
7439	3	5	4
* From Komarek (39)			

241 hours, and additional spores were liberated from the aggregate for many hours thereafter. After 302 hours, many of the spores had already enlarged and undergone one binary fission (arrows).

Figure 37 shows the development over a period of 229 hours of a group of three spores of strain 7432, typical of the remaining seven *Chroococcidiopsis* strains. The developmental sequence closely resembles that described above for strain 6712. The three spores included in the field of observation all enlarged at a similar rate, and underwent the first binary fission after attaining the same size. Small spores were released from the aggregate after 157 hours. After 208 hours, many spores had enlarged and undergone one binary fission (arrows). After 229 hours, an additional division of some of these spores had taken place, to produce tetrads (arrows).

Spore motility has never been observed in *Chroococcidiopsis* strains: when spore release occurs in a Cooper dish culture exposed to unidirectional illumination, the spores remain clustered evenly around the remains of the parental cells (Figure 15c). The absence of spore motility is, accordingly, the property which distinguishes *Chroococcidiopsis* from *Myxosarcina*.

The immotility of the spores of *Chroococcidiopsis* implies that synthesis of an F layer occurs in the course of multiple fission leading to spore formation. The spores of these organisms do not differ greatly in size from the vegetative cells (Table XV), which indicates that a rather small (and probably constant) number of spores is produced from each vegetative cell. Hence the distinction between spores and vegetative cells, either by light microscopy or by electron microscopy, is particularly difficult in the case of this group, and rests entirely on

the relatively small differences in cell size, evident in the light micrograph of a mass culture of strain 6712 (Figure 35a), and particularly in Cooper dish cultures (Figures 36 and 37).

Several representative electron micrographs of thin sections of *Chroococcidiopsis* aggregates are shown in Figures 38 and 39. Figure 38a is a section of an aggregate which we interpret as having just undergone a binary fission, producing a two-celled individual. The individual cells are of approximately the same dimensions as cells in a Cooper dish culture which have just undergone the first division (Figure 37; 61 hours). The cell aggregate shown in Figure 38b is of approximately the same size as that shown in Figure 38a, but contains many more cells. It can be interpreted as a two-celled aggregate, both the cells of which are in the course of multiple fission. It should be noted that the cleavage products are individually surrounded by an F wall layer, which is in accord with the observation that free spores are never motile. Figure 39 shows an electron micrograph of a thin section of a larger *Chroococcidiopsis* aggregate. The distinction between spores and vegetative cells in such preparations cannot be made with certainty.

All *Chroococcidiopsis* strains except 6712 sometimes produce an unusual type of aggregate in liquid cultures (Figures 40a and 40b). They consist of spheres of widely varying size, which always contain many cells, and are intermixed in the population with aggregates of normal form (Figure 40a). We have not been able to follow the development of these spherical aggregates, and the following interpretation of their nature is, therefore, hypothetical. They could arise from a single cell (or a pair of cells) which have undergone multiple fission, without

subsequent spore release. The growth and vegetative division of the spores entrapped within the F layer of the parental cell (or pair of cells) might then lead to the formation of a spherical aggregate. A configuration sometimes observed in electron micrographs of thin sections (Figure 40c) consists of a roughly spherical aggregate which could be an early stage in the formation of the larger spherical aggregates observed in light micrographs.

A diagrammatic representation of the developmental cycle of *Chroococcidiopsis*, based primarily on our observations of the growth of these organisms in Cooper dish cultures is shown in Figure 41. It should be explicitly noted that the interpretation portrayed in Figure 41 differs considerably from that offered by previous workers (28, 24, 39, 41), even though one of these workers (41) based his conclusions on the study of non-axenic cultures of seven of the eight strains which we have examined (i.e., all except 6712). Earlier workers have assumed that *Chroococcidiopsis* is a unicellular organism which divides only by multiple fission. This basic assumption necessitated the development of relatively complex explanations to account for the various types of cells and aggregates observable in mass cultures (24, 39, 41). The previous failure to recognize that *Chroococcidiopsis* can undergo both binary and multiple fission accounts for the errors which have long been made in the interpretation of the development of these organisms.

Figure 34

Photomicrographs of mass cultures of *Chroococcidiopsis* grown on agar plates of medium BG-11 showing the characteristic cell aggregates made by these strains. a. Strain 7203; b. Strain 7431; c. Strain 7432; d. Strain 7433; e. Strain 7434; f. Strain 7439. X1000.

Figure 35

Photomicrographs of mass cultures of *Chroococcidiopsis* grown on agar plates of medium BG-11. The aggregates visible in Figure 34, when crushed, break up into smaller cell clusters, typically containing from 2 to 8 cells, most of which are similar in size. a. Strain 6712, note the cells that have undergone multiple fission, resulting in the production of four spores per parental cell (arrows). b. Strain 7433; c. Strain 7434; d. Strain 7436; e. Strain 7439. X1000.

Figure 36

Development of *Chroococcidiopsis* strain 6712 on medium BG-11 supplemented with 0.3% sucrose in a Cooper dish culture. Successive photomicrographs of a single spore showing its development over a total period of 302 hours. The developmental pattern is identical to that of *Myxosarcina* (Figure 32) except that the spores released following multiple fission are immotile. X 500.

Figure 37

Successive photomicrographs of the development of three spores of *Chroococcidiopsis* strain 7432 on medium BG-11 supplemented with 0.3% sucrose in a Cooper dish culture over a total period of 229 hours. The developmental pattern is the same as that shown for strain 6712 in Figure 36. X 500.

Figure 38

Electron micrographs of thin sections of *Chroococcidiopsis* strain 7436 grown on a plate of medium BG-11. a. Two vegetative cells which have divided by binary fission. X19,500. b. Two cells which have undergone multiple fission after the completion of one binary fission. Both the size of the cells and the thickness of their individual surrounding F layers indicate that multiple fission has taken place. X25,000.

a

b

Figure 39

An electron micrograph of a thin section of *Chroococcidiopsis* strain 7432 grown in an agar overlay of medium BG-11. It reveals that small aggregates within a large colony are held together by their common surrounding F layers. The F layer material also appears to hold the aggregates together even after the common surrounding F layer has ruptured. Note the cell which has initiated binary fission by the centripetal ingrowth of the PG and LP wall layers, and characteristic nucleoplasm (N) with associated carboxysomes (arrows). X17,500.

Figure 40

a. and b. Photomicrographs of *Chroococcidiopsis* strain 7431. a. A field showing the two types of cell aggregates. X 500. b. A more detailed photograph of the spherical colonies produced by this strain. X 1000.

c. An electron micrograph of a thin section of strain 7432 revealing an individual which may be interpreted as having undergone multiple fission following the first binary fission. The many division planes that would be introduced if these spores developed within their common surrounding F layer might explain the production of the spherical colonies seen in Figures 40a and 40b. Note the carboxysomes (arrows) and the characteristic nucleoplasm (N). X 21,500.

Figure 41
Schematic Developmental Cycle of Chroococcidiopsis

A Symmetric spore enlargement to a predetermined size

B Repeated binary fissions in three regular planes

C Multiple fission of almost all the cells in the colony followed by the release of immotile spores

g. Pleurocapsa

i. Developmental patterns

The genus *Pleurocapsa* is represented in our collection by twelve strains (7310, 7314, 7317, 7319, 7320, 7321, 7322, 7324, 7327, 7440, 7506, and 7516). Characteristically their developmental cycles involve spore enlargement, followed by binary fissions in many irregular planes, producing cell aggregates which may vary widely in size and range from irregularly shaped clusters of cells to rather extensive filamentous assemblages. Eventually some of the cells in the aggregate undergo multiple fission, producing motile spores. The primary character common to all these strains that distinguishes them from *Myxosarcina* is the irregularity of the successive planes of binary fission, even early in vegetative growth. However, as will be described below, some strains can produce *Myxosarcina*-like aggregates under special conditions (growth at the expense of sugars).

When grown on solid mineral media, these strains display not only a wide variety of cell sizes and shapes but also a remarkable diversity with respect to the size and form of the cell aggregates. The examination of such mass cultures shows the presence of cells which have obviously undergone multiple fission, but reveals very little about the developmental cycles of the individual strains. Semicontinuous observations on Cooper dish cultures show that the strains of *Pleurocapsa* can be divided into two subgroups which differ in their mode of spore enlargement prior to the onset of binary fission.

In group I spore enlargement is symmetric; in group II it is asymmetric. The developmental features characteristic of the strains belonging to each subgroup will be separately described below. Special

TABLE XVI

STRUCTURAL PROPERTIES OF *Pleurocapsa* STRAINS

Strain	Diameter of spores in μm	Diameter of cell at time of first binary fission in μm	Diameter of filaments where present in μm	Approximate number of spores produced per cell
<i>Group I:</i>				
7310	2.5 - 3	- 10	some cells may reach 20 μm in diameter	32 - 64
7317	2 - 2.5	5 - 7	n.a.	n.d.
7320	2	6	6 - 7	30
7440	4	11	9 - 13	n.d.
7506	n.d.	n.d.	8 - 13	n.d.
7327	2 - 2.5	4 - 6	5 - 6	8 - 16
7322	2 - 3	6 - 8	6 - 10	16
7319	2.5	6 - 8	6 - 10	16 - 32
<i>Group II:</i>				
7314	2.5 - 3	n.a.	6 - 10	8 - 16
7321	2.5 - 3	n.a.	5 - 8	
7516	2.5 - 3	n.a.	6 - 10	8
7324	3	5	n.a.	4

n.a. : not applicable

n.d. : not determined

characteristics of the individual strains are presented in Table XVI.

Strains of Group I (7310, 7317, 7320, 7440, 7506, 7327, 7322, 7319)

Strains 7310 and 7317 typically produce small, undifferentiated cell aggregates (Figure 42). Their developmental cycles are initiated by symmetric spore enlargement, followed by binary fissions in many planes, producing compact, irregularly shaped clusters of cells which never produce extensive filamentous outgrowths (Figures 43 and 44). The two strains differ in cell size (Table XVI).

Strain 7320 also produces small cell aggregates (Figure 42 and 45) but unlike strains 7310 and 7317 the successive binary fissions follow a well-defined pattern resulting in the production of an aggregate of characteristic form. Following its symmetric enlargement, the spore elongates and divides transversely (Figure 45). One of the resulting daughter cells then divides at right angles to the first plane of division, while the other cell simply increases in width but does not divide. This asynchronous division is responsible for the production of the dichotomously branched cell aggregates seen in mass cultures (Figure 42f) which are typical of this strain.

Strains 7440 and 7506 both form irregularly branched cell aggregates (Figures 46a and 46b). Strain 7440 readily produces spores in pure culture which develop by symmetric enlargement (Figure 47). Strain 7506, on the other hand, has failed to reproduce by multiple fission since its isolation in pure culture, although spore formation was observed in unialgal cultures of this strain. Although its mode of spore enlargement has not been determined, its close general resemblance to strain 7440 suggests that it also belongs to Group I.

Strains 7327, 7322 and 7319 typically produce rather extensive, irregularly branched, filamentous cell aggregates (Figures 46c, 46d and 48-51). In these strains symmetrical spore enlargement is followed by binary fissions in many planes. Initially this results in the formation of a small, branched, filamentous aggregate which, following extensive growth, eventually forms a large irregularly shaped central cluster of cells with peripheral radiating branches. As the aggregate enlarges, it is able to maintain its filamentous habit because the cells at the distal ends of the branches divide transversely in one plane. As the branches elongate, the subapical cells divide either in two planes to produce dichotomous branches or in many planes to produce the large, irregularly shaped clusters of cells from which the branches radiate.

Despite their broadly similar patterns of development, strains 7327, 7322 and 7319 differ from one another with respect to the size of the vegetative cells, as shown in Table XVI. This difference is evident from the comparison of Figures 46c, 46d and 49). All these strains are facultative photoheterotrophs, as shown by their ability to grow in the light at the expense of sucrose in the presence of a concentration of DCMU which totally inhibits growth in a mineral medium. In strain 7327, the addition of sucrose to a mineral medium does not modify development. However, the development of both strains 7319 and 7322 is significantly altered in a sucrose-containing medium. Under such conditions, strain 7322 develops as cubical packets of cells which are devoid of filamentous outgrowths and indistinguishable from a *Myxosarcina* (Figures 49f and 52). The addition of sucrose to the medium does not change the gross structure of the aggregates of strain 7319. However, the filamentous

outgrowths, normally uniseriate at the tips, become multiseriate throughout their lengths (Figures 49c and 49d).

Strains of Group II (7314, 7321, 7516)

The spores of these strains are spherical and motile at the time of release, and cannot at this stage be distinguished from the spores of strains that belong to Group I. The difference is first apparent after the spores become immotile and start to enlarge. In the strains of Group II, enlargement occurs at first by elongation, producing a rod-shaped cell. Very rapidly, however, the enlarging cell broadens at one pole to become club-shaped or pyriform (Figures 53-57). At this stage, the first division occurs, typically in a transverse plane. The planes of subsequent divisions vary; the resulting aggregate is of irregular shape, with a number of filamentous outgrowths. Some cells of the aggregate subsequently undergo multiple fission to produce motile spores. Typical developmental sequences for the strains of group II are shown in Figures 55-57.

In both groups of *Pleurocapsa*, as in *Myxosarcina* and *Chroococcidiopsis*, the number of binary fissions which precede the onset of multiple fission is not fixed: the more favorable the growth conditions, the earlier is the onset of multiple fission. Indeed, some strains which normally produce filamentous aggregates may under favorable growth conditions undergo multiple fission after only one or two binary fissions (Figure 42e). This behavior is shown by strain 7320 (Group I) and by strain 7314 (Group II).

Schematic developmental cycles for Groups I and II of *Pleurocapsa* are shown in Figures 59 and 60 respectively.

Strain 7324

This strain, like the strains of Group I, is characterized by symmetrical spore enlargement (Figure 62). However, it is unusual in several respects. Binary fissions occur predominantly in two planes, forming relatively flat cell aggregates which may reach considerable size before undergoing multiple fission (Figure 61d). Nearly all the cells in the aggregate undergo multiple fission simultaneously, a property not possessed by any other strain which we have assigned to *Pleurocapsa*. In liquid cultures of strain 7324 that are several months old, some cells of the population become embedded in a mass of extracellular material which has the form of dichotomously branched threads with a diameter slightly less than that of the contained cells. The cells associated with this material are widely dispersed and are typically located at the apices of the threads (Figure 61e). Such structures, present only in old and presumably senescent cultures of this strain, resemble strikingly the classical illustrations of *Hormathonema*, a marine organism assigned to the Entophysalidaceae (order Chroococcales).

When growing under favorable conditions, the cells of strain 7324 are occasionally covered by an amorphous precipitate (Figure 61c) giving them an appearance characteristic of natural populations of *Entophysalis* (S. Golubic, personal communication).

ii. The endolithic habitat

Organisms which present the developmental features here described as representative of *Pleurocapsa* are traditionally assigned to two principal genera, *Pleurocapsa* and *Hyella*, distinguished solely by their growth habits in natural environments. The members of *Pleurocapsa* are

epiphytes ^{or} epiliths, whereas the members of *Hyella* are endoliths, which penetrate calcareous substrates. The eleven marine strains included in this study were all isolated from mollusk shells or rock chips, and could in principle have been either epiliths or endoliths. Except for strain 7516, observed to be endolithic in its natural habitat, the relations of these strains to their natural substrates were not determined at the time of their isolation. After pure cultures had been obtained, several strains (7314, 7319, 7321, 7322, 7324) were inoculated into petri dishes containing chips of oyster shell partially immersed in medium MN and incubated in the light for a total period of two years with periodic replacement of the culture medium. All grew profusely on the oyster shells; but none showed any penetration below the surface of this substrate. This experiment accordingly suggested that all the strains in question were epiliths and therefore assignable to the genus *Pleurocapsa*.

In the course of the more recent isolation of a member of this group (strain 7506) an attempt was made to determine its endolithic or epilithic nature by performing a similar experiment with a culture that was still unialgal and contained bacteria. Under these conditions, strain 7506 showed definite penetration of oyster shell chips after four months. Unfortunately, the behavior of this strain after purification could not be ascertained because in pure culture it has consistently failed to produce spores, and is thus unable to attach to solid substrates.

The fact that our first clear demonstration of endolithic growth in culture occurred with a strain that was not bacteria-free, suggested the possibility that penetration of calcareous substrates by members of

this group might be dependent on the activity of associated acid-producing bacteria. In order to test this hypothesis, the bacterial population associated with the unialgal culture of strain 7506 was screened by plating on a variety of glucose-containing media both defined and complex, all furnished with a pH indicator (phenol red). The strongest acid production was observed on plates of nutrient broth supplemented with 0.5% glucose, and a variety of acid-producing bacterial colonies which developed on plates of this medium were purified. One of these strains, selected on the basis of its marked acid-producing ability when grown in glucose-nutrient broth, was used in the following experiment.

Pleurocapsa strains 7319, 7440 and 7516 (the latter known to be endolithic under natural growth conditions), were grown in the light in two series of flasks of medium MN containing oyster shell chips. One series was inoculated with pure cultures of the cyanobacteria, the other series was inoculated in addition with the acid-producing bacterium. At the time of writing, this experiment has been underway for two months. Strains 7440 and 7516 have both penetrated the calcareous substrate in the culture which contain the acid-producing bacterium, but show no sign of penetration in the control cultures lacking the bacterium (Figure 63). However, strain 7319 has penetrated the substrate both in the mixed and in the pure culture. The latter result is in contradiction with the observations previously made on this strain. Thus, while this experiment appears to indicate that associated chemoheterotrophic bacteria can play a role in facilitating the penetration of calcareous substrates by members of the *Pleurocapsa* group, penetration by at least one strain in pure culture is apparently also possible. Further work on

this problem is required before any solid conclusions about the biological basis of the endolithic habit can be drawn.

Figure 42

Photomicrographs of mass cultures of *Pleurocapsa* grown on agar plates of medium MN. a. and b. Individual cells and compact cell aggregates of strain 7310. X1000. c. and d. Small cell aggregates of strain 7317. X1000. e. Strain 7320: the field includes cells (arrows) which have undergone multiple fission at the one or two celled stage. X1000. f. Typical small dichotomously branched cell aggregates of strain 7320. , X1000.

Figure 43

Successive photomicrographs of the development of a single spore of *Pleurocapsa* strain 7310 on medium MN in a Cooper dish culture over a total period of 350 hours. The spherical spore enlarges symmetrically and then begins to divide by binary fission in irregular planes to form a compact mass of cells. Eventually some of the cells in the aggregate undergo multiple fission and release spores. X 500.

Figure 44

Successive photomicrographs of the development of a single spore of *Pleurocapsa* strain 7317 on medium MN in a Cooper dish culture over a total period of 1104 hours. The developmental pattern of this strain is similar to that of strain 7310 (see Figure 43). X 500.

Figure 45

Development of *Pleurocapsa* strain 7320 on medium MN in Cooper dish culture over a total period of 290 hours. The spherical spore enlarges symmetrically and then divides transversely (hour 73). One of the two daughter cells produced then characteristically divides at a right angle to the plane of the first division. This second division is responsible for the dichotomously branched filaments formed by this strain (see Figure 42f). X500.

Figure 46

a. and b. Photomicrographs of mass cultures of two *Pleurocapsa* strains grown on plates of medium MN. a. Filamentous cell aggregates of strain 7440, some of the cells of which have undergone multiple fission. X1000. b. Typical filamentous cell aggregates of strain 7506. X1000. The boundary of the F layer is evident in both Figures 46a and 46b (arrows). c. and d. Characteristic cell aggregates of the thermophilic strain 7327 grown on medium BG-11 at 37°C. Some of the cells in the aggregate have undergone multiple fission (arrows). X1000.

Figure 47

Successive photomicrographs of a single spore of *Pleurocapsa* strain 7440 grown on medium MN in a Cooper dish preparation. Following symmetric spore enlargement the cell began to divide by repeated binary fissions, however, the cells of the aggregate failed to undergo multiple fission during the course of observation which lasted for a total of 423 hours. X 500.

Figure 48

Successive photomicrographs of the development of *Pleurocapsa* strain 7327 on medium BG-11 incubated at 37°C in a Cooper dish culture over a total period of 252 hours. The spherical spore enlarges symmetrically and then begins to divide in many planes to produce a filamentous cell aggregate. Some of the cells in the aggregate subsequently undergo multiple fission and release spores. X 500.

Figure 49

The influence of the medium on the development of two strains of *Pleurocapsa*. a. and b. Uniseriate branched filamentous aggregates of strain 7319 grown on mineral medium MN in the light. a. X 500; b. X1000. c. and d. Multiseriate filamentous aggregates of strain 7319 grown on medium MN supplemented with 0.3% sucrose in the light. c. X 500; d. X1000. e. Filamentous aggregates of strain 7322 grown on mineral medium MN in the light. The field includes many spores. X1000. f. Cubical cell aggregates characteristic of strain 7322 grown on medium MN supplemented with 0.3% sucrose in the light. X1000.

Figure 50

Successive photomicrographs of the development of *Pleurocapsa* strain 7319 grown on medium MN in a Cooper dish culture over a total period of 620 hours. The spherical spore enlarges symmetrically and then begins to divide in many planes to produce a filamentous cell aggregate. Eventually some of the cells in the aggregate undergo multiple fission and release spores. X 500.

Figure 51

Successive photomicrographs of the development of *Pleurocapsa* strain 7322 grown on medium MN in a Cooper dish culture over a total period of 527 hours. The developmental pattern is similar to that described for strain 7319 in Figure 50. X 500.

Figure 52

Successive photomicrographs of the development of strain 7322 grown in the light on medium MN supplemented with 0.3% sucrose in a Cooper dish culture over a total period of 278 hours. Under these conditions of growth, this strain forms cubical cell aggregates which are indistinguishable from those produced by strains of *Myxosarcina* (see Figure 32). X 500.

Figure 53

The outgrowth of spores of *Pleurocapsa* strains 7314 and 7321. An inoculation rich in spores was spread on a plate of medium MN, from which wet mounts were prepared after incubation for various periods in the light. a., b. and c. Successive stages in the development of spores of strain 7314. d., e. and f. Successive stages in the development of spores of strain 7321. X1000.

Figure 54

The outgrowth of spores of *Pleurocapsa* strain 7516, from a plate culture prepared as described in the legend of Figure 53. Some cells of the aggregates in Figure 54d have undergone multiple fission and produced spores (arrows). X1000.

Figure 55

Successive photomicrographs of the development of *Pleurocapsa* strain 7314 grown on medium MN in a Cooper dish culture over a total period of 289 hours. The initially spherical spore enlarges asymmetrically and then begins to divide in many planes producing a filamentous cell aggregate. Some of the cells in the aggregate have undergone multiple fission and released spores (starting at hour 198). In the photomicrographs taken at 246, 270 and 289 hours, early stages in the development of two spores (arrows) are shown. X 500.

Figure 56

Successive photomicrographs of the development of *Pleurocapsa* strain 7321 grown on medium MN supplemented with 0.3% sucrose in a Cooper dish culture over a total period of 206 hours. The developmental pattern of this strain is similar to that described for strain 7314 in Figure 55. X 500.

Figure 57

Successive photomicrographs of the development of *Pleurocapsa* strain 7516 grown on medium MN in a Cooper dish culture over a total period of 689 hours. The developmental pattern of this strain is similar to that described for strains 7314 (Figure 55) and 7321 (Figure 56). X500.

Figure 58

An electron micrograph of a thin section of *Pleurocapsa* strain 7314 grown in an agar overlay on medium MN. Each cell is enclosed by individual PG, LP and F wall layers. The group of cells is also enclosed by additional F layers synthesized during previous generations. As cell mass increases the outermost layers of F wall material become either stretched (as indicated by their loss of electron density) (arrows) or torn (arrows) to accommodate the increased volume. X14,000.

Figure 59

Schematic Developmental Cycle of Pleurocapsa Group-I

- A Symmetric spore enlargement
- B Binary fissions in many irregular planes
- C Multiple cleavage of some vegetative cells
- D Spore release
- E Period of spore motility

Figure 60

Schematic Developmental Cycle of Pleurocapsa Group-II

- A Asymmetric spore enlargement
- B Binary fissions in many irregular planes
- C Multiple cleavage of some vegetative cells
- D Spore release
- E Period of spore motility

Figure 61

Photomicrographs of mass cultures of *Pleurocapsa*

strain 7324 grown in medium MN. a. An early stage of

vegetative growth on an agar plate. X1000. b. A

later stage of development on an agar plate: some cell

aggregates are undergoing multiple fission (arrows).

X1000. c. Cell aggregates from a liquid culture; the

groups of cells are covered with an amorphous precipitate.

X1000. d. A large aggregate on an agar plate. X500.

e. A preparation from an old (2 months) liquid culture,

showing threads of extracellular material in which iso-

lated clumps of cells are embedded. Phase contrast

X500.

Figure 62 .

Successive photomicrographs of the development of *Pleurocapsa* strain 7324 grown on medium MN in a Cooper dish culture over a total period of 160 hours. The spore enlarges symmetrically and then divides by binary fissions predominantly in two planes to form a flat irregular cell aggregate. All the cells in the aggregate underwent multiple fission simultaneously. X 500.

Figure 63

Photomicrographs of three *Pleurocapsa* strains growing on fragments of oyster shell immersed in medium MN, and incubated in the light. Prior to photography, the oyster shells were scraped in order to remove the surface growth. The photomicrographs on the left side of the plate (a., c. and e.) show the appearance of oyster shells from pure cultures of the three *Pleurocapsa* strains. The photomicrographs on the right side of the plate (b., d. and f.) show the appearance of oyster shells from cultures which were simultaneously inoculated with a chemoheterotrophic acid producing bacterium. a. and b., Strain 7440; c. and d., Strain 7516; e. and f., Strain 7319. All X200.

In axenic culture, strain 7440 shows no penetration of the calcareous substrate (Figure 63a), but penetrates extensively (Figure 63b) in two-membered culture. The same behavior is shown by strain 7516; the cell aggregates in Figure 63c are growing on the surface whereas several aggregates in Figure 63d (arrows) have begun to penetrate. Strain 7319 has penetrated the calcareous substrate both in the axenic culture (Figure 63e) and in the two-membered culture (Figure 63f).

C. The Structure and Development of *Chamaesiphon*

Two strains in our collection, both of fresh water origin, are unicellular cyanobacteria which reproduce exclusively by budding. They appear to be representatives of the genus *Chamaesiphon*, although the cells are not invested by a distinct sheath open at one end ("pseudovagina"), reportedly characteristic of most *Chamaesiphon* spp. when growing on natural substrates (35).

On agar plates of medium BG-11, both strains form small compact colonies, consisting of cells embedded in an amorphous layer of slime, which are very difficult to disperse. In liquid cultures, the cells do not attach to the glass walls of the culture vessel.

The cells are spherical to ovoid, and rather variable in size (Figure 64a-d). The cells of strain 7430 are considerably larger than those of strain 6605 (Table XVII). For this reason, the unusual mode of division of strain 7430 is more obvious in light microscopic preparation than that of strain 6605. As shown in Figure 64c and 64d, mass cultures of strain 7430 contain many division stages in which the daughter cells are of markedly different size (arrows), a character less evident in strain 6605 (Figure 64a and 64b).

The fact that both these strains reproduce by bud formation was established by semi-continuous observations on slide cultures. Figure 65 shows the development in a slide culture of strain 6605. During the period of observation (151 hours) the cells labelled 1 and 2 each underwent approximately two doublings. The parent cell, initially spherical, elongated and produced a spherical bud from one pole. After detachment of the bud, a second bud developed at the same position on the mother cell. Hence bud formation appears always to occur at the same pole of

TABLE XVII

STRUCTURAL PROPERTIES OF *Chamaesiphon* STRAINS

Strain	Diameter of bud in μm	Greatest diameter of basal cell in μm	Length of cell just prior to division in μm
6605	2.5 - 2.8	- 3	3.8 - 4.2
7430	3	5	6 - 8

a mother cell, which we shall term the reproductive pole. Relative to the mother cell from which it arises, each bud has an "old" pole (i.e., that which originally existed on the mother cell) and a "new" pole (i.e., that which is formed at the site of cross wall formation). One of these poles must become the reproductive pole of the developing bud. The buds initially formed from the cells labelled 1 and 2 in Figure 65 had elongated and divided by the end of the period of observation. In both cases, buds arose from the "new" poles of these daughter cells. In other words, the newly formed poles of both the mother and the daughter cell are the reproductive poles.

Figure 66 shows the development in a slide culture of strain 7430. The arrows point to a cell which formed two buds over the period of observation (236 hours). Both developed successively from the same pole of the mother cell. Polarized bud formation is therefore likewise characteristic of this strain. The daughter cells did not develop sufficiently to permit a determination of the relationship between the reproductive poles of the mother and daughter cells.

The examination of thin sections of strain 7430 in the electron microscope reveals that its cell wall is composed only of the PG and LP layers. The absence of an F layer distinguishes this organism sharply from all members of the Pleurocapsales (Figure 64e and f).

As revealed by thin sections, cross-wall formation during budding closely resembles the same process during binary fission in members of the Chroococcales. At the onset of division, the thylakoids are dispersed throughout the cytoplasm (Figure 67), and become divided between the mother cell and the bud by the centripetal invagination of the cell wall, as is typical of other unicellular cyanobacteria. This

separation of pre-existing thylakoids between the mother cell and the bud differs from the internal membrane formation in other budding bacteria. In *Nitrobacter* sp. (77, p. 469) and *Rhodopseudomonas* sp. (59, p. 62) the internal membrane system of the mother cell does not enter the bud. In such bacteria, the internal membrane system of the bud arises *de novo* from invaginations of the cytoplasmic membrane.

The developmental cycle of *Chamaesiphon*, as inferred from the study of these two strains, is represented schematically in Figure 68.

Figure 64

a., b., c. and d. Photomicrographs of *Chamaesiphon* grown in liquid medium BG-11. a. and b. Strain 6605. Phase contrast, X 2000. c. and d. Strain 7430. X 2000. e. and f. Electron micrographs of thin sections of *Chamaesiphon* strain 7430 to show cell wall structure. Arrows point to the PG and LP wall layers. X 207,700.

Figure 65

Successive photomicrographs of the development of *Chamaesiphon* strain 6605 grown on medium BG-11 in a slide culture over a total period of 151 hours. This series of micrographs reveal that this strain divides by budding. Note that successive daughter cells are always cut off from the same pole of the basal cell. X1000.

Figure 66

Successive photomicrographs of the development of *Chamaesiphon* strain 7430 grown on medium BG-11 in a slide culture over a total period of 236 hours. The developmental pattern of this strain is similar to that of strain 6605 (Figure 65). X1000.

Figure 67

Electron micrographs of thin sections of strain 7430 grown in an agar overlay of medium BG-11. This series of electron micrographs shows several stages of bud development. a. X18,000, b. X16,500, c. X16,500.

Figure 68

Schematic Developmental Cycle of Chamaesiphon

D. Nutritional Properties of the Pleurocapsales and Chamaesiphonales

1. Ionic Requirements

A number of analyses of the ionic requirements of marine bacteria belonging to several different taxa (50, 51, 52, 53, 61) show that these organisms can be distinguished from members of the same taxonomic groups of non-marine origin by their relatively elevated requirements for sodium, magnesium, calcium and halides. Since the Pleurocapsales include members both of marine and of non-marine origin, it therefore appeared of interest to analyze their specific requirements for these ions. For comparative purposes, six marine representatives of the Chroococcales were also included in this study.

Table XVIII shows the minimum concentrations of sodium and chloride necessary to support continued growth of the strains examined. Some strains, which were all of terrestrial origin (*Dermocarpa* 7437 and 7438; *Chroococcidiopsis* strains) show no detectable growth requirement for either ion. The maximal levels of sodium and chloride in the medium employed (calculated from the analytical data furnished by the manufacturers for the salts which make up this medium) are 1.5×10^{-5} M and 3.4×10^{-6} M respectively. Consequently, if these strains do require either ion, their requirements are met by concentrations equal to or less than these values. All the remaining strains examined have readily detectable requirements for sodium, and in most cases also for chloride, although the minimal concentrations necessary for growth vary widely. With the exception of *Pleurocapsa* 7327 (isolated from a hot spring), these strains are all of marine origin. As a rule, any given strain requires concentrations of sodium and chloride that are similar. The requirements of these strains for a monovalent cation could in no case

TABLE XVIII

MINIMUM SODIUM AND CHLORIDE REQUIREMENTS FOR GROWTH OF
MARINE AND TERRESTRIAL CYANOBACTERIA

Strains	Sodium concentration in mol/l	Chloride concentration in mol/l	Growth in medium BG-11	Marine Isolates	Terrestrial Isolates
<i>Dermocarpa</i>					
7301	0.01	0.005		+	
7302	0.05	0.025		+	
7303	0.05	0.025		+	
7304	0.02	0.02		+	
7437	grew with no added Na ⁺	grew with no added Cl ⁻	+		+
7438	" " " "	" " " "	+		+
<i>Xanoxoccus</i>					
7305	0.2	0.2		+	
7306	0.2	0.2		+	
7307	0.05	0.005		+	
<i>Dermocarpella</i>					
7326	0.01	0.025		+	
<i>Myrosarcina</i>					
7312	0.02	0.05		+	
7325	0.05	0.1		+	
<i>Chroococcidiopsis</i>					
6712	grew with no added Na ⁺	grew with no added Cl ⁻	+		+
7203	" " " "	" " " "	+		+
7431	" " " "	" " " "	+		+
7432	" " " "	" " " "	+		+
7433	" " " "	" " " "	+		+
7434	" " " "	" " " "	+		+
7436	" " " "	" " " "	+		+
7439	" " " "	" " " "	+		+
<i>Plaurocapsa</i>					
7310	0.1	0.2		+	
7317	0.1	0.2		+	
7320	0.05	0.05		+	
7440	0.05	0.05		+	
7506	n.d.	n.d.		+	
7327	0.01	grew with no added Cl ⁻	+		+
7322	0.05	0.05		+	
7319	0.05	0.05		+	
7314	0.02	0.02		+	
7321	0.01	0.01		+	
7516	n.d.	n.d.		+	
7324	0.1	0.2		+	
<i>Synschoecoccus</i>					
7001	0.01	grew with no added Cl ⁻	+	+	
7002	0.01	0.005	+	+	
7335	0.05	0.1		+	
7336	0.2	0.2		+	
<i>Synschoecystis</i>					
7338	0.01	0.005	+	+	
7339	0.01	0.005	+	+	

n.d. : not determined

be met by substitution of potassium for sodium. Furthermore, the level of sodium necessary to support growth could not be decreased by an increase in the potassium concentration. These observations show that sodium is not required to maintain a minimum osmolarity, and suggest that it is a specific ionic requirement.

As also shown in Table XVIII, strains of terrestrial origin, whether or not they show a sodium requirement, can grow well in medium GB-11, which has a sodium concentration of 0.015 M. Strains of marine origin, on the other hand, fall into two categories with respect to growth in medium BG-11. *Synechococcus* strains 7001 and 7002, and *Synechocystis* strains 7338 and 7339 can grow well in this medium. All four strains have very low sodium and chloride requirements which are met by this medium. All remaining marine strains are unable to grow in BG-11. They also fail to grow when this medium is supplemented with 0.5 M sodium chloride, a concentration which exceeds the minimal requirements of the strains in question for both ions. Hence, for these marine strains medium BG-11 must be deficient in other ions than sodium and chloride. The nature of these additional ionic requirements is suggested by the fact that these strains grow well in medium ASN-III, which has far higher levels of magnesium and calcium than does medium BG-11. It is accordingly probable that these marine strains have elevated requirements for four ions: sodium, magnesium, calcium and chloride. Experiments designed to determine the minimum levels of magnesium and calcium required for growth revealed that the effects of these divalent cations are interrelated. The minimal level of either ion which supports growth is influenced by the level of the other one. It should be further noted that some of the marine strains (e.g., *Dermocarpella* 7326,

Pleurocapsa 7321) which have elevated requirements for magnesium and calcium have relatively low sodium and chloride requirements. They are indistinguishable in this respect from some strains able to grow in medium BG-11.

Selected marine representatives of the Pleurocapsales (7301, 7302, 7303, 7304, 7310, 7312, 7314, 7317, 7319, 7320, 7321, 7322, 7324, 7325) were examined to determine their halotolerance. All these strains could grow in medium ASN-III when the sodium chloride concentration was raised to 5% (w/v), but failed to grow when transferred to the same medium containing 10% (w/v) sodium chloride.

2. Vitamin Requirements

Although in most cases primary isolations from natural material were conducted by plating on media not furnished with vitamins, subsequent purification of nearly all strains both marine and fresh water was carried out on plating media supplemented with vitamin B₁₂. Consequently, it appeared desirable to screen our pure strains for a possible vitamin B₁₂ requirement. Such experiments showed that *Dermocarpa* 7301 and *Pleurocapsa* 7317 (both of marine origin) have an obligate requirement for this vitamin. Several other strains both marine (*Dermocarpa* strains 7302, 7303; *Pleurocapsa* 7310) and fresh water (*Chamaesiphon* 6605) are definitely stimulated by vitamin B₁₂ although they can grow in its absence.

3. Nitrogen Sources

With the exception of strains 7440, 7506 and 7516, which have not been tested, all members of the Pleurocapsales and Chamaesiphonales can use either ammonia or urea in place of nitrate as a source of combined nitrogen. Other possible combined nitrogen sources have not been

T A B L E X I X

THE ABILITY OF STRAINS BELONGING TO THE PLEUROCAPSALES AND CHAMAESIPHONALES
TO SYNTHESIZE NITROGENASE WHEN INCUBATED ANAEROBICALLY
IN THE LIGHT IN THE PRESENCE OF DCNU

Genera	Positive	Negative
<i>Dermocarpa</i>	7301, 7303	7302, 7304, 7437, 7438
<i>Xenococcus</i>	7305	7306, 7307
<i>Dermocarpella</i>		7326
<i>Myxosarcina</i>	7312	7325
<i>Chroococcidiopsis</i>	6712, 7203, 7431, 7432 7433, 7434, 7436, 7439	
<i>Pleurocapsa</i>	7320, 7327, 7322 7314, 7321, 7516, 7324	7310, 7317, 7440, 7506 7319
<i>Chamaesiphon</i>		6605, 7430

examined. None of the strains belonging to the Pleurocapsales or Chamaesiphonales can grow aerobically in the light in a medium devoid of combined nitrogen. The capacity for aerobic nitrogen fixation is consequently absent from these groups. However, when incubated anaerobically in the light in the presence of DCMU (see Methods) many strains develop nitrogenase activity as determined by the test of acetylene reduction (17, 65) (see Table XIX).

4. Heterotrophy

All strains were examined for their ability to utilize the three carbohydrates: glucose, fructose and sucrose, both as photosynthetic carbon sources (growth in the presence of DCMU in the light) and as sole carbon and energy sources for dark growth. As shown in Table XX, many strains (27 of 34) can develop photoheterotrophically at the expense of one or more of these sugars, sucrose being most commonly utilized. Certain sucrose-utilizing strains (11 of 23) fail to grow at the expense of one or both of the constituent monosaccharides, presumably because they lack specific permeases for the monosaccharides. In general, the sugars which can be utilized as photosynthetic carbon sources by a given strain are also capable of supporting its chemoheterotrophic growth in the dark. Although all strains of *Chroococcidiopsis* grow well at the expense of utilizable sugars in the dark, dark growth of organisms belonging to other genera is frequently either slow or not reproducible; such behavior is indicated by the symbol V (variable growth) in Table XX. The difficulty of obtaining reproducible growth on sugars in the dark may account for cases in which strains that grow with a given sugar in the light in the presence of DCMU are apparently unable to utilize the same sugar for dark growth (see data for strains 7310 and 7506).

TABLE XX

THE ABILITY OF MEMBERS OF THE PLEUROCAPSALES AND CHAMAESIPHONALES TO UTILIZE SUGARS FOR PHOTOHETEROTROPHIC AND CHEMOHETEROTROPHIC GROWTH

Strains		Photoheterotrophic Growth (DCMU - Light)			Chemoheterotrophic Growth (Dark)		
		Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
<i>Dermocarpa</i>	7301	+	+	+	V	V	V
	7302	-	-	-	-	-	-
	7303	-	-	-	-	-	-
	7304	+	-	+	+	-	+
	7437	+	-	+	+	-	+
	7438	+	+	-	+	+	-
<i>Xenococcus</i>	7305	-	-	-	-	-	-
	7306	-	-	-	-	-	-
	7307	+	-	+	V	-	V
<i>Dermocarpella</i>	7326	+	+	+	+	+	+
<i>Myxosarcina</i>	7312	-	-	+	-	-	+
	7325	+	-	+	+	-	+
<i>Chroococcidiopsis</i>	6712	+	+	+	+	+	+
	7203	+	+	+	+	+	+
	7431	+	+	+	+	+	+
	7432	+	+	+	+	+	+
	7433	+	+	+	+	+	+
	7434	-	+	-	-	+	-
	7436	+	+	+	+	+	+
	7439	+	+	+	+	+	+
<i>Pleurocapsa</i>	7310	-	-	+	-	-	-
	7317	+	-	-	+	-	-
	7320	+	+	+	V	V	V
	7440	-	-	-	-	-	-
	7506	+	-	-	-	-	-
	7327	-	-	+	-	-	+
	7322	+	+	+	V	V	V
	7319	-	+	+	-	+	+
	7314	-	+	+	-	+	+
	7321	-	-	+	-	-	+
	7516	-	-	+	n.d.	n.d.	n.d.
	7324	-	-	-	-	-	-
<i>Chamaesiphon</i>	6605	-	-	-	-	-	-
	7430	+	+	+	V	V	V

+ : growth

- : no growth

V : growth variable

n.d. : not determined

As shown in Table XX, heterotrophy has a highly sporadic distribution. Several genera (*Dermocarpa*, *Xenococcus*, *Pleurocapsa* and *Chamaesiphon*) contain some strains which are obligate photoautotrophs and others which are facultative heterotrophs.

5. Temperature Relationships

The study of temperature relationships has been limited to the determination of the maximum temperature at which photosynthetic growth can occur (Table XXI). *Pleurocapsa* 7327 was isolated from a thermal spring and consequently has a higher maximum temperature of growth (55°C) than any of the other strains examined. The temperature maxima of the remaining members of the Pleurocapsales range from 30 to 44°C, and most of them can grow at temperatures greater than 35°C. This may appear surprising in view of the marine origin of many of these strains. It should be noted, however, that they were isolated at temperatures of 20 to 25°C, which would have counter-selected any markedly psychrophilic members of the group. Both strains of *Chamaesiphon* have relatively low temperature maxima, possibly attributable to the fact that these organisms typically develop in cold streams and lakes.

With the exception of *Pleurocapsa* 7327, which cannot grow at temperatures below 37°C, all strains grow well at 20 to 22°C. The difficulty of measuring growth rates in the Pleurocapsales makes it impractical to determine temperature optima.

E. Chemical Characterization of the Pleurocapsales and Chamaesiphonales

1. Pigment Composition

The major photosynthetic pigments characteristic of these strains have been surveyed in a preliminary fashion by measurements of the

TABLE XXI
APPROXIMATE MAXIMUM TEMPERATURE PERMITTING GROWTH OF MEMBERS OF THE
PLEUROCAPSALES AND CHAMAESIPHONALES

Temperature °C	<i>Dermocarpa</i>	<i>Xenococcus</i>	<i>Dermocarpella</i>	<i>Myxosarcina</i>	<i>Chroococcidiopsis</i>	<i>Pleurocapsa</i>	<i>Chamaesiphon</i>
27							7430
30	7304	7306				7317	6605
35	7302	7305 7307	7326	7325		7310 7440	
37	7301 7303					7322 7314	7321
39				7312	6712	7320 7319	7324
40					7433 7436 7434 7439		
44	7437 7438				7431 7432		
55						7327	

absorption spectra of crude cell-free extracts. This was followed by a methanol extraction of chlorophyll and a closer spectral characterization of the phycobiliproteins in the supernatant after removal of the particulate fraction by high speed centrifugation.

Like other cyanobacteria (70) all these organisms show absorption maxima attributable to chlorophyll at 675 and 440 nm in crude cell-free extracts. After methanol extraction the position of the red peak shifts to 663 nm, typical of chlorophyll *a*

The phycobiliproteins show maxima both in crude cell-free extracts and in the supernatants after high speed centrifugation at approximately 618 nm and in some strains at 565 and 550 nm. The peak at 618 nm corresponds to the absorption maximum of phycocyanin and the peaks at 565 and 550 nm to the absorption maxima of one (or more) phycoerythrins.

It is known that certain phycoerythrin-producing cyanobacteria can undergo marked alterations of their phycobiliprotein complement in response to growth with light of different qualities, a phenomenon known as chromatic adaptation. Representative strains were screened for their ability to adapt chromatically during growth in red and in green light. In addition, some facultatively chemoheterotrophic strains were grown in the dark at the expense of sugars. The pigment compositions of cells grown under these different conditions are shown in Table XXII, where pigment concentrations are expressed as mg of pigment per 100 mg of cell protein. Chlorophyll was determined after methanol extraction, whereas phycocyanin and phycoerythrin were calculated from the absorbancies at two wavelengths of the supernatant fraction from cell-free extracts. These calculations were made from the simultaneous equations established by Bennett and Bogorad (5) on the basis of the absorption spectra of

purified phycocyanin and phycoerythrin from the filamentous cyanobacterium *Fremyella diplosiphon*. The numerical values obtained using these equations are correct only if the phycocyanin and phycoerythrin of the strains examined have absorption spectra identical with those of the corresponding pigments of *Fremyella diplosiphon*, an assumption which is not necessarily valid for all cyanobacteria, since it is known that cyanobacterial phycoerythrins do vary in spectral properties (12, 64). However, since the purpose of these experiments was to detect possible changes in the ratio between phycocyanin and phycoerythrin under different growth conditions, possible errors in the absolute numerical values for each pigment would not affect the conclusions which can be derived from them.

Table XXII shows that as a general rule the cellular chlorophyll content varies little in response to light quality. The chlorophyll contents of dark grown cells are slightly lower than those of cells grown in red and green light. For any given strain, the total phycobiliprotein content of the cells (sum of phycocyanin and phycoerythrin) also remains remarkably constant in red and green light, which suggests that the overall differential rate of synthesis of phycobiliproteins is not affected by light quality in members of these groups. Furthermore, the total phycobiliprotein concentration of dark grown cells is in most cases comparable to that of cells grown in red and green light. Notable exceptions are *Myxosarcina* 7312 and 7325 and *Pleurocapsa* 7327 where the dark-grown cells have roughly 50 percent of the phycobiliprotein content of light grown cells. An even more extreme reduction of cellular phycobiliprotein content as a consequence of dark growth occurs in *Pleurocapsa* 7314, where the value is only 12 to 15% of that for light grown cells.

T A B L E X X I I
CONCENTRATIONS OF THE MAJOR PHOTOSYNTHETIC PIGMENTS OF STRAINS OF THE PLEUROCAPSALES AND CHAMAESIPHONALES
GROWN UNDER DIFFERENT CONDITIONS OF ILLUMINATIONS

All values are expressed as mg pigment per 100 mg of total cell protein

Strains	Growth medium	RED LIGHT Ratio:					GREEN LIGHT Ratio:					DARK GROWTH Ratio:					Carbon source for dark growth
		Chl	PC + PE	PC	PE	PC/PE	Chl	PC + PE	PC	PE	PC/PE	Chl	PC + PE	PC	PE	PC/PE	
<i>Dermocarpa</i>																	
7301	MN	3.2	27	8	19	0.5/1	3.6	23	8	15	0.5/1						
7302	ASN-III	4.2	37	28	9	3/1	5.1	36	11	25	0.5/1						
7303	ASN-III	5.4	41	34	7	5/1	5.6	44	15	29	0.5/1						
7304	ASN-III	4.8	19	16	3	5/1	3.7	31	10	21	0.5/1	4.2	26	18	8	2/1	sucrose
7437	BG-11	5.3	32	28	4	7/1	5.2	29	14	15	1/1	4.1	25	19	6	3/1	sucrose
7438	BG-11	5.9	37	34	3	11/1	5.9	34	17	17	1/1	7.2	29	24	5	5/1	glucose
												5.4	28	24	4	6/1	fructose
<i>Xenococcus</i>																	
7305	ASN-III	4.0	57	51	6	8/1	3.8	54	14	40	0.33/1						
7307	MN	5.2	45	41	4	10/1	6.6	35	12	23	0.5/1	3.9	37	31	5	6/1	glucose
<i>Dermocarpella</i>																	
7326	MN	5.3	29	25	4	6/1	4.4	26	9	17	0.5/1	3.1	27	19	8	2/1	fructose
<i>Myxosarcina</i>																	
7312	MN	4.5	40	34	6	6/1	5.2	41	14	27	0.5/1	3.5	30	20	10	2/1	sucrose
7325	MN	4.4	41	35	6	6/1	3.3	30	12	18	0.66/1	3.2	19	9	10	1/1	sucrose
<i>Chroococcidiopsis</i>																	
(*)																	
6712	GN10SW	6.7	42	35	7	5/1	6.3	36	17	19	1/1	3.3	21	15	6	3/1	glucose
												3.6	22	17	5	3/1	fructose
7432	BG-11	2.8	16	16	0		2.8	18	18	0		5.4	39	26	13	2/1	glucose
<i>Pleurocapsa</i>												4.7	39	29	10	3/1	fructose
7310	MN	4.7	37	12	25	0.5/1	6.9	43	14	29	0.5/1	2.9	13	13	0		glucose
7317	MN	4.4	17	6	11	0.5/1	4.6	20	5	15	0.33/1						
7327	BG-11	5.3	37	19	18	1/1	6.2	30	16	14	1/1	2.5	16	6	10	0.6/1	glucose
7319	MN	5.2	35	29	6	5/1	5.2	39	14	25	0.6/1	4.0	14	8	6	1.3/1	sucrose
7314	MN	4.7	40	32	8	4/1	4.9	28	12	16	0.75/1	3.2	33	25	8	3/1	sucrose
<i>Chamaesiphon</i>																	
6605	BG-11	4.1	32	25	7	4/1	4.1	40	15	25	0.6/1	2.0	5	3	2	1.5/1	sucrose

(*) Medium GN10SW is medium BG-11 made with 10% (v/v) sea water.

Another interesting feature of the data is the very wide differences between strains grown under the same conditions with respect to phycobiliprotein content. Depending on the strain, these proteins may represent anywhere from 15 to over 50% of total cell protein in light grown cultures of different strains.

Although total phycobiliprotein content is little affected by light quality, many strains show a marked capacity for chromatic adaptation, evidenced by major changes in the phycocyanin/phycoerythrin ratio after growth in red and in green light. Some examples of this behavior are shown graphically in Figures 69 and 70. In every case where a response occurs, synthesis of phycoerythrin is favored by growth in green light. When such strains are grown in the dark, the cellular phycocyanin/phycoerythrin ratio resembles that characteristic of cells grown in red light (i.e., synthesis of phycocyanin is favored). Of the 18 phycoerythrin producing strains examined, only four failed to show a chromatic response (Table XXII). Figure 71 shows graphically the behavior under different growth conditions of one of these non-responding strains. It can be seen that the phycocyanin/phycoerythrin ratios are essentially unchanged by growth in green light, in red light and in the dark.

Failure to synthesize phycoerythrin appears to be rare among the Pleurocapsales. The only strains which lack this pigment are a group of *Chroococcidiopsis* strains which closely resemble one another in their phenotype and are exemplified (Table XXIII) by strain 7432. Of the two *Chamaesiphon* strains examined, one (6605) synthesizes phycoerythrin, whereas the other (7430) does not.

TABLE XXIII

MAJOR PATTERNS OF PHYCOBILIPROTEIN SYNTHESIS BY STRAINS OF THE PLEUROCAPSALES AND CHAMAESIPHONALES

Genera	A Phycoerythrin absent, Phycocyanin synthesis constitutive	B Phycocyanin and Phycoerythrin present and synthesized constitutively	C Phycocyanin and Phycoerythrin present, Phycocyanin to Phycoerythrin ratio variable depending on the light quality
<i>Dermocarpa</i>		7301	7302, 7303, 7304 7437, 7438
<i>Xenococcus</i>			7305, 7306, 7307
<i>Dermocarpella</i>			7326
<i>Myxosarcina</i>			7312 7325
<i>Chroococcidiopsis</i>	7203, 7431, 7432, 7433, 7434 7436, 7439		6712
<i>Pleurocapsa</i>		7310, 7317, 7321, 7324 7327	7320, 7440, 7322, 7319, 7314
<i>Chamaesiphon</i>	7430		6605

A graphic illustration of chromatic adaptation in *Pleurocapsa* strain 7319, evidenced by major changes in the phycocyanin and phycoerythrin levels following the growth of cells photoautotrophically in red and green light and chemoheterotrophically in the dark. Chlorophyll is not markedly altered under these conditions.

A graphic illustration of chromatic adaptation in *Dermocarpa* strain 7438, evidenced by major changes in the phycocyanin and phycoerythrin levels following the growth of cells photoautotrophically in red and green light and chemoheterotrophically in the dark. Chlorophyll is not markedly altered under these conditions.

A graphic illustration of a phycoerythrin producing strain of *Pleurocapsa* (7317) which does not show a chromatic response, as evidenced by the fact that the phycocyanin and phycoerythrin levels are essentially unaltered following the growth of cells photoautotrophically in red and green light and chemoheterotrophically in the dark. Chlorophyll is not markedly altered under these conditions.

2. Cellular Fatty Acid Composition

Previous studies have shown that the fatty acid composition of cyanobacteria varies widely among the representatives of the Chroococcales and Hormogonales (37, 38). Fatty acid analyses performed by Dr. C. N. Kenyon on cells of most of our strains shows a similar intra-group variability among the members of the Pleurocapsales (Table XXIV). In fact, the only common feature is the invariable presence of polyunsaturated fatty acids, which range from 5 to 50% of the total cellular fatty acids. One unusual feature of many members of the Pleurocapsales is the exceptionally high content of C_{16} fatty acids, which almost entirely replace C_{18} fatty acids and include polyunsaturated derivatives. This chemical feature is characteristic of *Dermocarpa*, *Xenococcus*, *Dermocarpella*, *Myxosarcina* and *Pleurocapsa* with the exception of the thermophilic strain 7327. It is not invariably correlated with a marine origin, being shared by two fresh water strains of *Dermocarpa* (7437 and 7438) and also encountered in one strain of the fresh water genus *Chroococcidiopsis* (6712). With this exception, *Chroococcidiopsis* strains have a very high content of the C_{18} polyunsaturated fatty acids 18:2 and 18:3 α , which account for approximately 50% of their total cellular fatty acids.

Since the order Chamaesiphonales is represented by only two strains, the data are insufficient to permit any generalizations about the fatty acid compositions in this group. Nevertheless, it is worthy of note that these two strains, which clearly belong to different species, share a closely similar fatty acid composition characterized by a high total content of polyunsaturated fatty acids, which include both C_{16} and C_{18} components.

T A B L E X X I V
CELLULAR FATTY ACID COMPOSITION OF STRAINS IN THE PLEUROCAPSALES AND CHAMAESIPHONALES (1)

	14:0	16:0	18:0	14:1	16:1	9(2) 16:2	10(2) 16:2	16:3	18:1	18:2	18:3 α	18:3 γ	% by weight of poly- unsaturated fatty acids
<i>Dermocarpa</i>													
7301	1	34	3		54		5		1				5
7302	6	28	1	1	49		13		1		1		14
7303	5	35	2	1	20		34		1	3			37
7304	7	24	3	1	43		13			3			16
7437	1	36	tr	1	58		2		1	1	1		4
7438	1	36	1	1	66		4		1	2			6
<i>Xenococcus</i>													
7305	6	38	1		33		16		1				16
7306	9	33	tr	2	34		8		tr		3		11
7307	2	31	1		56		8						8
<i>Dermocarpella</i>													
7326	2	26	1	tr	53		8		2	3			11
<i>Myxosarcina</i>													
7312	tr	23	1		62		6		5	1	3		10
7325	4	24	1		29		33		2	3			36
<i>Chroococcidiopsis</i>													
6712	26	9	7	3	27	2	7		3	2			11
7203	tr	29	1	tr	9				3	11	36		47
7431	tr	29	2	tr	7				6	15	36	tr	51
7432	tr	31	2	tr	7				6	12	37		49
7433	tr	28	1		8				7	16	33		49
7434	tr	27	1	tr	7				7	9	41		50
7436	tr	26	1		8				5	14	37	tr	51
7439	tr	31	2		7				7	14	35		49
<i>Pleurocapsa</i>													
7310	2	23	tr	tr	35	10	6	10	3	3	4		33
7317	4	34	1		28	1	20	3	1	2	2		28
7320	2	34	tr	tr	47		16			1			17
7440	2	22	1	tr	45	4	7	5	3	2	5		23
7506 n.d.													
7327	tr	47	2	tr	16		2		20	10		1	13
7322	1	32	1	tr	45		15		1		2		17
7319	2	30	tr		50		18						18
7314	5	23	tr		39		11	16	2				27
7321	4	28	tr		42		10	12	2				22
7516 n.d.													
7324	21	14			40		17		2	2			19
<i>Chamaesiphon</i>													
6605 (3)	6	20	2	1	2		7		3	43	4		54
7430	4	18	2		6	8	2	1	8	40	7	1	58

(1) Values given as percentage of total fatty acids as determined by gas-liquid chromatography.

(2) Elution peaks 9 and 10 are both 16:2 fatty acids that have not yet been identified.

(3) From Kenyon et al. (38).

n.d.: not determined

tr: trace

3. Mean DNA Base Composition

Prior to the present work, mean DNA base compositions had been determined for only two members of the Pleurocapsales, in both cases by buoyant density measurements. These values are shown in Table XXV, together with the present more extensive determinations based on T_m values.

The total span of DNA base composition within the order, is rather narrow: 40.7-46.5 moles percent guanine plus cytosine (G + C). Furthermore, the intrageneric ranges are in some cases almost as wide: 40.7-44.0 for *Dermocarpa*; 40.8-46.3 for *Chroococcidiopsis*; 42.8-46.5 for *Pleurocapsa*. This character is evidently of no value for making generic separations, although it may eventually prove useful to distinguish species.

Table XXV also shows the mean DNA base compositions of two strains of *Chamaesiphon*. The values are almost identical, and slightly higher than the highest values found in the Pleurocapsales.

IV. DISCUSSION

A. Physiological and Chemical Properties of the Pleurocapsales

The analysis of the physiological properties of the pure cultures of spore-forming cyanobacteria in our collection was undertaken primarily to ascertain the possible taxonomic utility of such properties, which can be determined only on axenic strains and have therefore not had any place in the traditional taxonomy of cyanobacteria. The results obtained show that such characters as: specific ionic requirements for growth, the ability to synthesize nitrogenase anaerobically, the ability to adapt chromatically, fatty acid composition and DNA base composition are

TABLE XXV

Mean DNA Composition of Members of the Pleurocapsales and Chamaesiphonales

Strains	Average Tm in °C	Moles % G+C	Mean Boyant density (g/cm ³)	Moles % G+C	Réf.
<u>DERMOCARPA</u>					
7301	71.93	44.0	1.703	44.0	19
7302	71.50	42.9			
7303	71.74	43.6			
7304	71.95	44.0			
7437	70.59	40.7			
7438	n.d.				
<u>XENOCOCCUS</u>					
7305	72.02	44.2			
7306	n.d.				
7307	71.76	43.6			
<u>DERMOCARPELLA</u>					
7326	72.38	45.1			
<u>MYXOSARCINA</u>					
7312	71.91	44.0			
7325	71.38	42.7			
<u>CHROCOCCIDIOPSIS</u>					
6712	70.34	40.2	1.700	40.8	70
7203	72.65	45.75			
7431	72.73	45.9			
7432	72.74	46.0			
7433	72.91	46.4			
7434	72.70	45.8			
7436	72.90	46.4			
7439	72.87	46.3			
<u>PLEUROCAPSA</u>					
7310	71.43	42.8			
7317	72.52	45.4			
7320	71.51	43.0			
7440	n.d.				
7506	n.d.				
7327	72.98	46.5			
7322	71.50	43.0			
7319	71.61	43.2			
7314	71.66	43.3			
7321	71.53	43.0			
7516	n.d.				
7324	71.64	43.3			
<u>CHAMAESIPHON</u>					
6605	n.d.		1.706	46.9	70
7430	73.06	46.7			

n.d. - not determined

for *Dermocarpa* 7437 and 7438, as well as for seven strains of *Chroococcidiopsis* (7203, 7431, 7432, 7433, 7434, 7436 and 7439).

5. Mean DNA Composition

In the cyanobacteria as a whole the span of mean DNA composition is wide, extending from 35 to 70 moles % guanine plus cytosine, a range almost as extensive as that for prokaryotes as a whole (29-74 moles % G + C). Since the mean base composition of the DNA can be significantly altered only by a very large number of base substitutions affecting most if not all segments of the genome, the wide dispersion of the values in cyanobacteria implies that this taxonomic group is an old one, within which a considerable amount of evolutionary divergence has occurred. In this context it is of interest to compare the ranges of DNA base composition characteristic of the major taxonomic subgroups among the cyanobacteria. Figure 72 is based on our data for the Pleurocapsales and Chamaesiphonales and on the data of other workers (19, 70, M. Herdman, personal communication) for the remaining taxa. Excluding the Chamaesiphonales, for which only two determinations are available, the values show an interesting general trend. For the Chroococcales and for the non-heterocyst-forming members of the Nostocales, DNA base composition spans an extremely wide range. On the other hand, the values for all heterocyst-forming cyanobacteria, whether true branchers (order Stigonematales) or not (order Nostocales) are much narrower, all falling between 39 and 48 moles % G + C. The values for the Pleurocapsales likewise fall into a narrow range (40 to 46 moles % G + C). In view of the relatively large number of strains which have now been analyzed among nostocalean and pleurocapsalean cyanobacteria,

Ranges of mean DNA composition among cyanobacteria. Data was taken from Edelman et al (19), Stanier et al (70), M. Herdman (personal communication) and the present study (Table XXV). Numbers in parentheses under each bar indicate the number of strains analyzed.

it is probable that the narrow ranges for these two groups do not reflect inadequate sampling but are true expressions of the degree of diversity in DNA base composition. The narrow ranges of DNA base composition of these two groups do not necessarily imply of course that they are of recent evolutionary origin, since evolutionary divergence may or may not be reflected by an overall change in DNA base composition. It is interesting to note that the three major groups which have comparatively narrow ranges of DNA base composition (Pleurocapsales, Stigonematales and heterocyst-forming members of the Nostocales) are precisely those characterized by the greatest degree of structural and developmental complexity.

B. The Taxonomy of the Pleurocapsales and Chamaesiphonales

1. Circumscription and Redefinition of the Orders Pleurocapsales and Chamaesiphonales and of Their Major Constituent Genera

The two orders Chamaesiphonales (synonym Dermocarpales) and Pleurocapsales, which are accepted in recent taxonomic treatments of the cyanobacteria (e.g., 7, 16) were originally proposed by Geitler in 1942 (29). It is therefore necessary to examine and evaluate the criteria upon which Geitler proposed these two orders, and the nature of the properties which distinguish their members.

Geitler's lengthy characterizations of the two orders (in effect, descriptions rather than definitions) contain extensive comments about the assumed course of structural evolution among cyanobacteria, and it is evident that the taxonomic proposals which he advanced were considerably influenced by these speculative considerations. The

following passage, taken from his description of the order Pleurocapsales (29, p. 79) is typical:

"Es sind dies Formen, welche die Weiterentwicklung von Entophysalidaceenartigen Chroococcalen-Typen zu ausgesprochenen Fadenalgen, allerdings Fadenalgen von nicht hormogonaler, sondern pleurocapsaler Organisation, darstellen; zudem treten typischerweise Endosporangien auf, die den Chroococcalen noch fehlen. Gegenüber den Dermocarpales s. str. dieser Bearbeitung, die ebenfalls Sporangien ausbilden, besteht der Unterschied darin, dass vegetative Teilungen ablaufen; während die Dermocarpales den typischen Protococcalen unter den Grünalgen entsprechen, sind die Pleurocapsales mit den Ulotrichalen vergleichbar.

Nach dem Gesagten sind Pleurocapsalen und Dermocapsalen gleichwertige Entwicklungsreihen. Sie stehen nicht in dem Sinn in einem näheren Zusammenhang, dass sich die eine Reihe von der anderen ableiten würde; vielmehr gehen beide auf die Chroococcalen zurück, nehmen aber von da aus verschiedene Entwicklung: die Pleurocapsalen gehen zur Fadenbildung über (wobei aber die durch den engen Zusammenhalt der Zellen gegebene Organisation der Hormogonalen noch nicht erreicht wird), während die Dermocarpalen unter Unterdrückung der vegetativen Zweiteilung, oder anders ausgedrückt, unter Betonung des einzelligen Zustands extrem protococcoide Organisation erreichen. Die Dermocarpalen stellen also eine Sackgasse der Entwicklung dar, die in den exosporinen Formen (*Chamaesiphon*) ihr Ende findet. Die Hormogonalen sind dagegen von den Pleurocapsalen abzuleiten."

A translation of this passage follows:

'These are the forms which represent the further development of Chroococcalean organization of the *Entophysalis* type into filamentous algae having, not a hormogonalean, but a pleurocapsalean organization. Furthermore, typical endosporangia occur, which are still lacking in the Chroococcales. In contrast to the Dermocarpales, as described here which also form sporangia, vegetative divisions occur in the Pleurocapsales. Whereas the Dermocarpales correspond to typical protococcalean forms in the green algae, the Pleurocapsales are comparable to the Ulothricales.

From what has been stated above, the Pleurocapsales and Dermocarpales are developmental sequences of equal importance. This does not mean that one series can be derived from the other. Rather, both are derived in divergent directions from the Chroococcales. The Pleurocapsales are transitional to filamentous forms (although they have not attained the close cellular association of the Hormogonales) while the Dermocarpales have undergone a suppression of binary fission, or in other words, have achieved an extreme protococcoid organization within the framework of unicellularity. The Dermocarpales therefore represent a blind alley of development, which terminates with the exospore-producers (*Chamaesiphon*). The Hormogonales on the other hand must be derived from the Pleurocapsales.'

T A B L E X X V I

CRITERIA EMPLOYED BY GEITLER TO SEPARATE THE ORDERS

DERMOCARPALES AND PLEUROCAPSALES (29)

<u>Differential Character</u>	<u>Order</u>	
	DERMOCARPALES	PLEUROCAPSALES
Structure:	1. Unicellular	1. Multicellular
Reproduction by Means of:	2. Exospores or Endospores	2. Endospores
Polarity of Organism:	3. Present	3. Absent
Major constituent genera:	{ <i>Chamaesiphon</i> <i>Dermocarpa</i> (which includes <i>Dermocarpella</i>) <i>Chroococcidiopsis</i>	{ <i>Xenococcus</i> <i>Myxosarcina</i> <i>Pleurocapsa</i> <i>Hyella</i>