tion and have found no evidence of specific patterns of impulses in different sensory nerves. It is most likely that each region of the skin has a local property which determines the specific central connection of its nerves.

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Fatty Acids in Blue-Green Algae: Possible Relation to Phylogenetic Position

Abstract. *Analyses of the lipids in five species of blue-green algae show that the fatty acids are largely the C_{16} and C_{18} acids. The only alga that could be grown heterotrophically,* Chlorogloea, *formed the triply unsaturated C_{18} acid in the light but only the doubly unsaturated C_{18} acid in the dark. Examination of these results and the results of others suggest that, except for one species, the more highly unsaturated acids are found in the morphologically more complex algae. The fatty acid compositions of blue-green algae are different from the fatty acid composition of the other prokaryotic organisms, the bacteria. It is speculated that the diversity of the patterns of fatty acid composition among the blue-green algae could be of phylogenetic significance.*

Several species of blue-green algae appear to be the only organisms now known that do not contain polyunsaturated fatty acids (*1–3*) but do carry out green-plant photosynthesis in which oxygen is evolved. Prior to our analyses of *Anacystis nidulans* (*1*), the correlation between the ubiquity of polyunsaturated acids and oxygen evolution in green plants suggested a relation of these acids to oxygen production (*4*), because in photosynthetic bacteria the polyunsaturated acids are absent and no oxygen is evolved during photosynthesis. Recent studies (*5*), in which the concentration of the polyunsaturated acid α-linolenate has been compared to photosynthetic evolution of oxygen by two species of the green alga *Chlorella,* have resulted in disagreement in the interpretation of results as to whether the content of α-linolenate is related to photosynthetic production of oxygen. It is clear that the functions of polyunsaturated acids in green plants are not yet understood, and further knowledge is desirable. Parker, Van Baalen, and Maurer (*3*) concluded that the blue-green algae were probably not the source of fatty acids found in the organic matter of sediments and noted that the 11 species that they studied could be divided into three categories on the basis of the content of oleic, linoleic, and linolenic acids. Our data confirm and extend these data and suggest a possible correlation of the classical phylogenetic position based on morphology with that suggested by the qualitative content of fatty acids.

Growth conditions used for the algae we studied are given in Table 1. Although the temperature was not rigidly controlled, we reported earlier that temperature affects quantitatively but not qualitatively the composition of fatty acids of *Anacystis* (*1*). Algae were harvested by centrifugation, washed in distilled water, lyophilized, and stored at −18°C until they were analyzed.

Lipids were extracted from 1 g of lyophilized algae and were saponified and esterified according to the procedures of Nichols, Harris, and James (*6*). A second saponification of the lipid fraction with methanolic KOH containing 5 percent water was done to insure complete saponification (*7*). Nonsaponifiable material was removed with peroxide-free ether and gas chromatographic analyses were made on the re-esterified acid fraction. To aid in the interpretation of the results, a portion of each ester mixture was reduced with hydrogen and platinum dioxide and analyzed in the same manner as the unreduced mixture.

As Parker *et al.* (*3*) noted in their survey of 11 species of blue-green algae, the fatty acid compositions of the algae we studied appear to be quite simple, but there are significant qualitative variations between one species and another (Table 2). The unicellular form *Synechococcus cedrorum* contains only the mono-unsaturated 18 : 1 (*8*) acid which has also been found in the other unicellular forms investigated, *Anacystis nidulans* (*1*) and *A. marina* (*3*). The simple filamentous species *Oscillatoria* contained both the polyunsaturated 18 : 2 and 18 : 3 acids, as had been shown by Schmitz (*9*) for *O. chalybea,* although only the 18 : 2 acid was present in marine filamentous species *O. williamsii* (*3*). We found polyunsaturated acids in *Nostoc muscorum,* as Parker *et al.* did (*3*).

The unexpected results involve two species. *Hapalosiphon laminosus* is a thermophilic blue-green alga found in many hot springs (*10*). Morphologically, it is more complex than any of the previously mentioned algae because it has true branches and specialized cells akinetes and heterocysts. Yet only mono-unsaturated acids were found in this organism.

Table 1. Growth conditions of algae. For medium D, see Kratz and Myers (*18*); medium A and A as given by Allen and Arnon (*19*) was supplemented with $10^{-2}M$ sucrose and 2 g of KNO_3 per liter; medium No. 11, see Hughes *et al.* (*20*). Cultures of the first four organisms were gassed with 0.5 percent CO_2 in air, *Oscillatoria* was grown in stagnant culture, and the *Hapalosiphon* was grown in shake culture. Au, autotrophic growth in the light; He, heterotrophic growth in the dark.

Organism*	Growth conditions		Gas phase
	Temp. (°C)	Medium	
Synechococcus cedrorum	26–30	D	0.5% CO_2
Nostoc muscorum A	26–30	D	.5% CO_2
Chlorogloea fritschii (Au)	26–35	A and A	.5% CO_2
Chlorogloea fritschii (He)	35	A and A	.5% CO_2
Oscillatoria sp.	25	No. 11	Air
Hapalosiphon laminosus	40	No. 11	Air

* Sources of the organisms were as follows: *Synechococcus* and *Nostoc,* Indiana University Culture Collection of Algae; *Chlorogloea,* Dr. G. E. Fogg, Westfield College, University of London; *Oscillatoria,* isolated from a soil sample from Knoxville; and *Hapalosiphon,* isolated by R. W. Holton (*10*).

Table 2. Total fatty acids of blue-green algae. Analyses were carried out by gas-liquid partition chromatography in an Aerograph 202 using a hot wire thermal conductivity detector. The column was packed with 20 percent diethylene glycol–succinate polyester coated on acid-washed Anachrom (60 to 70 mesh) with helium as the carrier gas (flow rate, 45 ml/min) and a column temperature of 175°C. The methyl esters were identified from a plot of the log of retention time versus the number of carbon atoms of known saturated and unsaturated esters (*1*). In addition to the acids given in the table, *Nostoc* contained 2.0 percent of a 16:2 acid; *Oscillatoria* also contained 1.5 percent of a 17:0 acid. Abbreviations: Tr, trace (less than 0.2 percent); N.D., not detected; Au, autotrophic growth in the light; and He, heterotrophic growth in the dark.

Organism	Percentages (by weight of total) of various fatty acids										
	10:0*	12:0	14:0	14:1	16:0	16:1	17:1	18:0	18:1	18:2	18:3
Synechococcus cedrorum	N.D.	0.2	0.5	1.0	47.0	38.8	0.5	1.4	10.0	N.D.	N.D.
Nostoc muscorum A	0.5	1.0	1.6	2.0	31.5	15.0	6.0	2.0	7.4	10.1	21.1
Chlorogloea fritschii (Au)	N.D.	0.2	0.6	0.3	41.2	16.5	0.6	1.8	13.9	13.0	12.0
Chlorogloea fritschii (He)	N.D.	Tr	.7	Tr	39.2	19.0	.5	1.5	25.8	13.0	0.4
Oscillatoria sp.	N.D.	0.2	1.3	N.D.	29.0	24.0	N.D.	1.5	25.6	10.2	6.8
Hapalosiphon laminosus	0.2	.4	1.0	N.D.	53.7	23.8	N.D.	2.9	18.2	N.D.	N.D

* See ref. (*8*).

A second interesting finding concerns *Chlorogloea fritschii* grown heterotrophically and autotrophically. In certain plants, including green and euglenoid algae and higher plants, the presence of the polyunsaturated 18 : 3 acid (α-linolenate) appears to be correlated with photosynthetic activity and this acid is absent (or present in very low concentrations) when these organisms are grown heterotrophically in the dark or are etiolated (*4, 5*). Only a few of the blue-green algae can be grown heterotrophically and these only with difficulty. Fay and Fogg (*11*) have shown that *Chlorogloea* can be grown in the dark by using sucrose as a source of energy. Data presented in Table 2 show that in *Chlorogloea* the only significant qualitative difference in fatty acid composition between the organisms grown in the light and those grown in the dark is the almost complete absence of the 18 : 3 acid in the dark-grown cells. The content of 18 : 2 acid remains the same while the 18 : 1 acid is much higher in the dark-grown cells. Although *Chlorogloea* was once considered a unicellular form on the basis of its morphology, it does form short filaments and heterocysts during its life history, fixes nitrogen, and probably belongs in the Nostocales according to Fay, Kumar, and Fogg (*12*). The presence of the polyunsaturated acids in this organism tend to confirm this suggestion.

Comparative biochemistry is useful in illuminating pathways of evolution in microorganisms because fossils are few and comparative morphological studies are limited. Fatty acid analyses have been particularly valuable because of sensitive new analytical techniques and the comparative studies of the biosynthetic pathways (*13*). Thus it is of interest to discover that within the blue-green algae there is a great variation in the content of polyunsaturated acids, which is not seen among other green plants.

Conventional taxonomy of the blue-green algae is based on cell arrangement (unicellular, colonial, and filamentous); the presence or absence of specialized cells (akinetes and heterocysts); and the presence or absence of branching, and, if present, whether false or true. We examined our fatty acid analyses and the few others reported in the literature and tried to ascertain if the analysis patterns bear any consistent relation to morphology and to certain physiological abilities.

Several conclusions can be drawn. (i) The three unicellular forms, *Anacystis nidulans* (*1, 3, 6*), *A. marina* (*3, 14*), and *Synechococcus,* lack morphological complexity, lack polyunsaturated fatty acids, and are unable to fix nitrogen or grow heterotrophically. (ii) The two colonial forms, *Coccochloris* and *Agmenellum* (*3, 14*), are both marine organisms, contain polyunsaturated acids, but cannot fix nitrogen or grow heterotrophically. (iii) The simple unbranched filamentous species that lack heterocysts, *Oscillatoria* (*3, 9*), *Lyngbya* (*3*), *Microcoleus* (*3*), and *Trichodesmium* (*3*), for the most part have triply unsaturated acids although two species have only doubly unsaturated ones. Physiologically, these organisms are unable to fix nitrogen or grow heterotrophically.

At the next most complex level of morphology, unbranched filamentous organisms with heterocysts, all four algae [*Chlorogloea, Anabaena* (*3, 6*), and two species of *Nostoc* (*3*)] contain polyunsaturated acids and fix nitrogen. *Plectonema,* which has false branching but lacks heterocysts and cannot fix nitrogen, also has polyunsaturated acids (*3*).

Finally, the lone exception to the general trend of the presence of polyunsaturated acids in the morphologically more complex forms is *Hapalosiphon.* This interesting and ubiquitous hot-springs alga was once suggested to be a relict organism from the time when warmer temperatures prevailed on the earth. This hypothesis has been seriously questioned (*15*) but quite recently has been revived (*16*). Our results can be interpreted to mean that it is a more primitive relict alga and can be expected to possess a less highly evolved pathway for the biosynthesis of unsaturated fatty acids. Of course, the *Hapalosiphon* data also can be interpreted as the loss of a biosynthetic pathway and as an indication of reduction rather than of primitiveness.

Blue-green algae are often grouped with the bacteria to comprise the prokaryotic organisms because they have in common certain cellular characteristics not present in the cells of the eukaryotic higher plants and animals. Does our knowledge of the fatty acid compositions aid our understanding of relations among the prokaryotic and of the eukaryotic organisms? In those photosynthetic bacteria that have been analyzed (*17*), polyunsaturated fatty acids are absent and the mono-unsaturated acid present is either completely or mostly the Δ-11 C_{18} vaccenic acid, which is also found in nonphotosynthetic bacteria. In contrast, the monoenoic acid found in blue-green algae is the Δ-9 compound oleic acid.

A specific comparison can be made between the blue-green alga *Oscilla-*

toria and its colorless counterpart, *Beggiatoa,* a gliding organism although not a eubacterium. Erwin and Bloch (*4*) report that an unspecified monounsaturated acid is present in *Beggiatoa,* but doubly and triply unsaturated fatty acids were found in all *Oscillatoria* species examined. The limited data available appear to support Shaw's conclusion (*13*) that there is no evidence from fatty acid composition of a close relation between blue-green algae and bacteria.

The diversity of the fatty acid compositions among the blue-green algae is curious when the ubiquity of the polyunsaturated acids in other algae and lower and higher green plants is considered. Does the acquisition of desaturation enzymes involved in the synthesis of polyunsaturated acids represent a trivial or significant event in the evolution of this group? It is reasonable to speculate that increased morphological complexity among the blue-green algae was accompanied by increased biochemical abilities and that the fatty acid composition is an indicator of this. Such speculation could tempt one to the conclusion that the blue-green algae represent a more phylogenetic diverse group than we now consider them. However, the role of polyunsaturated acids in the cell is largely unknown and, therefore, evaluation of the significance of the diversities in fatty acid patterns must await further knowledge.

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Melanocyte-Stimulating Hormone: Activity in Thermal Polymers of Alpha-Amino Acids

Abstract. *Thermal polymers of arginine, glutamic acid, glycine, histidine, phenylalanine, and tryptophan have melanocyte-stimulating activity. The fact that similar polymers lack such activity indicates that the effect is related to the specific amino acid residues. The active polymers are discussed as a model of an evolutionary precursor of contemporary melanocyte-stimulating hormone.*

We have found that several thermal anhydropolymers of α-amino acids possess melanocyte-stimulating activity. A number of laboratories have reported various catalytic activities (*1*, and bibliographies) in thermal proteinoids [thermal heteropolyanhydro-α-amino acids (*2*)]. Photosensitization yielding faster reactions (*3*), growth support (*4*), and a tendency to form easily structures having many of the properties of contemporary cells (*5*), as well as the ability of the organized unit to participate in reproduction of its own likeness in a presumably primitive mode (*6*), have been reported. Reactions of basic proteinoids with polynucleotides, to yield morphologies characteristic of complexes of histones and RNA or DNA, have been observed (*7*). To these properties is now added one of hormonal activity.

The polymers tested in this study were produced by this typical process: An equimolar mixture of L-arginine hydrochloride (28.2 g), L-glutamic acid (19.7 g), glycine (10.1 g), L-histidine hydrochloride monohydrate (28.1 g), L-phenylalanine (22.2 g), and L-tryptophan (27.6 g), in a total mass of 136 g, was heated in a reaction tube (22.5 by 5.6 cm) for 5 hours at 180° ± 3°C. The temperature was measured within the reaction mixture, which was heated always under an atmosphere of nitrogen. The resultant brown glassy polymer was extracted with four 1-liter portions of hot water, and the extract was filtered from a small amount of solid after cooling to room temperature. The filtrate was dialyzed against distilled water for 2 days before the solution was lyophilized to yield 7.33 g (5.4 percent) of polymer having a key lime-yellow color. A companion polymer lacking tryptophan in the reaction was white.

A hydrolyzate of a sample of the polymer thus prepared gave an amino acid analysis showing 13 to 19 percent of each amino acid, including tryptophan. Assessment of the heterogeneity of the hexatonic polymers, from observation of elution patterns from a Bio-Rad AG50W-X2 column, revealed nine principal peaks. This finding may be compared with elution patterns of a 1:1:1-proteinoidamide fractionated on DEAE-cellulose (*8*); the latter yielded

Table 1. Activity of melanocyte-stimulating hormone (MSH) in thermal polyanhydro-α-amino acids.

Polymer	Activity (units per gram)
Polyanhydro (Ala,Asp,Glu,Leu,Lys,Phe)	0.0
Polyanhydro (Arg,Glu,Gly,Hsd,Phe,Try)	2.2×10^4
Corresponding free amino acids	0.0
Polyanhydro (Arg,Glu,Gly,Hsd,Phe)	1.4×10^3
Polyanhydro (Glu,Gly,Hsd,Phe,Try)	0.0
L-Glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine	2.2×10^5
α-MSH	3.3×10^{10}