available glycoproteins, it may be suggested that the alpha-2-globulins are important in the nutrition of mammalian cells.

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## Distribution of Labeled Carbon in Reef-Building Corals with and without Zooxanthellae

*Abstract.* The uptake and distribution of carbon-14 in two reef-building corals, *Manicina areolata* and *Montastrea annularis*, were studied by radioautographic methods. Experiments on colonies with and without zooxanthellae were run in light and in darkness. We suggest that corals cannot derive any effective nutrition from their zooxanthellae, and that the physiological response of corals to the presence of zooxanthellae is due to the secretion of trace amounts of vitamin-like or hormone-like substances by the algal symbionts, and not to their food value per se.

Using a radioautographic method, Muscatine and Hand (*1*) reported transfer of photosynthetically fixed material from the zooxanthellae into the host tissues of the sea anemone *Anthopleura elegantissima* (Brandt). Although these investigators obtained no data about the nature and quantity of the material transferred, they suggested that the actiniarian host could derive at least a part of its nutrition from its commensal algae. Muscatine and Hand also claimed that their conclusions support the inference that the hermatypic, or reef-building, corals, which also contain zooxanthellae, could likewise derive food from the algae.

The point of view that hermatypic corals are herbivorous as well as carnivorous is a controversial one which has recently been resurrected by Sargent and Austin (*2*) and Odum and Odum (*3*) to explain results of their studies on the oxygen balance of Pacific atoll reefs. On the other hand, the experimental evidence of Yonge *et al.* (*4*, *5*) demonstrated that corals appear to be specialized carnivores which cannot utilize their zooxanthellae for food. These investigators also showed that the zooxanthellae are nevertheless very important in the bioeconomy of their hosts in that the metabolic efficiency of the coelenterate is greatly enhanced through the *in situ* absorption of waste products and the liberation of oxygen. Our own unpublished observations confirm these conclusions.

It is now almost certain that, among the Coelenterata, zooxanthellae do play an important role in the nutrition of some of the Alcyonaceae, notably the Xeniidae (*6*), and possibly in some of the Zoanthidea as well. There appears to be great variation throughout the various groups of the coelenterates, some of which are purely carnivorous even though they contain zooxanthellae, whereas others are wholly or in part herbivorous. The role played by the zooxanthellae in the bioeconomy of their coelenterate hosts appears to vary in specific and unpredictable ways from one group to another, and it is unjustified to extrapolate results from the Actiniaria to the Scleractinia, as was done by Muscatine and Hand.

Investigations are now in progress in this laboratory to determine the role of the zooxanthellae in calcium deposition and nutrition in the hermatypic scleractinia, or reef-building corals (*7*, *8*). In view of the possible importance of our findings in helping to resolve the controversy over zooxanthellae in reef corals, we present here some preliminary results on the fixation and migration of labeled carbon in corals with and without zooxanthellae, both in light and darkness.

Two species of West Indian reef-building scleractinian corals were used in our experiments, *Manicina areolata* (Linnaeus) and *Montastrea annularis* (Ellis and Solander). The latter is one of the most important framework builders of Western Atlantic reefs (*9*). Half of the colonies used were first kept in complete darkness for 3 months to cause complete extrusion of the zooxanthellae from the coenosarc; the other colonies used were normal and contained large numbers of zooxanthellae. One series of colonies, both with and without zooxanthellae, was run in light, 1 foot under a double bank of 40-watt daylight fluorescent tubes. Another series, containing the same types of coral, was run simultaneously under identical conditions, except that the colonies were kept in lightproof jars. $Na_2C^{14}O_3$ at *p*H 9.0 was added to the experimental vessels to give a final activity of approximately 1 $\mu$c/ml. The water was stirred with a gentle stream of moist air to prevent anaerobiosis and *p*H changes, especially in the dark jars. The experiments were run for 50 hours at a temperature of 27 ± 1.5°C and were terminated by transferring the corals into "cold" fresh-running sea water for 2 hours to rinse out any activity not incorporated into tissue constituents. The specimens were fixed in modified Carnoy's fluid (1 part glacial acetic acid to 3 parts absolute ethyl alcohol), decalcified *in vacuo* with 10 percent glacial acetic acid, washed, dehydrated, embedded, and sectioned at 5 and 9$\mu$. Radioautography was carried out by the stripping film method of Pelc (*10*) with Kodak Ltd. (England) A.R. 10 plates. The emulsion was exposed for 10 days at 4°C and developed.

Intense exposure was observed over the zooxanthellae of those corals which were kept in the light, whereas there was virtually none in those which had been kept in darkness to prevent photosynthesis. The amount of activity incorporated was three to five times greater in zooxanthellae located in the gastroderm of the oral disk and column wall than in those located in deeper tissues such as the mesenteries and calicoblast, where the available light is of much lower intensity and the rate of photosynthesis is thereby reduced. Very little activity was seen in zooxanthellae located in the excretory zone of the mesenteries, possibly because these algae were either moribund or dead and in course of extrusion from the coral (*5*).

No significant algal uptake of radiocarbon was observed in those corals which had been kept in darkness. The tissues of both normal and zooxanthella-less corals kept in darkness during the experiment showed identical but faint background activity, probably due to the heterotrophic $CO_2$ fixation in the animal tissues. The tissue background activity of the zooxanthella-less corals kept in the light was equally low. In normal corals kept in light to allow algal photosynthesis, preliminary grain counts indicated that the tissue background activity was about five times higher than in the controls kept in darkness. This activity was confined to the cell masses of the epidermis and gastrodermis, and was reduced or absent over mucous glands and the mesoglea, which is nearly acellular. The activity was somewhat greater in tissues that contained zooxanthellae than in those that did not, but the difference was small. These observations indicate that some of the radioactivity fixed by algal photosynthesis was transferred and became incorporated in the tissues of the animal host.

The level of such transfer seems

surprisingly low, especially if the possibility that the zooxanthellae supply the coral host with food materials is considered. But if this were indeed the case, then the rate of transfer of labeled products from algae to coral should have been far greater, and a much higher level of tissue radioactivity than was actually found would have been expected. The activity observed in the tissues of our normal corals, which were kept in the light for 50 hours at 27°C, was much lower than that in the anemone section shown in Fig. 3 of Muscatine and Hand's paper (*1*, p. 1262), which came from a specimen run for only 18 hours at 14°C. It should be pointed out that the specific activities, tissue-section thickness, and exposure times of Muscatine's experiments were roughly the same as ours.

Under the conditions of our experiments, the results suggest that there is some movement of labeled photosynthetic products from the zooxanthellae to the coral, but that this occurs at a very low level. Work is now in progress to determine the quantitative relations of this process. It appears probable that the amount transferred from the algae to the animal host can at best satisfy only a very small proportion of the coral's total nutritional requirement. This preliminary conclusion would be in agreement with the observations of Yonge *et al.* (*5*), who demonstrated that reef corals starved of animal food, but still containing zooxanthellae, could not use their algal commensals as a substitute source of food.

If the corals cannot utilize the zooxanthellae as a source of food, what then is the role of the zooxanthellae in the bioeconomy of the coral host, particularly in relation to the algal products which do reach the coral tissues and are incorporated there in small amounts? Elsewhere we have shown that the zooxanthellae exert an enormous potentiating influence on the calcium deposition rate of those hermatypic corals we have tested (*8*). A possible mechanism for this has been suggested, and evidence has been presented that the zooxanthellae may have a general stimulating action on the coral host's metabolism, possibly mediated through vitamin or hormone-like trace factors which are secreted in small amounts by the algae but which by themselves do not contribute significantly to the nutrition of the coral (*7*, *11*).

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## Antibacterial Activity of Acyclic Decapeptide Analogs of Gramicidin S

*Abstract.* Three acyclic decapeptide analogs of gramicidin S, although found to possess antibacterial activity, apparently have modes of action which differ from that of the naturally occurring cyclic antibiotic. In contrast to the immediate action of gramicidin S, one of the decapeptides produced complete bacteriostasis only after several cell divisions had occurred. Furthermore, mixtures of gramicidin S with either of two of the acyclic peptides were synergistic. Some implications of these findings are discussed.

In an earlier communication (*1*), the antibacterial activity of a decapeptide analog of gramicidin S was described. The compound (Fig. 1, decapeptide I) (*2*), possessed all the amino acids of the naturally occurring antibiotic in their proper sequence but lacked the cyclic structure. It was found to be approximately 1/10 as active as gramicidin S against *Escherichia coli* and 1/40 as active against *Staphylococcus aureus*.

The synthesis of two additional decapeptide analogs has recently been completed (*3*). Their structures and that of gramicidin S and decapeptide I are shown in Fig. 1. In one of them (decapeptide II), D-tyrosine residues replace the D-phenylalanine residues of the natural product. In the other (decapeptide III), L-lysine residues replace L-ornithine residues. All the synthetic analogs are acyclic.

The antibacterial activities of gramicidin S and the synthetic analogs against a strain of *E. coli* B are shown in the first four rows of Table 1. The conditions of the assay are described in the legend. Neither decapeptide II nor decapeptide III is as active as decapeptide I, which, in the earlier experiments (*1*), was shown to be about 1/10 as active as gramicidin S. Apparently both the L-ornithine and D-phenylalanine residues contribute to the activity of acyclic decapeptide I. This finding would appear to conflict with that of Schwyzer and Sieber (*4*), who reported that the *cyclic* lysine analog of gramicidin S (that is, the cyclic analog of decapeptide III) was as active as gramicidin S. We now have evidence, however, that the mode of action of the acyclic analogs differs from that of gramicidin S, a possibility which was mentioned but not considered seriously in our earlier paper (*1*).

First of all, it was observed that decapeptide I and gramicidin S differ in the manner in which they inhibit *E. coli* B. Gramicidin S, at all effective concentrations, produces immediate bacteriostasis; decapeptide I, on the other hand, at concentrations between 12 and 25 μg/ml, inhibits growth only after the organism has undergone several cell divisions.

Second, it was possible to show that mixtures of gramicidin S with either decapeptide I or decapeptide III exhibit a synergistic effect which is lacking when the two decapeptides are combined. The experimental data are shown in the last three rows of Table 1.

We may infer, therefore, that the mode of action of gramicidin S differs from that of either decapeptide and that presumably it is dependent upon the unique cyclic structure of the antibiotic.

The original purpose of this program (*5*) was to determine the influence of the cyclic structure of gramicidin S upon its activity. The finding that deca-

Table 1. Minimal concentration of peptides required to inhibit *E. coli* B (24 hours at 37°C). Medium: 1 liter contains 7 gm of $K_2HPO_4$, 3 gm of $KH_2PO_4$, 0.5 gm of sodium citrate, 0.1 gm of $MgSO_4 \cdot 7\ H_2O$, 1 gm of $(NH_4)_2SO_4$, and 2 gm of glucose (*p*H adjusted to 7.0). Inoculum: 300,000 organisms per milliliter (stationary phase).

| Compound | Concn. (μg/ml) |
|---|---|
| Gramicidin S | 1.85 |
| Decapeptide I | 15 |
| Decapeptide II | 107 |
| Decapeptide III | 66.7 |
| Gramicidin S + I | 0.5 + 3.8 |
| Gramicidin S + III | 0.5 + 12.8 |
| I + III | (7.6 + 33)* |

* Only slight inhibition occurred with this combination, as determined by nephelometric measurements during the first 10 hours of the run.