ENERGY EXCHANGE IN CORAL REEF ECOSYSTEMS

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INTRODUCTION

Marine productivity begins with photosynthesis of metabolic intermediates and storage of energy-rich compounds. Of these, the lipids possess the most energy on a weight basis, they provide the greatest buoyancy on a volume basis, and they provide for the energy needs of the organism over a long time basis.

The major energy content of photosynthetic diatoms or dinoflagellates lies in the amphipathic lipids of their chloroplast lamellar membranes. The electron microscope reveals membranes as double dark lines in osmium-stained sections. These are actually "energy pictures" of the cell because it is the lipids of membranes which stain most effectively. The chloroplasts of algae, then, produce and contain their most valuable energy stores. In many cases the blue light environment of the sea engenders chloroplast replication (Vesk and Jeffrey, 1974) and thereby accumulation of the polyunsaturated galactolipids of their lamellar membranes. Lipid production and utilization are dominant in the metabolism of both marine plants and animals.

The density of lamellar lipoprotein is over 1.20, and were it not for a regulatory relationship in algae, one might expect such dense organisms to sink into darkness. Nakamura and Yamada (1975) have observed that the light intensity required by algae for lipid synthesis from acetate is lower than that required for photosynthesis with carbon dioxide. This relationship must provide the avenue for phytoplankton to return themselves to light levels supporting further CO_2 fixation. Synthesis of fatty acids leads to triglyceride accumulation as oil globules with densities of 0.89, whereas the acetate is derived from the cell's metabolites and structures having densities up to 1.23. Condensation of acetate molecules to produce fatty acids utilizes ATP and NADPH produced in the light by the photosynthetic apparatus. The fact that optimum light intensity for fatty acid synthesis by condensation of acetate was lower than that for CO_2 fixation indicates that there may be limiting factors for CO_2 fixation different from those for fatty acid synthesis. Nature appears to have designed a mechanism for protection of her photosynthetic organisms from starvation and demise in the depths of the sea. The non-photosynthetic organisms of the depths, too, maintain well-developed reserves of lipid which insure survival in a world where their probabilities as predators are low.

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WAX PRODUCTION IN COLD WATERS

Energy reserves of cold water animals include two major lipid types. Both have relatively the same energy contents, densities, and viscosities, but they differ strikingly in their availability to the metabolic systems. These are the triglycerides (fats) and the fatty acid esters of long chain alcohols (waxes) (Benson and Lee, 1975). By selecting two substances for energy storage, animals which may have to survive long periods of starvation have an important advantage. They can more carefully regulate their fuel supply with a rapid (fat) metabolism and a slow (wax) metabolism. Throughout Nature there appear examples of two-level regulation (insulin-glucagon somatostatine calcitonin-parathormone, glycogen (amylopectin) - amylose, etc.). Copepods of cold waters accumulate both wax ester and triglyceride which provide them with the potential for regulation of fuel consumption (Lee et al., 1971), necessary for survival in polar or abyssal waters. The lipid content (64% dry wt basis) of the eggs of the copepod Euchaeta japonica for example, (Lee et al., 1974) includes 58% of wax ester. This provides the naupliar stages 1 to 6 and the copepodite I with almost all of their energy supply.

The polyunsaturated fatty acids of phytoplankton lamellar lipids are converted by copepods to triglycerides and to wax esters. Up to 70% of the animal's dry weight may be wax ester in the case of Calanus plumchrus of British Columbia (Lee, 1974b) and C. hyperboreus of the Arctic Ocean (Lee, 1974a, 1975a); these animals must survive nearly ten months and considerable activity without further food. Although the mechanisms for synthesis and storage of wax are not yet clearly delineated, the work of Holtz et al., (1973); Sargent and Lee (1975); Gatten and Sargent (1973); Sargent et al., (1973); Sargent and McIntosh (1974); Sargent, Lee, and Nevenzel (in press) demonstrated that wax esters are synthesized de novo in copepods and other marine animals which accumulate wax ester. These are not passed through food chains (Lee and Puppione, 1972) but are hydrolyzed and oxidized directly or utilized for construction of new lipid molecules by metabolic processes in each predator. Usually the polyunsaturated fatty acid moieties are conserved by the predator and, as in the case of Pacific Salmon, incorporated into phospholipids of essential membrane systems such as that of their erythrocytes. The fatty alcohol, being more saturated, is less essential and more likely to be oxidized to the corresponding fatty acid and then further degraded by metabolic transport and oxidative processes.

WAX STRUCTURE AND BIOCHEMISTRY

The term "wax" has long been used to describe substances with properties of bee or plant waxes. They are chemically very different from "paraffin wax" which possesses some similar properties. Natural waxes are esters comprised of long chain fatty alcohols esterified by a long chain fatty acid (Nevenzel, 1970). The simplest and most

widespread wax ester is cetyl palmitate so named because of early recognition of cetyl alcohols in cetacean oils and of palmitic acid from palm oils. Both are saturated C_{16} compounds.

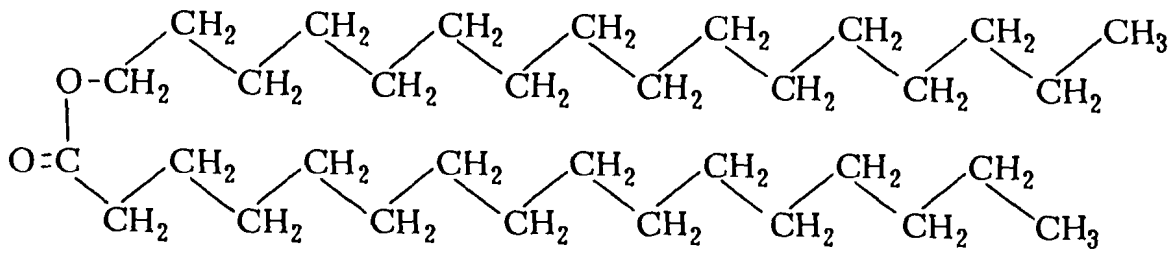
Cetyl palmitate (Figure 1) (melting point, 50-50.5°C) differs from the polyunsaturated wax esters of copepods which may have up to ten double bonds and therefore are liquids near zero degrees in marine organisms.

Storage fats on the other hand (Figure 2), are similarly liquid or solid but are triesters of glycerol instead of a single long chain alcohol.

It can be seen that the linkages of both type of esters are similar but that some physical properties of the two will be dramatically different. Densities of the two are very nearly the same. Accessibility of the hydrophilic ester groups, each with two oxygen atoms, to enzymatic attack will be very different. The wax has an oxygen atoms to fatty chain ratio of 2:2 while the triglycerides have a corresponding raio of 6:3, a difference of 300%! The surface properties of the two reflect this difference, especially when the oils are immersed in water which can interact only with their oxygen atoms by hydrogen bonding. The six oxygens of the triglyceride have a far greater tendency to be exposed to the aqueous phase than the two oxygen atoms of the wax ester. It is not surprising that lipase enzymes act ten times faster on triglycerides than they can on the same types of bonds in wax esters. Utilization of wax in marine organisms requires adaptations to facilitate attack at the relatively hydrophobic surface of the wax. It is such properties of wax esters which contribute to their unique lubricant and surface adhesive properties long appreciated by watchmakers and cosmetics formulators.

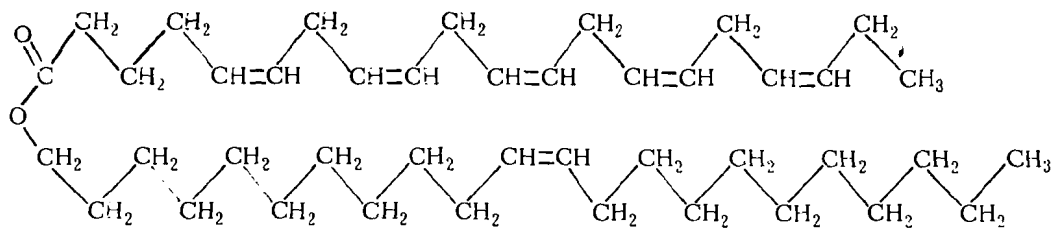
TROPICAL WATERS

Wax and triglyceride accumulation in copepods of tropical waters (Lee and Hirota, 1973; Lee *et al.*, 1971) revealed their difference from related zooplankton of arctic and temperate seas and the colder mesopelagic and bathypelagic zones in the lower latitudes. Wax accumulation was related to the animal's need for fuel during long starvation periods, whether they be the result of unproductive seasonal periods or of the low probabilities of finding food in the cold depths. Zooplankton in subtropical and tropical regions have a more constant and reliable source of food. Although the standing stock of food is known to be low, the supply is constant and the grazers need store less energy than those in the high latitudes. Epipelagic (upper 250 m) copepods accumulated 3-37% (median,14%) of lipid containing a trace of wax ester, while deep-living species accumulated 18-68% (median, 29%) lipid containing 11-72% (median, 63%) wax ester. Most epipelagic tropical copepods, with the exception of *Calanus*, *Euchaeta*, and *Eucalanus*, did not possess oil sacs for wax ester storage. Consistent with these conclusions was the

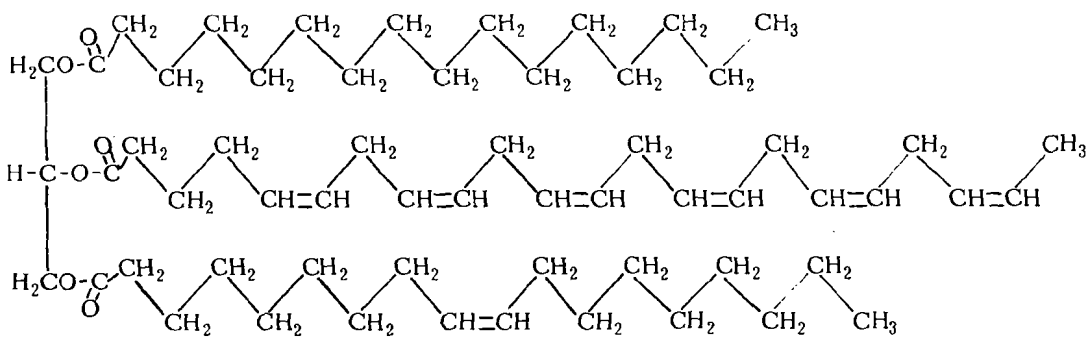


'Coral Wax' , Cetyl palmitate , C_{32}

Figure 1. Cetyl palmitate, coral wax ester.



WAX ESTER C_{42} 20:5 Acid, 22:1 Alcohol



TRIGLYCERIDE 16:0, 22:6, and 18:1 Fatty Acids.

Figure 2. Typical polyunsaturated wax ester and triglyceride structures found in the British Columbia copepod, Calanus plumchrus.

observation by Lee (1975b) that the parasitic copepods associated with marine fishes do not accumulate wax esters. Their constant food supply apparently makes wax storage unnecessary for such copepods.

Most tropical waters possess nutrient levels considered "unproductive," and although effective light levels may penetrate up to 500 meters, phytoplankton populations are severely limited by low prevailing levels of phosphorus and nitrogen compounds. Pilson's (1974) recognition of adaptations to the low phosphate levels (Johnson and Pilson, 1972) of tropic waters dramatically emphasized the obstacles to productivity. Sargasso sea waters having phosphate levels of the order of 10^{-8}M contained arsenate in levels of 10^{-6}M. Both are known to be transported through cell membranes by the same mechanisms, and arsenate is an effective inhibitor of photosynthetic carbon dioxide reduction and phosphorylation. Marine bacteria (Johnson, 1972) and corals (Pilson, 1974) have found effective solutions to the problem by evolution of processes for reduction of the arsenate to arsenite. In doing so, the corals may even have learned to produce phosphite from phosphate with which to synthesize their ubiquitous phosphoric acid analogs of the phospholipids (phosphate derivatives).

With ammonia and nitrate concentrations as low as 10^{-8}M and vanishingly low phosphate levels, it is no wonder that the principles of thermodynamics have guided the evolution of systems capable of survival and productivity. To concentrate nutrients from sea water levels to those in living cells involves considerable work. The minimum effort would be described as $\Delta G = -RT \ln \frac{C_{sw}}{C_{cell}}$. This reduces to an effort on the part of the algal cell approximating 20 minutes of maximal photosynthesis to collect its N and P.

"Entropy of dilution" is a term used by Lewis and Randall to quantify the work wasted when a solute is dispersed into a solution. By avoiding loss of N and P, corals and some other reef organisms have learned to avoid this waste and thereby to conserve their available capabilities for growth and ecological accomplishments.

The work of Muscatine and his collaborators has revealed the coral's capabilities in avoiding "the entropy of dilution" of ammonia. Instead of excreting ammonia into the sea where it would require a free energy expenditure of 10.9 Kcal/mole for ammonia to be reconcentrated for the cells needs, the zooxanthellae avidly absorb the animal's ammonia and convert it to alanine and other protein components of use to both plant and animal.

Phosphate, too, becomes a conserved commodity. Although phosphate is usually associated with insoluble organic and inorganic structures, its preservation dispersal is deftly avoided by most organisms. Their phosphorus, largely concentrated in bones and membranes, appears to be transferred from producer to predator in a solid state with minimal danger of dilution by the sea. Membrane phospholipids secreted by mucus of many organisms are carefully collected by others which reuse its phospholipid components. The countless mucus feeders, from fish

cutaneous mucus to that exuded by corals, sponges and most other organisms, collect and use the phosphate which might otherwise be lost to "entropy of dilution." It appears axiomatic that dilution with the sea is scrupulously avoided in the nutrient-poor waters of tropic seas and that the most scrupulous species can be the most productive.

Algal symbiosis provides tropical animals with many mechanisms for insuring success. The internal energy source, alone, is exceedingly important, but the adaptations for survival only begin there. The energy conservation inherent in nutrient and product exchange between host and alga is a most elegant example of thermodynamic efficiency.

LIPID AS A MEDIUM FOR METABOLIC ENERGY EXCHANGE

Most growing algae contain 3% lipid on a dry weight basis. The amount is small compared to normal levels of protein and the diurnally fluctuating levels of polysaccharides. Under conditions of nitrogen stress or heterotrophic growth on substrates other than carbon dioxide, algae often accumulate globules of lipids. These "osmiophilic globules" observed in fixed and stained sections with the electron microscope can possess many times as much available energy as the rest of the cell's contents. The long chain hydrocarbon components of the fatty acids of the lipids possess 11.15 Kcal per gram of CH_2 and certainly are the most compact and buoyant of all sources of metabolic energy (Table 1).

Lipids are neither water-soluble nor low molecular weight, but since the complex lipids make up half or more of the cell's membranes they are exposed to the environment. They are thus accessible to predatory proteins capable of clearing or converting them. The coral host therefore has a source of fatty acid or lipid in contact with its lipolytic enzymes. Although no direct evidence of such attack is available, it is known that zooxanthellae become "leaky" upon exposure to the animal enzymes. This phenomenon is consistent with the presumption that the structure of zooxanthellar membranes is affected by their environment.

WAX ACCUMULATION IN HERMATYPIC CORALS

Corals have long been known as sources of neutral lipid (Silliman, 1846). When extracted by organic solvents, the wax crystallizes upon evaporation of the solvent. When corals are heated in water, wax floats to the surface. The wax ester structure of this lipid was recognized by Lester and Bergmann in 1941 (Figure 1). More recent studies have revealed the presence of other simple wax esters in related organisms such as myristyl myristate in the tropical anemone, Condylactis gigantea. Appropriate values for wax content of corals are not available, because there has been no accepted form from which the data might be compared. The wax content of coral tissue must be stated in terms of the amounts of coral tissue present. Either protein content or algal chlorophyll content could be used for a base, and the result could be given in terms

Table 1. Energy content and densities of marine metabolic energy sources.

Energy Source	Kcal/gram	Density g/cc
Hydrocarbon (21:6)	10.2	0.78
Lipid (wax ester, sperm oil)	10.0	0.88
Lipid (triglyceride, lard)	9.3	0.93
Protein	5.8	1.4
Carbohydrate	4.1	1.28
Seawater	-	1.025

of grams wax ester per gram chlorophyll. The amount of cetyl palmitate recoverable from Goniastrea retiformis (Lamk) was approximately 3 mg/cm^2 coral surface (Benson and Muscatine, 1974). It is obvious that wax contents based upon wet weights of coral would be misleading or useless.

The massive storage of wax ester in eggs and nauplii of Euchaeta japonica (Lee et al., 1974) provides for energy requirements of growth and development. It seems probable that coral planulae, too, may contain wax ester derived from their parent to maintain the organism until its own symbiotic metabolism is established. Although planulae have not been examined for wax ester content, it seems reasonable to expect that their copious oil globules may indeed be stores of wax ester.

WAX UTILIZATION BY CORAL FEEDERS

For several years we have been studying the digestion of wax by a variety of marine organisms. A comparative study of wax ester digestion by reef fish showed that oxidation of wax alcohol occurs rapidly in the intestine and the resulting fatty acid is incorporated into typical acyl lipids (Patton and Benson, 1975). In this study wax was assimilated well only when it was of high specific activity and accompanied by unsaturated triglyceride. A timed feeding study with live anchovies, whose diets were rich in wax, showed that digestion and absorption of triglyceride occurred four times faster than wax (Patton et al., 1975). Additional in vitro studies using intestinal fluid also showed slow hydrolysis of wax.

One fact has become eminently clear--wax ester is a difficult compound to digest even for those species whose diets include considerable amounts of wax. Biochemists and physiologists have long studied the pancreatic enzyme that digests fat in terrestrial animals. This enzyme, pancreatic lipase, will hydrolyze wax but at a rate 10-20 times slower than its rate on typical fat (triglyceride). Reasoning that terrestrial animals encounter little dietary wax, we sought to find a "wax lipase" in fishes which consume a lot of wax. We discovered a major fat-digesting enzyme in fish and showed that a fat digestion in a vertebrate can be accomplished by an enzyme other than pancreatic lipase (Patton et al., 1975; Patton, 1975a and b; Patton et al., in press). This unique lipase does not cleave wax any better than the classic pancreatic lipase, although it does hydrolyze esters of polyunsaturated fatty acids which are so abundant in marine organisms. Numerous attempts to facilitate wax hydrolysis by both lipases have been unsuccessful. Commercially available lipases from microorganisms are also poor wax digestors. Thus, although wax esters yield as much or more energy than triglycerides upon combustion, their physical properties appear to impose limitations on their rapid utilization.

Even though no specific "wax lipase" seems to exist, coral reef grazers and other wax-eating fish still have at least three digestive strategies for exploiting the hard-to-digest wax. (1) They can increase the amount of digestive lipases secreted into the intestine, and/or (2)

they can increase the time food is exposed to digestive enzymes, and/or (3) they can increase the amount of food taken in. Coral grazers are fortunate in this last regard as they really do have unlimited food supply. Varying degrees and combinations of these three strategies show up in different fish species.

Internally, the most distinctive characteristic of coral grazers is the absence of a stomach. Acidic conditions in any region of the alimentary tract would create gas problems for an organism injesting quantities of $CaCO_3$. The intestines of the triggers, puffers and filefish are coiled unbranched tubes. Pieces of intact coral pass intact and lose only their surface coat of living tissue. The parrotfish possesses a unique digestive system, large chunks of coral are pulverized by a pharyngeal mill in the rear of the oral cavity, the crushed coral then enters a coiled intestine which appears like a long string of pouches. Gut contents cannot be extruded because of restricted connection between the pouches. A ligament runs the length of the intestine and serves as a support for the massive system of "stomachs." As compared to other coral eaters, the distinctive morphology of the parrotfish gut increases the surface area of absorption and greatly prolongs the residence time of food particles. Among fish it is one of the most unusual systems observed and may account for the great size attained by parrotfish compared to other reef grazers. Whereas the triggers, puffers and filefish have relatively high lipolytic activity in the intestinal fluid, the parrotfish contents show only weak lipolytic activity (Patton, 1975b; Letourneuk and Bagnis, 1973).

Pelagic filter-feeding fish like the anchovy, whose diet consists of wax-containing copepods, face a fluctuating, unpredictable food supply. In addition to high levels of lipase, these fish possess numerous blind pouches extending from the alimentary tract; these greatly prolong the residence time of food. We feel that these pyloric caeca have arisen during fish evolution as a direct consequence of high wax diets.

Acanthaster ellisii and A. planci digestive tissues hydrolyzed wax ester and triglyceride much more rapidly than those of other asteroid species studies (cf. Endean, 1973; Yamaguchi, 1973). The rate of wax ester hydrolysis, however, appears to be less in these animals than that for triglyceride hydrolysis. The study of Benson et al. (1975) with A. ellisii gave hydrolytic yields which indicated more rapid wax hydrolysis. Subsequent experiments with the same species yielded results indicating greater triglyceride hydrolysis. In one other study with a mollusc, (Patton and Quinn, 1973) wax was hydrolyzed at 1/10 the rate of triglyceride by the digestive lipases.

In conclusion, there appears not to be a specific wax lipase. The classic pancreatic lipase and the newly described shark nonspecific lipase both hydrolyze wax very slowly compared to typical fat. This apparent inaccessibility of the wax ester linkage to enzymic cleavage has profound implications to the biology of marine organisms. Thus a copepod with energy reserves of both triglyceride and wax ester quickly

depletes the triglyceride during starvation, then exists by slow depletion of its wax reserve until another plankton bloom occurs (Lee et al., 1971). The occurrence of wax in coral, however, remains a mystery and it is not known whether corals possess a specific wax lipase.

ALGAL PHOTOSYNTHESIS AND PHOTORESPIRATION

Like all photosynthetic plants, coral zooxanthellae fix carbon dioxide by condensation with ribulose diphosphate in a process (Figure 3) catalyzed by a major protein of the plant. This protein, "Fraction I Protein" or ribulose diphosphate carboxylase, binds the substrate and either carbon dioxide or oxygen with an effective avidity ratio of 4:1. Since marine concentrations of carbon dioxide are relatively constant and that in intra-chloroplast oxygen is higher in the high light intensity environment of reef corals, the effect of photorespiration in reducing photosynthetic yields is greater than for algae living at lower intensities.

The reaction between oxygen and ribulose diphosphate yields phosphoglycolate which passes from the chloroplast with concommitant dephosphorylation to the cell's peroxisomes where it is oxidized to glyoxylate with oxygen consumption and then in the forms of glycine or serine is oxidized further by mitochondria. The extent of photorespiration in zooxanthellae was examined by Tolbert et al. (1976) and by J. E. Burris (personal communication). Such internal cycles of respiration and photosynthesis are evaluated only with difficulty but may involve half of the carbon reduced by photosynthesis. Evaluation of glycine-serine fluxes from zooxanthella to host have not been made, but these fluxes are an order of magnitude smaller than that of alanine. The exchange of alanine from photosynthetic source to the sites of transamination and further carboxylation of the pyruvate to oxalacetate resembles the efficient process adopted by C_4 plant species denoted as "PCK-Type" and "NAD-ME-Type" by Hatch (1975). Present evidence does not preclude carbon dioxide transport by malate or aspartate from host to zooxanthella. The modified transport capabilities of the in situ zooxanthellae must be examined in the light of the classic works by Hatch and Slack on the transport processes of C_4 photosynthesis. Photorespiration in C_4 plants (sugar cane, etc.) is reduced to a minimum by concentration of CO_2 at the site of ribulose diphosphate carboxylation. The carbon dioxide is transported in the form of the β-carboxyl groups of malic or aspartic acids from their cytoplasmic sites of synthesis to the sites of release of carbon dioxide by specific enzymatic decarboxylation.

The products of zooxanthellar photosynthesis which may be translocated readily to the coral cannot include the high molecular weight substances widely used by animals. Proteins and polymeric carbohydrates are not likely to be excreted by algae. The low molecular carbohydrates, glycerol, glucose, and maltose, however, were recognized by Muscatine (1967) as "energy carriers" from zooxanthella to host. Likewise the low molecular weight protein component, alanine, is also exuded by the

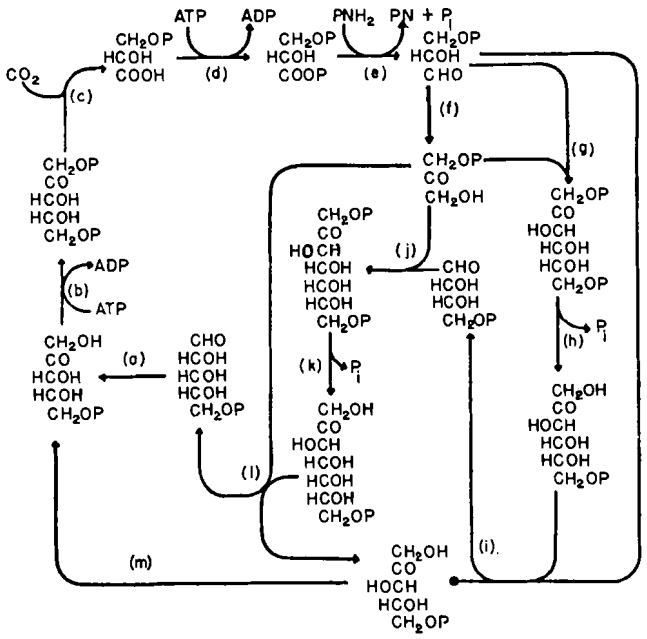


Figure 3. Photosynthetic CO_2 reduction cycle.

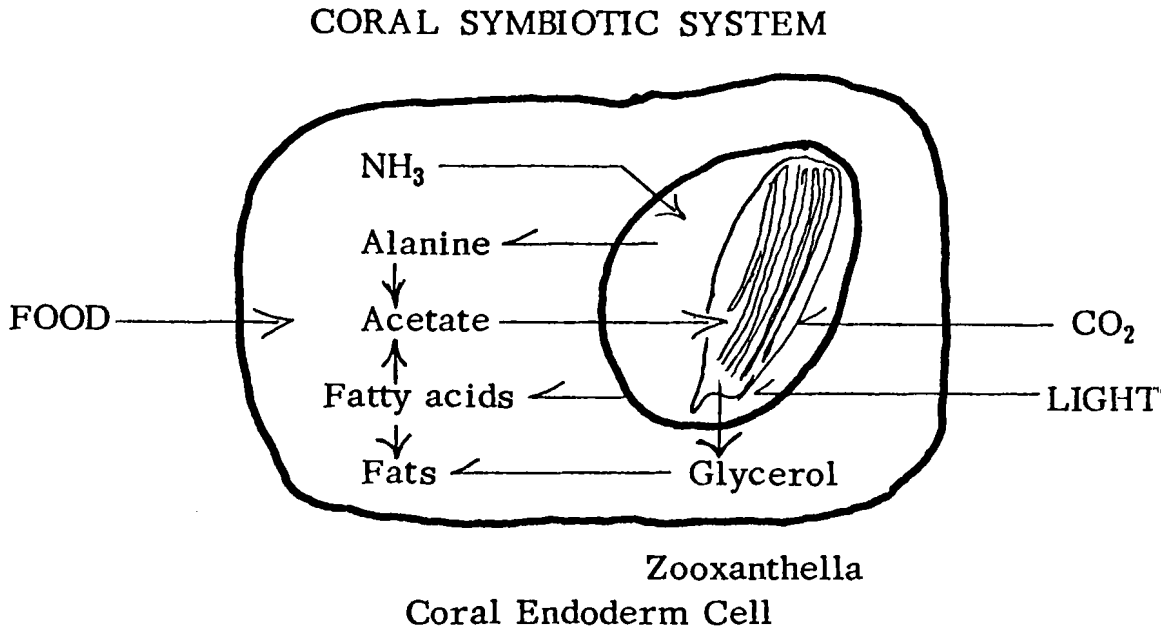


Figure 4. Coral symbiotic system.

zooxanthella as a metabolic substrate for the animal's growth and respiration. The remaining major medium for metabolic energy exchange is lipid.

The evidence of Abraham, Patton, and Benson (unpublished) indicated that fatty acid and triglyceride synthesis occurs mainly in the zooxanthellae where an excess of photosynthetically generated ATP and reducing power is harnessed for the energetically costly process of lipid synthesis. In this process the animal host releases two-carbon products of its own oxidative metabolism in the form of acetate to the zooxanthellae where long chain fatty acid and triglyceride syntheses take place. The zooxanthellae then "leak" the newly synthesized lipid, probably in the form of membrane components, back to the host where it is converted to other forms including wax ester and stored or catabolized. If this proposed process is correct, the transfer of acetate becomes an important process in facilitating symbiotic efficiency.

The elegance of the coral-zooxanthella symbiosis is further revealed in considering their structural association. Zooxanthellae are located within the basal portion of digestive cells of the endoderm. In this position they have access to acetate produced upon degradation of foodstuffs by enzymatic processes. They may release lipoproteins to the host circulation, a process not unlike the release of lipoproteins by the intestines of mammals. The typical marine fatty acids (polyunsaturated) which are undoubtedly part of the coral's carnivorous diet are not found in tissue lipids, presumably because they are oxidized to acetyl CoA in the distal portion of the animal's digestive cells. The acetate is then absorbed by the zooxanthella and reconverted to fatty acids of low diversity and a high degree of saturation. Evidence for this process stems from the unique propensity of zooxanthellae for synthesis of saturated fatty acids instead of the normally unsaturated acids of marine algae. It is thereby possible for the host to acquire saturated lipids directly without having to synthesize them *de novo*, a process which would require expenditure of costly energy. The generalized scheme for exchange of metabolites in the coral endoderm cell is presented in Fig. 4. While not fully established, these relationships are consistent with available information.

Fatty acid synthesis in plant systems (Stumpf, 1975) usually leads to formation and storage of polyunsaturated fatty acid derivatives by a cooperative effort of cytoplasmic and chloroplast systems. The chloroplast-associated assembly of acetyl and malonyl CoA esters leads directly to palmitic acid (16:0). Further addition of malonyl CoA to produce stearic acid (18:0) and the essential polyunsaturated acids of chloroplast lamellae seems to be bypassed in zooxanthellae in favor of transfer of the C_{16} acid to the host and its subsequent conversion to wax ester. Acetate metabolism studies by Abraham *et al.* (to be published) reveal what appears to be an important mechanism for energy transfer from alga to host and an important difference in fatty acid synthesis in zooxanthellae. The formation of saturated C_{16} and C_{18} acids in major amounts reveals a remarkable adaptation in the captive algae. The nature of the adaptation or regulation remains to be elucidated.

ACETATE-1-^{14}C UPTAKE BY CORALS AND ZOOXANTHELLAE

When growing tips of Pocillopora capitata (Isla Clarion, Mexico) were provided with carboxyl-labeled acetate, they synthesized considerable amounts of wax esters having equal amounts of ^{14}C in their acid and alcohol moieties. Zooxanthellae, exposed to the same concentrations of labeled acetate, produced about 1% as much wax ester as did the intact coral and, of that wax ester, the predominant ^{14}C label was found in the fatty acid (Table 2).

The lipids synthesized by isolated zooxanthellae from acetate-1-^{14}C were largely triglycerides and amphipathic lipids (Table 3). It is clear that the triglycerides are a reservoir for fatty acids to be utilized later for synthesis of cetyl palmitate in the animal.

WAX ESTERS AND OTHER LIPIDS IN EPIDERMAL MUCUS

Epidermal mucus secretion by a number of invertebrates (Storch and Welsch, 1972) serves a variety of purposes, including collection and transport of food particles, removal of foreign particulate matter, protection of eggs, carrier for substances for olfactory enhancement purposes (Todd et al., 1967) and protection from desiccation. Mucus may also carry enxymatically functional proteases or glycosidases (Christie, 1974). In most cases the extruded mucus includes residual membrane material of the mucus cell (Pedersen, 1963) or membrane-bound granules derived usually from golgi membranes (DeLuca and Wolf, 1972; Harris et al., 1973). The phospholipid content of coral and fish cutaneous mucus (Lewis, 1970, 1971) is derived from membranes surrounding the muco-proteins during their production and storage. Lewis (1976) has correlated mucus viscosity and consequent stability with membrane protein and phospholipid content. Observations of Smith (1968), correlating mucus production with carotenoid pigmentation in sponges, are in accord with the recognized function of vitamin A in mucus cell function (Lewis, 1973).

Jakowska (1965) emphasized the importance and variety of mucus function in invertebrates. Her efforts to integrate the interests of zoologists and the research on mucus secretions related to human disease stimulated interest in the role of mucus in marine biology.

Coral surface mucus production is stimulated by the irritating effects of sand particles or of agitation by small fish striving to remove it from coral surfaces. While not enough is known of diurnal variation in mucus productivity or in mucus feeding (Hobson, 1968, 1974, 1975; Losey and Margules, 1974), it is apparent that mucus production is not continuous. Benson and Muscatine (1974) observed mucus feeding by reef fishes and examined its lipid content by thin layer chromatography. The mucolipids of most reef coral species examined contained 60% wax ester (cetyl palmitate) and 30% triglyceride with lesser amounts of glycerol ether and phospholipid. Tracer experiments with intact corals and $^{14}CO_2$ revealed the rapid incorporation of labeled triglyceride into

Table 2. Distribution of ^{14}C activity in wax ester synthesized from 1-^{14}C-acetate by isolated zooxanthellae and intact coral*

Preparation		Total ^{14}C in Wax Ester	% of Total ^{14}C in Alcohol	% of Total ^{14}C in Fatty Acid
Zooxanthellae	3	670	20.	80.
Zooxanthellae	2	1,130	9.1	91.
Coral tip	1	87,100	50.	50.
Coral tip	2	91,400	45.	55.

* Pocillopora capitata. Incubation time under fluorescent light was two hours.

Table 3. Types of lipid synthesized from acetate by intact isolated zooxanthellae.[a]

Lipid Class	% of Total Lipid Synthesized[b] Prep. 2	Prep. 3
Wax esters	1.6	1.9
Triglycerides	73.	66.
Fatty acids	10.	11.
Sterols	3.2	4.6
Phospholipids[c]	13.	16.

[a] From Pocillopora capitata

[b] 1-^{14}C-acetate incubations were carried out under fluorescent light (1000 f.-c.) during 1.5 to 4.0 hours in filtered seawater at 24°.

[c] Includes galactolipids of chloroplast lamellae.

exuded mucus while formation of labeled wax ester was very slow, being impeded by the massive amounts of endogenous wax ester. After 48 hours the ratio of labeled triglyceride to wax ester was only 2:1.

POTENTIAL APPLICATION OF IMMUNOCHEMICAL TECHNIQUES IN ASSAY OF MUCUS FEEDING BY REEF FISHES

Antibodies to mucus are readily obtained and exceedingly species-specific. It appears possible to use the fluorescent (Kent, 1963) or labeled antibody technique for assay of ingested mucus in fish gut contents (Reynoldson and Davies, 1970; Davies, 1969). Published information on reef fish gut contents has left the identity of major amounts very much in doubt. Identification of mucus and its source (Jakowska, 1963) has not been a subject of investigation until recently. Being glycoprotein in nature, the specificity of its recognition by antibody reaction is particularly high.

UTILIZATION OF WAX ESTER BY COMMENSAL ORGANISMS IN CORAL

For many types of organisms the branched corals become a haven and a source of nutrient as well. Although there are many true commensal organisms, many others appear to be mucus feeders (Nigrelli, 1969) and therefore derive some of their energy from the mucolipids. Recent experiments by L. McCloskey (unpublished) with commensal groups on Pocillopora capitata (Isla Clariōn, Mexico) clearly ascertained which of the commensal creatures were mucus feeders. Coral heads were freed of their commensal populations and labeled with ^{32}P during ten hours in the light. The corals were then placed in fresh seawater; the commensal population returned. After 24 hours or more in seawater tanks or in situ, the commensal population was again removed and their ^{32}P measured. Some were clearly labeled while others were clearly unlabeled. Since coral mucus contains phospholipids the use of this source of phosphorus by the organism clearly delineated those which were purely parasitic and those which were completely commensal.

UTILIZATION OF WAX ESTER BY ALGAE AND BACTERIA

The most striking indication of energy-containing material in coral is revealed after death of the coral. When Acropora palmata is killed by invasion of the parasitic blue-green alga, Oscillatoria submembranacea, the substrate is rapidly colonized by other algae. The rapid growth of these algae precludes their development by photosynthesis alone; other substrates do not support such growth. It appears probable that the invading algae derive at least some of the nutrient necessary for growth from the coral skeleton and its stored nutrient. Since cetyl palmitate is the major of these, one must suspect that the developing algae derive energy from their utilization of wax ester.

In living Porites and other massive corals, the internal band of Ostriobium (green algae) (Odum and Odum, 1955) may also derive its energy from wax ester. It is clear that nutrition of these parasites by labeled wax esters must be investigated.

DISCUSSION

It is not yet clear why corals have adapted their metabolism for storage of wax ester. If one were to relate their wax storage to that in organisms of colder seas, one would conclude that the coral must be subjected to starvation periods. Evidence for this is not clear.

The needs of coral planulae for a long-term source of energy suggested the possibility of their wax accumulation. Certainly the micrographs of Pocillopora damicornis planulae published by Vandermeulen (1974) attest to the probability that the ITP spherical inclusions throughout the gastrodermis may indeed be wax ester. A. E. Lambert (unpublished) has come to similar conclusions based upon electron micrographs of similar planulae. The energy requirements by coral planulae for swimming and substrate exploration are considerable and could best be provided by metabolism of stored lipids derived from the parent.

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