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DIRECT EVIDENCE FOR THE TRANSFER OF MATERIALS FROM SYMBIOTIC ALGAE TO THE TISSUES OF A COELENTERATE

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This paper takes issue with a number of inferences that have been drawn on the subject of animal-algal relationships and seeks to resolve a question that seems not to have been answered well. Specifically, it deals with the subject of unicellular symbiotic algae and their bearing on the bioeconomy of the animals which they inhabit. No attempt will be made here to summarize the literature on the subject. We refer the reader to the reviews of Buchner[1] and Yonge[2] for historical details. Attempts to delineate the role of symbiotic algae from a nutritional standpoint have resulted in a wide range of conclusions. These are (1) the animal digests whole or fragmented algal cells; (2) nutritional substances may diffuse from algae to animal; (3) there is no nutritional role whatever on the part of the algae. These conclusions are not based on direct experimental evidence; rather, they are largely drawn from circumstantial or negative evidence.

Ignoring for the present time the other important features of a balanced mutualistic association such as the exchange of gases and minerals, we have investigated the possibility that the algae do have a nutritional role and have sought to demonstrate this with direct experimental evidence. These investigations received impetus from the recent studies on coral-algal relationships by Odum and Odum[3] at Eniwetok. Their conclusions stand out in sharp contrast with those drawn by Yonge and Nicholls[4] in their now classical studies carried out on the Great Barrier Reef. Yonge and Nicholls state that "there is no evidence whatsoever of any . . . transference of material from the plants to the tissues of the animal." Contrary to this, the Odums[3] have found that, unless the nutrition of corals is regarded as partly herbivorous, the trophic structure of the coral reef community cannot be resolved. In any event, neither opinion is supported by direct evidence. A further inference in support of the Odums' viewpoint can be drawn from such studies as those of Krogh, Lange, and Smith.[5] They find that certain algae may yield up to 10 per cent of their synthesized organic matter to the external medium, which, in the instance of the coral-algal association, would be the cells of the animal. Krogh, Lange, and Smith[5] also warn that losses of organic matter to the medium may be the result of dead and decaying algal cells. More recently, Allen[6] has shown that 10–45 per cent of the organic material formed by cultures of *Chlamydomonas* appears in soluble form in the culture medium.

In considering these opposing points of view, it seemed to us that, by the use of radioisotopes and standard autoradiographic techniques, it should be possible to

demonstrate rather conclusively whether or not symbiotic algae contribute to the nutrition of the host animal.[7] Our method of attack was to place animals with symbiotic algae in sea water containing radioactive carbon dioxide. Under illumination, the algae incorporated the labeled carbon into organic molecules, whose initial location and subsequent movement was determined by autoradiographs of sections of the animal. Animals kept in labeled sea water but in the dark served as controls and indicated to us, by the same techniques, any effects independent of the algae, such as animal fixation of the radiocarbon.

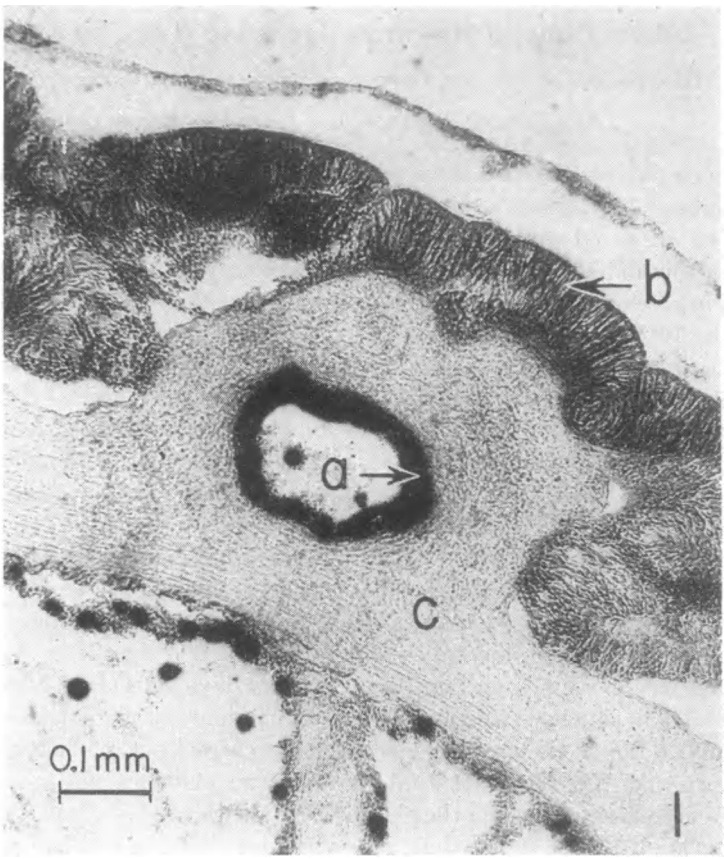

FIG. 1.—Photomicrograph of autoradiograph of section through verruca of anemone kept under illumination for 4 weeks in sea water labeled with $C^{14}O_2$. *a:* Gastrodermis and algal cells; *b:* outer epithelium; *c:* mesoglea. Photomicrographs focused to show both the section and the film exposures.

The animal chosen for this experiment was the intertidal sea anemone *Anthopleura elegantissima* (Brandt, 1835)—a small gregarious anemone which ranges from Alaska to Southern California and whose gastrodermal tissues abound with symbiotic, yellow-brown zoöxanthellae. Two millicuries of the isotope were obtained from Oak Ridge as $BaC^{14}O_3$, converted to $Na_2C^{14}O_3$, and thoroughly mixed with 2 liters of filtered, aerated sea water, resulting in a final isotopic concentration of 1 μc/ml. Twenty anemones were placed in separate 60-ml. containers of labeled sea water at 14°C; ten were held under constant illumination from two 40-watt fluorescent

tubes, and ten were kept in the dark. In both groups, five were left continuously in tagged sea water while the other five were removed after 48 hours and placed in "cold" sea water. A specimen from each group was then removed at the end of each week over a 5-week period. As a fixative, we used Carnoy's fluid to minimize the fogging effects on the film encountered with the use of aromatic fixatives. Carnoy's also dissolves out low-molecular-weight compounds, leaving only the larger organic molecules, thereby reducing the effects on the film from tagged atoms which may have diffused directly into the animal from the medium. Sections were cut

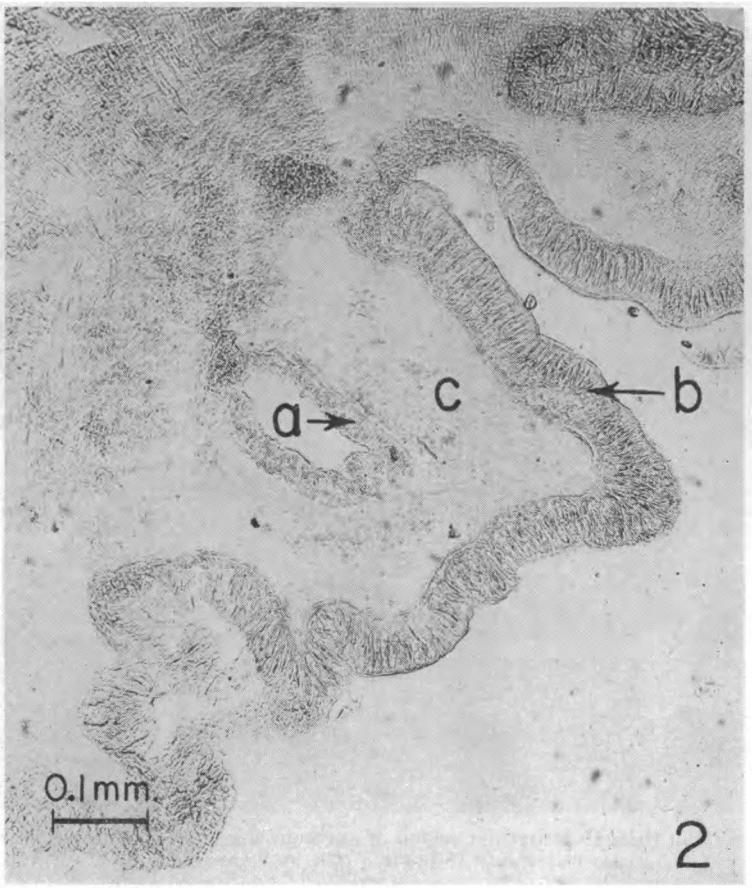

Fig. 2.—Similar section of dark control, 4 weeks. *a*, *b*, and *c* as in Fig. 1.

at 10 μ, autoradiographed with Kodak AR-10 stripping film (Kodak, Ltd., London, England), and allowed to expose the film for 9 days.

The presence of labeled material in the animal tissues of the illuminated anemones, as shown by autoradiographs of animals exposed to labeled sea water for 1–5 weeks indicated clearly that transfer from the algae had taken place. Effects independent of the algae were ruled out, in view of the absence of any tagged material in the dark controls, not withstanding some scattered exposures, not localized to any cells, which may have been the result of animal fixation. Previous experiments of shorter duration had shown that the algae incorporate enough labeled carbon over a period of 18 hours to produce a readable autoradiograph, but trans-

fer from algae to animal was not detectable. Figure 1 is a photomicrograph of an autoradiograph of a transverse section through a verruca on the column of an anemone kept in radioactive sea water for 4 weeks. The dark circular area represents exposures due to radiation from algal cells abundant in the gastrodermis. The light-gray area immediately surrounding it marks the mesoglea, only moderately labeled by comparison. The broad wavy band at the outer edge of the section delineates the outer epithelium. Judging from the density of photographic exposures, this tissue contains a moderate amount of tagged material. The thin black line beyond this represents labeled mucus. Figure 2 is a similar section of a dark control, previously described. Figure 3 is a section through the tentacular

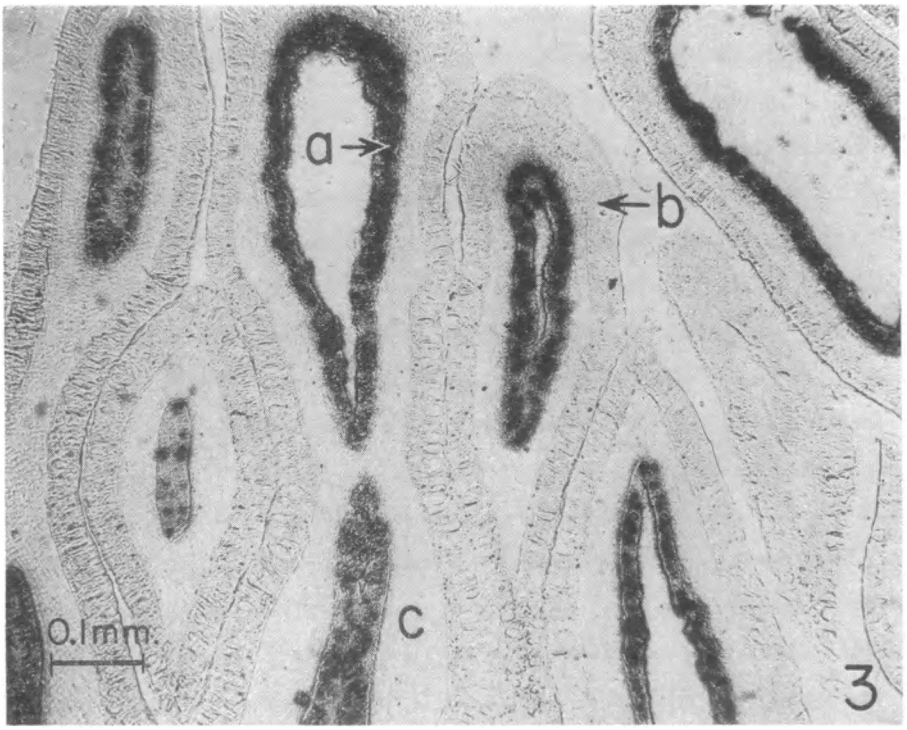

Fig. 3.—Section through tentacular region of anemone kept under illumination in labeled sea water only 18 hours. *a*, *b*, and *c* as in Fig. 1.

region of an anemone kept in labeled sea water for only 18 hours. The dark inner rings again mark the presence of algae in the gastrodermis which have incorporated labeled carbon. The relatively brief exposure time in sea water was sufficient only to effect transfer of tagged material to the gastrodermis. Mesoglea and outer epithelium are as yet unlabeled. Again, as in the 4-week dark controls, these latter tissues show scattered exposures, barely above the density of background fogging and probably due to animal fixation.

The evidence obtained from these experiments is consistent with the hypothesis that there is a transfer of material from these symbiotic algae to the host animal tissues. The probability that the same system exists in corals supports the Odums'[3] hypothesis that the algal-coelenterate complex is actually mutualistic. The recep-

tion of algal products at the higher-energy level more clearly defines the corals' role as partial herbivores. We wish to emphasize that "herbivore" is used here in the same sense as used by the Odums,[3] that of animal tissues receiving the products of algal symbionts by diffusion rather than by ingestion of particulate plant material. It should be recalled, however, that 94 per cent of the plant material in a coral colony of the type studied by the Odums[3] consists of filamentous green algae imbedded in the skeleton, the rest being zoöxanthellae. We do not wish to imply at this time, on the basis of our findings, that these filaments are involved in the same economic system; but if corals are dependent on plant symbionts for part of their nutrition, then the settlement of coral planulae in shallow, illuminated waters, the limitation of their growth to the upper photic zone, the positively phototactic orientation, and the invariable presence of zoöxanthellae in reef-building corals now appear to be more than a coincidence. Initiation, growth, and form of coral reefs must be the result of a long evolutionary history, during which adaptations favoring the association were selected for, in view of their survival value to the partners from a mutalistic nutritional standpoint.

Sea anemones with symbiotic algae have been exposed to sea water labeled with $C^{14}O_2$. By means of autoradiographs we have been able to show that after fixation of the labeled carbon by the algae there is a subsequent movement of labeled materials to all the tissues of the host anemone. In view of these results, it is suggested that the nutrition of anemones with symbiotic algae, and probably reef-building corals, is at least in part derived from materials passed to these animals as excesses of the photosynthetic activities of the symbiotic algae.

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