Wax in coral mucus: Energy transfer from corals to reef fishes[1]

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Abstract

Mucus from a variety of reef corals has been found to contain wax ester (cetyl palmitate) and triglycerides. Observation revealed extensive mucus feeding by many species of reef fishes. When coral mucus is artificially dispersed fish assemble and avidly ingest it. Coral mucus could be an energy source linking the coral producer and small fish consumers in reef communities.

Reef-building corals are dominant and highly productive life forms in tropical coral reef communities. Their symbiotic dinoflagellate algae (zooxanthellae), which, for example, constitute from 45–60% of the protein biomass of *Pocillopora damicornis*, provide the coral animal with products of their photosynthesis, notably glycerol, alanine, and glucose (Muscatine and Cernichiari 1969; Lewis and Smith 1971). The animal, in turn, provides the algae with ammonia for production of amino acids and protein (Kawaguti 1953; Muscatine and D'Elia unpublished). This symbiotic exchange of reduced carbon and nitrogen occurs within the coral tissues, thereby minimizing dilution with the ocean and ensuring the survival of corals in an environment relatively poor in dissolved and particulate nitrogen compounds.

Since the zooxanthellae in corals are among the major primary producers in reef communities, questions arise as how and to what extent their energy-rich products are made available to other members of the reef community. Feeding on coral polyps by reef fishes has been reported as one type of predation on corals (Hiatt and Strasburg 1960). Perhaps more novel is the observation by Johannes (1967) of substantial mucus release by corals on windward reefs in Eniwetok and the subsequent formation of organic aggregates from this released mucus downstream in the lagoon. These aggregates were viewed as potential food sources for filter feeders and fishes. Coles and Strathmann (1973) reported elemental analyses of coral mucus flocs and discussed the enrichment of these flocs with extrinsic particulate material. They comment that (p. 677) "despite the potential food value of coral mucus, few animals are known to feed on it."

In this report, we describe the isolation and identification of wax ester and triglyceride in mucus exuded by reef corals and the feeding by coral reef fishes on this mucus. Our observations emphasize the general role of wax ester, along with other mucus constituents, in energy transfer in marine communities and support the contention of Johannes (1967) and Coles and Strathmann (1973) that coral mucus is an important food source for reef inhabitants.

Methods

Field observations were made on the fringing reefs of Lizard Island and nearby islands as well as on reefs adjacent to Cook's Passage, The Great Barrier Reef, Australia. Analytical procedures were carried out in the laboratories of the RV *Alpha Helix*. Mucus exuded by scleractinian (*Pectinia lactuca*, *Acropora* sp.) and alcyonacean (*Sarcophyton trocheliophorum*) corals and *Tridacna* mantles was initially collected underwater by wiping the tissues gently with pieces of clean gauze. This method was abandoned in favor of lesser

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yields of purer mucus obtained by directing a gentle jet of seawater at the coral tissues with a pipette and collecting bubble-laden mucus produced in response to this mechanical irritation. Mucus from about 200 kg of cracked *Acropora* sp. was obtained by draining the seawater and collecting about 1,100 ml of clear viscous mucus solution over a 4-hr period. Mucus samples were precipitated by addition of minimal amounts of ethanol and separated from the aqueous phase by centrifugation. The precipitate was separated into mucopolysaccharide and mucolipid fractions by treatment with two volumes of ethanol and one of chloroform. The suspension of insoluble mucopolysaccharide was removed from the organic solvent by filtration. Addition of water to the filtrate produced a separate chloroform phase. It was washed twice with water and dried, when necessary, with anhydrous sodium sulfate. Evaporation of the chloroform under a stream of nitrogen freed the mucolipid as a residual yellow oil. Separation of mucus from dilute suspensions in seawater was simplified by addition of diatomaceous earth, (Celite, 2 g liter^{-1}). In 3 hr the suspension of Celite and absorbed mucus had settled and the water could be decanted. Mucolipids were readily obtained by chloroform-ethanol extraction of the Celite, filtration, and procedures as above. The mucolipid was fractionated into wax and sterol esters and triglyceride by thin layer chromatography on silica gel G plates using a solvent system of petroleum ether : ethyl ether, 10 : 1, and visualizing the fractions by treatment with iodine vapor or by spraying with 40% sulfuric acid and heating. Because wax esters and sterol esters cochromatograph on silica gel, it was necessary to separate them by passing a solution of the mucolipids in petroleum ether : ethyl ether 10 : 1 through a column of Florisil. The effluent solution was evaporated to dryness under nitrogen to yield a colorless oil, suitable for gas chromatographic analysis on a column (JXR Supelco, Inc., Bellefonte, Pa.) programed at 300–350°C. This proved to be pure wax ester.

^{14}C-labeled mucus was prepared by illuminating a small frond of "lettuce coral" (*Pectinia lactuca*), known for its copious mucus production, in a minimal volume of seawater, freed of carbonate by acidification to pH 4 with dilute HCl, flushed with N_2, adjusted to pH 7.8 with NaOH, and containing up to 10 μCi ml^{-1} ^{14}C-$NaHCO_3$. Periodically, mucus was allowed to drip from the exposed, inverted head, collected with a pipette, and fractionated as above. This technique takes advantage of the translocation of photosynthetic products of zooxanthellae to host tissues. In this case the products were ^{14}C-labeled and utilized by the host in its own anabolic processes, including the synthesis of mucus (Smith et al. 1969). Continuous "milking" of the coral resulted in the synthesis of new, labeled mucus of relatively high specific activity (cf. Trench et al. 1972).

Observations and results

Labeled mucus

A small (6 cm^2) specimen of *Pectinia lactuca* was illuminated in blue light at 25°C in ^{14}C-labeled seawater which had been acidified, aerated, neutralized to pH 7.8, and labeled by addition of 2 mCi ^{14}C-$NaHCO_3$. After a 32-hr incubation, mucus release was stimulated with a fine water jet and allowed to settle to the bottom of the beaker. It was collected with Celite and separated into mucolipid and mucopolysaccharide fractions. The ^{14}C in each was determined by liquid scintillation counting. The mucolipid fraction contained 840,000 cpm and the mucopolysaccharide contained 1,080,000 cpm. The ratio of labeled mucolipid to labeled mucopolysaccharide produced was 0.75.

Bulk isolation of mucus components

A colony of the soft coral *Sarcophyton trocheliophorum*, 25-cm diameter, was suspended in air and 35 ml of mucus was collected in 6 hr. The mucus was treated as described in methods and yielded 14 mg of brownish oil (mucolipid) and 85 mg of white powder (insoluble mucopolysaccharide), giving a weight ratio of 0.16.

Table 1. Percent incorporation of ^{14}C-CO_2 by Pectinia lactuca *in mucolipid components by thin layer chromatography.*

	Photosynthesis period (hrs)				
Mucolipid	16	20	24	36	48
Wax ester	8.3	9.5	9.9	14	16
Triglyceride	31	50	35	23	34
Sterol	20	20	19	10	7.2
Phospholipid	40	21	35	52	43
Wax ester/ Triglyceride	.26	.19	.28	.60	.47

Mucus produced from cracked *Acropora* contained the least mucolipid with a mucolipid : mucopolysaccharide ratio of 0.10. This mucus was evolved from the freshly broken surfaces of the perforate skeleton rather than strictly from its external surface. The relatively low lipid content of this mucus revealed its difference from that produced during exudation from the surface of intact corals.

Relative rates of formation of wax ester and triglyceride in *Pectinia lactuca* mucolipid

Kinetic analysis of mucolipid labeling by *P. lactuca*, incubated as above, showed that the triglyceride portion was earliest labeled, the ratio of wax ester to triglyceride activity increasing from about 0.2 in 16 hr to about 0.5 after 48 hr (Table 1). Approximate relative rates of labeling of wax ester, triglyceride, sterol, and phospholipids are given by the figures in Table 1. Since the mucus samples collected were not necessarily from the same areas of the coral one cannot expect complete numerical continuity in the data. However, it appears that the wax ester fraction increases while the sterol components decrease with time. These figures were obtained from the thin layer chromatograms of the labeled mucolipids by counting ^{14}C in the radioactive areas on the TLC plates defined by radiograms prepared with Eastman Kodak Co. single-coated blue-sensitive X-ray film. A large diameter G.-M. counter was used for this measurement. The phospholipid fraction remained at the origin (Fig. 1) in the solvent used for wax and triglyceride resolution.

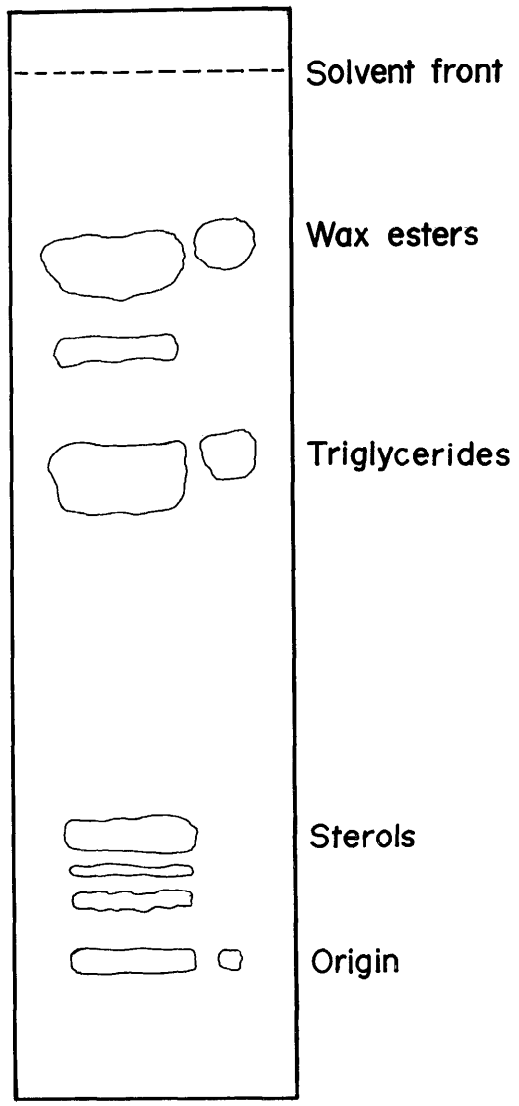

Fig. 1. TLC separation of mucolipid components (*Pectinia lactuca*) on silica gel G. Solvent: petroleum ether : diethyl ether, 10 : 1. Sample at right was a mixture of triglycerides and long chain wax esters. Phospholipids remain at the origin in this system. Unidentified spot between wax ester and triglyceride is probably glycerol ether, a fatty alcohol ether of a 1,2-diglyceride.

Isolation of cetyl palmitate from coral

A 12-cm-diameter *Goniastrea retiformis* (Lamk.) coral was extracted with ethanol : chloroform, 1 : 1, during 24 hr at room temperature. The lipids were obtained in the

chloroform phase by addition of water. Upon evaporation of the dark chloroform solution the residue crystallized nearly completely. The product (0.83 g) was removed by filtration and recrystallized from ethanol to yield white crystals. They were identified as cetyl palmitate by thin layer chromatography on silica gel G plates. Unequivocal identification as cetyl palmitate was achieved by mass spectrometric analysis (kindly performed by W. H. Fenical).

The 480 molecular weight component, cetyl palmitate, was clearly predominant. The entire mass spectrum was identical with that of a sample of authentic cetyl palmitate. Small amounts of C_{30} (5%) and C_{34} (1%) wax esters were revealed by high temperature gas chromatography (kindly performed by J. C. Nevenzel). Taking into account the possible presence of triglycerides, glycerol ethers, sterol esters, and hydrocarbons, we estimate that cetyl palmitate comprised over 80% of the lipid extract.

Fishes feeding on mucus

Many species of fish were observed feeding on many types of coral (both scleractinian and alcyonacean). In accord with the taxonomic information provided by Hiatt and Strasburg (1960), we recognized members of the families Chaetodontidae (butterfly fishes), Monacanthidae (file fishes), and Pomacentridae (damsel fishes), among others. We observed small blue fishes (*Chromis caeruleus*) and yellow damsel fish (*Pomacentrus sufflavus*) feeding on *Pocillopora* sp. by gently pecking at its surfaces. The large brain corals (*Platygyra lamellina*) were most regularly sought by the butterfly fishes (including *Chaetodon vagabundus*), having a pronounced snout, which seemed to feed consistently at the clefts between skeletal structures where the mucus was accumulating. Although we saw no evidence of polyp feeding or damage to delicate coral structures this possibility is not excluded.

During the course of investigations on corals we routinely made collections by dislodging small heads underwater and bringing them to the surface in a bucket. The result of this general disturbance (mechanical irritation, turbulence, etc.) was to cause these corals and those in the immediate vicinity to exude and slough off flocs of mucus. We observed fishes ingesting these flocculent mucus clusters as they rose from the corals and floated off in the prevailing currents. When a cloud of mucus was released by stroking certain alcyonacean corals with a glove, large groups of small fish would immediately appear and avidly ingest the mucus until it was gone. We observed fishes feeding on mucus similarly produced by *Tridacna* mantles.

Discussion

Cetyl palmitate, a well known marine wax ester, has been previously identified as a major component of the lipids of coral tissue and coral skeletal matrix (Lester and Bergmann 1941; Young et al. 1971). The ^{14}C-labeling experiments reported here reveal the effect of the massive pool of cetyl palmitate within the coral on its labeling pattern and the kinetics of labeling of this compound in exuded mucus. Since the pool sizes of triglyceride and membrane-associated phospholipids and sterols are relatively small they are most rapidly labeled with ^{14}C. They are only slowly exceeded by the labeling of the wax ester in the coral-mucolipid system.

The importance of lipids in mucus of marine animals was pointed out by Lewis (1970) who found high concentrations of cholesterol and phospholipids in fish skin mucus. We now recognize that wax ester and triglyceride are secreted as major lipid components of the mucus exuded by corals. Further, it is clear that this mucus is energy-rich by virtue of its wax and triglyceride content. We have shown that wax ester is hydrolyzed and digested by fishes. The digestibility of coral mucopolysaccharide, however, has not been established. Although wax ester is a main medium for energy storage in many copepods of polar and temperate waters, cope-

pod species of tropical waters store very little wax (Lee and Hirota 1973).

It has been reported that small reef fishes feed on coral polyps directly (Hiatt and Strasburg 1960). We suspect that this activity by butterfly fishes (Chaetodontidae) and others includes the process of nibbling at masses of coral mucus. It is possible that some fishes are primarily mucus feeders while others take polyps directly. The cleaning activities of the barberfish *Heniochus nigrirostris* (Gill), observed by Hobson (1968), may be related to coral mucus feeding by other Chaetodontidae populations. Johannes (1967) observed coral mucus release in situ in Eniwetok, Marshall Islands, and Puna, Hawaii, and *Spratelloides delicatulus* and *Chromis* sp. feeding on mucus aggregates. We can confirm the observation that some fishes feed on coral mucus, both at the coral surface and on dispersed mucus flocs. It is entirely possible that a major source of energy for fishes like the cleaning wrasses and for surface commensal "parasites" may be the lipids of skin and gill mucus exuded by fishes. Wickler (1960) and Hobson (1968) have reported observations of this activity by blennioid fishes. The relative invisibility and structureless nature of mucus may have precluded its recognition as an important medium for energy exchange in these systems.

Ingestion of coral mucus by reef fishes indicates one route by which the energy-rich products of coral metabolism may be transferred to the reef fish population. Although its actual energy content and the extent to which coral mucus represents coral productivity are not yet clearly established, the process provides a novel pathway for utilization of a major product of coral metabolism. In this process, wax ester becomes an energy-rich link in the coral reef food chain. From a metabolic standpoint, tropical reef community energetics may resemble those in colder waters, where wax ester provides many small fishes with an important fraction of their energy requirement.

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