termined by the energy and duration of the light pulse, the absorbancy properties of the irradiated substance or substances (*5*), and the amount of cellular material exposed to irradiation. The major effect of the absorbed radiation results from transformation of light energy to heat (*5, 21*). This is evident from the charring of the cells at the site of irradiation. Other less likely or less well-documented physical effects may include shock waves and secondary radiations, but these have not been described for biologic preparations of the type used. The possibility that shock waves created by laser impact caused disruption of the liquid crystalline structure of the polymerized hemoglobin molecule and reversion to the nonsickled state is not compatible with the relatively long periods of time (seconds) required for the observed changes. Whatever the exact nature of the biophysical injury to the sickled cells, it seems that subsequent events occurred as the result of interchanges between contents of the cell and its environment dictated by alteration of the intervening cell membrane. The more likely explanations seem to be those based on the size of membrane defect. Large defects allow immediate loss of hemoglobin from the cell while smaller defects preclude the direct loss of hemoglobin but allow increased exchange of electrolytes and water. The subsequent swelling of the cell may cause formation of a sphere or may proceed beyond the critical hemolytic volume resulting in osmotic lysis. These observations may have application to other types of deformed cells such as acanthocytes, burr cells, and schizocytes (*22*).

From our observations, we propose that sickled cells are subject to at least two fates following injury in vivo by normal circulatory dynamics. The first follows avulsion of processes of sickled erythrocytes allowing escape of hemoglobin and intravascular hemolysis; the second follows the loss of smaller amounts of cell membrane with creation of "leaky cells" which become spherocytes and escape immediate lysis only to undergo erythrophagocytosis.

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Fatty Acids in Eleven Species of Blue-Green Algae: Geochemical Significance

Abstract. *Analyses of the total lipids of 11 species of blue-green algae showed a simple but qualitatively variable fatty acid composition. The species can be grouped in three categories on the basis of their oleic, linoleic, and linolenic acid content. One species was unusual in that the ten-carbon acid accounts for one-half of its total fatty acid. Branched chain acids are absent in the algae, but are major components of marine bacteria. The geochemical significance of the data is discussed.*

Algae are often pointed to as possible major source materials for the organic matter found in sediments (*1*). Blue-green algae are especially interesting because of their ability to grow in environments which favor the preservation of organic matter, such as hypersaline and reducing environments. The total fatty acid composition of 11 species (8 marine) of blue-green algae were measured in the hope of learning something about organism-sediment interactions. Very little data on the fatty acid composition of blue-green algae is available (*2*). The fatty acid composition of four marine bacteria were also measured for comparison with the blue-green algae. The simple and qualitatively variable fatty acid patterns found in this study are geochemically useful and it is felt they may be of interest to others.

The conditions used to grow the pure cultures of algae are given in Table 1. *Trichodesmium* was collected from large unialgal surface blooms which occurred near Port Aransas in the Gulf of Mexico. The results in Table 2 were obtained on samples collected in August and October 1965; the same results were found with a sample collected in July 1966.

The bacteria were isolated from sediment collected in Baffin Bay, about 30 miles (48 km) south of Corpus Christi, Texas. The isolation medium was aged 80 percent sea water with 0.1 g of yeast extract, Trypticase, and Soytone per liter, and 1 μg of vitamin B_{12} per liter, solidified with 1 percent Difco agar. The cells used for analysis were grown in shake cultures on 80 percent sea water containing 0.5 g of yeast extract, Soytone, and Trypticase per liter, and 1 μg of vitamin B_{12} per liter. The four organisms were Gram-negative small rods.

The analytical procedure was designed to detect fatty acids containing from 10 to 20 carbon atoms. The moist sample was treated with methanol for 15 minutes while being stirred and subjected to ultrasonic vibrations. The methanol was recovered by filtration. The sample was treated with chloroform and again stirred and subjected to ultrasonic vibrations. The recovered chloroform was combined with the methanol, and the mixture was almost completely dried with a rotating evaporator. This residue was taken up in chloroform and washed with 1*N* HCl to remove inorganic salts. The chloroform was evaporated under a

Table 1. Growth conditions of blue-green algae.

Organism	Code name	Growth conditions	
		Temp. (°C)	Medium*
Trichodesmium erythaeum	Trico	†	Bloom
Coccochloris elabens	17a	39	ASP-2
Microcoleus chthonoplastes	Ba-1	39	ASP-2
Nostoc muscorum G.‡	NM	30	C
Anabena variabilis‡	Anb. v.	30	C
Agmenellum quadruplicatum	PR-6	30	ASP-2
Plectonema terebrans	SP-31	30	ASP-2
Oscillatoria williamsii	Mev	30	ASP-2
Lyngbya lagerhaimii	Mont	30	ASP-2
Anacystis marina	WH-20	30	ASP-2
Anacystis nidulans‡	Ana. n.	39	C

* For medium C, see ref. *12*; for medium ASP-2, see ref. *13*. All organisms were grown in test tubes with continuous gassing with 1 percent CO_2 in air. Illumination was provided by 300 ft-ca (3300 lu/m²) from fluorescent lamps. At harvest, cell concentrations were approximately 0.5 mg dry weight per milliliter. † Natural. ‡ Obtained from Jo-Ruth Graham, University of Texas, Austin. The other cultures are marine isolates of our laboratory (*13*).

stream of dry nitrogen. After saponification with potassium hydroxide in methanol, impurities were removed by extracting the basic alkaline solution with hexane. Finally, the alkaline solution was acidified, and the fatty acids were extracted into hexane. The methyl esters were prepared with BF_3 (*3*). The methyl esters were identified and measured by gas chromatography on columns of diethylene glycol succinate (DEGS) and Apiezon L. A linear-log plot of the DEGS retention values was used to further confirm the identification (*4*).

The fatty acid compositions of the blue-green algae were simple but had a pronounced qualitative variation among the different species (Table 2). The absence of polyunsaturated acids in *Anacystis nidulans* reported by Holton, Blecker, and Onore (*2*) is confirmed and extended to *A. marina*. *Oscillatoria* and *Lyngbya* contain the polyunsaturated 18:2 but no 18:3 acid. The presence of polyunsaturated acids in *Anabaena*, as reported by Nichols, Harris, and James (*5*), is confirmed and extended to seven others. Perhaps the most striking fatty acid pattern is that of *Trichodesmium*, which in one bloom contained 50 percent C10:0 acid. Organisms with this high concentration of the acids of low molecular weight are rare. *Trichodesmium* may be the source of the C10:0 found in Gulf of Mexico sea water by Slowey, Jeffrey, and Hood (*6*). An abundant organism like *Trichodesmium* could supply a geochemically significant amount of a relatively rare fatty acid to sediments.

The blue-green algae contain a measurable amount of odd carbon acids but not enough to enrich sediment in these molecules. The branched-chain fatty acids are almost completely absent in the blue-green algae. The branched-chain acids found in sediments by Leo and Parker (*7*) almost certainly do not come from blue-green algae, if the algae reported here may be taken as representative. The branched-chain acids are probably due to bacteria such as those in Table 3. Of the four bacteria species isolated, three are rich in branched-chain acids while one had the more common straight-chain acids. Although the abundance of the bacteria in the sediment is not known, it seems probable that they account for the branched-chain acids. An exception would be phytanic acid, reported by Eglington *et al.* (*8*), which is probably derived from phytol. Kates (*9*) and Kaneda (*10*) have reported bacteria which have branched-chain acids as their major acid. As expected, none of the bacteria contain polyunsaturated acids.

In paleobiochemistry the fatty acids are especially interesting because traces of them persist in rocks for geologically long periods of time (*11*). Our data suggest very simply that organic matter in an ancient sediment derived only from blue-green algae and preserved without modification would not contain branched-chain iso- and anteiso-fatty acids, and that the presence of such acids is probably due to bacteria.

Table 2. Total fatty acids of blue-green algae (percentage composition). Abbreviations: tr (trace), less than 0.5 percent; nd, not detected.

Organism*	12:0†	14:0	14:1	15:0	16:0	16:1	16:2	18:0	18:1	18:2	18:3
Trico‡	2.2	21.	tr	1.4	17.	3.7	nd	2.6	2.8	4.2	19.
Trico‡	5.0	7.0	tr	2.3	11.	6.7	nd	2.6	7.4	1.3	6.3
17A	tr	1.1	0.5	0.8	49.	12.	nd	1.2	13.	17.	2.6
Ba-1	1.5	5.0	tr	2.5	37.	13.	nd	3.7	14.	5.0	18.
NM	tr	2.7	2.1	2.2	27.	20.	nd	3.1	16.	14.	11.
Anb. v.	tr	1.8	1.1	0.9	32.	15.	nd	4.4	14.	14.	17.
PR-6	tr	2.0	1.2	1.8	34.	15.	3.8	2.6	16.	14.	5.2
SP-31	tr	1.4	0.8	1.1	35.	13.	5.1	2.5	20.	11.	6.0
Mev	0.6	2.0	1.2	1.7	36.	24.	14.	1.9	11.	3.9	nd
Mont	tr	2.0	0.8	1.1	40.	15.	tr	1.9	31.	7.4	nd
WH-20	tr	21.	1.4	1.4	32.	36.	tr	1.6	4.1	nd	nd
Ana. n.	tr	0.5	0.6	tr	49.	34.	nd	1.7	13.	nd	nd

* See Table 1 for code name equivalents. † The numbers before the colon indicate the numbers of carbon atoms; the numbers after the colon, the numbers of double bonds. ‡ The two samples of *Trichodesmium*, from two different blooms, contained 27 and 50 percent 10 : 0; this acid was barely detectable in the other organisms.

Table 3. Total fatty acids of four marine bacteria (percentage composition). Abbreviations: i, iso; a, anteiso; tr, trace.

Acid	K-2	E	L	G*
i 14:0	1.6	2.7		
14:0	2.1	3.4	4.2	3.
(i+a) 15:0	47.	57.		
15:0	tr	tr	tr	3.7
i 16:0			6.1	1.5
16:0	18.	15.	20.	24.
16:1	10.	11.	48.	38.
(i+a) 17:0	13.	5.4		
17:0		tr		3.
18:0	3.2	2.0	tr	2.
18:1	5.	4.1	21.	12.

* Bacteria G contained 1.3, 1.6, and 9.8 percent 14 : 1, 15 : 1, and 17 : 1, respectively.

In paleobiochemistry the fatty acids are especially interesting because traces of them persist in rocks for geologically long periods of time (*11*). Our data suggest very simply that organic matter in an ancient sediment derived only from blue-green algae and preserved without modification would not contain branched-chain iso- and anteiso-fatty acids, and that the presence of such acids is probably due to bacteria.

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