A method for analysis of fatty acids in coral

Abstract—A method using gas-liquid chromatography was developed to analyze the total fatty acid composition of animals having carbonate skeletons and used to determine the fatty acids in a scleractinian coral and a milleporine coral. The major acid in both samples is palmitic acid, and marine-type polyunsaturated fatty acids were not detectable in either type of coral.

We have developed a method to measure the distribution of total fatty acids in invertebrates having skeletons composed of calcium carbonate. Our procedure uses gas-liquid chromatography (GLC), thus allowing high resolution of small amounts of fatty acids, and can easily be adapted to other types of organic compounds that can be analyzed by this technique. The results of our analysis of members of two classes of cnidarians are reported here.

We thank H. P. Jeffries and J. W. Porter for reviewing this manuscript and offering many helpful comments.

The whole animal samples were outer branch pieces from *Acropora palmata*, a true coral (class Anthozoa), and *Millepora* sp., a fire coral (class Hydrozoa), both taken from the reef bordering the entrance to the lagoon at South Caicos Island in the Caribbean Sea. Samples were refrigerated at 4°C or frozen at –20°C from the time of collection until analysis in the laboratory. The samples were collected on 10 June 1970 and arrived at the laboratory in Rhode Island 1 week later. Analysis began on 23 June 1970.

Portions of the frozen samples were crushed, weighed, and placed under 3 N hydrochloric acid at room temperature for 18 hr to dissolve the carbonate matrix. A 20-g and a 1-g sample of each coral was used. Moisture content of the finely divided, crushed samples was determined by drying small subsamples to constant weight at 105°C. Agitation and shaking of the acidic mixtures were avoided to minimize foaming and possible loss of sample. Methanolic KOH (2 N) was added to the carbonate solutions to give basic solutions (pH > 10), and then the samples were refluxed for 1 hr to hydrolyze combined fatty acids. The fatty acids and other lipids freed by acidifying the solutions to pH 2 with 3 N HCl were extracted into chloroform (Quinn and Meyers 1971). These organic extracts were filtered through Whatman No. 1 filter paper to remove traces of water and evaporated to dryness under vacuum on a rotary evaporator at 40°C. The residues were transferred to Teflon-lined screwcap tubes with 1 ml of methanol and 1 ml of benzene. Fatty acids were methylated by adding 2 ml of boron trifluoride-methanol, flushing the tubes with nitrogen, sealing, and heating at 100°C for 5 min. The methyl esters and other lipids were isolated after adding distilled water by extracting with petroleum ether.

The methyl esters were separated from other classes of lipids in the extract by preparative thin layer chromatography (TLC). The petroleum ether extract was evaporated under vacuum and the residue dissolved in a small volume of chloroform and applied to a predeveloped (chloroform: methanol, 1:1) 0.5 mm thick Merck silica gel G plate (20 × 20 cm). After development in petroleum ether:ethyl ether: acetic acid (90:10:1), the methyl ester band was visualized by brief exposure to iodine vapors, scraped from the plate, and the esters extracted from the silica gel with chloroform:methanol (9:1). It should be noted that while brief exposure to iodine vapors does not measurably alter hydrocarbons (Farrington et al. 1973*b*) or fatty acids (our experiments), long exposure can result in appreciable losses of polyunsaturated acids (Nichaman et al. 1963). The solvents were removed under reduced pressure, a small amount of carbon disulfide added, and the methyl esters analyzed by GLC by procedures described elsewhere (e.g. Meyers and Quinn 1971).

Table 1. Fatty acid weight percent composition of coral. A—20-g sample of whole coral from South Caicos; B—1-g sample of whole coral from South Caicos; C—coral tissue from Eniwetok.

	Acropora sp.			*Millepora* sp.		
Fatty Acid*	A	B	C	A	B	C
myristic (14:0)	7.2	6.9	9.6	3.7	2.6	3.8
(15)+	2.8	3.3	--	--	--	--
palmitic (16:0)	57.5	57.2	60.9	46.3	34.1	43.8
palmitoleic (16:1)	4.3	--	--	--	--	--
stearic (18:0)	15.3	16.4	20.4	32.8	40.5	38.3
oleic (18:1)	9.7	12.3	4.3	6.1	10.3	--
linolenic (18:3)	3.2	3.9	--	--	--	--
arachidic (20:0)	--	--	4.8	11.1	12.5	14.1
total concn (mg g^{-1})	0.60	--	--	3.24	--	--

*fatty acids designated by carbon chain length: number of double bonds.
+unresolved mixture of normal, iso, and anteiso 15 plus 14:1 fatty acids.

The components in the samples were qualitatively identified by comparing their relative retention times on polar and nonpolar columns to those of standards and by coinjecting samples of authentic methyl esters. The amount of each component present was determined by comparison of its peak area to that of an internal standard added to the sample container before dissolution of the carbonate skeleton. The internal standard (heptadecanoic acid, 17:0) was chosen after a preliminary analysis indicated that this odd-chain fatty acid was below detectable levels in both cnidarian samples.

All chemicals used in this study were A.C.S. reagent grade, and the organic solvents were distilled before use in an all-glass still fitted with a 30-cm Widmer column. The purity of the internal standard was >99% by TLC and GLC. A blank done on the entire procedure showed negligible quantities of fatty acids.

The composition and total concentration of major fatty acids in the whole animal samples are reported in Table 1. The 20-g and 1-g samples of each coral correlate well. Most of the acids in both samples are saturated, with palmitic acid (16:0) the predominant component and lesser amounts of myristic (14:0) and stearic (18:0) acids. The predominance of palmitic acid in these Cnidarian samples agrees with the high concentrations of the palmitic acid ester cetyl palmitate found in corals by Lester and Bergmann (1941), Pasby (1965), and Young et al. (1971). The *Millepora* species contains 11.1% arachidic acid (20:0), which is absent from *A. palmata.* Three unsaturated acids account for about 17% by weight of the total fatty acids of this true coral, while the fire coral contains 6.1% of oleic acid (18:1), the only unsaturated acid detected in this sample. Total fatty acids in *Millepora* sp. (3.24 mg g^{-1}) are five times those of *A. palmata* (0.60 mg g^{-1}). This difference is reasonable, because living tissue in hydrozoan corals is distributed through much of the skeletal mass whereas in anthozoan corals it is limited to the outer surface of the skeleton. Therefore, more *Millepora* sp. tissue than *A. palmata* tissue was probably present in a given weight of whole animal sample.

Also shown in Table 1 is the fatty acid composition of coral tissue from samples collected on Eniwetok Atoll on 19 June 1971. The material arrived in Rhode Island on 29 June. The tissue had been separated from the coral skeleton by the Water Pik method of Johannes and Wiebe (1970). The fatty acid composition was determined on 15 August 1971 by established extraction and chromatographic procedures (Farrington et al. 1973*a*). For the *Millepora* sp.,

the individual fatty acids in the tissue sample and whole coral are in fairly good agreement, with the exception of the oleic acid values. The corresponding samples of *Acropora* sp. show somewhat greater differences in fatty acid composition but are similar. In both the whole coral and tissue samples, the ratios of palmitic to stearic acid contributions are significantly greater in the *Acropora* than in the *Millepora*. This was also found by Pasby (1965) and may represent an important distinction between anthozoan and hydrozoan corals.

Although a small amount of linolenic acid (18:3) was found in the whole *A. palmata* sample, the longer chain polyunsaturated acids typical of marine animals were not detected in any of the corals we examined. We believe that this indicates their absence from the tissues of these animals, since the analytical method is capable of identifying these acids in organisms (Farrington et al. 1973*a*). Furthermore, analysis of polyunsaturated fatty acid mixture No. 1 (Supelco, Inc., Bellefonte, Pa.) by the whole coral method showed no destruction or loss of marine-type polyunsaturated fatty acids. However, it is quite possible that these acids comprise such a small amount of the total acids that they cannot be detected by our procedure. Polyunsaturated acids were detected in a gorgonian (class Anthozoa) by Hooper and Ackman (1972), although in very low amounts. They suggested that these low levels may indicate some oxidation and loss of the acids between collection and analysis or that the relatively high environmental temperatures (20–30°C) of these organisms could reduce their need for polyunsaturated fatty acids.

We are expanding our analyses to other corals and plan to examine classes of lipids in an attempt to identify polyunsaturation. Meanwhile, it is evident that even though biochemical as well as morphological distinctions exist between these classes of corals, the Atlantic and Pacific samples of each genus are similar despite their different geographical sources.

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Submitted: 9 November 1973
Accepted: 11 March 1974