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INTRA-COLONIAL TRANSPORT OF ORGANIC COMPOUNDS AND CALCIUM IN SOME ATLANTIC REEF CORALS

Dennis L. Taylor
Rosenstiel School of Marine and Atmospheric Science
University of Miami
4600 Rickenbacker Causeway
Miami, Florida 33149

ABSTRACT

Intra-colonial transport of soluble organic compounds, and Ca^{++}, is examined in the hermatypic corals Acropora cervicornis (Lamarck) and Montastrea annularis (Ellis and Solander), using isotopic ^{14}C-labelled HCO_3^-, protein hydrolysate, alanine, glycerol and glucose and $^{45}Ca^{++}$. Results support earlier contentions that these materials can be transported over considerable distances, and that the direction of transport is toward zones of maximum growth and calcification.

KEY WORDS: Intra-Colonial Transport, Organic Compounds, CA^{++}, Acropora cervicornis, Montastrea annularis, Calcification

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Introduction

Light-enhanced calcification in hermatypic corals is known to be dependent upon the photosynthetic activity of micro-algal endosymbionts (1-4). The rate of calcium deposition in the light will exceed that observed in the dark, or in photosynthetically inhibited animals, by a factor of 3-4 (2). Translocation of symbiont photosynthetic products is usually invoked as the most widely accepted mechanism capable of satisfying the energetic demands of the phenomenon as it is presently known (5, 6). Beyond this generalization, little is known about the role of specific, symbiont-derived metabolites affecting calcification rates. In fact, the major components of the organic complement excreted by symbionts in situ (e.g., glucose, glycerol and alanine), fail to stimulate the dark (or inhibited) calcification rate in experimental studies of Pocillopora damicornis (7), and Acropora cervicornis (8). At present, this matter is unresolved.

Analysis of growth and form in dendritic and massive corals, suggests the existence of a strong directional component in skeletogenesis that is due to differential growth rates within colonies. Among dendritic species (e.g., A. cervicornis), this manifests itself as an enhanced rate of calcification near the tip or terminal polyp (3). Among massive species, obvious variations in growth form exist. These are directly attributable to ambient light intensities, and corresponding differences in intercolonial growth rates (e.g., Montastrea annularis). Usually the result is a progression in growth form beginning with a hemisphere in shallow depths, and ending in a flat plate-like form in deeper fore-reef areas (9).

In studies of A. cervicornis, Pearse and Muscatine (3) observe that relatively few symbionts occur in tissues associated with the terminal polyp. Nevertheless, the highest rates of calcification occur in this region. They conclude that symbiont photosynthate is translocated through the animal tissues and utilized in this region of maximal demand. Some data are presented which supports this hypothesis.

Accumulated data therefore suggest: (1) that differential rates of calcification occur within individual colonies of hermatypic corals, and that maximal zones of calcification exist; (2) the maximal zone in A. cervicornis is the terminal polyp; (3) the maximal zone in M. annularis varies as a function of ambient light intensity and effects gross colonial morphology; (4) symbiont photosynthate and/or other materials must be translocated within the colony if maximal rates are to be sustained in these zones. The data which follow result from studies designed to provide basic information relative to these suggestions.

Materials and Methods

The development of methods for in situ measurements was pursued over several years beginning in 1971. Experiments reported here were carried out in the latter half of 1975, and early 1976, when techniques were perfected. Experimental sites were located near Churchill Beach, Grand Bahama Island, Bahamas and Dancing Lady Reef, Discovery Bay, Jamaica. Work with A. cervicornis was done at the former site, while work with M. annularis was done at the latter site.

Experiments on intra-colonial transport were performed using ^{14}C-labelled sodium bicarbonate (40mCi/mM), protein hydrolysate (45mCi/milliatom of C), glucose (2mCi/mM), glycerol (5mCi/mM) and alaline (10mCi/mM), and $^{45}CaCl_2$ (40mCi/mM). Colonies were prepared and labelled in situ using SCUBA, and the basic plastic bag method employed by Barnes and Taylor (10), modified to suit experimental requirements (see below). Incubations were timed to occur between 1000 and 1400 hours and lasted 4 hours. A. cervicornis was sampled and incubated at a depth of 20 meters. M. annularis was sampled and incubated at a depth of 35 meters.

Differences in physical form require different methods to establish conditions for discrete labelling of areas on the colony.

a. A. cervicornis: Labelling of the terminal 1 cm of individual branches was achieved by attaching 5 x 10 cm clear plastic bags to the tip using elastic bands to form a triple seal. Labelling of distal regions of branches was accomplished similarly, by cutting 12 cm lengths of branch and attaching bags to the distal 1 cm. These were then suspended off the bottom using underwater floats.

Following incubation, bags were removed, and the branches were collected and rapidly frozen for analysis. Frozen branches were cut in 1 cm lengths, the 1 cm enclosed by the bag during labelling was discarded, and the remaining pieces processed for counting (see below).

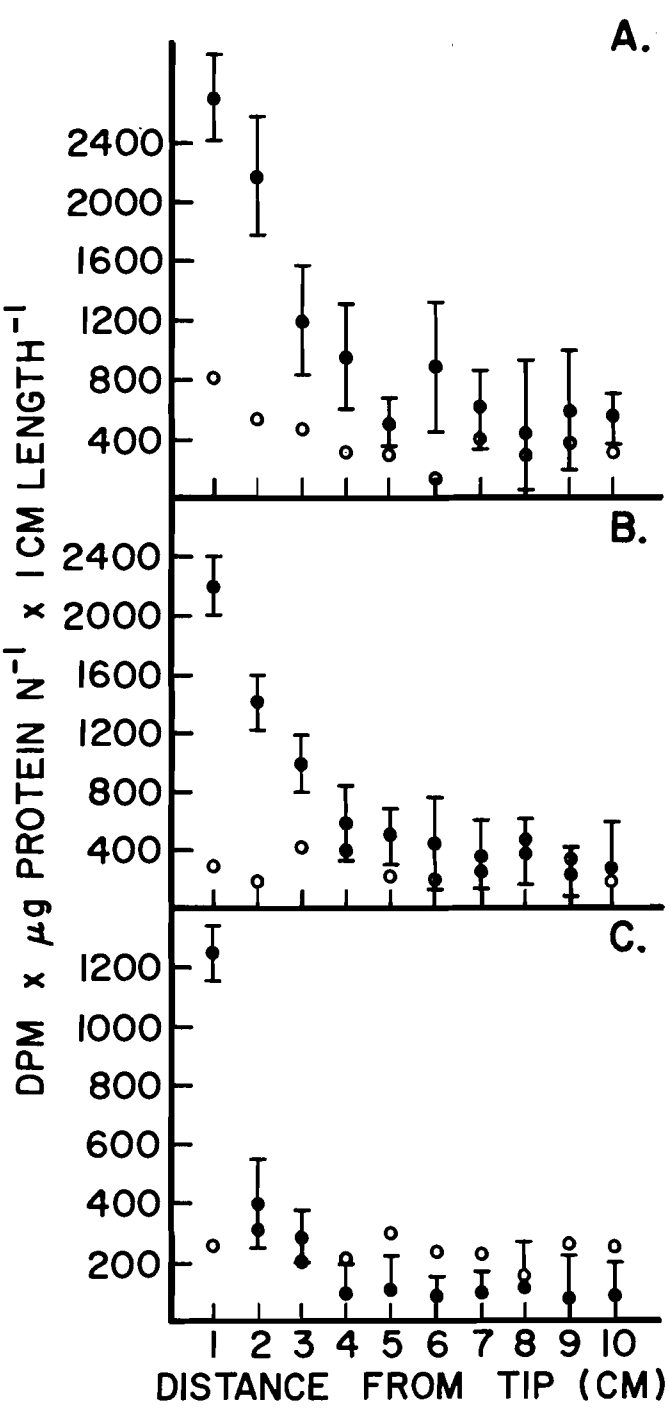

Figure 1. Labelling of A. cervicornis at the tip. A. $^{14}CO_3^{=}$ (2.6 µCi/ml final activity), B. ^{14}C-protein hydrolysate (2.7 µCi/ml final activity), C. $^{45}Ca^{++}$ (1.5 µCi/ml final activity). Mean $\pm$ 1 standard deviation. Closed circles are experimental, open circles are formalin control.

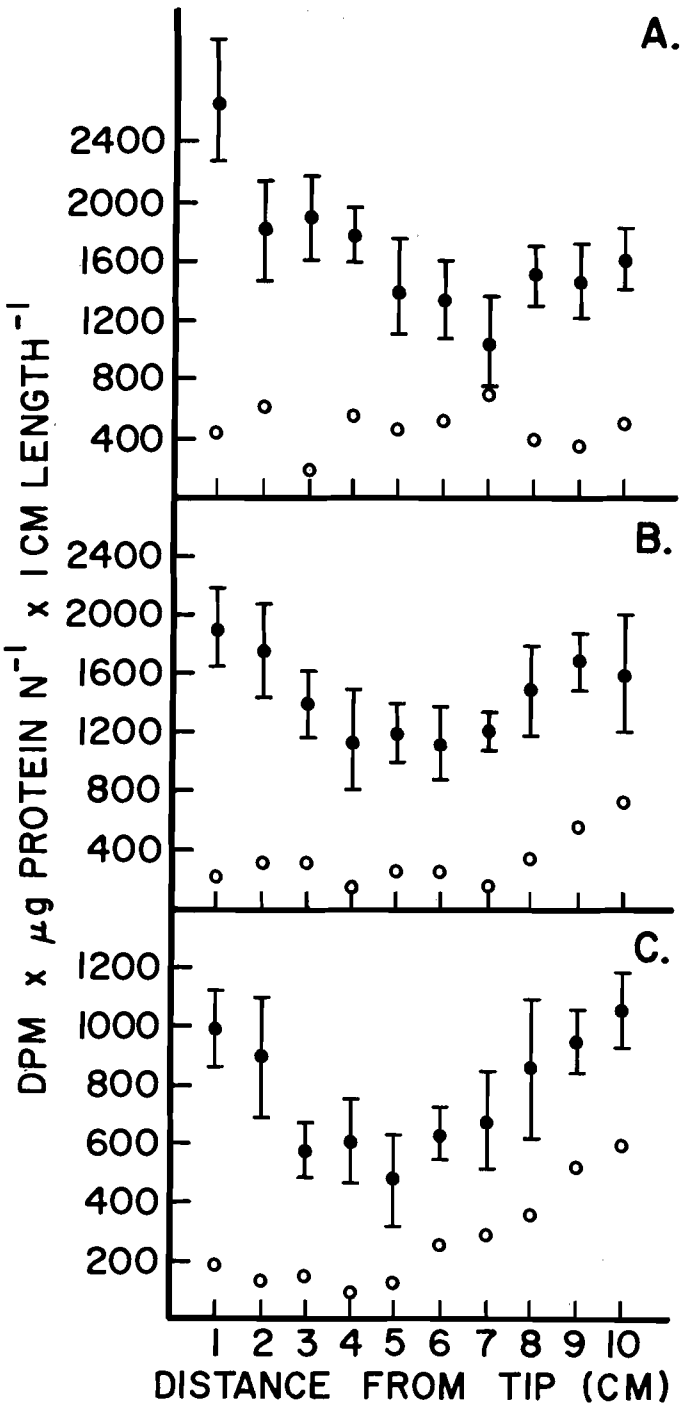

Figure 2. Labelling of A. cervicornis at the distal end. See Figure 1 for description.

Separate experiments to determine leakage during incubation indicated that less than 1.5% of the label was lost, and that this had no effect on observed fluxes.

b. M. annularis: Discrete labelling of areas near the center and edge of plates was accomplished by mounting plexiglass rings (5 cm high, 2 cm inside diameter) on the plate with semi-cured silicone aquarium cement as a gasket, and elastic hold-downs to keep the ring secure. Plastic bags were then mounted on the ring using elastic bands.

Following incubation, bags and rings were removed, and the plates collected and rapidly frozen for analysis. Frozen plates were cut with a surgical bone saw as 2 cm wide strips along the vectors indicated in Figs. 3 and 4. Labelled areas under the ring were discarded, and the strips cut into 1 cm pieces for processing and counting (see below).

Separate experiments determined that there was no detectable leakage from this system during incubation.

Prepared samples of both species were processed for counting by (1) extraction in hot 70% ethanol/water for 1 hour in the case of ^{14}C-labelled specimens, or (2) in the case of ^{45}C-labelled material, cleaning in 50:50 Clorox:seawater for 4 h to remove tissue, and dissolution of the skeleton in concentrated HCl + 0.1 ml 1-butanol to retard foaming (2). Samples were counted by liquid scintillation counting using Aquasol (New England Nuclear) as a gel following previous methods (2).

Observations

Data from experiments employing ^{14}C-labelled sodium bicarbonate, protein hydrolysate and $^{45}CaCl_2$ are reported graphically in Figs. 1-4. Data from experiments with ^{14}C-labelled glucose, glycerol and alanine are essentially identical, and will not be treated further for this reason.

Analysis of A. cervicornis labelled at the tip (Fig. 1), shows that ^{14}C-labelled compounds (i.e., photosynthate, and protein hydrolysate) are confined to this region and those areas adjacent to it. They apparently are not transported more than 2-3 cm from the tip. Localization of $^{45}Ca^{++}$ in the skeleton is similar. However, it does not move appreciably when compared to the ^{14}C-labelled materials noted. Formalin-killed controls show no significant labelling or transport. Comparison with branches labelled at the distal end is dramatic. In these experiments an obvious movement of materials can be demonstrated (Fig. 2). ^{14}C-labelled compounds show a strong preferential movement towards the tip, and tend to accumulate there. $^{45}Ca^{++}$ in the skeleton behaves similarly.

Similar, and analogous results are obtained in experiments on the flat growth form of M. annularis (Figs. 3 and 4). Labelled compounds applied at the forward "growing" edge of plates move preferentially in the zone of maximal growth, and show little tendency to move towards the center of the plate (Fig. 3). Analysis of total DPM along each vector shows that both the direction and magnitude of flux is confined to the "growing" edge. Application of labelled materials 9 cm back from the forward "growing" edge (Fig. 4), provides further information on their transport into zones of maximal growth. Again, both direction and magnitude of movement is towards the forward edge.

Discussion

These experiments provide evidence for the existence of a system for intra-colonial transport of organic compounds and Ca^{++} in hermatypic corals. Both the direction and magnitude of movement favors transport towards zones of maximum growth and calcification in the species studied. These are regions which may be expected to have the highest apparent demand for metabolites and materials.

Transport of organic compounds within coral colonies is not surprising, and has been suggested in the work of others (3, 10). Data presented here provide support for this earlier speculation, although they do not provide insights into the transport mechanism itself. In contrast, Ca^{++} movement within colonies seems surprising, if not unnecessary. It is difficult to envision a requirement for Ca^{++} that would exceed sources of direct supply at or near the site of utilization. Nevertheless, the data indicate that Ca^{++} is transported at least 12 cm in M. annularis, and 10 cm in A. cervicornis. These are considerable distances, and they are indicative of an active mechanism satisfying demand for materials.

Intra-colonial transport of materials and metabolites provides a means of expanding the availability of resources, and concentrating their effect in limited zones of demand. In some respects the system may be modelled after a simple, linear food chain: a portion of the resource being utilized at the site of acquisition, and the remainder being passed on to the next cellular complement in the gradient leading to maximal demand. It should be possible to calculate rates of transfer and determine the energetics of the system on this basis. Such calculations would be an integral part of any carbon budget developed for a hermatypic coral and would provide information on the dynamics of growth within the colony.

Existence of transport, and the concept of an intra-colonial "food chain", with its suggestion of differential utilization of resources,

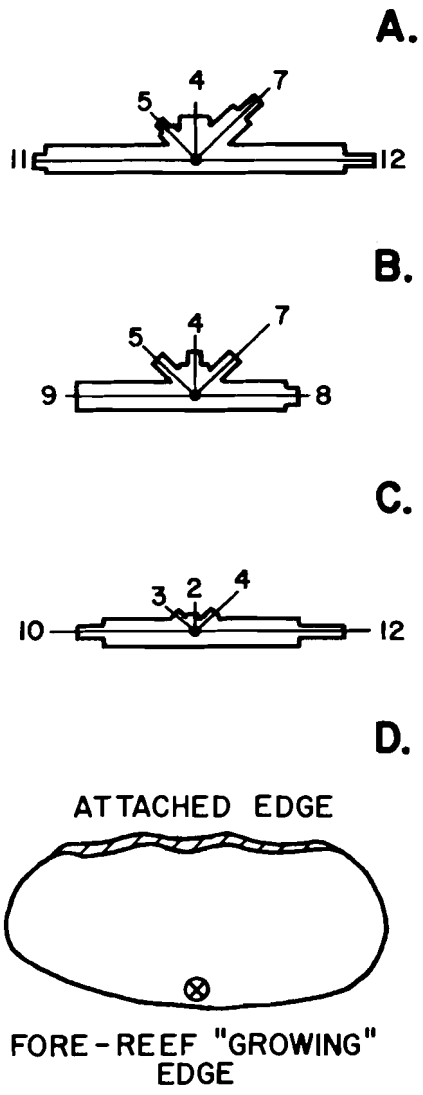

Figure 3. Labelling of M. annularis at the edge. A. $^{14}CO_3^{=}$ (2.6 μCi/ml final activity), B. ^{14}C-protein hydrolysate (2.7 μCi/ml final activity), C. $^{45}Ca^{++}$ (15 μCi/ml final activity). Closed circle indicates origin. Not less than 50 DPM ; 50-150 DPM ; greater than 150 DPM . D. Diagram of plate, ⊗ = origin. All distances in cm.

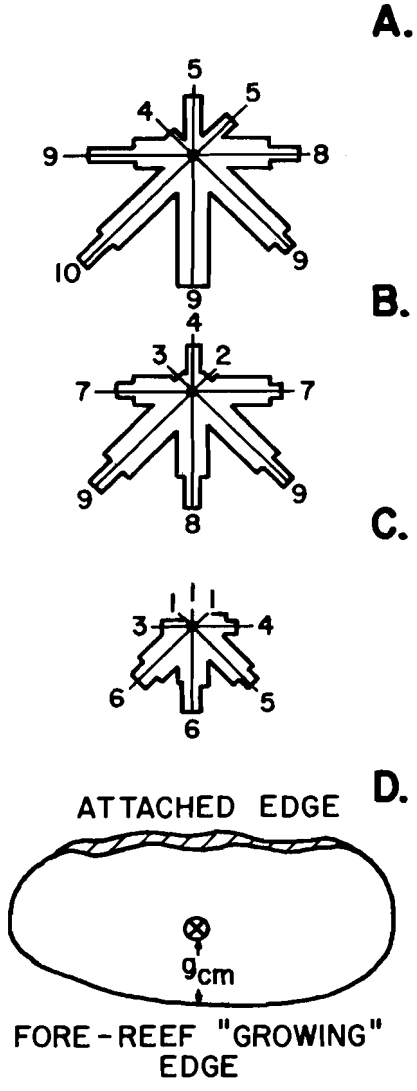

Figure 4. Labelling of M. annularis near center. See Figure 3 for description.

potential gradients of materials and metabolites and inter-cellular partitioning of resources, raises some serious questions about the way in which stable isotopes (e.g., oxygen and carbon) may find their way into the tissues and skeleton of hermatypic species. These questions have not been approached, yet the transport of organic compounds and inorganic ions may have lasting effects on the isotopic ratios observed, and their interpretation may be seriously effected.

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