effluent, containing only the desired amino acids, was evaporated to dryness. After this pure fraction was converted to highly purified CO_2, it was dated in a proportional counter to within an accuracy of at least one standard deviation (*9*).

Analysis by gas chromatography of hexane extract of a portion of the purified acids showed no petroleum contamination. Amino acid analysis of small samples gave results consistent with those of Ho (*7*) for the same species. Each sample required 3 to 4 days for completion of the separation process. The dates for bone samples from pit 3 and the previously obtained dates for wood from pit 3 are given in Table 1.

This method may be used to date bone from several other Brea deposits in California (*10*) and Peru (*11*). It may also prove satisfactory for the removal of contamination due to preservatives and humic acids. One further refinement is possible which will guarantee that all of the dated carbon is derived from bone collagen. Hydroxyproline is a major constituent of collagen and is rarely present in other proteins (*12*). Purified hydroxyproline may be separated chromatographically from the other amino acids present for dating. About 10 percent of bone collagen is hydroxyproline; therefore a sample ten times as large as that used in this study would be required to obtain sufficient carbon for dating (*7*). Such large samples are available from single bones at La Brea. Sufficient agreement of the dates for bone and wood and the repeatability of dating of the same specimen have indicated that this additional refinement was not required for dating La Brea bone.

In general, our procedure allows for greater utilization of one of the richest collections of mammals in the world and permits, for example, studies on morphological changes within the same animal species with time during the Late Pleistocene.

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Fatty Alcohols (Normal and Isoprenoid) in Sediments

Abstract. *Normal long-chain alcohols were isolated from Recent marine sediments from several environments. The isoprenoid alcohol dihydrophytol, which is thought to originate from phytol, the side chain of chlorophyll, by hydrogenation in the reducing environment of the sediment, was also present in most of the samples. Both the normal and isoprenoid alcohols were found in the Green River shale (Eocene). Geochemical implications are considered.*

Biochemicals and recognizable degradation products of biochemicals, although present only in trace amounts in sediments, are in most cases the only source of data on the chemical composition of ancient organisms. It is useful to study the organic compounds contained in Recent sediments and to note biological sources for various compounds and some of the chemical transformations that specific biochemicals undergo in geologically short periods of time (*1*). We now report the finding of normal and isoprenoid fatty alcohols in sediments from several environments. Although the fatty acids (*2*, *3*) and corresponding long-chain hydrocarbons (*4*) have been found in marine sediments, the finding of alcohols has not been reported.

Results of analyses on fatty alcohols in samples from three different Recent environments and three ancient sediments are presented in Table 1. The sample of Miocene age is from an undescribed outcrop in the Philippine Islands. The sample of shale from the Green River (Eocene) is from the U.S. Bureau of Mines oil-shale mine near Rifle, Colorado. The Eagleford shale (Upper Cretaceous) is from an outcrop near Austin, Texas (*5*). Baffin Bay is a hypersaline arm of the Laguna Madre located about 30 miles (48 km) south of Corpus Christi, Texas. The core from the Gulf of Mexico was taken in 25 m of water off Port Aransas, Texas. The sample from the San Nicholas Basin is a grab sample taken off the coast of southern California (*5*). The fatty-acid composition of some of these samples has been reported (*1*, *2*, *6*). All of the Recent samples of sediment were frozen soon after the time of collection and were kept frozen until used.

The following analytical procedure yields a sample suitable for gas-chromatographic analysis, but it does not exclude any type of long-chain alcohol. The sample was treated with dilute hydrochloric acid to remove carbonate and was washed with water to remove inorganic salts. After filtration, the moist mud was treated with methanol for 20 minutes while being stirred and subjected to ultrasonic vibrations. The methanol extract was recovered by filtration. The mud was again extracted with chloroform while being stirred and subjected to ultrasonic vibrations. The methanol and chloroform filtrates were combined and dried on a rotary evaporator. The organic residue was taken up in chloroform and washed with 1*N* hydrochloric acid to remove any last traces of inorganic salts. The chloroform was then dried, and the residue was weighed. The residue was saponified with 0.5*N* potassium hydroxide in methanol for 1 hour on a steam bath. The nonsaponifiable fraction containing the hydrocarbons, alcohols, and sterols was then separated from the saponifiable fatty-acid fraction by extraction of the alkaline solution with benzene. The nonsaponifiable benzene fraction was dried under a

stream of dry nitrogen and then was taken up in 1 ml of *n*-hexane. This was carefully placed on a chromatography column (2.5 by 20 cm) packed with prewashed silica gel (Woelm, activity I). Straight-chain hydrocarbons were eluted from the column with 75 ml of hexane. Elution of the column with 75 ml of benzene removed most of the aromatics of high molecular weight. The alcohols were eluted with 75 ml of methanol. The methanol fraction was then dried under a stream of dry nitrogen and was taken up in a known volume of benzene. This benzene solution was used for preliminary identification of the alcohols by means of gas-liquid chromatography (GLC).

A gas chromatograph (F & M Biomedical model 400) with a hydrogen flame-ionization detector, operated isothermally, and a copper column, 1.5 m long, packed with 10-percent FFAP coating (Varian) on 60- to 80-mesh Chromosorb G support were used. The column oven was operated at 215°C for chromatographing alcohols of lower molecular weight, and at 250°C for alcohols of high molecular weight. Fatty alcohol standards consisting of alcohols containing an even number of carbons from C_{14} to C_{28} (no double bonds) and dihydrophytol were used for calibration. The alcohols containing 14 to 22 carbon atoms and dihydrophytol were easily identified and measured by this procedure.

Since retention times in gas-liquid chromatography are not unique, the identifications were verified by several methods. A second set of retention times was obtained by use of a nonpolar SE-30 column. The infrared spectra of the isolated alcohol fractions (methanol fraction off silica gel) were taken in CCl_4 for several of the more alcohol-rich samples. Absorption bands due to the OH functional group were present. Finally, the fatty alcohols were converted to their acetate esters by refluxing with acetic anhydride; the retention times of the alcohol peaks shifted to those expected for the corresponding esters (Fig. 1). The alcohol fraction was further purified by column chromatography on aluminum oxide, but this was proved unnecessary for most samples.

The Recent sediments contain normal saturated alcohols with 12 to 26 carbon atoms (Table 1). Alcohols with both even and odd numbers of carbon atoms are present. In addition, normal, unsaturated alcohols are present in the

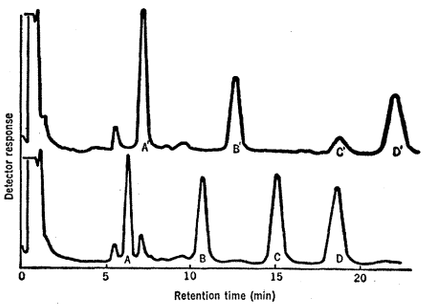

Fig. 1. Gas-liquid chromatogram of the fatty alcohols of lower molecular weight from the Eagleford shale on a FFAP substrate. The upper curve shows peaks for the free alcohols; the lower curve is for the acetate esters. In the lower curve peak *C* is enlarged due to the addition of pure dihydrophytol acetate spike. *A*, C_{14} (no double bonds); *B*, C_{16} (no double bonds); *C*, dihydrophytol; *D*, C_{18} (no double bonds).

Recent muds but are absent in the ancient sediments. The isoprenoid, dihydrophytol (3,7,11,15-tetramethylhexadecanol), is present in substantial concentrations in both Recent and ancient sediments. Several unidentified peaks present in the GLC traces may be isoprenoid alcohols of lower molecular weight.

Normal, monomethyl branched-chain and isoprenoid long-chain hydrocarbons, alcohols (Table 1), and fatty acids are present in sediments (*1, 2, 4, 7, 8*). The relations among these three different types of carbon skeletons involving three different functional groups are beginning to emerge. We confirmed (*9*) the presence of the C_{20} isoprenoid acid, phytanic, and the C_{19} isoprenoid acid, pristanic, in Recent sediments (*7*). Eglinton *et al.* (*8*) found phytanic acid and pristanic acids in the Green River shale. Blumer (*7*) found that, whereas the C_{19} isoprenoid hydrocarbon, pristane, is present in Recent sediment, the C_{20} isoprenoid hydrocarbon, phytane, is absent. Both these hydrocarbons are present in ancient sediment and crude oil (*10*). The C_{20} isoprenoid alcohol, dihydrophytol, present in both Recent and ancient sediments (Table 1), may be the first reaction product in the conversion of phytol into phytane and phytanic acid. We did not determine whether the C_{19} isoprenoid alcohol was present in our samples.

Because, in the biosphere, fatty alcohols occur less frequently and generally in much lower concentration than the corresponding fatty acids, it was expected that the concentration of fatty alcohols would be much lower than that of fatty acids in Recent sediments. However, the amounts of alcohols found in Recent sediment were from one order of magnitude less to the same order of magnitude as the concentrations of fatty acids. Baffin Bay mud contained 9.7 μg of total alcohol per gram of dry sediment (Table 1). Parker (*6*) reported 40 μg of fatty acid per gram of sediment over the same range of molecular weight. These high concentrations are surprising but difficult to explain. In order to be sure that the sonification used in the extraction procedure did not generate fatty alcohols from other organic material present in the sediment, sev-

Table 1. Concentration (parts of alcohol per million parts of dry sediment) of the normal alcohols and dihydrophytol in sediments.

Alcohol*	Samples from					
	Baffin Bay	Gulf of Mexico	San Nicholas Basin	Miocene age	Green River shale	Eagle-ford shale
12:0	1.00					
14:0	1.40	0.36	3.00	1.75	3.20	1.30
15:0	0.94	.52	1.50	0.31	2.10	0.88
16:0	.99	1.08	2.20	.14	0.50	1.10
16:1	.08		0.54			
17:0	.55	0.18	.80	.07	.35	0.92
Dihydrophytol	.39	.32	1.57	.08	1.10	.09
18:0	.64	.82	1.75	.17	0.96	.90
18:1	1.06		3.25			
20:0	0.17	.60	3.90	.20	2.00	.65
21:0			1.35	.14		.42
22:0	.85	1.35	7.05	.15	9.50	.32
24:0	.61	0.48				
26:0	1.05					
Total	9.7	5.7	26.9	3.0	19.7	6.6
	Percentage of total organic carbon					
	1.8	1.0	6.7	4.2	18.	15.

* The numbers before the colon indicate the numbers of carbon atoms; the numbers after the colon indicate the numbers of double bonds.

eral control experiments were run. In one experiment, 50 mg of palmitic acid was added to a sample of Baffin Bay sediment which had been extracted with organic solvents. The mixture was extracted with methanol by the use of sonification. Fatty alcohols and hydrocarbons were analyzed according to the standard procedure. No alcohols or hydrocarbons were detected. In other control experiments split samples were extracted by Soxhlet extraction and sonic energy, and the alcohol patterns were compared. In general, the total yield of alcohols was slightly higher with sonification. In qualitative composition the recovered fatty alcohols were not significantly different. The extraction procedure based on sonic energy does not create any artifacts.

Fatty alcohols in sediments probably have their origin in the marine life of the environments studied. Because Baffin Bay normally receives very little fresh water as runoff, it is often twice as saline as seawater. Such restricted runoff probably could not transport enough terrestrial organic matter to account for the uniform concentrations of alcohols observed in the bay sediments. Inasmuch as the same types and concentrations of alcohols are present in all the Recent samples, we assume Baffin Bay to be typical. We cannot specify which marine organisms are producing the normal fatty alcohols, although the bacteria may be involved. The isoprenoid alcohol, dihydrophytol, probably is derived from phytol, the side chain of the chlorophyll molecule. The reducing environment of the sediment must be sufficient to hydrogenate the double bond of phytol. Likewise, reduction of the corresponding fatty acid would yield the fatty alcohols. However, our data do not allow us to evaluate this mechanism.

Hoering (*11*) has isolated and identified fatty alcohols in Recent sediment while we were conducting our investigations. His results, based in part on mass spectra, are qualitatively similar to ours. The fatty alcohols promise to be a rewarding subject for organic geochemical studies. Dihydrophytol, in particular, should serve as a useful biological marker.

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Sterile Soil from Antarctica: Organic Analysis

Abstract. *Soils from the dry-valley region of Antarctica can be sterile by the usual microbiological criteria and yet contain significant amounts of organic carbon. Examination of one such soil shows that the organic material is finely divided anthracite coal. These findings have significant implications for the biological exploration of Mars.*

The ice-free valleys of Victoria Land, Antarctica, are among the world's most hostile deserts. No higher plants or animals inhabit these regions. Dense microbial populations are found where glacial melt makes liquid water available during part of the year, but the driest parts of the valleys are sterile or nearly so (*1*). To the extent that low temperatures and scarcity of water limit life in the dry valleys, these valleys may resemble the planet Mars; we have found these valleys useful as a model environment for the investigation of problems related to the search for life on Mars.

There is little correlation between the microbial count of dry-valley soils and their content of organic carbon as determined by the standard Allison wet-combustion method (*2*). Particularly anomalous are soils which show no microorganisms but which contain a significant amount of organic carbon. A possible explanation of the anomaly is that these soils harbor an undetected population of microorganisms; we have carried out an intensive study of one of the abiotic soils in order to determine the nature and origin of the organic matter.

The soil chosen for study (No. 542) was collected during the austral summer of 1966–67 on a gentle slope facing northeast about 1.5 km west of Lake Vida, near the junction of the Victoria and McKelvey valleys. The sample was collected aseptically (*3*) at a depth of 15 to 25 cm and has since been stored at a temperature below −25°C. This sample was selected because it had been collected in metal or poly(chlorotrifluoroethylene) containers, rather than in standard commercial soil bags. The latter, constructed of canvas with a polyvinyl chloride liner, introduce minor amounts of solvents and plasticizers which are detectable by the sensitive method involving pyrolysis, gas chromatography, and mass spectrometry that we have developed for the organic analysis of desert soils (*4*, *5*).

The microbial count on soil No. 542 was below the detectable limit of approximately one organism per gram in all media tested. These included the following commercially available media: trypticase soy agar, trypticase soy broth with and without added sodium chloride, lactose broth, fluid thioglycollate, Rose Bengal agar, actinomycete isolation agar, deoxycholate agar, nitrate broth, anaerobic agar with and without glucose, and Brewer anaerobic agar. The following special media were also used: simulated Taylor Valley salts (*6*), Thornton's algal medium (*7*), Burk's agar (*7*), Van Delden's agar (*7*), and yeast agar (*8*). Samples were incubated from 15 days to 3 months, usually at 20°C; some samples were incubated at 2°, 25°, 37°, or 55°C. Anaerobic incubations were carried out under both N_2 and CO_2.

Unlike some Antarctic soils which are toxic because of their high boron content (*6*), soil No. 542 did not inhibit growth when mixed in a 1:1 ratio with another dry-valley soil containing 1500 organisms per gram. As a further check of the sterility of soil No. 542, its capacity to generate metabolic CO_2 from labeled glucose and amino acids was determined. The results are shown in Table 1, together with comparable data for other dry-valley soils, including the entire profile (ground level to