The cells of the sponge *Tethya aurantia* var. *californiana* were separated on a Ficoll density gradient and the fractions analyzed for cell types and their lipids. Major cell types were choanocyte, archeocyte, and symbiont. Major differences in archeocyte and choanocyte fatty acid composition were noted for 20:4, 26:1 and 26:2: The fatty acids 26:1, 26:2, and 28:3 were dominant in the phosphatidylcholine fraction. Archeocytes had highest concentrations of 4,7,10,13-20:4 and 5,8,11,14-20:4 (arachidonic) acids which could be derived from symbionts, as odd-chain and methyl-branched fatty acid were also present. Sterol which could be derived from symbionts, as odd-chain and 4,8,12-trimethyltridecanoic acid.

Dissociated sponge cells are known to retain the potential for mitogenesis, proliferation and differentiation (1–3). Muller et al. (4) recently demonstrated that when a sponge's aggregation factor inserts into its membrane receptor, a series of biochemical events are initiated, which include an increase in extracellular calcium and increased turnover of phosphoinositides. Weissman et al. (5) further established the synergistic formation of diacylglycerol and presented experimental evidence for its role as a second messenger and an activator of protein kinase C, an event that may ultimately lead to DNA replication. Both studies draw analogy to intercellular signaling events in higher animals (as reviewed by Berridge, (6)).

A sponge is a collection of cells of two fundamental types. First, the mobile ameboid cells of the mesohyl, the archeocytes and cells of this type with apparent special functions (i.e., spongocytes), are characterized by an “embryonic” nature that allows them either to differentiate into other cell types or to temporarily take on specialized functions (7). These relatively large cells are characterized by a large nucleus in a rounded, finely granulated nucleus, a well developed Golgi apparatus, and rough endoplasmic reticulum, and large intercellular spaces (8) resembling gap junctions (between 300–1000 Å) which are believed to be important for cells to be electrically coupled and for the passage of small molecules (9,10). Second, the fixed cells of the surfaces, the choanocytes and pinacytes, are terminally differentiated and are collectively characterized by a smaller nucleus (a choanocyte nucleus, which may be haploid, is about 60% that of an archeocyte) with no nucleus, a greatly reduced number or no mitochondria or Golgi membranes, close intercellular association, and a relatively small size (a choanocyte is usually half the size of an archeocyte) (8). Pinocytes are not adapted for active transport since the deficiency in mitochondria and their thinness make them unsuited for actively maintaining a gradient between the external medium and the mesenchyme. The scarcity of tight junctions makes them ineffective in providing a barrier to ions and water (11). The apparent lack of a nucleolus and a poorly organized rough endoplasmic reticulum suggest that there is less protein synthesis. Although Golgi bodies can be found in choanocytes, glycogen production is greatly reduced (8). In a first approximation, more than 90% of the cells of the sponge can be seen as being of two types (12)—mobile cells of the mesohyl and fixed cells of the surface.

Our first report (13) on lipid differences between cell type was concerned with a Great Barrier Reef sponge *Pseudaxinyssa* sp. This sponge contains long-chain fatty acids and a very unique sterol composition (99%) with two triple alkylated 24-isopropyl sterols. In spite of heavy infestation with cyanobacterial symbionts, these sterols were strongly suspected to be from sponge cell membranes. An asymmetrical distribution of fatty acids and sterols was observed: small, surface-fixed cells (ca. 4×2 μm) contained larger quantities of very long-chain fatty acids (>24 carbons) and smaller quantities of sterols than were present in the large mobile sponge cells. We considered it important to examine in detail a second sponge with “conventional” sterols, but unusual fatty acids. For this purpose, we selected a local sponge, common to the California coast, *Tethya aurantia*, which has an unusually high content of small cells (choanocytes) and long-chain fatty acids, but a sterol content that is common among sponges of the Monterey Bay (USA) and whose symbionts appear to be mainly bacterial and fungal (or red algal).

**EXPERIMENTAL PROCEDURES**

*Sponge sample.* *Tethya aurantia* var. *californiana* ranges in color from orange to yellow and is commonly found on rocky outcrops, pinnacles, and under ledges of the Monterey Bay. Some sponges are known to display seasonal variations in lipid content, as has been shown for *Microciona prolifera* (14). The present cell fractionation was done on sponges collected in the fall of 1988.

*Chemicals and materials.* The protease enzyme (no. P-2143) and Ficoll (type 400) were purchased from Sigma Chemical Co. (St. Louis, MO). The calcium-magnesium-free artificial sea water (CMF-ASW) was prepared from doubly distilled water and contained 27 g/L NaCl, 1 g/L Na₂SO₄, 0.8 g/L KCl and 0.18 g/L NaHCO₃.
**Electron microscopy.** Aliquots of sponge cell fractions fixed in 3% glutaraldehyde and Millonig's phosphate buffer (15) were washed (three times) with 0.1 M phosphate buffer for 10 min. Samples were then postfixed in 1% OsO₄ and 0.1 M phosphate buffer and stained after washing (distilled water) with 1% uranyl acetate. After ethanol dehydration, the samples were embedded in VCD-HXSA resin (16). Sections were stained with Reynolds (17) lead citrate and examined with a Philips 410 electron microscope (Philips, Cincinnati, OH) at 60 or 80 KV.

**Ficoll gradients.** Two types of Ficoll gradients (Fig. 1) were prepared for fractionations by layering the designated (w/w, listed below) concentrations using CMF-ASW. Type I was prepared by layering 5 mL aliquots of 23, 20, 17, 13, 10, and 5%. Type II contained 35, 30, 26, and 23% Ficoll solutions, respectively. The Ficoll gradients were prepared prior to use and stored at 0°C.

**Dissociation of sponge cells.** This was done according to Thompson et al. (18) with the following modifications: The wet sponge (50 g) was washed four times in CMF-ASW, cut into cubes 0.5 cm on a side, soaked in a bath of 500 mL of CMF-ASW, to which 126 mg of protease had been added, and was aerated at room temperature for 2 hr. The resulting cloudy mixture was then filtered through nylon mesh (30 μ) and centrifuged at 650 × g (International Equipment Co., Boston, MA, model HR-1) for 10 min to produce an orange-red sponge pellet. The pellet was suspended in 10 mL CMF-ASW and applied to the Ficoll gradients as described.

**Separation of cells.** Ten mL of the resuspended pellet was added to the top of a type I Ficoll gradient (Fig. 1) and the system was spun 5 min at 23°C and 650 × g in a swing bucket centrifuge. The cells were collected separately in each Ficoll layer and at each interface. The material from the lightest four fractions was combined, washed free of Ficoll in CMF-ASW (50 mL × 2) and centrifuged (650 × g/10 min). The supernatant was discarded and the cells were added to the top of another Ficoll type I gradient. The cellular material between the different Ficoll concentrations was collected, with the interface of each layer collected as a separate fraction and labeled as the T fraction of the more concentrated layer. All other fraction numbers are the Ficoll concentration from which the cells were collected, as summarized in Figure 1. Fractions derived from Ficoll concentrations 17–23% were obtained from the original gradient. The pellet from the original gradient was reapplied to the top of another Ficoll gradient of heavier concentrations (Type II) and the system was centrifuged as before. After this procedure was run twice, the following combined fraction weights were realized: Zero (0) fraction (31.5 mg), T5 (8.7 mg), 5 (8.6 mg), T10 (6.1 mg), 10 (16.4 mg), T13 (21.7 mg), 13 (33.2 mg), T17 (20.8 mg), 17 (16.1 mg), T20 (36.0 mg), 20 (31.5 mg), T23 (10.8 mg), 23 (32.3 mg), T26 (8.3 mg), 26 (16.5 mg), T30 (12.4 mg), 30 (35.4 mg), T35 (36.0 mg), 35 (36.0 mg), pellet (0.9 mg).

**Lipid analysis of cell fractions.** The cell fractions were separately extracted with CHCl₃/CH₃OH (1:1, v/v). The soluble material was separated from other cellular debris by filtration through a small plug of glass wool and sand. The extract was dried, 10 mL of 1.5 N HCl (CH₃OH) added, and the mixture heated under reflux for 30 min. The solvents and reagents were removed azeotropically with 10 mL toluene. The resulting esterified material was dissolved in 5 mL hexane/diethyl ether (2:1, v/v), and passed through a florisil column (0.5 g) using (5 × 1 mL) hexane/diethyl ether (2:1, v/v) as solvent. After evaporation of the solvent (N₂), the resulting fatty acid methyl esters and sterols were collectively analyzed by capillary gas chromatography (GC) using a Hewlett Packard 5790A series gas chromatograph equipped with a 25 m (0.3 mm i.d.) SE-54 coated fused silica column programmed between 170–320°C at 5°C/min (injector, 250°C; detector, 300°C; automatic injector system, Hewlett Packard sampler model 7672A; injector, model 3392A, sampler/event control module, Hewlett Packard model 19405A).

Fatty acid methyl esters (C₉–C₂₀) were identified based on the Hewlett Packard Peak Library, AEROBE, which can assign 119 fatty acid methyl esters common to microbes and sponges. Partially resolvable peaks are described as mixtures in Table 1. For the localization of double bonds or branching, N-acyl pyrrolidides were prepared and analyzed by GC/MS (19). For this purpose, fatty acid methyl esters were reacted with pyrrolidine/acetic acid (10:1, v/v) in a capped vial (1 hr, 100°C), folowed by extraction with diethyl ether from the acidified solution and purification by preparative TLC (Merck, Ltd., West Germany, aluminum backed sheets precoated with silica gel 60 F₂₅₄) using hexane/diethyl ether (1:4, v/v) as developing solvent. The samples were analyzed using a Ribermag GC-MS-DS system which combines a Ribermag R 10-10 quadrupole mass spectrometer with a Carlo-Erba series 4160 Fractovap.