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Nutritional potentials in *Zoanthus sociathus* (Coelenterata, Anthozoa)*

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KURZFASSUNG: **Ernährungsmöglichkeiten bei *Zoanthus sociatus* (Coelenterata, Anthozoa).** Die Krustenanemone *Zoanthus sociatus* (ELLIS) lebt in Symbiose mit Dinoflagellaten der Gattung *Gymnodinium*. Feinstrukturelle, physiologische und biochemische Aspekte dieser symbiotischen Partnerschaft wurden untersucht. *Z. sociatus* zeichnet sich durch eine autotrophe Ernährungsweise aus, indem Photosyntheseprodukte der intrazellulär lebenden Zooxanthellen verwertet werden. Darüber hinaus ernährt sich *Z. sociatus* auch heterotroph durch die Aufnahme von Detritus, Bakterien und gelösten organischen Substanzen. Freiland- und Laboruntersuchungen erbrachten jedoch keine Hinweise, daß lebendes Zooplankton verwertet wird, obgleich in bezug auf das Verhalten gegenüber Nahrungsstoffen, die Ultrastruktur der Mesenterialfilamente und die Art der Verdauung keine wesentlichen Abweichungen gegenüber anderen planktonfressenden Coelenteraten festgestellt werden konnten. *Z. sociatus* nimmt offensichtlich – ebenso wie andere wirbellose Riffbewohner – eine polytrophe Stellung innerhalb des Ökosystems Korallenriff ein. Die Vielseitigkeit der Ernährungsweise wird unter dem Gesichtspunkt der ökologischen Stabilität des Lebensraumes Korallenriff erörtert.

INTRODUCTION

Zoanthids possess endosymbiotic dinoflagellates (zooxanthellae). They share this property with many other reef-dwelling coelenterates such as the hard and soft corals (Scleractinia and Alcyonacea) and the anemones (Actiniaria), as well as other invertebrate phyla, for example the giant clams (Lamellibranchia) (see DROOP 1963, MCLAUGHLIN & ZAHL 1966, MUSCATINE 1971). In a wide range of marine invertebrates harbouring photosynthetic endosymbionts, evidence points to the direct movement of photosynthetic products from the symbionts to the host's tissues, and subsequent utilization of these substances by the animals (see SMITH et al. 1969, MUSCATINE & CERNICHIARI 1969, TRENCH et al. 1969, TRENCH 1970, 1971a, b, c, GREENE 1970, GREENE & MUSCATINE 1972, LEWIS & SMITH 1971, MUSCATINE et al. 1972, TRENCH et al. 1970, 1972, GOREAU et al. 1973, TRENCH 1973, TRENCH et al. 1973b, 1974, TAYLOR 1973). Because of the direct utilization of photosynthetically derived organic matter, these associations can be viewed as having an autotrophic mode of nutrition.

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It is not necessary at this point to query whether primary production by the endosymbionts satisfies the total energy requirements of the host animals. That movement of photosynthetic products to the animals is accomplished, at least initially, without disruption of the endosymbionts, implies that the association does not represent an herbivore consuming its plant food. Functionally these "plant-animal" associations should be viewed as primary producers.

In the past, a great deal of controversy has been generated over the question of whether coelenterates, traditionally viewed as specialized carnivores (YONGE 1940), should be regarded as autotrophs because of the presence of symbiotic algae, or as heterotrophs (for recent review see GOREAU et al. 1971), since the animals retain their "normal" heterotrophic mode of nutrition. The implication has been that the two modes of nutrition are mutually exclusive. I would like to propose instead that organisms such as corals or other invertebrates with photosynthetic endosymbionts are nutritonally plastic, and may simultaneously occupy several trophic levels, deriving nutrients through a variety of mechanisms from several different sources. The relative amounts of the total nutrient requirements of the animals supplied by each different mode of nutrition is at present unresolved, and would probably be very difficult to quantify. The point is that these organisms possess an autotrophic mode of nutrition complementary to heterotrophic modes.

The relationship between *Zoanthus sociatus* and its flora of zooxanthellae has in the past been regarded as different from that demonstrated by corals (GOREAU 1964, GOREAU et al. 1971). This view was based on the following observations: (1) it could not be demonstrated that *Z. sociatus* showed any feeding response when offered homogenates of animal matter, e.g. crab, clam or lobster muscle, (2) the mesenteric filaments appeared to be undifferentiated and (3) the algae were not expelled by *Zoanthus* under stressful conditions that caused corals to expel their zooxanthellae (GOREAU 1964). In addition, the nematocysts in *Zoanthus* appeared to be non-functional (GOREAU, personal communication). The concept has been proposed then, that *Zoanthus,* like some of the xeniid alcyonarians, e.g. *Xenia* (ASHWORTH 1898, 1899, GOHAR 1940, 1948), are nutritionally more dependent on the photosynthetic products of the zooxanthellae than are corals; in the latter case the importance of the algae was thought to be involved in enhancing calcification (GOREAU 1961, 1963), or perhaps contributing trace substances or vitamins (GOREAU & GOREAU 1960).

VON HOLT & VON HOLT (1968a) provided evidence for the transfer of photosynthetic products from the zooxanthellae in *Zoanthus flos-marinus (= Z. sociatus)* to the animal, and found (VON HOLT & VON HOLT 1968b) that the isolated algae released a wide variety of ^{14}C-labelled metabolites when allowed to photosynthesize in vitro in $NaH^{14}CO_3$. However, it was not clear from their data to what extent the substances detected in the media were released through a selective process or through indiscriminate liberation resulting from cell lysis.

Although previous studies on *Z. sociatus* had been conducted as early as 1963 (GOREAU & TRENCH, unpublished), a re-investigation was begun in March, 1971, with the hope of clarifying some aspects of the feeding behaviour and of the biochemical interactions between the algae and the host animals. These preliminary experiments suggested that the animals could ingest material of animal origin. Indeed,

experiments by REIMER (1971a, b) showed that other species of *Zoanthus,* previously thought not to feed, demonstrated a characteristic feeding behaviour. Of course, that *Zoanthus* engulfed materials of animal origin did not demonstrate that such materials were digested and assimilated.

This study was undertaken in the hope of providing evidence on the possible different modes of obtaining nutrients potentially utilized by *Z. sociatus.* The feeding behaviour, and ability of the animals to digest and assimilate exogenously supplied protein were investigated, as well as their capacity to utilize exogenously supplied amino acids (^{3}H-leucine) and sugars (^{3}H-glucose). In addition, the movement of photosynthetic products from the algae in vivo and the release of photosynthetic products in vitro were reinvestigated.

The results of laboratory experiments show that *Z. sociatus* has the ability to derive nutrients in several distinct ways. The animals are able to utilize photosynthetic products from their zooxanthellae, they are able to absorb dissolved organic matter and to digest and assimilate exogenously supplied proteins. However, it remains unclear how much nutrition is procured by the latter two mechanisms under natural conditions, particularly since field and laboratory observations show that *Z. sociatus* continues to be totally unresponsive to live zooplankton or other potential living prey. What is apparent is that *Z. sociatus,* like many other reef-dwelling invertebrates with photosynthetic endosymbionts, possesses the ability to derive nutrion from several different sources, indicating a possible polytrophic status within the ecosystem.

MATERIALS AND METHODS

Collection and maintenance of specimens

Zoanthus sociatus (ELLIS 1767; Fig. 1) was collected from the reefs off Discovery Bay, Jamaica and from Whalebone Bay, Bermuda, from depths of 1.5 to 5 meters.

Since a certain degree of uncertainty exists on the taxonomic identity of the animals, a short discussion of the problem is probably justified. Zoanthid taxonomy is currently in a state of chaos. Y. NEUMANN (personal communication) has suggested that *Z. sociatus* (E.) and *Z. flos-marinus* (DUCHASSAING & MICHELOTTI) are synonymous. I have found no major distinguishing characters between these two and *Z. proteus* (VERRILL). I have therefore selected to treat the zoanthids collected from Jamaica and Bermuda as identical. This is justified since results of the same experiments carried out on the two groups of animals were essentially the same.

Specimens used for experimental work were maintained in running sea water aquaria for not more than one week. Some specimens which were used for either histology and histochemistry or electron microscopy were sometimes kept for longer periods before fixation. Animals used in experiments were always in a healthy state as indicated by their tactile responses. In experiments involving incubations with isotopes, only specimens which remained fully expanded during the "pulse" period of labelling were selected for the continued "chase" experiments.

Animals which were to be used in feeding experiments were maintained for a

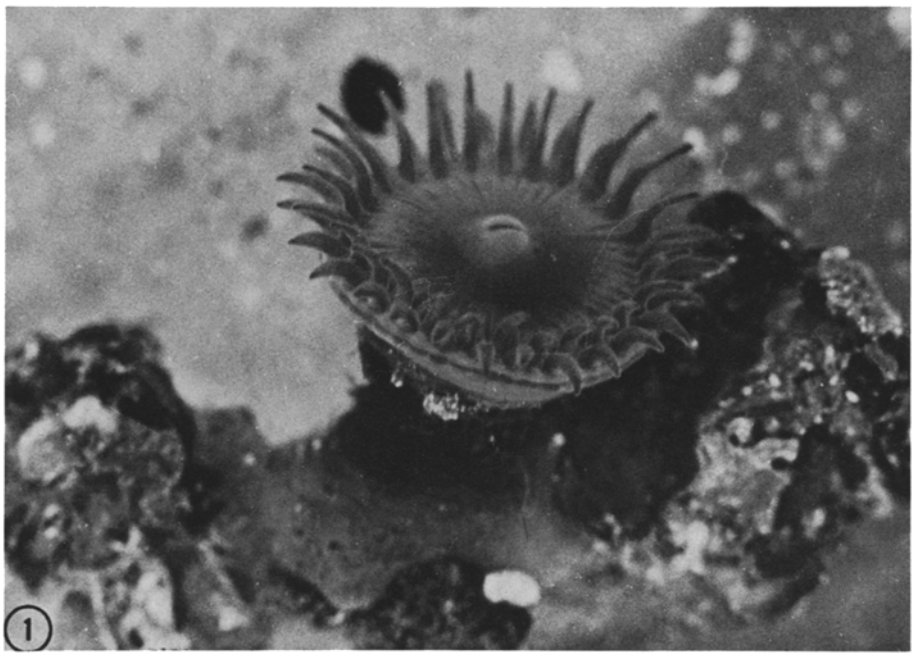

Fig. 1: Underwater photograph of *Zoanthus sociatus* taken at 3 meters on the reef at Discovery Bay, Jamaica. (1.6:1)

minimum of 72 hours before experiments were begun, in closed aquaria under aeration in Millipore-filtered (porosity 0.25 μm) sea water, to offset the slightest possibility of feeding. It subsequently became evident that this precaution was unnecessary.

Experimental

Pulse-chase experiments with $NaH^{14}CO_3$ in vivo

Specimens were incubated in finger bowls (4 inch diameter) containing about 250 ml Millipore-filtered (porosity 0.25 μm) sea water. The animals were allowed to aclimatize for about two hours before $NaH^{14}CO_3$ was added, usually to a concentration of 1 to 5 μC/ml, depending on the experiment. Pulse incubations were conducted for from thirty minutes to four hours depending on the nature of the experiment. Temperature was 24–25° C and light intensity 1×10^5 ergs cm^{-2} sec^{-1}. In all experiments, dark-incubated and DCMU-treated (3-[3,4-dichlorophenyl]-1,1-dimethyl urea, 5×10^{-5}M) controls were included (see VANDERMEULEN et al. 1972). After the initial incubation period, the animals were transferred to running sea water aquaria under Grolux fluorescent lights (0.5×10^5 ergs cm^{-2} sec^{-1}) until they were fixed for autoradiographic analysis.

Isolation and incubation of zooxanthellae in vitro

Algal cells were isolated from *Zoanthus sociatus* by homogenization of the animals (after removal of the cuticle by dissection) with a Virtis blender, followed by filtration through bolting silk and centrifugation at 500 xg for 20 minutes (TRENCH 1971b). Suspensions of algae (2×10^6 cells/ml) were prepared in the homogenate of animal tissue recovered after centrifugation of the algae (TRENCH 1971b, c, MUSCATINE et al. 1972). To 2 ml algal suspension, $NaH^{14}CO_3$ was added to give an initial activity of 25 μC/ml. Incubations were conducted in stoppered 10 ml erlenmyer flasks at 23–24° C, with an illumination of 5×10^4 ergs cm^{-2} sec^{-1} delivered by Grolux fluorescent tubes.

Suspensions were incubated for 30 minutes to 2 hours. At the end of the incubation period, the cells were centrifuged at 1000 xg for 10 minutes at 10° C and the supernatant medium withdrawn and frozen. The pellet was extracted in 2 ml boiling 80 % ethanol, and extract and debris were stored at – 20° C until analysed.

Identification of photosynthetic products of zooxanthellae

The media in which zooxanthellae were incubated were evaporated to dryness in a Buchler Evapomix under reduced pressure at 40° C. Organic compounds were recovered from the salt by extraction with absolute ethanol followed by chloroform, accounting for about 97 % of the ^{14}C present.

The radioactive compounds in the cell extracts and from the media were separated by two dimensional paper partition radiochromatography. Labelled compounds were then eluted from the paper chromatograms and rechromatographed along with authentic standards either on Brinkman precoated Silica Gel plates by ascending chromatography or by one dimensional descending paper chromatography (see TRENCH 1971b for details).

Light microscopic autoradiography

After incubating animals in solutions containing the different isotopes, they were fixed in Bouin's solution for 24 hrs. Specimens were dehydrated with methyl cellosolve (ethylene glycol monomethyl ether), cleared in methyl benzoate and benzene and embedded in vacuo in paraplast. Sections were cut at 7 μm in the case of specimens incubated with ^{14}C and at 4 μm in the case of those incubated with ^{3}H.

Radioactive tissues were exposed to Kodak AR-10 stripping film or Kodak NET-2 liquid emulsion for from 10 to 30 days, developed in Kodak D-19 developer and fixed in Kodak rapid fixer (see ROGERS 1969). Tissues were stained through the emulsion with toluidine blue to enhance contrast for photography.

Electron microscopy

For routine electron microscopy, tissues were fixed in cold (3° C) cacodylate buffered (pH 7.4) glutaraldehyde (4 %) made iso-osmotic to sea water with glucose,

for 6 hours, washed overnight in buffered glucose (pH 7.4) and post fixed in 2 % OsO_4 for two hours at 3° C. Tissues were dehydrated through serial changes of ethanol and embedded in either araldite or epon (KAY 1965).

Ultrathin sections were prepared on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, viewed and photographed with a JEM-6C electron microscope.

In order to detect histochemically the presence of phosphomonoesterase II (acid phosphatase) in animal tissues, the tissues in question were dissected out under cold fixative and subsequently fixed for 6 hours at 3° C, washed overnight in cacodylate buffer (pH 7.2) and then separated into three groups:

Group I. Experimental: Tissues were incubated in fresh-filtered Gomori (1950) medium at 37° C for two to three hours.

Group II. NaF control: Tissues were incubated as above, but NaF (enzyme inhibitor) was added to a final concentration of 0.5 % (see FANKBONNER 1971).

Group III. Substrate control: Tissues were incubated as in I above but in Gomori medium lacking β-glycerophosphate.

After incubation, the tissues were washed in acetate buffer (pH 5.0, 30 min), acetic acid (5 min) and then cacodylate buffer (pH 7.4, 15 min), followed by post osmication, dehydration and embedding as described above. These tissues were viewed and photographed without staining.

Feeding experiments

Feeding behaviour

In order to investigate the response of *Zoanthus sociatus* to live prey, suspensions of freshly hatched *Artemia salina* nauplii or recently captured zooplankton, were pipetted into a finger bowl containing 5 or 10 polyps. The behaviour of the animals was observed with a dissecting microscope, either with a microscope lamp (which tended to concentrate the *Artemia* or the zooplankters in the path of the beam) or in a room with subdued diffuse lighting. In several cases these experiments were continued for up to 6 hours in order to enhance the possibility that organic compounds diffusing from the zooplankton may influence a feeding response by lowering the threshold for nematocyst discharge.

In addition to intact organisms, experiments were conducted using fresh suspensions of tissue from a range of invertebrates. In each case the tissue in question was homogenized in a glass tissue grinder and aliquots of the suspension were pipetted on to the surface of the oral disc, care being taken not to disturb the animals mechanically during this process.

In order to readily follow food particles into the stomodeaum, some suspensions were mixed with india ink, and offered to the animals. After predetermined periods, the animals were fixed in Bouin's solution for subsequent histological examination. Controls offered india ink alone were always included in these experiments, but the india ink was always rejected.

Attempts were also made to investigate the effect of reduced glutathione (GSH) (see LENHOFF 1968a, b, LINDSTEDT 1971, REIMER 1971a) in eliciting a feeding response in *Z. sociatus*. Specimens were placed in feshly prepared solutions of GSH ranging from 10^{-6} to 10^{-2}M kept in the reduced state with dithioerythritol (10^{-6}M) and observed under a dissecting microscope. In addition, pieces of filter paper imbibed with different concentrations of GSH were placed on the oral discs and the animal's response observed. Other substances tested were glycine, proline, alanine, aspartic and glutamic acids.

Preparation of ^{35}S and ^{3}H-protein

Protein labelled with ^{35}S or ^{3}H was prepared using the method of COWIE et al. (1952). *Escherichia coli* (B) were grown in media in which $Na_2{}^{35}SO_4$ (New England Nuclear) was the sole form of sulphur, in order to produce high specific activity ^{35}S-protein, or on nitrogen deficient media to which ^{3}H-leucine (New England Nuclear) was added.

After attaining stationary growth, the bacteria were pelleted by centrifugation and extracted in 80 % ethanol. A portion of the ethanol insoluble residue was hydro-

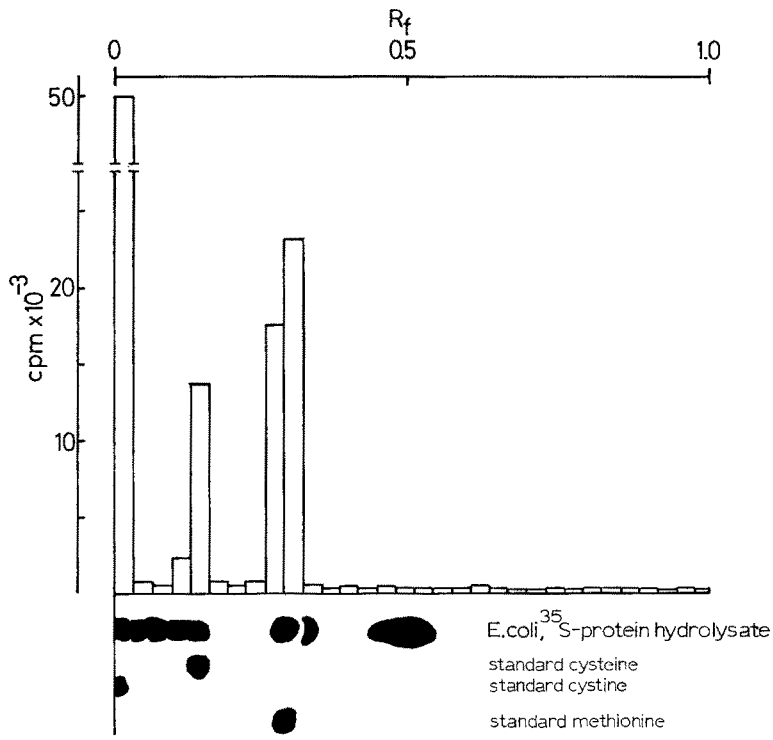

Fig. 2: Distribution of ^{35}S in amino acids derived from 6N HCl hydrolysis of proteins from *Escherichia coli* grown on $Na_2{}^{35}SO_4$

lysed with 6N HC1 in sealed tubes at 100° C, and the hydrolysis products separated by ascending thin layer chromatography using Eastman Kodak Cellulose strips and n-butanol:propionic acid:water (142:71:100 v/v) as solvent system. The gel was scraped from the strips at 0.5 cm intervals into scintillation vials, and the radioactivity measured using Aquasol scintillation fluid (New England Nuclear) and an Intertechnique SL-30 liquid scintillation spectrometer. Standards run along with the hydrolysates were visualized with ninhydrin.

Figure 2 shows that the ^{35}S was incorporated into the bacterial protein as cystine, cysteine and methionine, while Figure 3 shows that the ^{3}H was incorporated as ^{3}H-leucine. The dried powdered bacterial proteins were stored in a vacuum dessicator over P_2O_5 at – 20° C until used.

Digestion and assimilation experiments

Specimens were fed aliquots of homogenates of *Echinometra* eggs mixed with either ^{35}S or ^{3}H-protein. At intervals of up to 48 hours after feeding, specimens were sampled in duplicate. In the case of those experiments conducted with ^{35}S-protein,

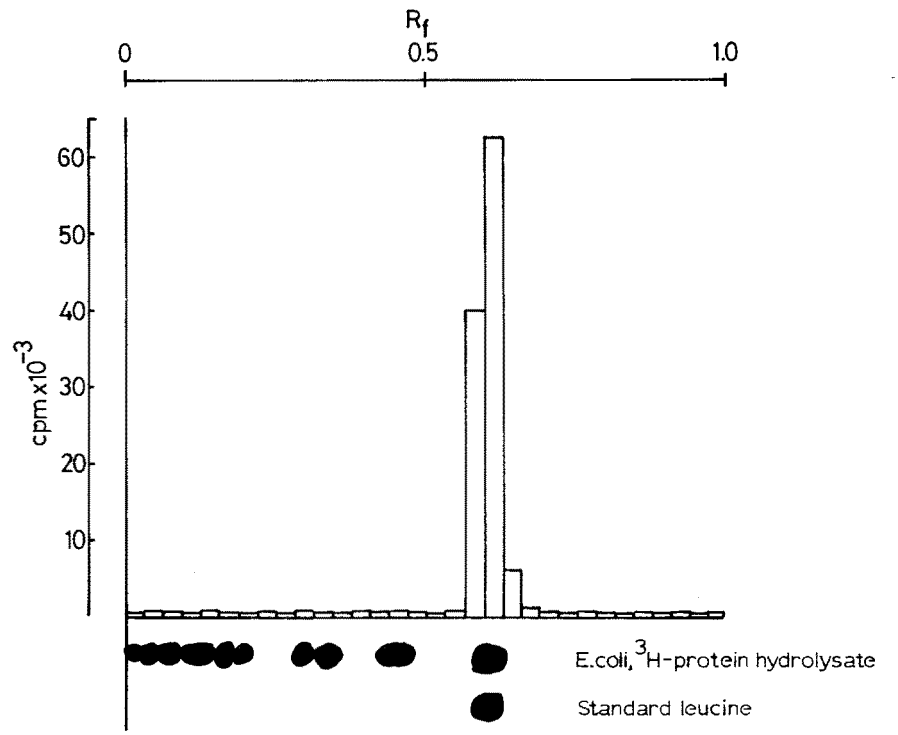

Fig. 3: Distribution of ^{3}H in amino acids derived from 6N HCl hydrolysis of proteins from *Escherichia coli* grown on ^{3}H-leucine

the animals were homogenized in cold distilled water and cold trichloroacetic acid (TCA) added to a final concentration of 10 % to precipitate protein. The precipitate was then collected on Millipore filter discs (porosity 0.25 μm), and an aliquot of the filtrate recovered. The radioactivity in each was measured and the percentage TCA-soluble ^{35}S was calculated.

In the case of experiments conducted using ^{3}H-protein, the animals were fixed in Bouin's fixative and subsequently analysed by autoradiography. Combination of the two methods provides an estimate of the rates of digestion and assimilation as well as the morphological sites for assimilation and subsequent dispersal of assimilated food throughout the animal.

Pulse-chase experiments with ^{3}H-leucine and ^{3}H-glucose

To investigate the potential of *Zoanthus sociatus* for uptake of dissolved organic matter from solution, specimens were incubated in Millipore filtered (porosity 0.25 μm) sea water to which ^{3}H-leucine or ^{3}H-glucose (New England Nuclear) were added, for up to 4 hours, and then transferred to isotope free media for chase periods of up to 14 days. Animals were sampled, fixed in Bouin's fluid and analysed by autoradiography.

RESULTS

The photosynthetic component

The morphology of the association of Zoanthus sociatus with zooxanthellae

The zooxanthellae in *Zoanthus sociatus* are located exclusively in the gastrodermal cell layer (Figs 4–6). This is similar to the situation found in corals, alcyonarians and sea anemones (see DROOP 1963, MCLAUGHLIN & ZAHL 1966, TRENCH 1971a), but is in contrast to that found in some other zoanthids, e.g. *Protopalythoa grandis, Palythoa caribbea, Palythoa* sp. and *Isaurus* sp. which have zooxanthellae in the epidermis and in gastrodermal cells lining the lacunae of the mesoglea as well (TRENCH 1969, 1971, HERBERTS 1970).

In *Z. sociatus* the highest concentration of algae is found in the tentacles and the oral disc. However, zooxanthellae can also be found in the cells of the gastroderm of the column, and in the "hypertrophied" gastroderm cells of the mesenteries proximal to the mesenteric filaments, where "degenerate-appearing" (pycnotic) zooxanthellae are especially abundant (Fig. 19).

The ultrastructure of the phycobionts

The zooxanthellae are intracellular (Figs 4–8) as determined by light and electron microscopy combined with tissue masceration techniques. They divide by binary fission

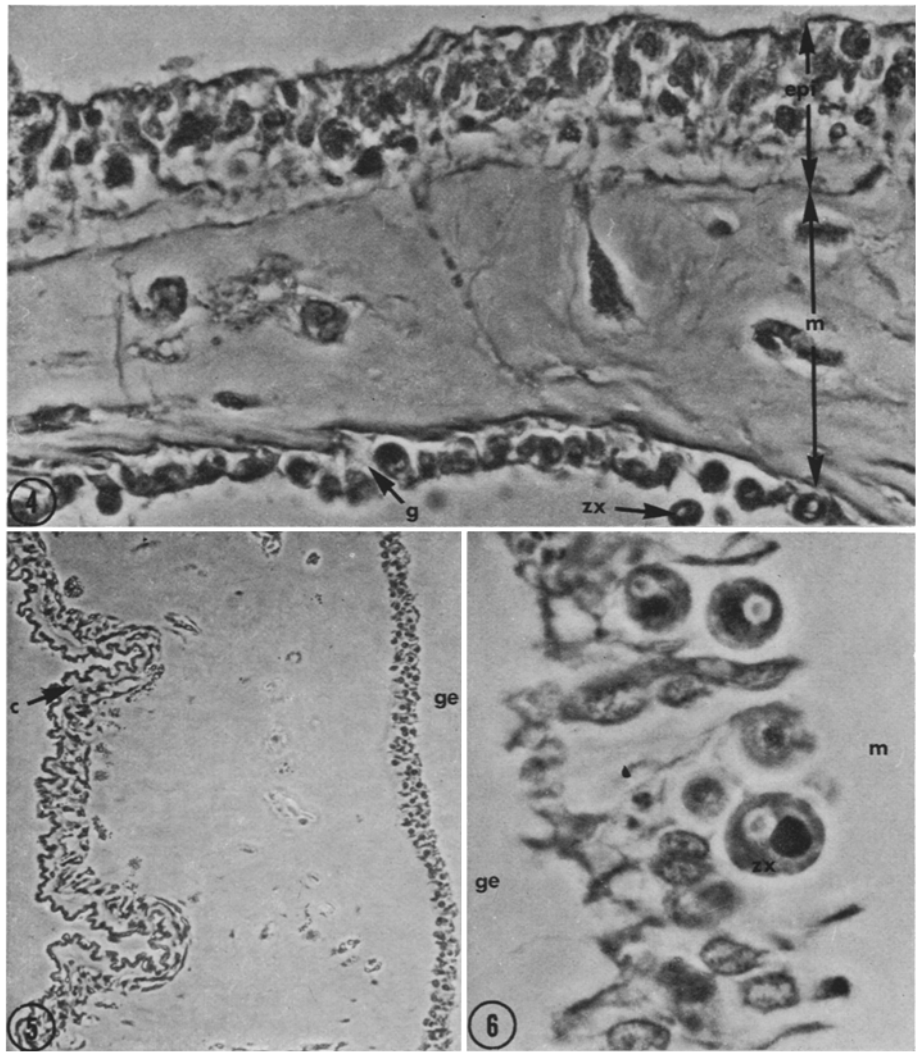

Fig. 4: Light micrograph of the oral disc of *Zoanthus sociatus*. The epithelium is ciliated and electron microscopy shows the presence of extensive microvillation. epi = epidermis; m = mesoglea; g = gastroderm; zx = zooxanthella. (660:1)

Fig. 5: Light micrograph of the body wall of *Zoanthus sociatus*. c = cuticle; between the cuticle and the very thick mesoglea is a very thin epidermis. ge = gastroenteron, boardered by the gastroderm. (130:1)

Fig. 6: Light micrograph of the gastroderm cells of *Zoanthus sociatus* containing zooxanthellae. m = mesoglea; ge = gastroenteron. (1500:1)

(Fig. 8). The algae show typical gymnodinioid morphology (TAYLOR 1968, 1969c, KEVIN et al. 1969) and, based on morphology alone, appears to be *Gymnodinium microadriaticum* (F).

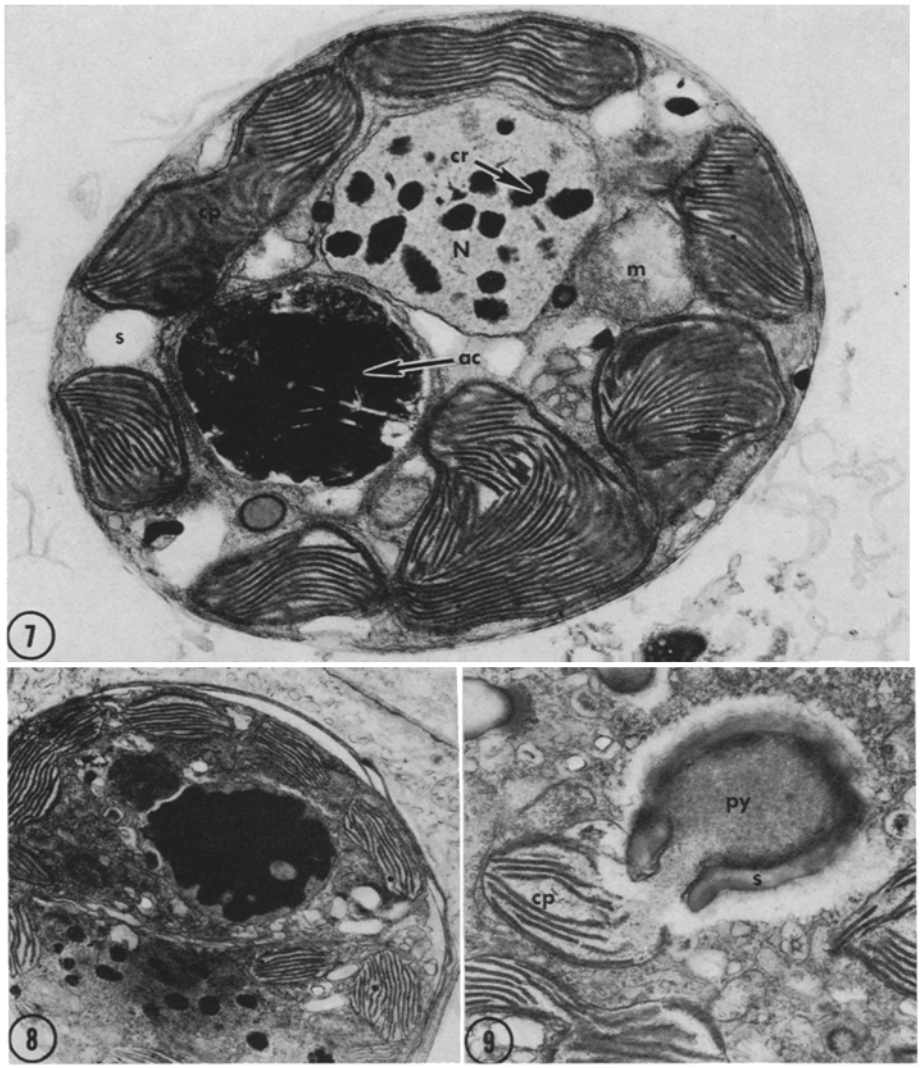

Fig. 7: Electron micrograph of *Gymnodinium microadriaticum* (?) in a gastrodermal cell in the tentacle of *Zoanthus sociatus*. N = nucleus; cr = polytene chromosomes; m = mitochondrion; cp = chloroplast; s = starch; ac = "accumulation body". (12 250:1)

Fig. 8: Electron micrograph of a dividing zooxanthella in a gastroderm cell of the oral disc of *Zoanthus sociatus*. (6700:1)

Fig. 9: The single-stalked pyrenoid of a zooxanthella in *Zoanthus sociatus*. py = pyrenoid; cp = chloroplast; s = starch cap. Magnification (15 500:1)

Figure 7 shows the general plan of the phycobiont. The reticulate chloroplast is peripherally located, contains thylakoids in groups of three (Figs 10, 12) and is bounded by an envelope comprised of three unit membranes (Fig. 10). No connections

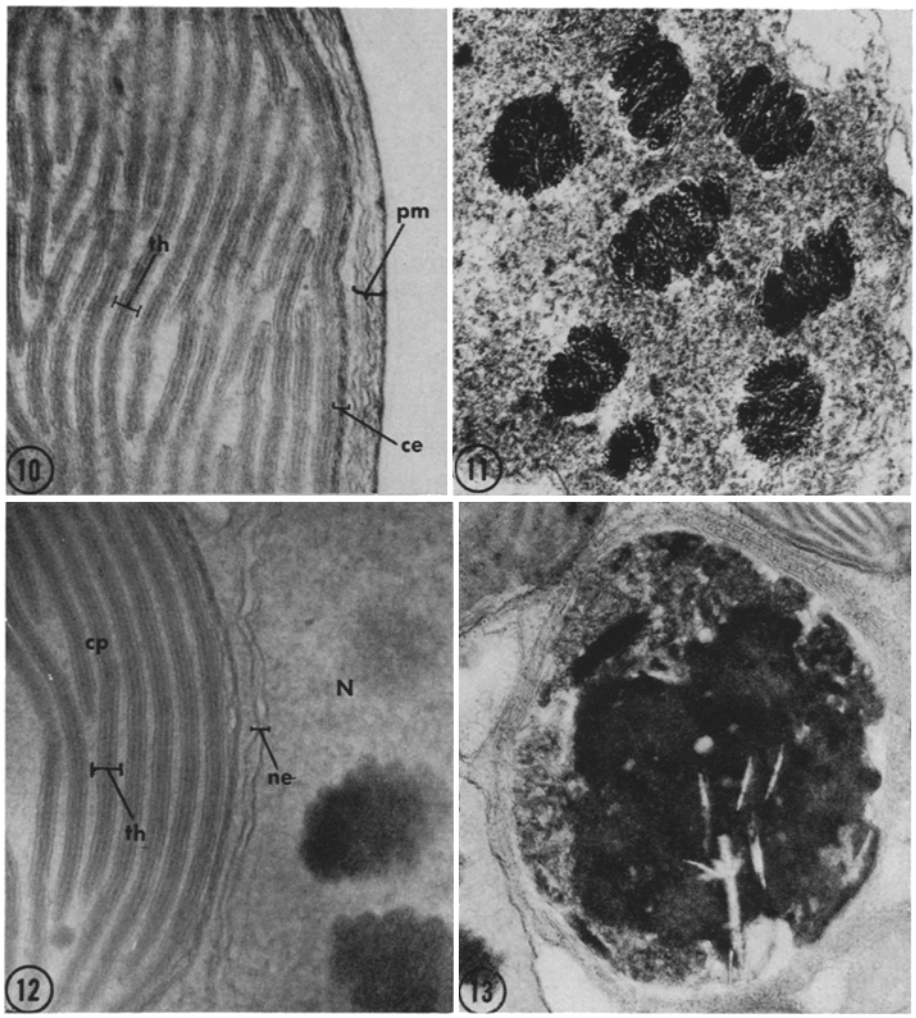

Fig. 10: The periplast membrane system (pm) composed of five unit membranes, the chloroplast envelope (ce) composed of three unit membranes, and chloroplast thylakoids (th) in groups of three. (33 500:1)

Fig. 11: The nucleus of a zooxanthella showing the polytene chromosomes. OsO_4 fixed. (27 500:1)

Fig. 12: The chloroplast (cp) and nucleus (N) of a zooxanthella. The nuclear envelope (ne) is composed of two unit membranes. Th = thylakoids. (28 000:1)

Fig. 13: The "accumulation body" of a zooxanthella in *Zoanthus sociatus*. Note the abundance of membranes and "crystalline-appearing" particles. (20 650:1)

between chloroplast and nuclear envelopes have been observed (cf. TAYLOR 1969c). Evidence suggests a single reticulate mitochondrion. The nucleus is large, bounded by a double nuclear membrane (Fig. 12) and contains condensed chromatin (polytene

chromosomes) (Fig. 11), a characteristic feature of dinoflagellate nuclei (DODGE 1966, 1971). A prominent granular nucleolus has also been observed. The pyrenoid (Fig 9) is vase shaped and its stroma is continuous with that of the chloroplast to which it is attached, most often at a single point, but frequently with double attachments (see TAYLOR 1968). No pyrenoid lamellae are present. In addition to the starch cap associated with the pyrenoid, several free starch grains are distributed through the cytoplasm (Fig. 7). Each alga is bounded by a system of 5 or 6 unit membranes, the periplast membrane system (Fig. 10), which can more readily be discerned during division (see TAYLOR 1968). The major inclusion is the "accumulation" body (Fig. 13) which appears to be much more complex than has heretofore been recognized, containing an abundance of smooth endoplasmic reticulum and other unrecognizable structures. The precise functions of this organelle remain obscure, although accumulation of waste (TAYLOR 1969c) and storage of material for subsequent reassimilation (SCHMITTER 1971) have been proposed.

Transfer of photosynthetic products from algae to hosts in vivo

When specimens of *Zoanthus sociatus* were incubated in $H^{14}CO_3$ in the light and analysed for incorporation of ^{14}C by tissue autoradiography, it readily became apparent that incorporation occurred in two distinct zones, (a) in the gastrodermis where the zooxanthellae are abundant and (b) on the surface of the cuticle (Fig. 14).

Radioactivity associated with the zooxanthellae was high even after very brief "pulse" exposures of the animals to $H^{14}CO_3$ in the light (see Fig. 18). Control incubations conducted in the dark, or in the light in the presence of DCMU, showed that non-photosynthetic fixation of ^{14}C by zooxanthellae was negligible (Fig. 17).

During "chase" experiments, the radioactivity was found to diffuse to different regions of the polyps, including those regions towards the base of the coelenteron, where very few zooxanthellae are found, and within 48 hours could be found associated with the cuticle, particularly that portion of the cuticle juxtaposed to the epidermis. This process is very rapid, and does not have any correlation with breakdown, degeneration or digestion of zooxanthellae. In the hypertrophied regions of the mesenteries, no algae incorporated ^{14}C during the "pulse" labelling period (Fig. 19, see also p. 202).

It was surprising to find very intense radioactivity associated with the cuticle after short exposure of animals to ^{14}C in the light (Fig. 15). The cuticle is a structure (a glycoprotein) which lies exterior to the epidermis and is secreted by the epidermis using substrates derived from zooxanthellar photosynthesis (TRENCH 1970). Electron microscopy revealed the presence of aggregates of bacteria (Fig. 16) which appeared to be embedded in some form of gelatenous matrix on the surface of the cuticle. Epizoic blue-green algae are also present. The fixation of ^{14}C by the epizoic bacteria and blue-green algae is photosynthetic, since no evidence for fixation was observed during dark incubations or incubations in the light in the presence of DCMU. It was impossible to determine whether photosynthetically fixed ^{14}C moved from the bacteria or blue-green algae to the animals.

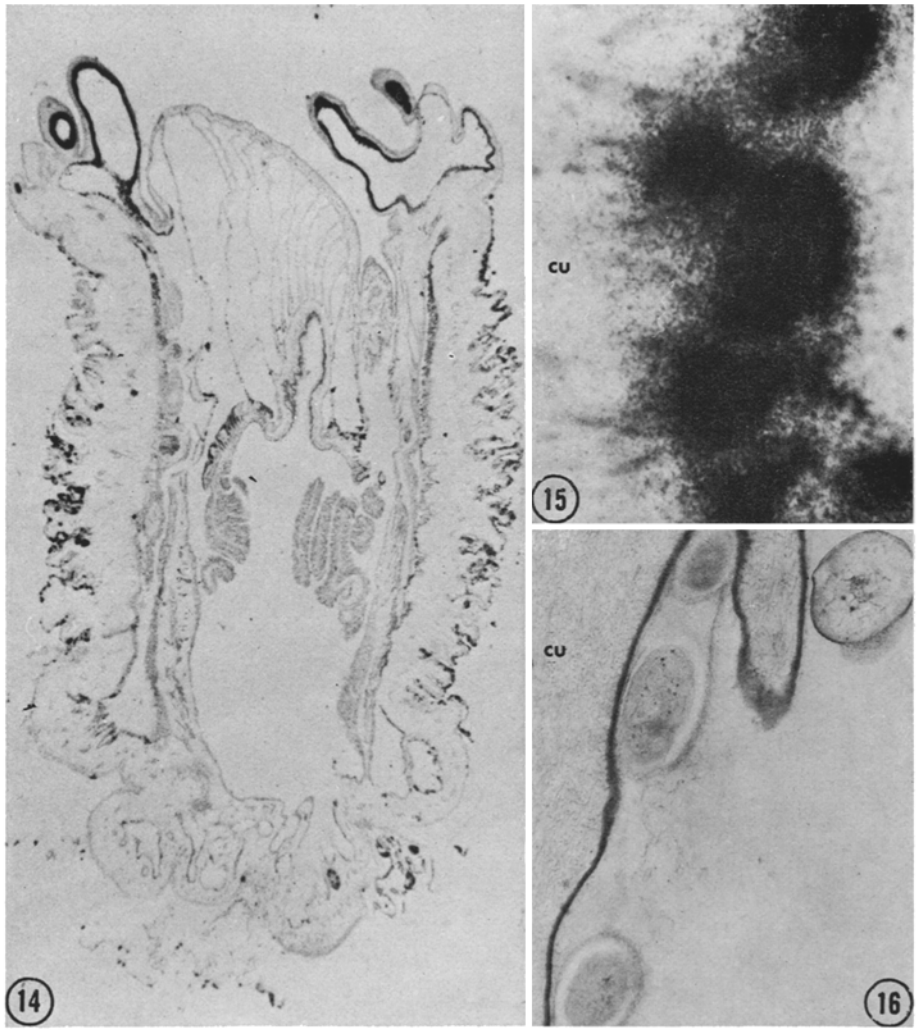

Fig. 14: Light micrograph of an autoradiograph of *Zoanthus sociatus* after incubation in sea water containing $NaH^{14}CO_3$ in the light for 45 minutes. Note the presence of intense labelling over the gastroderm of the tentacles and oral disc as well as over the surface of the cuticle. Magnification (3.3:1)

Fig. 15: Light micrograph of an autoradiograph of the cuticle of *Zoanthus sociatus* showing intense labelling after 60 minutes incubation in $NaH^{14}CO_3$ in the light. cu = cuticle. Magnification (4660:1)

Fig. 16: Electron micrograph of the cuticle of *Zoanthus showing* the bacteria associated with it. These organisms are partly responsible for the intense labelling associated with the cuticle shown in Figure 15. (14 600:1)

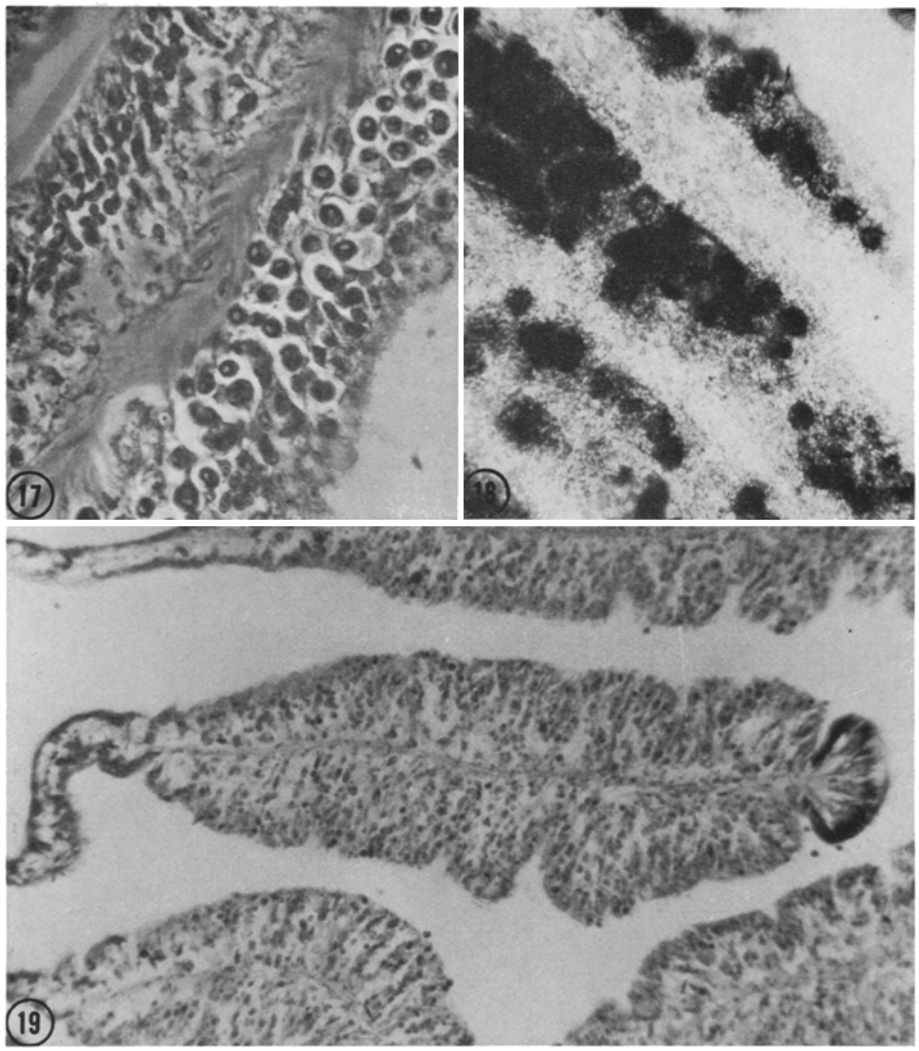

Fig. 17: Light micrograph of an autoradiograph of *Zoanthus sociatus* incubated in the light in the presence of $NaH^{14}CO_3$, and DCMU (5×10^{-5}M). Note the absence of silver grains over zooxanthellae. Tissue stained with toluidine blue through the emulsion. (960:1)

Fig. 18: Light micrograph of an autoradiograph of *Zoanthus sociatus* incubated in $NaH^{14}CO_3$ in the light for 4 hrs. Tissue unstained. (960:1)

Fig. 19: Light micrograph of an autoradiograph of mesentery of *Zoanthus sociatus* incubated in $NaH^{14}CO_3$ in the light for 4 hrs. Tissue stained through the emulsion with toluidine blue. Note the absence of silver grains over the tissue and over the pycnotic zooxanthellae contained there in. (800:1)

Since the results obtained through the use of the autoradiographic technique were only qualitative, a study was conducted in order to quantify the level of translocation between the zooxanthellae and the animal hosts. The technique used was essentially that described by TRENCH (1971a). The epizoic photosynthetic bacteria and blue-green algae on the cuticle of *Z. sociatus* initially caused very erratic results. The reason was that it was not possible to separate the bacteria and blue-green algae from the host

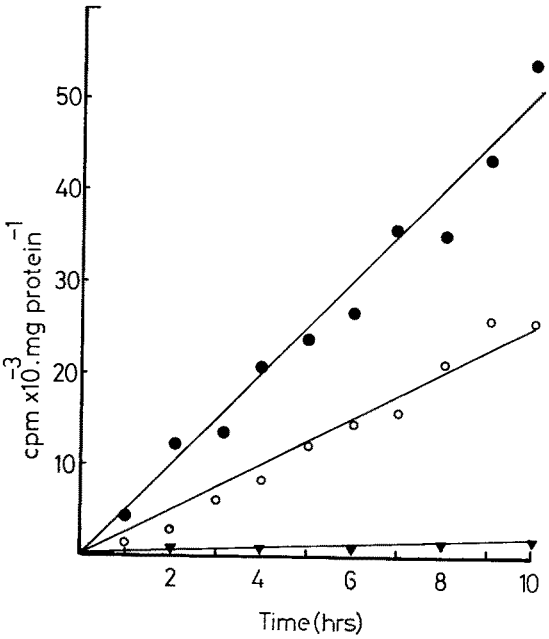

Fig. 20: Photosynthetic fixation and translocation of ^{14}C from zooxanthellae to animal tissues in *Zoanthus sociatus*. ●——●, ^{14}C in algae; ○——○, ^{14}C in animal tissues; ▼——▼, ^{14}C in whole DCMU-treated controls

tissue during centrifugation, and observation of the algal pellet showed varying amounts of contamination with bacterial aggregates and filaments of blue-green algae. It was obvious therefore that epizoic photosynthetic ^{14}C was contributing to both "zooxanthellar" and "host" fractions in a non-reproducible manner. I therefore decided to first treat the animals with an antiboitic mixture*. Autoradiographic analysis showed that after six hours of pre-treatment the antibiotics did not eliminate the bacteria nor the blue-green algae, but did eliminate their photosynthesis without affecting that of the endozoic zooxanthellae. Antiboitic treated specimens were therefore used in subsequent experiments.

Antibiotic-treated animals were incubated in filtered sea water containing $NaH^{14}CO_3$ for up to 10 h. Control specimens were incubated in the presence of

* Antibiotic mixture (mg/50 ml) – polymixin, 1.2; streptomycin, 330; tetracycline, 13; pennicillin, 150; neomycin, 20; erythromycin, 100.

DCMU. Sample specimens were withdrawn at hourly intervals and homogenized in a Virtis blender. The algae and animal tissues were separated by sucrose density gradient centrifugation (see FRANKER 1971). The supernatant was regarded as animal cell debris. The radioactivity in the algae and in the animal tissues was measured by liquid scintillation spectrometry. Protein was determined by the method of LOWRY et al. (1951).

Figure 20 shows that the rate of net ^{14}C fixation by the experimental organisms was essentially constant throughout the experiment. DCMU-treated organisms incorporated very low levels of ^{14}C. The proportion of photosynthetically fixed ^{14}C translocated to the animals under these conditions was 36–41 %.

The distribution of ^{14}C in hot 80 % ethanol soluble and insoluble fractions in algae and in animal tissues (see Table 1), shows that in the algae, the ratio of soluble to insoluble ^{14}C remained relatively constant, while in the animal tissues the ratio

Table 1

Assimilation of photosynthetic ^{14}C from zooxanthellae by *Zoanthus sociatus*

Incubation time (hrs)	% ^{14}C translocated	ethanol-soluble / ethanol-insoluble ^{14}C in algae	animal
1	38	1.6	3.5
3	41	1.8	2.9
5	39	1.5	2.4
7	36	1.7	1.6
10	37	1.8	0.7

decreased. This decrease is a result of the increase in the insoluble ^{14}C in the animal cells. No estimate of the loss of $^{14}CO_2$ through animal respiration was made. The major insoluble ^{14}C in the animal appears to be protein or muco-protein (see MUSCATINE & CERNICHIARI 1969, TRENCH 1971a). These data demonstrate that photosynthetic ^{14}C from the algae is incorporated by the animal hosts (see also VON HOLT & VON HOLT 1968a).

Release of photosynthetic products by zooxanthellae in vitro

When incubated in vitro in the presence of an homogenate of animal tissues, zooxanthellae from *Z. sociatus* fixed ^{14}C photosynthetically at a constant rate. These algae also released organic ^{14}C to the incubation media at a constant rate, representing 45–48 % of the total ^{14}C fixed. These results are similar to those obtained in previous studies on zooxanthellae from other coelenterates (see MUSCATINE 1967, MUSCATINE & CERNICHIARI 1969, MUSCATINE et al. 1972, TRENCH 1971b, c).

Analysis of the media (Fig. 21) showed that ^{14}C was incorporated principally into glycerol and glucose, with less incorporation into leucine, alanine, glycolic acid and three other unidentified compounds. These results are similar to those reported by MUSCATINE (1967), MUSCATINE & CERNICHIARI (1969), MUSCATINE et al. (1972)

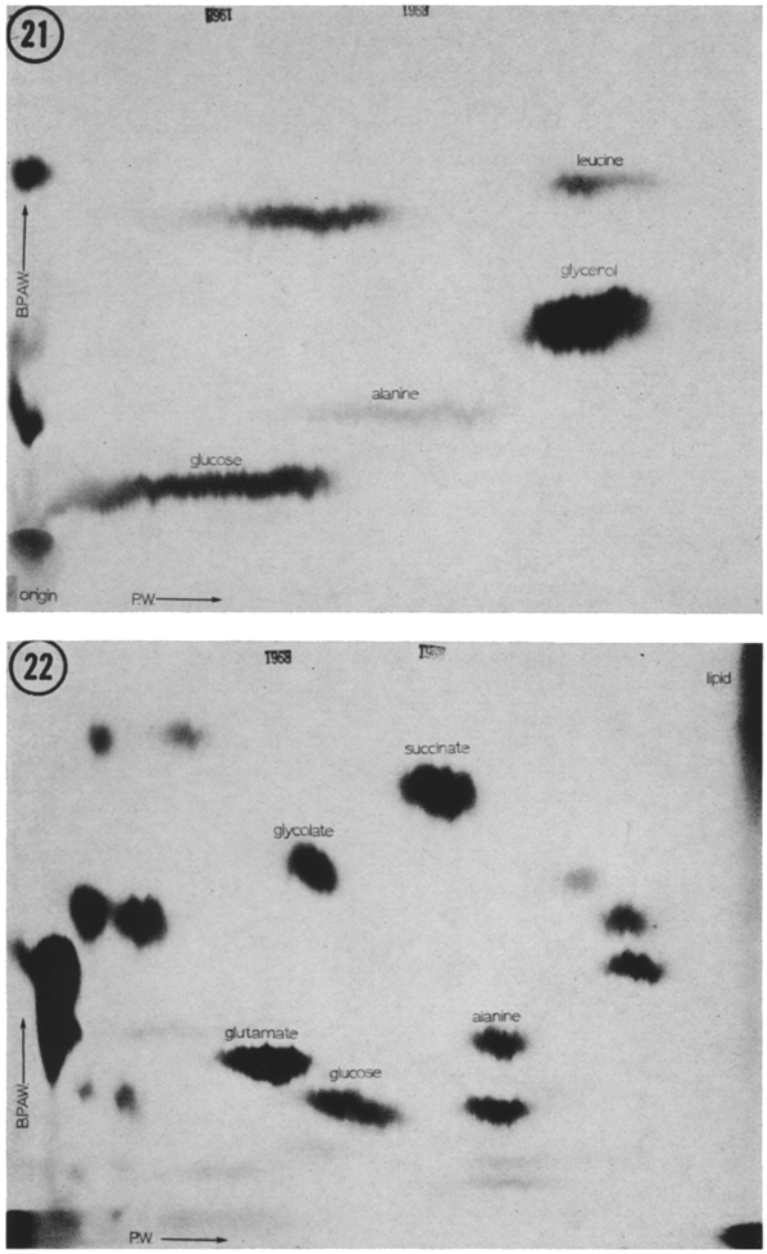

Fig. 21: Autoradiograph of a two-dimensional radiochromatogram of the soluble ^{14}C compounds released to the incubation medium by zooxanthellae form *Zoanthus sociatus*. P.W. = phenol-water; B.P.A.W. = butanol-propionic acid-water

Fig. 22: Autoradiograph of a two-dimensional radiochromatograph of ethanol soluble intracellular ^{14}C compounds of zooxanthellae from *Zoanthus sociatus*

and TRENCH (1971b, c), but are different from those of VON HOLT & VON HOLT (1968b).

Analysis of the ethanol soluble photosynthetic products synthesized by the algal symbionts (Fig. 22) demonstrates that several soluble compounds are synthesized but not released. Comparison of Figures 21 and 22 shows that the appearance of photosynthetic products in the incubation media was not as a result of indiscriminate release brought about through cell lysis, but was more likely a selective process. The assumption is being made, based on similar previous studies, that the zooxanthellae release the same or similar photosynthetic products in vivo and in vitro, during short term experiments (see TRENCH 1971c).

The heterotrophic component

The feeding behaviour of Zoanthus sociatus

The feeding behaviour of *Z. sociatus* has been a paradox for several years. In early experiments, freshly collected specimens of *Z. sociatus* showed no response to live brine shrimp or homogenates or particles of other invertebrates (see GOREAU et al. 1971). However, in a series of experiments conducted in 1971 at Discovery Bay, what

Table 2

Feeding response of *Zoanthus sociatus* to homogenates and particulate matter derived from various marine organisms[1]

Origin of homogenate and/or suspension	Percent *Z. sociatus* giving a positive response[2]	Percent *Z. sociatus* giving a negative response[3]	No. of experiments
Fresh Lobster muscle[4] (particulate)	0	100	7
Fresh crushed crab muscle (*Pachygrapus* sp.)	0	100	13
Fresh clam *(Donax)* (particulate)	0	100	7
Artemia nauplii (live)	0	100	15
Artemia nauplii (fresh homogenate)	0	100	20
Sea urchin eggs *(Echinometra)* (fresh homogenate)	99.5	0.5	17

[1] 10 specimens of *Zoanthus* were used in each experiment.
[2] A positive response is defined as the sum of all feeding activities (REIMER 1971b) culminating in the uptake of particles into the cells of the digestive epithelium.
[3] A negative response is defined as the sum of all activities resulting in the removal of food particles from the region of the oral disc, and never resulting in uptake of material into the coelenteron.
[4] GOREAU & TRENCH (1963, unpublished observations).

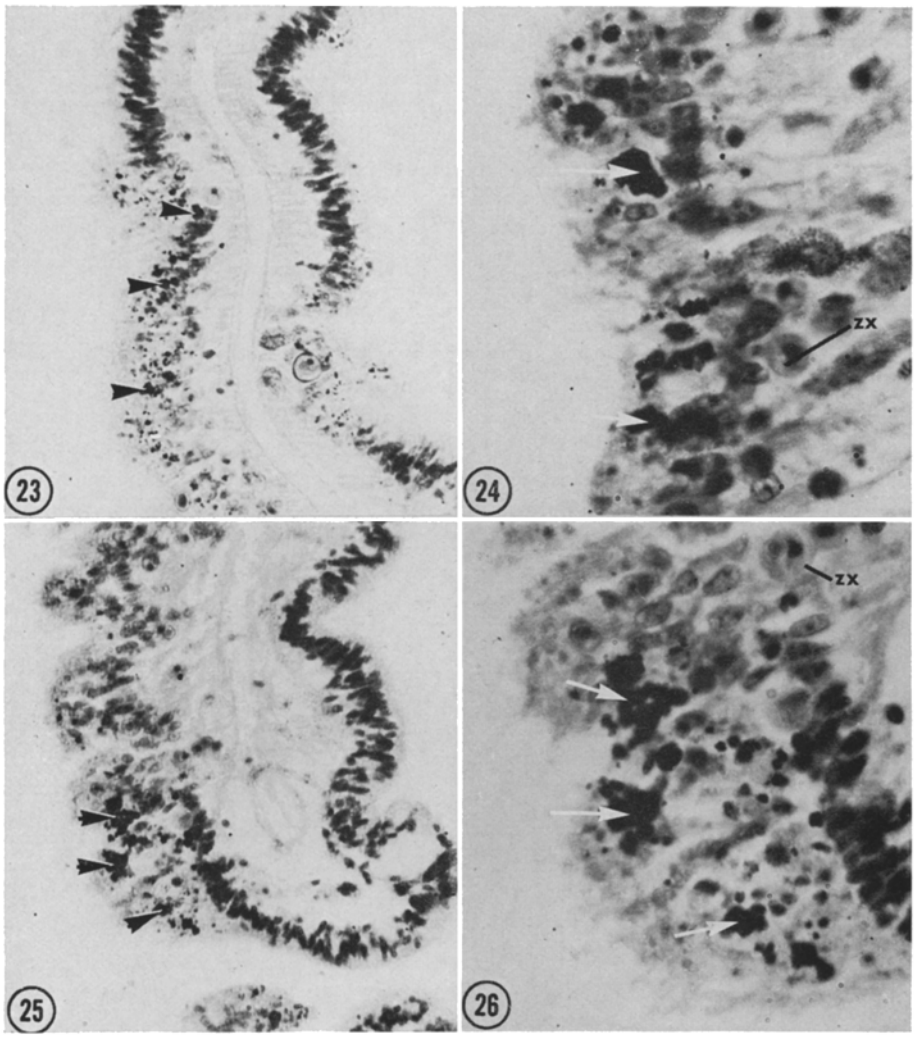

Fig. 23: Longitudinal section of a mesentery of *Zoanthus sociatus* fixed 20 minutes after the animal had fed on a mixture of sea urchin eggs and india ink. Arrows indicate india ink particles in the cells of the "digestive-excretory" zone of the mesentery. (660:1)

Fig. 24. Higher magnification micrograph of tissue in Figure 23 showing india ink particles within the cells of the mesentery. Arrows indicate two such aggregates. A few zooxanthellae can be seen in this tissue (zx). (16 700:1)

Fig. 25: Longitudinal section of a mesentery of *Zoanthus sociatus* fixed 6 hours after having been fed with a mixture of sea urchin eggs and india ink. Arrows indicate india ink particles. (800:1)

Fig. 26: Higher magnification micrograph of tissue in Figure 25 showing india ink particles within the cells of the mesentery. Arrows indicate india ink particles. (1,6700:1)

appeared to be a discriminatory feeding behaviour was observed (see Table 2), in that the specimens of *Zoanthus* in question rejected homogenates and particulate matter from a variety of sources, but accepted homogenates of *Echinometra* eggs with a high degree of reproducibility. The significant point was that the eggs of *Echinometra* could be followed into the cells of the "digestive-excretory" lobes of the mesenteries (see Figs 23–26), which strongly suggested phagocytosis and intracellular digestion.

The inital response of *Z. sociatus* to homogenates of *Echinometra* eggs was elevation of the tentacular margins of the oral disc and the collection of the particles into mucus-bound aggregates. Ciliary activity of the oral disc initially began to move the particles towards the edge, but very abruptly reversed direction and simultaneously the mouth opened and the mucus-laden particles then moved towards the mouth and into the actinopharynx.

In instances of rejection, the tentacular margins were depressed to form a convex oral disc, and particles were moved to the edge and allowed to fall off.

Since the data in Table 2 suggested some form of taste discrimination to be operating, a series of experiments were designed in which homogenates of an acceptable food and those of a non-acceptable food were offered separately and simultaneously. The results (Table 3) show that *Z. sociatus* accepted homogenates of *Echinometra* eggs at all times and rejected the homogenates of *Artemia* nauplii. When the *Echinometra* homogenates were offered first, the normal response was elicited and the mucus laden particles began moving towards the mouth and into the actinopharynx. At this point an aliquot of *Artemia* homogenate was applied to the oral disc and this too began moving towards the mouth. However, as soon as the *Artemia* particles made contact with the mouth, a violent contraction, reversal of the direction of ciliary beat and regurgitation even of the *Echinometra* homogenate was observed.

On the basis of these results, it was concluded that *Z. sociatus* could feed on exogenously supplied materials, and that the organism may have a well-developed taste discriminatory mechanism. It was nontheless difficult to explain this apparent

Table 3

Response of *Zoanthus sociatus*[1] to homogenates of sea urchin eggs and of *Artemia* nauplii offered simultaneously and separately

Experiment	Percent *Z. sociatus* giving positive response	Percent *Z. sociatus* giving negative response	No. of experiments
Mixture of *Artemia* and sea urchin egg homogenate	0	100	5
[2] Homogenate of sea urchin eggs first applied ...	100	0	7
followed by aliquot of *Artemia* homogenate	0	100	

[1] 5 specimens used in each experiment.
[2] A positive response in this case is defined as the sum those activities culminating in the movement of food particles through the mouth and into the coelenteron.

preference for sea urchin eggs, since it was not possible to conceive how sea urchin eggs could be important as a source of nutrition under natural conditions on the reef.

On returning to Discovery Bay in 1972, these experiments were repeated, and this time, in addition to the other homogenates tested, live and freshly homogenized zooplankton taken at dusk on the shallow reef, were offered to specimens of *Zoanthus* that were previously collected and held in running sea water aquaria for four days. It was not surprising to find that the animals continued to show no response to any live prey offered, but it was surprising to observe them ingest homogenates of zooplankton (predominantly crustaceas, but also containing small worms and a variety of invertebrate larvae), of *Artemia* nauplii as well as *Echinometra* eggs. Since the *Artemia* eggs used in these later experiments were the same batch as those used in the previous experiments, it is difficult to explain these contradicting observations. Nontheless, these data do show that these coelenterates possess a co-ordinated feeding response which appears to be more closely allied to muco-ciliary feeding as in small polyped, short tentacled corals (YONGE 1930a), than to the tentacle-feeding coelenterates, e.g. *Palythoa* sp. which catch live prey (*Artemia* nauplii) with nematocysts and subsequently transfer the prey to the mouth (GOREAU & TRENCH 1963 unpublished, see also GOREAU et al. 1971 & REIMER 1971).

Since it is now well established that some amino acids and/or the tripeptide glutathione (reduced) elicits the feeding response in several coelenterates (see LENHOFF 1968, 1971, LINDSTEDT 1971, REIMER 1970, 1971) a study was conducted to determine the response of Z. *sociatus* to GSH and a few selected amino acids. Since REIMER (1971a, b) had already studied the behaviour of Z. *pacificus*, attempts were made to determine to what extent Z. *sociatus* and Z. *pacificus* were similar in their feeding behaviour.

The results in Table 4 show that filter paper imbibed with GSH at concentrations of 10^{-4}M to 10^{-5}M elicited a positive response which eventually resulted in the swal-

Table 4

Response of *Zoanthus sociatus* to filter paper imbibed with GSH, glycine or proline at the concentrations indicated

Chemicals and concentrations		No. of polyps tested	Percentage positive response	Type of response
GSH	10^{-2}	15	20	mouth opened only
	10^{-3}	15	60	mouth opened only
	10^{-4}	20	83	mouth opened-swallowed paper
	10^{-5}	25	99	mouth opened-swallowed paper
Glycine	10^{-2}	10	15	slight mouth opening
	10^{-3}	10	0	—
	10^{-4}	10	0	—
	10^{-5}	10	0	—
Proline	10^{-1}	15	23	mouth opened-tentacles
	10^{-2}	10	13	closed over paper
	10^{-3}	10	0	—
	10^{-4}	10	0	—
	10^{-5}	10	0	—

lowing of the paper. Untreated filter paper was always rejected. When the animals were placed in sea water containing GSH at 10^{-5}M and a suspension of carmine in sea water pipetted on to the oral disc, the carmine was always taken into the mouth. In sea water alone, the carmine was rejected.

Filter paper experiments with glycine ($10^{-2} - 10^{-5}$M) and proline ($10^{-1} - 10^{-5}$M) gave similar results. Both substances caused mouth opening, and in some instances the paper was closed over by the oral disc and tentacles. The filter paper was however never taken into the mouth. When animals were placed in sea water containing glycine or proline separately, each at 10^{-2}M, the polyps responded by opening the mouth, but when carmine was pipetted on to the oral disc it was always rejected. Animals never responded positively, to alanine, aspartic acid or glutamic acid at any concentrations tested.

The morphology and histochemistry of the mesenteries

Figure 19 shows the general morphology of the mesentery. The mesenteries are supported by a "midrib" of mesoglea. Distally, a zone of secretory cells is located, and these comprize the mesenteric filament. Proximally are found the gastroderm cells which show active phagocytosis. These gastroderm cells appear hypertrophied, and form the zone referred to as the "digestive-excretory" zone of the mesenteries (see YONGE 1931).

Electron microscopy conducted on individually dissected mesenteries demonstrated that this tissue is well differentiated. The filaments are composed of several flagellated cells, the flagella being associated with flagellar pits (see Fig. 27) similar to those observed in corals by GOREAU & PHILPOTT (1956), VANDERMULEN (1972) and GOREAU, N. I. & TRENCH (unpublished). Prominent are also large flagellar root fibers (see Figs 28 and 29). Two types of granular secretory cells are present (Fig. 28). In one form, the granules are about half the diameter of those of the other cells. These smaller granules are orthochromatic when stained with toluidine blue; the larger granules stain green. There are also mucus cells (Fig. 27), and some of these show strong γ-metachromasia with toluidine blue while others are orthochromatic. In Weigert's Trichrome some granules stain orange, others blue or green.

The filaments also contain nematocysts (Fig. 29), which appear to be functional and completely differentiated holotrichous isorhizas. These nematocysts were however only very infrequently encountered.

The cells of the "digestive-excretory" zone are microvillated and flagellated (see Figs 30–32), and are highly vacuolar. Similar flagellar pits and flagellar root fibers are found in this tissue.

Tests for the presence of acid phosphatase in this tissue showed that the enzyme can be located within some vacuoles (Fig. 30), associated with the microvilli (Fig. 31), and within possible lysosomes (Fig. 32). Acid phosphatase activity was never detected associated with the pycnotic zooxanthellae found in these cells, but was sometimes detected in association with vacuoles containing unrecognizable conglomerates.

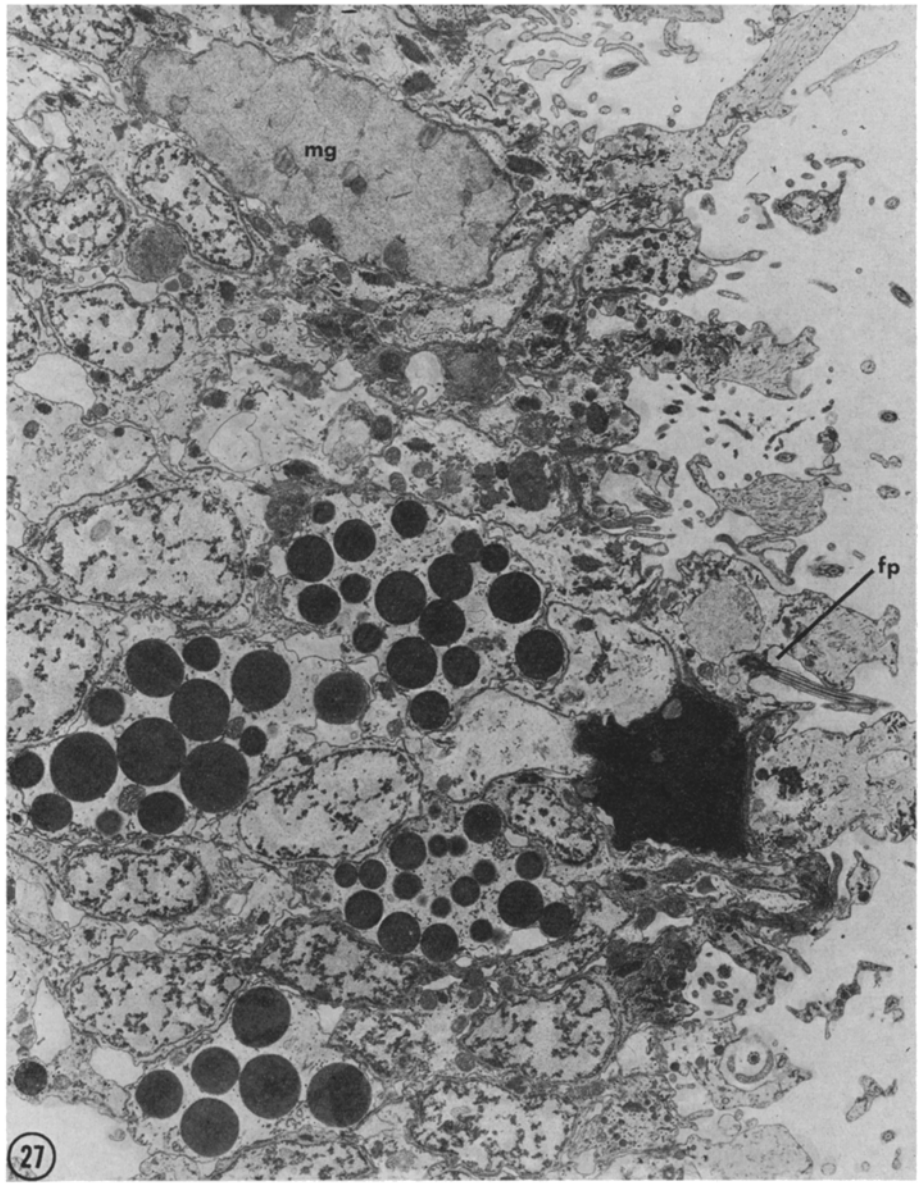

Fig. 27: Electron micrograph of a portion of a mesenteric filament of *Zoanthus sociatus* showing cellular differention. Mucus glands (mg) and "zymogen" granules can be seen in different cells. Flagellar pits (fp) are very common. Note the tight junction between cells. (4,650:1)

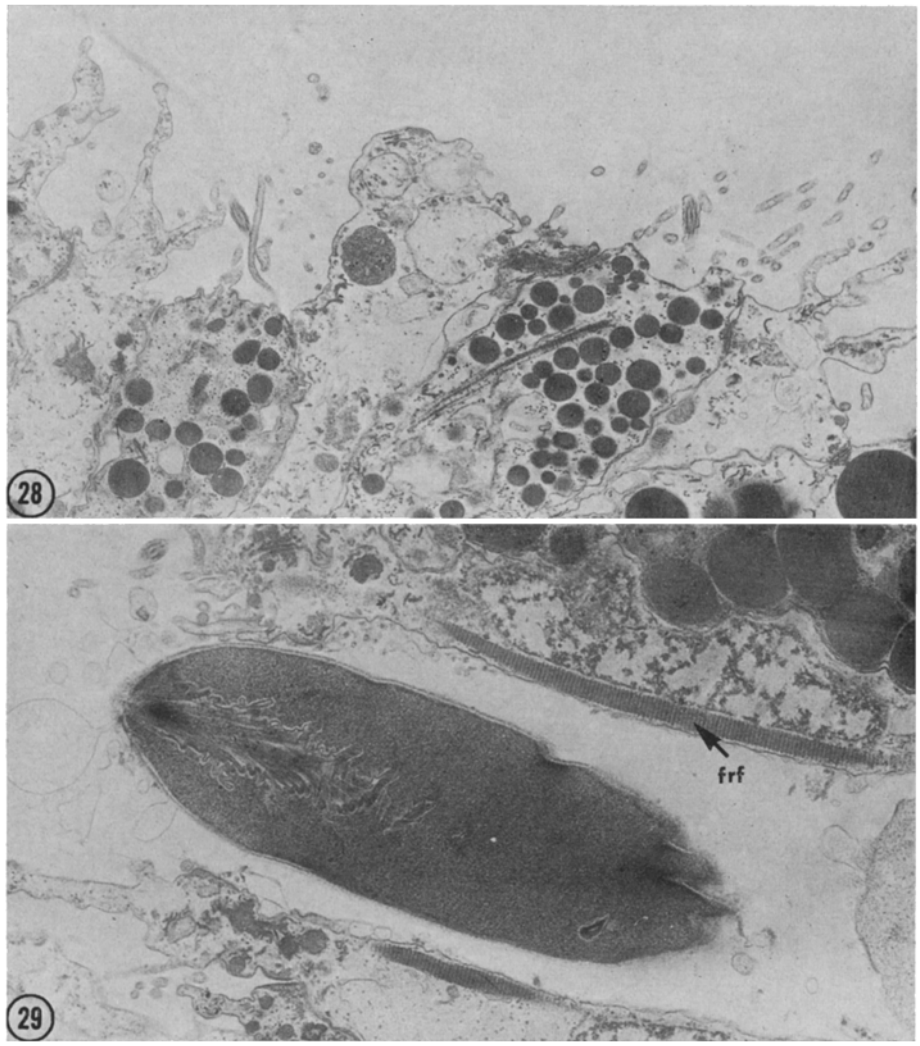

Fig. 28: Electron micrograph of a portion of the mesenteric filament of *Zoanthus sociatus* showing the two size categories of the "zymogen" granules within secretory cells. 5,000:1)
Fig. 29: Electron micrograph of a nematocyst (holotrichous isorhiza) in the mesenteric filament of *Zoanthus sociatus*. Note also the flagellar root fibers (frf) which are very common in this tissue (10,500:1)

Digestion and assimilation of exogenously supplied protein

When specimens of *Zoanthus sociatus* were offered suspensions of homogenized *Echinometra* eggs mixed with india ink, the suspensions were collected on the oral disc and ciliary activity effected their transport towards the open mouth and into the

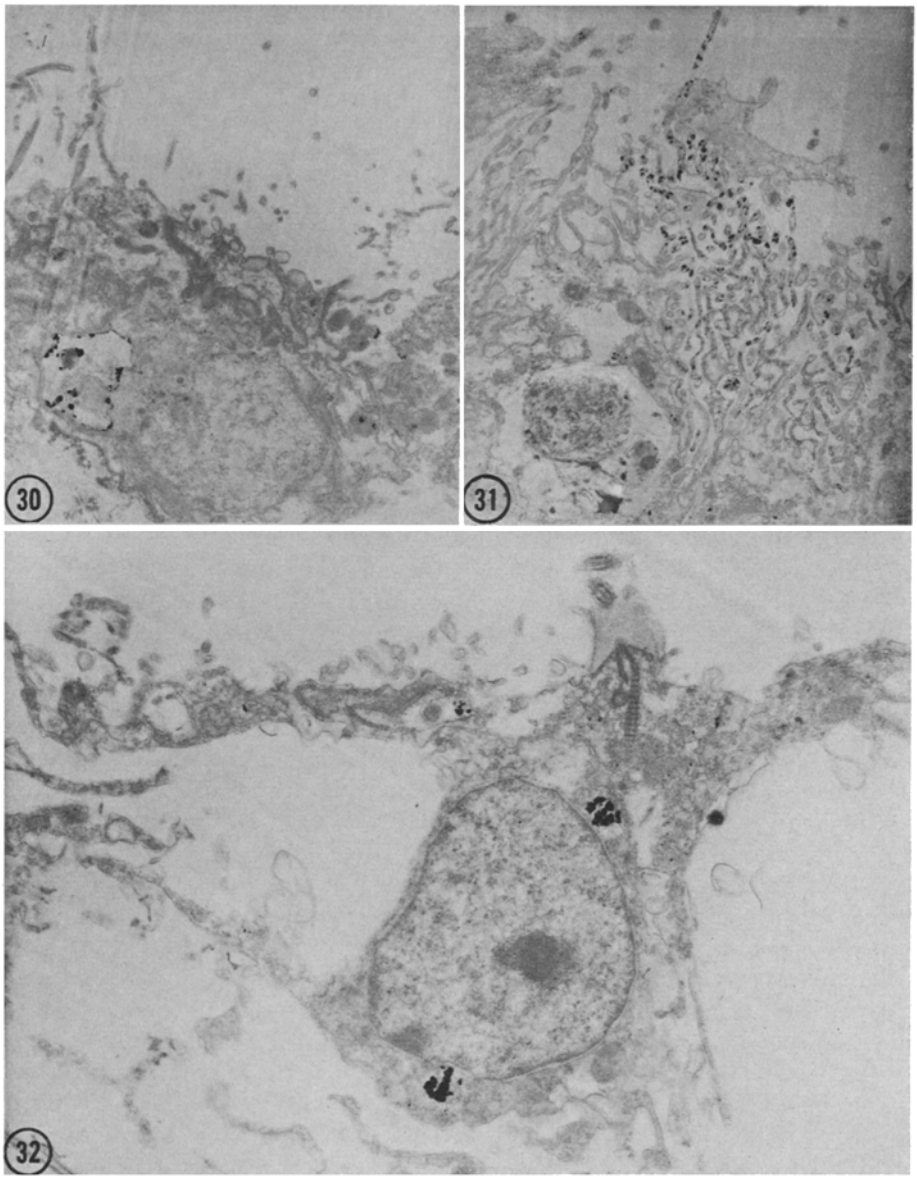

Fig. 30: The distribution of acid phosphatase in the mesenteries of *Zoanthus sociatus*. Acid phosphatase associated with animal cell vacuole. (3,350:1)

Fig. 31: Acid phosphatase associated with the microvilli of the cells of the "digestive-excretory" zone of the mesentery of *Zoanthus sociatus*. (5,200:1)

Fig. 32: Acid phosphatase positive bodies in cells of the "digestive-excretory" zone of the mesentery of *Zoanthus sociatus*. Note also the presence of the flagellar pit-flagellar root fiber system. (8,850:1)

stomodeaum. Initial experiments were therefore conducted in which animals were prepared for light microscopy at intervals after the completion of food intake ranging from a few minutes to 12 hours.

Examination of the specimens by light microscopy showed that the food bolus was taken into the gastric cavity, and within 30 min. india ink particles (presumably with adsorped organic matter) could be found in the "digestive-excretory" zone of the mesentery. India ink suspensions alone were always rejected. This zone of cells (see Figs 23–26) continued their phagocytic activity up to 12 hours after feeding, by which time most of the aggregates of food particles in the coelenteron had been converted to amorphous masses. The gastroderm cells of the column showed no phagocytic activity.

In several instances between 8 and 12 hours after feeding, india ink particles, aggregated in mucus balls, were found in the actinopharynx. These probably represent particles exocytosed by the "digestive-excretory" cells after removal of organic matter which had been sorbed. This sequence of phagocytosis and expulsion is consistent with that described by YONGE (1931) in corals.

Since the results described above indicated that *Z. sociatus* was capable of feeding on exogenously supplied materials, experiments were designed to determine the rate of digestion and assimilation of exogenously supplied protein.

Animals were fed with ^{35}S-protein, (prepared as described in Materials and Methods) mixed with homogenates of *Echinometra* eggs. After given intervals, the animals were assayed for cold TCA-soluble and insoluble ^{35}S.

Figure 33 shows that the TCA-insoluble proteins fed to the animals were rendered soluble rapidly. This process very likely represents the hydrolytic conversion of proteins to amino acids or peptides, and probably takes place predominantly in the coelenteron as extracellular digestion. The reconversion of soluble to insoluble ^{35}S, representing the assimilation of ^{35}S-amino acids was a much more gradual process, and continued over the 48-hr period of the experiment.

These data are interpreted as demonstrating that *Z. sociatus* is capable of digesting and assimilating proteins, and that digestion is a rapid process followed by a slower process of assimilation.

In other experiments, the animals were fed protein labelled with ^{3}H-leucine, and at given intervals after feeding they were fixed and subsequently prepared for light microscope autoradiography.

Examination of the animals showed that radioactivity could be detected very rapidly in the "digestive-excretory" zone of the mesenteries where phagocytosis of labelled particles had taken place. However, within 3 hrs after feeding, radioactivity could be detected within the gastroderm cells of the column, and later (8–12 hrs) could be found in other gastroderm cells including those of the tentacle. Radioactivity accumulated in the "digestive-excretory" zone of the mesenteries throughout the experiment.

Since the autoradiographic method employed in this experiment removes small molecular weight substances (e.g. amino acids), the initial process of digestion could not be detected, and only when the animal had converted these labelled substrates into insoluble matter, i.e. the process of assimilation, could the label be detected. The rapid

appearance of label in the gastroderm cells of the column indicates that although these cells do not appear to phagocytize particles, they may take up small molecular weight substances from the coelenteron. Therefore, a portion of digestion in *Zoanthus sociatus* must be extracellular. In fact, when the results of the two experiments are taken to-

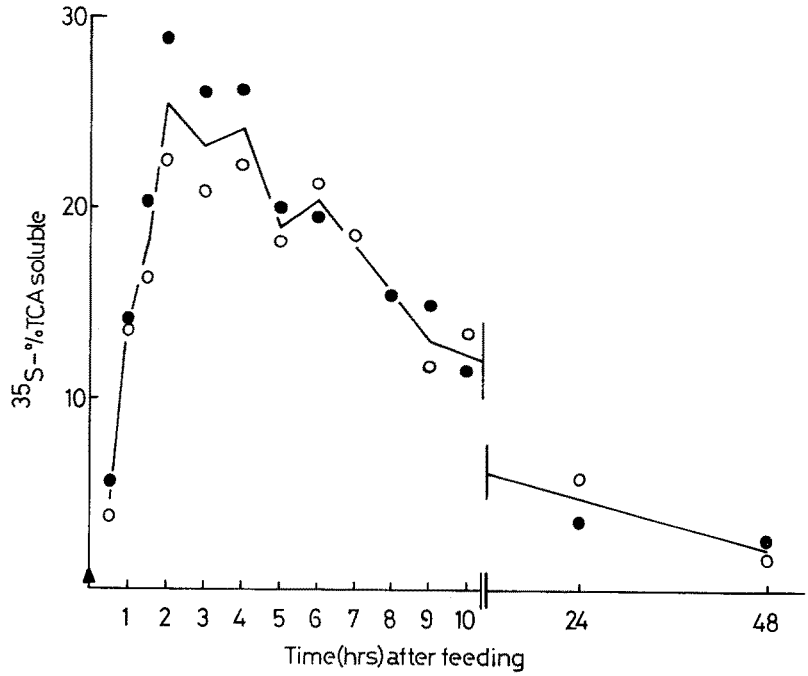

Fig. 33: The transformation of insoluble ^{35}S-protein fed to *Zoanthus sociatus*. ▲ = % soluble ^{35}S protein in the food offered to the animals. Open and closed circles represent respectively data from two separate experiments

gether, it appears that both extracellular and intracellular digestion occurs. It also seems reasonable to conclude that the products of extracellular digestion are transported throughout the organism probably under the influence of the large ciliary tracts of the mesenteries.

Uptake and incorporation of ^{3}H-leucine and ^{3}H-glucose

When specimens of *Z. sociatus* were incubated in sea water containing ^{3}H-leucine or ^{3}H-glucose and subsequently analysed for uptake and incorporation of label, it was found that with both substrates, uptake was demonstrable, and that the principal site of uptake appeared to be the epithelium of the oral disc, which electron microscopy showed to be composed of highly microvillated cells.

After short incubations (e.g. specimens exposed to the labelled substrate for 4 hrs only) most of the radioactivity detected was associated with the immediate region of

the oral disc. It was surprising to find however, that shortly after the start of the "chase"period, radioactivity was found associated with gastrodermal cells deep within the coelenteron. This observation was only made in the case of animals incubated in leucine, where, by the end of the "chase" period, the radioactivity was distributed essentially throughout the polyp. In several instances, the specimens used were found to be in a reproductive state, mostly producing sperm, but in two instances also producing eggs. In either case, the sites of egg or sperm production on the mesenteries accumulated ^{3}H-leucine. This was not observed with ^{3}H-glucose.

The apparently rapid transport of ^{3}H-leucine through the polyp is probably not a diffusion process. The most logical explanation is that during the "pulse" period some stimulant in the sea water caused the organisms to open their mouths, and the coelenteric currents would tend to transport ambient sea water into the coelenteric cavity from which the amino acid was absorbed randomly by the gastrodermal cells. This is analogous to the gastroderm cells absorbing the products of extracellular digestion (p. 200).

Although these studies are not quantitative, they do demonstrate that *Z. sociatus*, like a variety of other marine invertebrates (particularly reefdwelling invertrebrates) possess the necessary morphological and physiological adaptations to effect utilization of nutrient resources in the form of dissolved organic matter.

The ultimate fate of zooxanthellae in Zoanthus sociatus

The observation that the "digestive-excretory" zone of the mesenteries contained large numbers of "pycnotic" zooxanthellae suggested that a careful analysis of the morphological and functional aspects of these algae should be undertaken. Therefore the ultrastructure and capacity for ^{14}C fixation by "pycnotic" zooxanthellae was determined.

Electron microscope observations on the "pycnotic" algae found in the cells of the mesenteries showed a wide range of morphological integrity, from completely "normal" appearing algae to totally disorganized cells (see Figs 34–37). Several attempts were made to demonstrate acid phosphatase activity associated with these degenerating algae using different substrates (e.g. β-glycerophosphate and p-nitrophenylphosphate). In no instance could a phosphatase-specific reaction be detected in or closely associated with the "pycnotic" algae (cf. FANKBONNER 1971).

A reconstruction of the morphological changes occurring in zooxanthellae in the "digestive-excretory" cells of the mesentery showed that the earliest sign of degeneration was the development of large vacuoles within the algal cytoplasm (Fig. 34). As degradation progresses, the vacuoles appear to coalesce (Fig. 35) and the mitochondrion and chloroplast begin to become disorganized. Very often, myelin figures and crystalline bodies can be found within the vacuoles (Fig. 38). Eventually, the chloroplast thylakoids and other membrane systems become disorganized (Fig. 36). In all these stages, the algal periplast membrane system remains intact, suggesting that the process of degradation originates within the zooxanthellae. Finally, at some undetermined stage, the periplast membrane system ruptures and the algal diameter becomes reduced

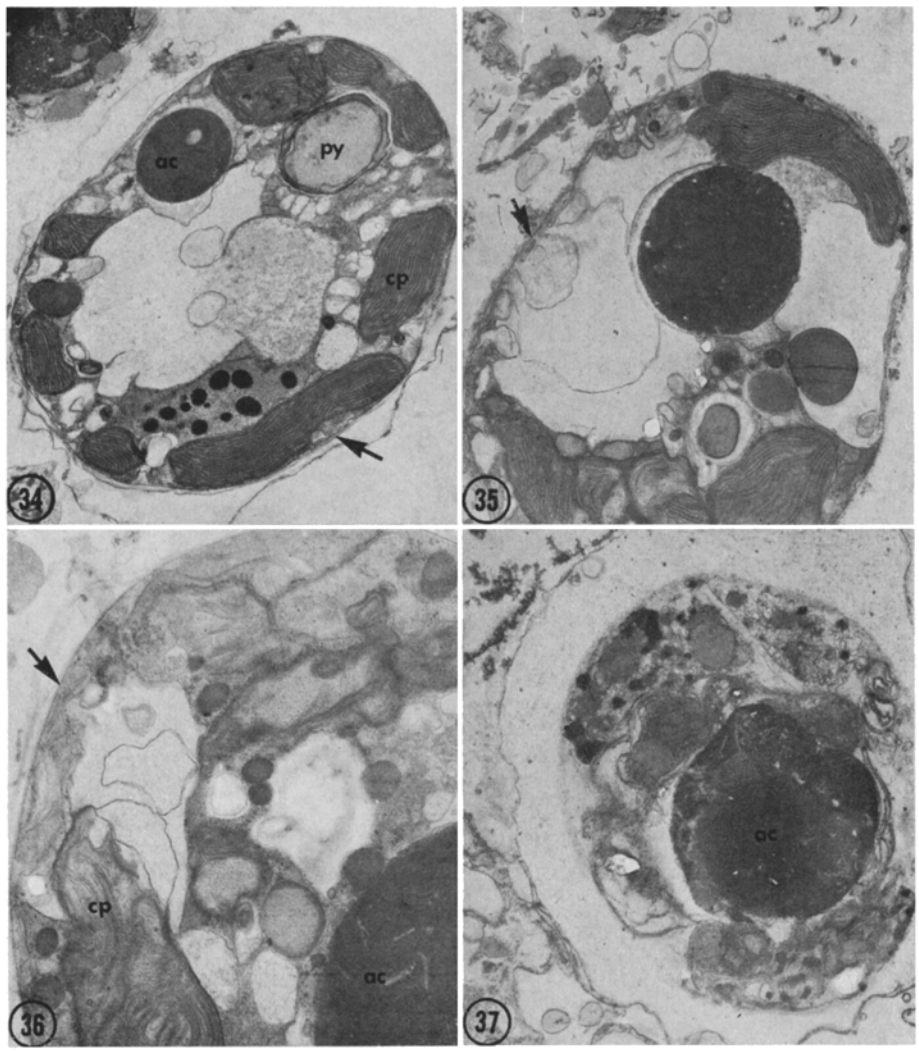

Fig. 34: Possible sequence of events in the degradative process in zooxanthellae in the "digestive-excretory" zone cells of the mesenteries of *Zoanthus sociatus*. Arrows indicate intact perialgal membrane system. The development of vacuoles within the alga. At this stage the chloroplasts are still intact, but the mitochondrion has begun to be degraded. ac = "accumulation body"; py = pyrenoid; cp = chloroplast. (6,000:1)

Fig. 35: The enlargement of vacuoles within the algal cytoplasm and the development of "myelin" figures within the vacuoles of the mesenteries of *Zoanthus sociatus*. Note that the chloroplast still appears intact. (6,330:1)

Fig. 36: Onset of chloroplast degradation. Note the disarray of the chloroplast thylakoids and the chloroplast envelope membranes. The mitochondrion is now also disorganized, but the perialgal membrane system is still intact. (10,000:1)

Fig. 37: The totally disorganized remains of a zooxanthella. Note that the "accumulation body" (ac) has remained. (6,000:1)

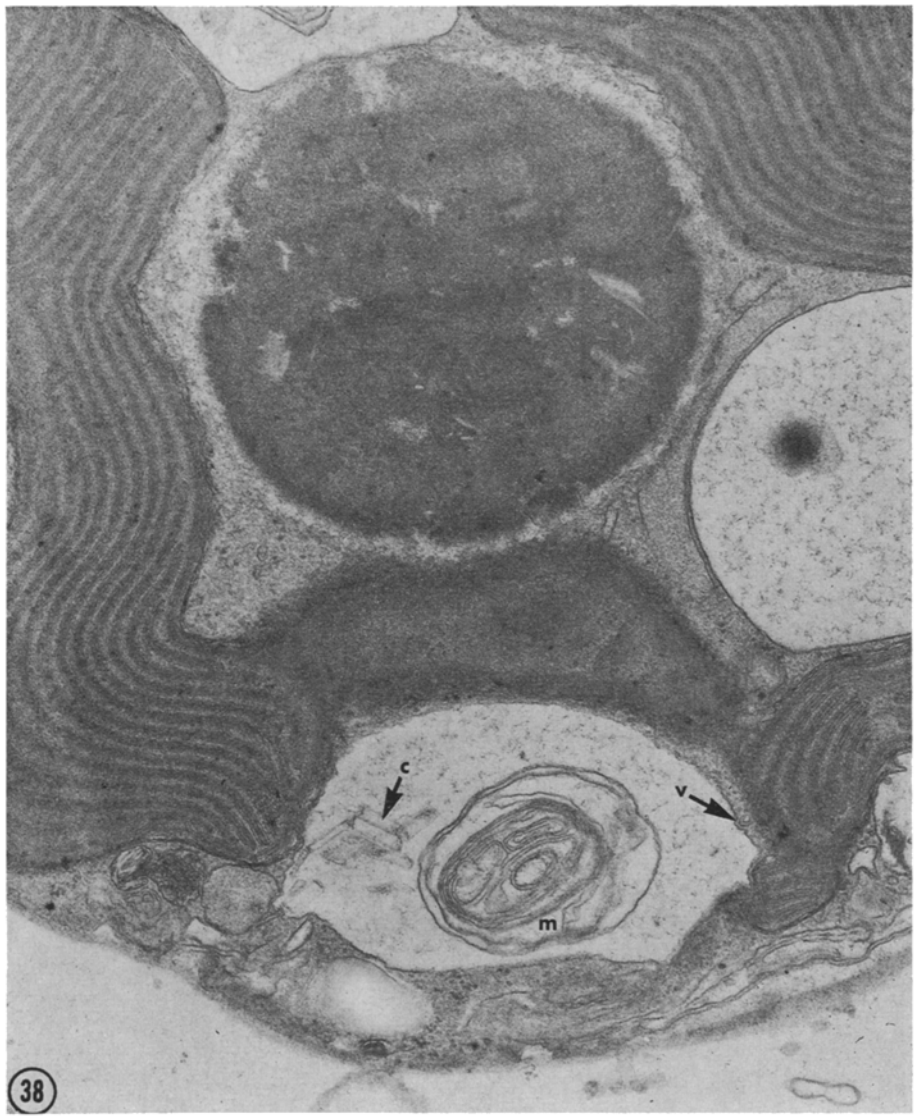

Fig. 38: Electron micrograph of a portion of a zooxanthella in the early stages after the onset of degradation showing some newly formed vacuoles, a myelin figure and crystalline bodies frequently found within vacuoles. (30,000:1)

to approximately half the original (Fig. 37), and at this final stage, the alga contains a conglomerate of membranes derived from cell organelles, and the "accumulation body" (see also TAYLOR 1969c).

In an attempt to determine the origins of the "pycnotic" algae, animals were "pulse" labelled in $NaH^{14}CO_3$ for 4 hours in the light and "chased" in unlabelled

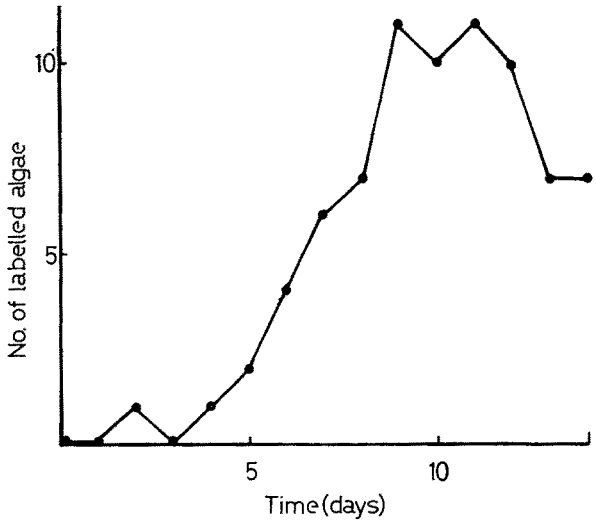

Fig. 39: The appearance of ^{14}C-labelled zooxanthellae in the "digestive-excretory" zone of the mesenteries of *Zoanthus sociatus* after a "pulse" incubation of 4 hours

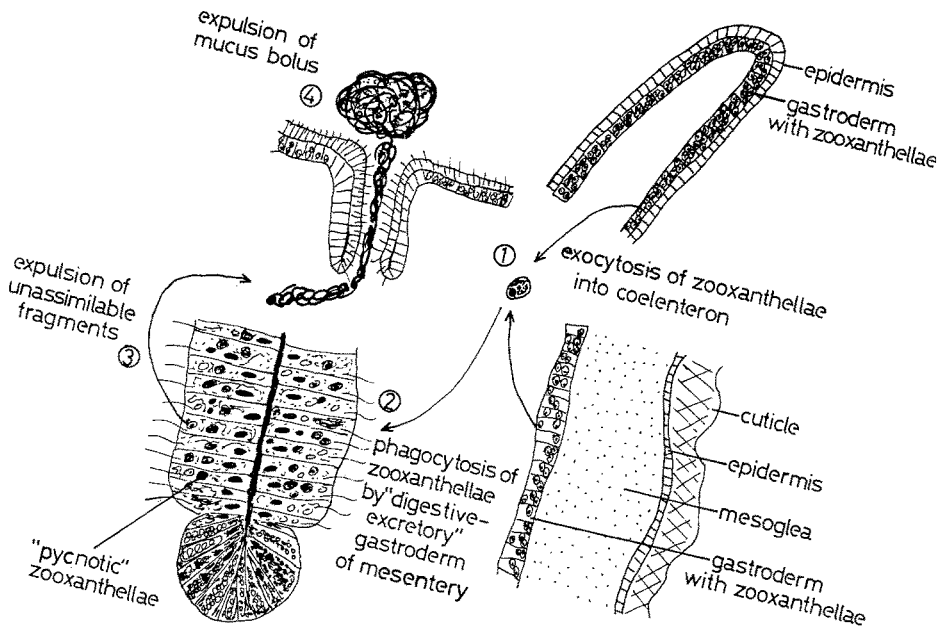

Fig. 40: Diagramatic representation of the pathway of zooxanthellae during the onset of senescence and degradation. (1) exocytosis of zooxanthellae by the gastroderm cells of column and tentacles. (2) phagocytosis of zooxanthellae by the cells of the "digestive-excretory" zone of the mesentery. (3) degradation of zooxanthellae within the mesentery cells, exocytosis of unassimilable remains and (4) expulsion from the animal.

sea water for up to 15 days. At daily intervals, specimens were fixed and processed for analysis by light microscopic autoradiography. The number of zooxanthellae containing radioactivity within the cells of the "digestive-excretory" zone of the mesenteries were counted.

The results (Fig. 39) show that the number of zooxanthellae in the mesenteries containing radioactivity is initially low and increases to a maximum about 10–12 days after the initial pulse of ^{14}C. In the early stages, all the radioactivity in the mesenteries was associated specifically with the zooxanthellae. However, progressively more activity became associated with the animal cells.

Since initially almost no algae in the mesenteries fixed ^{14}C, it was concluded that the algae which were finally found containing label were derived from other regions of the polyp where the ^{14}C fixation originally occurred. A reconstruction of the possible pathway of zooxanthellae is depicted in Figure 40 (see also HADDEN 1968).

DISCUSSION

The results of this study indicate that *Zoanthus sociatus* possesses the potential to derive energy in the form of reduced carbon and nitrogen from several different sources. In this respect, *Z. sociatus* is not unlike many other reef-dwelling invertebrates, e.g. corals and *Tridacna,* which have been shown to derive and utilize photosynthetically fixed carbon from their endozoic zooxanthellae (MUSCATINE 1967, MUSCATINE & CERNICHIARI 1969, TRENCH 1971b, GOREAU et al. 1973), while retaining the ability to utilize reduced carbon and nitrogen from other sources (YONGE 1940, PORTER 1973) such as detritus or dissolved organic matter (GOREAU et al. 1971) or possibly bacteria (DISALVO 1971a, SOROKIN 1973). It has so far only been possible to determine to what extent these organisms possess the mechanisms necessary to make use of these various resources potentially available to them. No evidence of a quantitative nature is available to estimate how much of the total nutritional requirements of corals or any other reef-dwelling invertebrate is satisfied by these different sources. A detailed analysis needs to be conducted on the nutritional requirements of corals and other animals with symbiotic zooxanthellae in order to ascertain their nutritional needs, and the extent to which these needs are met by the resources available. To say that corals are specialized carnivores and therefore the photosynthate provided by their zooxanthellae is of little or no significance, is unwarranted in the light of present evidence (GOREAU et al. 1971). Similarly, the implication that because the availability of zooplankton to corals on a reef might be insufficient to satisfy the respiratory needs of the corals, they must therefore depend more on the zooxanthellae, is probably unjustified (JOHANNES et al. 1970). The caloric value of a given resource is on its own not very meaningful in the context of total nutrition, since small quantities of trace substances and vitamins are absolute necessities (PROVASOLI, personal communication), and are frequently overlooked in ecological nutritional studies. At this time it is not possible to state in any meaningful way whether the autotrophic mode of nutrition is relatively more important to corals, etc. than the heterotrophic mode. What is apparent is that the two modes of nutrition are complementary to one another, and

the symbiotic unit can therefore potentially exploit more resources, thus occupying a polytrophic status within the ecosystem.

In the case of *Z. sociatus*, several factors have played important roles in fostering the concept that the nutrition of this organism was different from that of corals. It was difficult to demonstrate a feeding response in *Zoanthus*, and the morphological attributes of a typical carnivorous coelenterate, i.e. the presence of nematocysts and differentiated mesenteric filaments with digestive function, were thought to be either absent or poorly developed. The analogy was therefore made that *Zoanthus*, like *Xenia*, was principally dependent on the photosynthetic products of its zooxanthellae as a source of nutrition. However, it now appears that *Zoanthus* in fact has the behavioural and physiological capabilities to utilize exogenous substances just as well as some corals do, and like these corals, still utilizes the photosynthetic contribution made by their zooxanthellae.

The autotrophic mode of nutrition

The zooxanthellae in the cells of *Zoanthus sociatus* function in a similar manner to chloroplasts in a plant cell, in that they fix carbon photosynthetically, and release a large proportion of this reduced carbon to the animal tissue. The proportion of photosynthetic carbon released was estimated as 36–48 %. This value is within the range of that reported by VON HOLT & VON HOLT (1968a), who calculated that this level of production could be of great significance to the animal hosts. It is very clear that the animals utilize the carbon contributions of the algae.

From in vitro studies of the release of products by the algae from *Zoanthus*, it is clear that these products in no way differ from those reported for zooxanthellae from other hosts (see MUSCATINE 1967, MUSCATINE & CERNICHIARI 1969, TRENCH 1971b, c), except for the release of leucine. On this basis then, it must be concluded that the numerous released substances found by VON HOLT & VON HOLT (1968b) are probably artefacts of over-extended incubations or cell lysis during incubation.

The synthesis and release of photosynthetic products by zooxanthellae is not the sole mechanism whereby algal products become available to the animals. Several different authors have suggested that coelenterates may digest their zooxanthellae (BOSCHMA 1925a, b, 1926, HADDEN 1968). These conclusions were based on the appearance of pycnotic algae in the "digestive-excretory" zone of the mesenteries. However, until clear evidence of the production of hydrolytic enzymes by the animal host which acts on algal material is produced, the problem remains unresolved (see MUSCATINE 1973). An alternative possibility is that the algae undergo autolysis within this tissue after they have been expelled from other gastrodermal cells. Regardless of the actual mechanism, it is apparent that materials from the defunct zooxanthellae can pass to and become assimilated by the animals. The process of transfer of algae to the mesenteries appears to be a continuous one, and may well represent the phenomenon of "pruning" as described by TAYLOR (1969b) in the anemone *Anemonia sulcata*. It is now suggested, however, that this latter process should be distinguished from that wherein animals "expel" their zooxanthellae when under metabolic stress.

For example, it has been observed (TRENCH, unpublished), that in *Palythoa* sp. expulsion of zooxanthellae occurs when the animals are experimentally placed under osmotic stress (see also GOREAU 1964). The algae are rapidly expelled in mucus strands via the mouth. Examination of these extruded algae by electron microscopy, and exposure to $H^{14}CO_3$, showed that they retained both morphological and biochemical integrity. By contrast, the brown mucus masses intermittently produced by *Zoanthus* under normal conditions contained pycnotic zooxanthellae similar in appearance to that shown in Figure 37. Such defunct algae were never observed in the gastroderm cells of the column or the tentacles. Again, in the marine hydroid *Myrionema amboinense*, pycnotic algae occur in the gastrodermal cells, but these are always distally located, while the intact zooxanthellae are proximally displaced (TRENCH unpublished). COOK & MUSCATINE (personal communication) have found a similar situation in *Hydra viridis* (see also PARDY & MUSCATINE 1973). Apparently senescence and death of the zooxanthellae occur in morphologically distinct regions in *Zoanthus*, while in the hydroids, the compartmentalization of functional from non-functional algae occurs within the same cell.

FANKBONNER (1971) interpreted results of electron microscopic histochemistry as demonstrating intracellular digestion of zooxanthellae by blood amoebocytes in *Tridacna*. The presence of acid phosphatase near pycnotic zooxanthellae was regarded as evidence in support of digestion. In this study, no enzyme specific reaction was detected in association with the pycnotic algae in the cells of the mesenteries. Nonetheless, the algae did appear to undergo degradation, this process apparently being initiated from within. The breakdown products of autolysis, excluding the "accumulation body" and the numerous membrane systems of the algae, may become assimilated by the animals.

It is interesting that although zooxanthellae are rejected by *Zoanthus* when offered orally, when within the coelenteron, they are phagocitized by the cells of the mesenteries. HADDEN (1968) injected ^{14}C-labelled zooxanthellae into the coelenteron of *Z. sociatus* and later, by autoradiography, detected these labelled algae in the cells comprising the "digestive-excretory" zone of the mesenteries. Similarly, the "pulse-chase" experiments reported here show clearly that zooxanthellae which initially fix carbon in one region of the polyp may be translocated to the mesenteries where they undergo autolysis. This interpretation is, from the point of view of mechanism, different from that proposed by YONGE (1968), who suggested that the algae in corals were transported from cell to cell in the gastroderm and finally to the mesenteries by wandering amoebocytes. Such amoebocytes have not been observed, and their progression through the tissues could only be effected if the tissue were syncytial, which electron microscopy has clearly demonstrated it is not.

The sequence of organelle degradation within senescent zooxanthellae is not unlike that described in photosynthetic tissues of higher plants (see DODGE 1970). The mitochondrion and chloroplast appears to be the first organelles to show signs of degradation, and the nucleus appears to be the last structure to be destroyed before the loss of the periplast membrane system. It must be assumed that the "accumulation body" contains substances which are not assimilable by the animals, and so are finally expelled (see TAYLOR 1969b).

The heterotrophic mode of nutrition

It is quite apparent that *Zoanthus sociatus* possesses the necessary morphological behavioural and biochemical mechanisms to utilize exogenous food resources in the form of protein. The feeding behaviour is, as in several other coelenterates, based on chemical activation by the reduced form of the tripeptide glutathione (see LENHOFF 1968, REIMER 1971a, b, c, d, LINDSTEDT 1971). Unlike *Z. pacificus,* however, *Z. sociatus* showed a feeding response with glycine (REIMER 1971a) as well as proline (cf. LEHMAN & PORTER 1973, FULTON 1963, MARISCAL & LENHOFF 1968), but in both instances, although mouth opening was elicited, particles placed on the oral disc were removed by the ciliary currents. Neither aspartic, glutamic acid nor alanine elicited a positive feeding response. It is therefore possible to conclude that *Z. sociatus,* like so many other coelenterates tested, does possess the sensory mechanisms which are important in the capture of prey (see LENHOFF 1968a). However, the mechanism for food collection used by *Zoanthus* is more similar to that used by small polyped-short tentacle bearing corals such as some species of *Fungia, Herpetolitha, Polyphyllia, Coeloseris* and *Pachyseris* (YONGE 1973), which involves ciliary reversal mechansims. It is assumed that the latter organisms feed on detrital material of animal origin which falls out of suspension. The extent to which this might apply to *Z. sociatus* is open to speculation. The following observations bear on this topic. During exhaustive observations on the reef crest where *Z. sociatus* occurs as extensive carpets (see GOREAU 1959), and elsewhere on the reef, *Z. sociatus* were never observed feeding. On the reef crest the water mass is constantly in motion, and such turbulence reduces the possibility that particles would settle out of suspension. In reef lagoons where the sediment may be almost at the level of the oral discs of the polyps, it might be possible that sediment borne detritus could come into contact with the animals' feeding apparatus. However, to test the possibility that *Zoanthus* might utilize detritus, zooplankters were repeatedly extracted in sea water, and the residue offered to the polyps. Such preparations were always rejected. Again, it could be argued that the animals fed at night. Numerous observations at night, using underwater lights, showed that the zooplankters which concentrated in the light beam often came into contact with the tentacles of *Zoanthus,* but were never captured. This is consistent with laboratory experiments in which *Z. sociatus* showed no response to the presence of live *Artemia* nauplii nor live zooplankton. It is therefore not possible to state how these organisms acquire animal protein in nature.

Regardless of what the natural food source might be, that *Z. sociatus* can engulf, digest and assimilate exogenously supplied protein is now demonstrated. Digestion is rapid, and the rate in *Zoanthus* compares favourably with that reported for the sea anemone *Aiptasia* (MURDOCK & LENHOFF 1968, see also PORTER 1973). In both organisms, the process of assimilation appears to be slow. It is apparent that both extracellular and intracellular digestion occurs in *Zoanthus.* The products of extracellular digstion are distributed within the coelenteric cavity, probably through the influence of the large ciliary tracts on the mesenteries. In this manner, small molecules, e.g. amino acids and peptides, products of extracellular digestion, are transported throughout the polyp. The enzymes that function in extracellular digestion are

very likely produced by the "zymogen" containing cells of the mesenteric filaments (Fig. 27). Phagocytosis and intracellular digestion is the exclusive function of the cells of the mesenteries proximal to the filament, the same cells wherein the pycnotic zooxanthellae occur. This situation is therefore identical to that reported in the Scleractinia and Actiniaria (YONGE 1968, NICOL 1959). It is then no wonder that many investigators interpreted the presence of these pycnotic algae in the cells of the mesenteries as indicating digestion by the animals (see BOSCHMA 1926, HADDEN 1968).

The other food resource that *Z. sociatus* may exploit is dissolved organic matter. Since the original work of PÜTTER (1909), several investigators have studied the uptake and utilization of dissolved organic matter by marine invertebrates (see STEPHENS 1967, 1968). SCHLICHTER (1973) demonstrated the uptake of dissolved amino acids in the anthozoan *Anemonia sulcata*. GOREAU et al. (1971), FANKBONNER (1971) and JOHANNES (1967) have proposed that such organic resources are of great importance to reef-dwelling invertebrates.

Morphologically, *Z. sociatus* possesses the necessary adaptation for the uptake of dissolved organic matter, i.e. a microvillated epithelium on the oral disc. This structure is usually associated with transport systems (e.g. see RAMSEY 1971, TRENCH & GOODAY 1973, TRENCH et al. 1974, GOREAU et al. 1973). From autoradiographic analysis the site of uptake of dissolved glucose and leucine appears to be the oral disc. The efficiency of uptake might well be high, since the animals were able to remove these metabolites from very low concentrations indeed ($10^{-6} - 10^{-7}$M). TAYLOR (1974) found half saturation constants of 7.7–8.2 μM for the uptake of glucose, glycerol and alanine by the anemone *Aiptasia pallida*. Although similar parameters have not been measured for *Z. sociatus*, the prediction is that these organisms would probably do as well.

In summary, *Z. sociatus* possesses all the mechanisms described in other reef-dwelling coelenterates with endosymbiotic zooxanthellae for the utilization of primary photosynthate, animal protein and dissolved organic matter. Although this may assist in a clearer understanding of the place of *Z. sociatus* within the trophic structure of the reef ecosystem, the nutritional limits that define the niche occupied by this species still remain unclear.

The significance of the polytrophic habit in coral reef ecosystems

Reef-dwelling coelenterates and other invertebrates with photosynthetic endosymbionts, e.g. *Tridacna* or *Tridachia*, by virtue of their symbioses possess an autotrophic mode of nutrition. Nonetheless these organisms retain their "normal" abilities to derive nutrition heterotrophically. It has not been possible to determine quantitatively which nutritional mode contributes most to the total requirements of the animals concerned. What is apparent is that these different potentials tend to act in a complementary and synergistic manner.

Studies on primary production by individual corals using respirometry have shown P:R ratios of 2–5 (KANWISHER & WAINWRIGHT 1967, ROFFMAN 1968). Taken

together with data on photosynthetic carbon translocation from the intact algae to the animals, and their subsequent utilization by the hosts (MUSCATINE 1973) there should now be no doubt that these organisms possess an autotrophic mode of nutrition (SMITH et al. 1969).

The availability of zooplankton to reef corals appears to be less than can satisfy their respiratory demands (JOHANNES et al. 1970, GLYNN 1973). Even if corals only use the dermersal zooplankton (PORTER 1973), the conclusion appears to be still that this resource is on its own not sufficient. No quantitative data are available on the contributions of dissolved organic matter. What conditions in a coral reef would tend to promote selection for nutritional plasticity?

Coral reefs have come to be regarded by some authors as stable (or predictable) environments (see GRASSLE 1973). Such environments are usually typified by high species diversity and high specialization (see HESSLER & SANDERS 1967, SANDERS 1969, SLOBODKIN & SANDERS 1969). It is quite apparent that coral reefs fit the criterion of high species diversity, but it is difficult to see specialization expressed in the great deal of morphological polymorphism and nutritional plasticity demonstrated by the organisms central to the ecosystem, i.e. the corals themselves. Coral reef ecosystems are probably also predictable with respect to temperature, salinity and insolation, but within habitat predictability might be low, due to such factors as food availability, structural undermining and slumping (GOREAU & HARTMAN 1963) or competition for space (LANG 1973). It seems possible then that the term stability could be applied to coral reefs within the geological time scale, but instability or unpredictability may very well exist within such a system, based on more small scale perturbations.

If the positive correlation between environmental homogeneity and low genetic polymorphism is real, it should be possible to determine the homogeneity of the reef from the expressed polymorphism of the organisms which live therein. Some data are available which strongly suggest that many reef-dwelling invertebrates are highly polymorphic, e.g. different varieties of certain coral species are now being recognized (DUSTAN & LANG, personal communication; LEHMANN & PORTER 1973); *Z. sociatus*, a dominant coelenterate, is recognized as having possibly six variants (HADDEN 1968, Y. NEUMANN, personal communication); genetic polymorphism in *Tridacna maxima* apears to be very high (AYALA et al. 1973). POWELL (1971) has shown that nutritional heterogeneity alone can bring about genetic polymorphism. Given that coral reefs are very patchy environments and that many of the organisms therein demonstrate a high degree of nutritional plasticity, it would be reasonable to predict high levels of genetic polymorphism in reef-dwelling invertebrates.

The preponderance of symbiotic associations involving photosynthetic organisms (or organelles) in coral reef ecosystems may lend stability to the system through the creation of discrete "micro-ecosystems" within the ecosystem at large. Evidence is available to support the notion that each plant-animal association may represent a system of "tight" nutrient recycling (LEWIS & SMITH 1971) superimposed on the larger scheme of nutrient recycling within the overall system (TRENCH 1973) or the dynamic interchange between reef and "boundary water" (GOREAU et al. 1971).

SUMMARY

1. The tropical coral reef-dwelling coelenterate *Zoanthus sociatus* (ELLIS) lives in mutualistic symbiosis with dinoflagellates of the genus *Gymnodinium*. These algae are intracellular.
2. Analysis of the photosynthetic contribution of these endosymbionts shows a direct transfer of photosynthate from algae to animal and utilization of such substances by the animal. Such transfer does not involve destruction of the algae. In vitro studies of the photosynthetic products of the algae show that they synthesize a wide range of metabolites, but selectively release only a few including glycerol, glucose and alanine. These data indicate that such organisms possess an autotrophic mode of nutrition.
3. *Z. sociatus* shows a well defined feeding behaviour when offered homogenates of *Echinometra* eggs. A similar behaviour may be elicited with reduced glutathione. Proline and glycine produced "mouth opening" responses but not the complete feeding response. Alanine, glutamic acid and aspartic acid gave no response.
4. The ultrastructure of the mesenterial filaments shows that the tissue is well differentiated and possesses nematocysts (holotrichous isorhyzas). Several distinct cell types including mucus secreting and "zymogen" cells have been recognized. These cells probably play an important role in extracellular digestion.
5. The rate of digestion of exogenously supplied proteins by *Z. sociatus* compares favourably with that of sea anemones and corals. Digestion is both extracellular and intracellular, the latter process taking place after particle phagocytosis in the "digestive-excretory" cells of the mesenteries proximal to the filament.
6. *Z. sociatus* can absorb dissolved amino acids and sugars from very low concentrations ($10^{-6} - 10^{-7}$M), and may incorporate such metabolites particularly into reproductive tissue.
7. The pycnotic zooxanthellae found in the cells of the "digestive-excretory" zone of the mesenteries are probably derived from other areas of the animal gastroderm. These algae appear to undergo senesence in this tissue, but are not digested by the animals. This is very likely a normal phenomenon, in which case the "digestive-excretory" zone of the filament could be regarded as a "grave yard" for old defunct members of the algal population.
8. The polytrophic habit of reef-dwelling invertebrates with photosynthetic endosymbionts is viewed as an important parameter of coral reef nutrition, lending a great deal of nutritional versatility to animals. Such plasticity is probably a reflection of microinstability within an overall stable ecosystem.

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