megacycle alternating current free of a d-c component.

It appears reasonable to attribute the high effective electrical permittivity of the living cells, in part at least, to the known existence of an easily distortable electric double layer across the cell membrane.

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Branched-Chain Fatty Acids in Sediments

Abstract. *Branched-chain (iso and anteiso) fatty acids were isolated from marine sediments from several environments. The relatively high ratio of branched-chain to straight-chain fatty acids for the even-numbered carbon molecules suggests a bacterial origin for the branched-chain isomers. The branched-chain fatty acids are present in the Green River shale. Possible geochemical implications are suggested.*

A small but significant fraction of the biochemicals produced by marine organisms is preserved in sediments. Most of this material quickly loses its biochemical identity and reappears as an insoluble material (kerogen) or as breakdown products. The fatty acids are among the chemical species best preserved in sediments. Cooper and Bray (*1*) reported straight-chain saturated acids, having even and odd numbers of carbon atoms, from recent and ancient sediments. Abelson *et al.* (*2*) and Parker and Leo (*3*) found even-numbered carbon unsaturated acids in addition to the saturated ones. We now report the finding of a homologous series of iso and anteiso methyl branched-chain acids in sediments from several environments.

Results of fatty acid analyses of samples from three different recent environments and the Green River shale are presented in Table 1. The Green River shale was collected near Rifle, Colorado, from the Mahogany Ledge outcrop (*4*). Baffin Bay is a hypersaline arm of the Laguna Madre located about 30 miles (48 km) south of Corpus Christi, Texas. The average results for nine samples of surface mud taken from the bay are reported. The Gulf of Mexico core was taken in 25 meters of water off Port Aransas, Texas. Since no significant variation of the acids was observed in the core, average values for seven samples are given. The British Honduras samples were from a 3.6-meter core, consisting mostly of carbonates, taken from a typical back-reef environment (*4*).

All of the recent sediment samples were frozen soon after the time of collection and kept frozen until used, except the British Honduras samples which were preserved in alcohol during transportation.

The analytical procedure developed to avoid excluding any type of fatty acid has been described (*3*). The urea adduction used by Cooper and Bray was avoided because it excludes branched-chain acids. Since the branched-chain acids have not been reported to occur in sediments, special care was taken to confirm their identification. The methyl esters were identified and measured by gas chromatography on columns of diethylene glycol succinate (DEGS) and of Apiezon L. The gas-liquid chromatography peaks identified as methyl-branched chains were not removed by bromination, whereas the unsaturated peaks were removed. A linear-log plot of the DEGS retention values of the methyl-branched peaks yielded two straight lines, corresponding to the iso and anteiso acids (*5*). Confirming evidence was obtained from the infrared spectra of the combined iso and anteiso C_{15} peaks. The peaks were collected at the exit port of an Aerograph 202 in a capillary tube and redissolved in carbon tetrachloride. Although the collected peaks contained some normal C_{15}, the "isopropyl splitting" of the band at 1380 cm^{-1} was apparent (*6*).

All the recent sediments examined thus far contain appreciable amounts of branched-chain acids. Figure 1 is a typical gas chromatogram of the relative positions of the branched- and straight-chain acids. The iso and anteiso peaks are not well resolved from each other, but the curve of the logs of the retention values indicates that both series are present in many samples. The branched-chain isomers with odd numbers of carbon atoms are more abundant than those with even ones (Table 1). The 15 carbon branched-chain acids are especially abundant, often as abundant as the normal C_{14} acid. The C_{15}

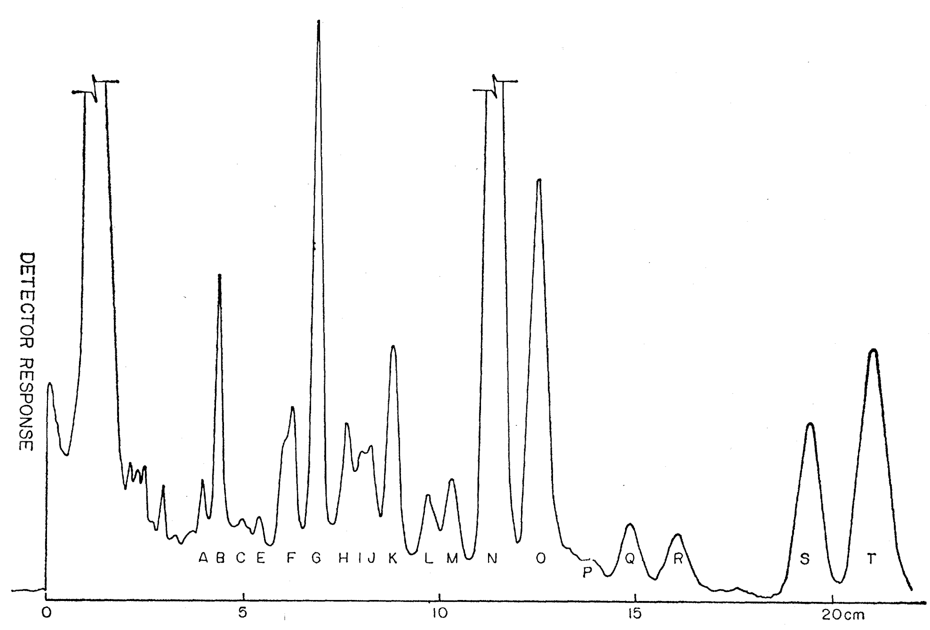

Fig. 1. Gas-liquid chromatogram of a fatty acid extract on a DEGS substrate. Key: A, iC_{12}; B, $C_{12}(0)$; C, iC_{13}; E, $C_{13}(0)$; F, iC_{14}, aC_{14}; G, $C_{14}(0)$; H, $C_{14}(1)$; I, iC_{15}; J, aC_{15}; K, $C_{15}(0)$; L, $C_{15}(1)$; M, iC_{16}; N, $C_{16}(0)$; O, $C_{16}(1)$; P, iC_{17}; Q, $C_{17}(0)$; R, $C_{17}(1)$; S, $C_{18}(0)$; T, $C_{18}(1)$. The numbers in parentheses indicate the number of double bonds; i, iso; a, anteiso.

Table 1. Concentration (parts of fatty acid per million parts of sediment) of the branched-chain and straight-chain saturated fatty acids in sediments.

Fatty acid	BB (9)*	GM (7)	BH (8)†	GRS
iC_{14}	0.97	0.4	32	0.6
C_{14}	2.6	2.9	130	3.4
$(i + a)C_{15}$	3.1	0.6	61	0.5
C_{15}	1.3	1.9	76	2.0
iC_{16}	1.3	0.4	38	0.5
C_{16}	11	15	560	12.2
$(i + a)C_{17}$	1	0.1	13	n.d.‡
C_{17}	1	1.0	39	1.6
iC_{18}	0.84	n.d.	n.d.	2.5§
C_{18}	3.0	3.5	140	8.4

* Numbers in parentheses indicate numbers of samples averaged. See text for sample locations. † Calculated on a carbonate-free basis. ‡ Not detected. § Identification tentative.

iso and anteiso acids are about equally abundant. Usually the even-carbon acids have only the iso acid. The iso C_{12} acid and several monounsaturated acids are present although not listed in Table 1. The relatively high ratio of branched-chain acids to straight-chain saturated acid for the various carbon numbers (especially the even ones) raises a problem concerning possible origins.

Sediments receive a small fraction of every type of fatty acid produced by its community. The fatty acid pattern of a sediment will depend (i) on the nature of the acids supplied by the community, and (ii) on the relative survivability, both biological and chemical, of the different acids. Branched-chain acids are chemically as stable as the normal acids and are certainly no more subject to biological degradation than straight-chain acids. Therefore the most important factor is the nature of the acids being produced by the organisms which make up the marine community.

The ratios of the major straight-chain saturated acids to the corresponding branched-chain isomer is lower in sediments than the same ratio is in higher organisms which we have analyzed. This comparison holds for a variety of higher marine organisms reported by Ackman and Sipos (*7*). For their organisms the ratios of straight-chain to branched-chain (iso) for C_{16} and C_{14} are between 100 and 500. The same ratios for sediments are between 1 and 20. This suggests that higher organisms are not the major source of the branched-chain acids in sediments. One should make the same ratio comparisons for phyto- and zooplankton but scarcity of data does not seem to justify doing so. Williams (*8*) studied the fatty acids of six phytoplankton and reported no branched-chain acids. Only minute traces of branched-chain acids were found in ten species of blue-green algae studied in this laboratory. While we do not know the contribution made by plankton to the branched-chain acids in sediments, we would like to suggest a possible source. Bacterial lipids are noted for being rich in branched-chain acids (*9*). Kaneda has shown that the major fatty acids of *Bacillus subtilis* are the iso and anteiso acids (*10*). Two of the five ciliated protozoa studied by Erwin and Bloch (*11*) contained significant amounts of iso acids (23 and 12 percent of the total acids); both bacteria and protozoa live in the upper sediment, deriving food from organic detritus and perhaps producing enough branched-chain acids to account for the enrichment observed.

The branched-chain fatty acids promise to be an interesting group of compounds for organic geochemists. They are present in at least one ancient sediment, the Green River shale (Table 1). It remains to be seen whether they will be found in the variety of ancient sediments which contain straight-chain acids (*1*). The Ponca City crude oil contained 1.8 percent of *n*-decane, 0.3 percent of 2-methylnonane, 0.1 percent of 3-methylnonane, 0.1 percent of 4-methylnonane, and no 5-methylnonane (*12*). The relationship of the iso paraffin being the second most abundant isomer holds for the nonanes and octanes; unfortunately data is not available for the higher paraffins. This correspondence between the methyl branched-chain fatty acids and paraffins suggests either a genetic relationship or simply that organisms make paraffin skeletons much as they make fatty acid skeletons.

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Prolonged Immunosuppression and Tumor Induction by a Chemical Carcinogen Injected at Birth

Abstract. *The injection of 60 micrograms of 9,10-dimethyl-1,2-benzanthracene into newborn mice gave rise to a very high incidence of malignant thymomas. The tumor incidence was directly related to the dose of the carcinogen. The neonatal injection of the carcinogen also resulted in a depression in the immune response when the animals were challenged with an antigen as early as 4 weeks or as late as 11 weeks after administration of the carcinogen.*

Neonatal injection of chemical carcinogens into mice induces a variety of tumors (*1, 2*). The strain of mouse used in our experiments (*3*) was extremely sensitive to the induction of malignant lymphomas when the animals were injected with 9,10-dimethyl-1,2-benzanthracene (DMBA) at birth. The number of lymphomas induced was related to the dose of carcinogen. In addition, the neonatal injection of DMBA resulted in a prolonged depression of the immunological response.

A colloidal suspension of DMBA prepared according to the method of Pietra *et al.* (*1*) was injected subcutaneously in the intrascapular region of mice that were less than 24 hours old. Care was taken to prevent leakage of the carcinogen at the injection site. One-half of the mice of each litter were injected with carcinogen and the other half received an equal volume of 1-percent aqueous gelatin. Both treated and control mice were kept together with their mothers for 1 month, at which time they were weaned, separated according to sex, and thenceforth observed for the appearance of tumors.

Our results show that the number of lymphomas induced is related to the dose of carcinogen. Of 120 mice injected with 60 μg DMBA, 90 percent developed lymphomas, whereas the tu-