ENDOCYTOSIS OF THE SYMBIOTIC
DINOFLAGELLATE Symbiodinium
Microadriaticum Freudenthal by
Endodermal Cells of the Scyphistomae of
Cassiopeia xamachana and Resistance of
The Algae to Host Digestion

William K. Fitt and Robert K. Trench
Department of Biological Sciences and Marine Science Institute, University of
California, Santa Barbara, California 93106, U.S.A.

Summary

The ingestion and fate of four types of particles by endodermal cells of the scyphistomae of Cassiopeia xamachana were investigated by scanning and transmission electron microscopy. Ferritin was endocytosed pinocytotically by invagination of the plasmalemma. These small pinocytotic vesicles fuse with other similar vesicles to form larger ferritin-containing vacuoles, which eventually fuse with lysosomes. Such secondary lysosomes exhibit acid phosphatase activity. The co-occurrence of acid phosphatase activity and ferritin in secondary lysosomes achieved maximum frequency within 2 h of uptake of ferritin and was evident for at least 4 h following uptake. Artemia particles, live freshly isolated symbiotic algae (Symbiodinium microadriaticum), and heat-killed S. microadriaticum are phagocytosed by endodermal cells. Ferritin-labelled lysosomes fused with food vacuoles containing particles of Artemia. Vacuoles containing heat-killed S. microadriaticum also showed evidence of phagolysosome fusion. S. microadriaticum in situ (i.e. in host cells) after 3 days exposure to the photosynthetic inhibitor, 3-(3-4-dichlorophenyl)-1,1-dimethylurea, appeared degenerate, and were found in loose-fitting host vacuoles, many in mid and apical portions of the host cell. More than 70% of these vacuoles with moribund algae contained the ferritin label, indicating that lysosome fusion had occurred. In contrast, live S. microadriaticum in control animals were almost always found at the base of the host cell in individual tight-fitting vacuoles with no evidence of lysosome fusion.

Live S. microadriaticum apparently escape host digestion by prohibiting the fusion of lysosomes with the vacuole in which they reside. Vacuoles containing defunct algal symbionts, in contrast, were subject to lysosomal attack.

Introduction

Entry of a genetically foreign entity into a host cell during the establishment of a symbiotic association involves a number of steps or 'obstacles', which may be summarized as: coming together, uptake (endocytosis), resistance to or avoidance of host cellular defences, and sequestration in the appropriate location in the host cell (cf. Muscatine, Cook, Pardy & Pool, 1975a; Trench, 1979, 1980a; Smith, 1980). In a previous paper (Fitt & Trench, 1983) a number of mechanisms of 'coming together' of marine hosts and the symbiotic alga Symbiodinium microadriaticum were
addressed experimentally. In the present study we place emphasis on the two following steps: namely, endocytosis and resistance to host digestive attack.

Previous studies on uptake of algal symbionts by coelenterate hosts have centred on the *Hydra–Chlorella* symbiosis. Phagocytosis in *Hydra* is initiated by contact of algae with the digestive cell surface (Cook, 1980), followed by engulfment by microvilli or pseudopods that possess a diffuse glycocalyx (Cook, D'Elia & Muscatine, 1978). McNeil (1981) characterized two different modes whereby freshly isolated algae were taken into digestive cells of hydra. Live algal cells were phagocytosed via either a funnel-shaped extension of the plasmalemma or a meshwork of microvilli. Heat-treated symbionts were only phagocytosed by the ‘funnel’ method. These two methods of entry are in contrast to the ‘multiple overlapping folds’ mode found in the uptake of *Artemia* (food) particles. Following phagocytosis of algae, the newly formed vacuoles contained only one alga per vacuole, with no evidence of the coated vesicles or diffuse glycocalices that were observed on the unchallenged plasmalemma (Cook *et al.* 1978). Algae were detected within phagosomes in less than 1 min after injection into the coelenteron (Cook *et al.* 1978), illustrating the rapidity of phagocytosis.

In the only other intracellular algal symbiosis in which endocytosis has been studied, ingestion of *Chlorella* by *Paramecium bursaria* differed from algal uptake into coelenterates in that more than one alga can initially be taken into a vacuole. Sequestration of one alga per vacuole was the usual end result of phagocytosis of the live symbionts (Karakashian & Karakashian, 1973).

There are few studies on the acquisition of algae in marine symbioses. The phenomenon is important, in the light of the growing list of symbiotic species that do not pass their algae directly to their sexual progeny via the egg (Trench, 1980a, 1983; Fitt, 1982). These species must acquire symbionts from the ambient environment. Present evidence indicates that *S. microadriaticum* are phagocytosed by coelenterate endodermal cells (Fitt, 1982; Colley & Trench, 1983).

The fate of particles endocytosed by coelenterate endodermal cells has only recently been investigated. Previous investigators noted acid-phosphatase activity at the apical ends of *Hydra* digestive cells and deduced that food particles in vacuoles in these portions of the cell were being digested (Lentz, 1966). Algal symbionts, in contrast, are apparently able to resist digestion and they persist, grow and divide inside the digestive cells. Studies with unicellular parasites and other algal–invertebrate associations (see Muscatine *et al.* 1975b, for a review) have shown several ways in which an intracellular symbiont may avoid digestion, survive and persist inside a host cell. These include escape from the phagosome to lie free in the host cell’s cytoplasm (see Trench, 1980b), movement of the vacuole and algae to areas away from the digestive zones of the cell (e.g. see Weis, 1976; Cooper & Margulis, 1977; McNeil, Hohman & Muscatine, 1981), prevention of phago–lysosome fusion (e.g. see Hohman, McNeil & Muscatine, 1982; O’Brien, 1980; Karakashian & Rudzinska, 1981), and resistance to or inactivation of lysosomal enzymes.

In this study we analysed the mechanism of ingestion, and the intracellular fate of food particles and of live and dead *S. microadriaticum* within endodermal cells of the scyphistomae of *Cassiopeia xamachana*. 
**Endocytosis and persistence of zooxanthellae**

**MATERIALS AND METHODS**

**Maintenance of experimental animals**

The host animals used in all experiments were scyphistomae of *Cassiopeia xamachana* cloned from asexual buds. Standard maintenance conditions for aposymbiotic animals were constant darkness at 25 ± 1 deg. C. They were fed *Artemia* nauplii 2–3 times per week. During incubation with algae, food particles or ferritin, the animals were kept at light intensities of 20 μEinstein m⁻² s⁻¹ at 25°C and were starved for 2 days before injection.

**Particles**

Ferritin (2X recrystallized, Sigma) was diluted approximately 8:1 with 0.45 μm Millipore-filtered sea-water (MFSW) to a concentration of about 10 mg/ml.

Food particles were prepared by homogenizing *Artemia* nauplii in a glass tissue-grinder and filtering the homogenate through a 0.22 μm Millipore filter. *S. microadriaticum* (clone no. 313) were isolated from 1- to 3-month-old *C. xamachana* jellyfish. These algae were originally introduced to *C. xamachana* scyphistomae from cultures maintained in ASP-8A (see Fitt, Chang & Trench, 1981). The algal symbionts were obtained by homogenizing jellyfish in a glass tissue-grinder; the resulting slurry was strained through four layers of cheesecloth to remove larger pieces of animal tissue, and then centrifuged at 433 g and rinsed three times with MFSW. Algae were heat-killed by exposure to boiling water for 5 min.

All particles were introduced into the mouths of 48-h-starved aposymbiotic scyphistomae via a micropipette. Algae, *Artemia* and ferritin were incubated in scyphistomae for various periods of time in sea-water containing the same concentration of particles as that injected into the coelenteron, before being rinsed with filtered sea-water and fixed for electron microscopy.

Time-course experiments of phagocytosis of algal cells into endodermal cells of scyphistomae were done for periods extending from 5 min to 24 h using algal densities of 10⁵–10⁸ algae per ml. After incubation for the prescribed period of time scyphistomae were transferred to MFSW, their bases cut off with a razor-blade to remove any adhering algae and the non-phagocytosed algal cells remaining were rinsed out of the coelenteron with micropipette-injected MFSW. The number of algae remaining was determined by homogenizing each scyphistoma in 0.2 ml MFSW in a tissue-grinder and taking the average of four replicate haemacytometer counts.

**Electron microscopy**

Scyphistomae were fixed in Karnovsky's (1965) fixative for 1 h, rinsed in 0.2 M-cacodylate buffer (pH 7.4), and post-fixed in cacodylate-buffered 1% osmium tetroxide. After dehydration in ethanol, animals for transmission electron microscopy (TEM) were embedded in Spurr's medium, sectioned and observed on a Siemens Elmiskop I electron microscope. Some sections were left unstained to enhance unambiguous recognition of ferritin labelling. Other sections were stained with uranyl acetate and lead citrate. Animals for scanning electron microscopy (SEM) were critical-point dried, cut in half with a razor-blade, sputter-coated with gold/palladium, and observed with a JEOL LSM-2 SEM.

Acid phosphatase was assayed after glutaraldehyde fixation by a modified Gomori technique (Barrett & Heath, 1977), using B-glycerophosphate as substrate, in acetate buffer (pH 6.1) at 37°C for 20 h. Animals were rinsed in buffer and post-fixed in 1% osmium tetroxide. Control animals were incubated without the substrate.

**Assay for phago—lysosome fusion**

Binding of secondary lysosomes containing ferritin to phagosomes was assayed by methods similar to those of Armstrong & Hart (1971). Ferritin (10 mg/ml) was injected into the mouths of 2-day-starved scyphistomae and the whole animal was allowed to incubate in sea-water containing the same concentration of ferritin. After 15 min the coelenteron of the scyphistomae and surrounding solution was flushed with MFSW three to four times over a 15-min period. The animals were then maintained for 1.5 h in MFSW to allow all residual ferritin to be taken into cells before algae or food particles were injected into the coelenteron. Scyphistomae were fixed for electron microscopy 2 h after injection with live or dead *S. microadriaticum* or *Artemia* particles.
The kinetics of formation of secondary lysosomes containing ferritin were established by incubation of scyphistomae in ferritin for 15 min, rinsing in MFSW three to four times and fixing scyphistomae periodically between 2 min and 4 h after the initial ferritin injection. Acid phosphatase was assayed as previously described.

For some experiments scyphistomae already containing clone no. 313 S. microadriaticum were placed in $10^{-6}$M-DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) for 3 days before ferritin was injected as previously described. Ferritin was allowed to incubate for 4 h, then washed out of the coelenteron. After 1 h post-incubation in sea-water these scyphistomae were fixed and processed for electron microscopy.

RESULTS

Ferritin-labelling of lysosomes

The endodermal cells of the scyphistomae of C. xamachana pinocytosed ferritin into small vacuoles (Fig. 1A), which appeared to fuse to form larger vacuoles (Fig. 1D). Lysosomes fused with vacuoles containing ferritin (Fig. 1B, C) so that the percentage of vacuoles containing both acid phosphatase and ferritin increased from less than 5 % at 5 min to maximum detectable levels of 65-70 % 2 h after the injection of ferritin (Fig. 2).

Phagocytosis of Artemia particles

Food particles (Artemia) were engulfed phagocytically (Figs 3A, 4). Small vacuoles (Fig. 3B) containing particles were present in the apical portions of the endodermal cells within 5 min of injection. Scanning electron micrographs showed that Artemia particles were often found attached to the flagella of endodermal cells shortly after injection into the coelenteron (Fig. 4A) and phagocytosed in groups by overlapping pseudopodia (Fig. 4B). The fused pseudopods containing newly phagocytosed 'packets' of Artemia particles (Fig. 4C) were prevalent in most endodermal cells after 1 h (Fig. 4D).

When animals had been preincubated with ferritin to label secondary lysosomes, and then provided with Artemia particles, lysosomes fused with phagosomes (Fig. 3c) and emptied their contents into the phagosomes (Fig. 3D).

Endocytosis of S. microadriaticum

Live and dead S. microadriaticum appeared to be phagocytosed by mechanisms involving engulfment by extensions of the plasmalemma (Figs 5, 6, 7). Algae were seen adjacent to host endodermal cells (Fig. 6A), often in contact with host cell flagella (Figs 5B, 6B), shortly after injection into the coelenteron. After initial contact with a host cell (Figs 5A, 6A), pseudopods surrounded the alga (Figs 5C, 6C, 7A, B). Engulfment by microvilli was not seen. Live freshly isolated S. microadriaticum were sequestered in individual tight-fitting vacuoles and were commonly seen at the base of endodermal cells after 1 day (Fig. 5D). Heat-killed freshly isolated S. microadriaticum were usually seen in loose-fitting vacuoles (Fig. 7D, E) and only rarely near the base of the host cell. Dead algae were not seen in macerations of scyphistomae 48 h after injection. Neither coated vesicles nor diffuse glycocalices were seen during uptake of S. microadriaticum.
Fig. 1. Endocytosis and fate of ferritin particles in endodermal cells of scyphistomae of *C. xamachana*. A. Pinocytosis of ferritin 5 min after injection into the coelenteron (c). Small vacuoles (small arrows) fuse to form larger vacuoles. Ferritin is also found between endodermal cells (large arrows). Unstained. ×20 500. B. Large secondary lysosomes (l) 2 h after injection of ferritin. Unstained. ×30 800. C. Small secondary lysosomes (large arrow) formed by the fusion of a primary lysosome (small arrows) containing acid phosphatase and a vacuole containing ferritin (f) 5 min after injection of ferritin. Unstained. ×63 600. D. Large phagosomes containing ferritin (f), after injection of ferritin. Stained. ×14 900.
Phagocytosis of live freshly isolated algae is a rapid process. Fig. 8 shows that at concentrations of $10^6$ and $10^8$ algae per ml phagocytosis saturates at 1000–2000 cells per scyphistoma after only 30 min. At $10^8$ algae per ml uptake is slower, probably because of the clumping of cells characteristic of this clone of *S. microadriaticum* at high concentrations. Maximum levels of uptake reached 10 000 cells per scyphistoma. The actual concentration of algae in the coelenteron changed in an undetermined
Endocytosis and persistence of zooxanthellae

Fig. 3. Endocytosis and fate of *Artemia* particles in endodermal cells of scyphistomae of *C. xamachana*. Unstained. c, coelenteron. a. Phagocytosis of *Artemia* particles by cellular extensions of the plasmalemma (arrows) 5 min after injection of *Artemia*. ×22700. b. Phagosomes containing one or more *Artemia* particles (arrows) 5 min after injection of *Artemia*. ×28 000. c. Fusion (?) of a secondary lysosome (f) to a phagosome (p) containing *Artemia* particles 30 min after injection of *Artemia*. ×19 300. d. Ferritin label, indicating lysosomal contents in phagosome containing *Artemia* particles (arrows) 2 h after injection of *Artemia*. ×22 000.

fashion as the scyphistomae concentrated algae by ciliary action, which may explain some of the variability in the data.

Intrinsic acid-phosphatase activity was found in 21–36 % of live algal cells freshly
Fig. 4. Scanning electron micrographs of endocytosis of *Artemia* particles by endodermal cells of scyphistomae of *Cassiopeia xamachana*. a. Spherical *Artemia* particles (arrows) adjacent to and attached to flagella of endodermal cells (d) 5 min after injection of *Artemia*. ×16 700. b. Phagocytosis of a mucus-bound 'packet' of *Artemia* particles (p) by pseudopods (arrows) 10 min after injection of *Artemia*. ×16 600. c. Phagosome (arrows) in the apical portion of an endodermal cell enclosing *Artemia* particles (p) 5 min after injection of *Artemia*. ×3300. d. Bulbous-appearing endodermal cell surface after 60 min of phagocytosis of *Artemia* particles. ×2200.

Fig. 5. Endocytosis and fate of live freshly isolated *S. microadriaticum* in endodermal cells of scyphistomae of *C. xamachana*. Unstained. a. Contact of host plasmalemma (p) with algal cell (s) 5 min after injection of algae into coelenteron. ×14 000. b. Algal cell in contact with host-cell flagellum close to endodermal cell surface. Note root fibre of flagellum (arrow). ×11 000. c. Extension of plasmalemma (large arrows) around algal cell during phagocytosis. Note host-cell flagellum (small arrow). ×9 400. d. Newly phagocytosed algal cell in tight-fitting vacuole (arrows). Note nearby lysosome containing ferritin (f) and lack of ferritin in phagosome. ×14 700.
Endocytosis and persistence of zooxanthellae

Fig. 5
isolated from medusae of C. xamachana (Table 1). After phagocytosis, slightly higher incidence (between 33 and 59%) of acid-phosphatase activity was associated with the algae within the phagosome. These data suggest that between 12 and 23% of the algae phagocytosed were unable to prevent lysosome fusion. Intrinsic acid phosphatase was apparently denatured in heat-killed algae, but was found associated with all heat-killed algae after phagocytosis, indicating that host lysosomes had fused with the phagosomes.

Similarly, Table 2 shows that while ferritin is seldom found in vacuoles containing newly phagocytosed, live freshly isolated S. microaadriaticum (Fig. 5D), it commonly occurs in vacuoles with heat-killed algae (Fig. 7c, d, e). These observations suggest that some property of the live alga inhibits lysosome fusion with algal phagosomes. However, perialgal material, which often coats the surface of freshly isolated S. microaadriaticum (Fig. 7a, c), may also be responsible for this phenomenon (Trench,

Fig. 6. Scanning electron micrographs of phagocytosis of live freshly isolated S. microaadriaticum by endodermal cells of scyphistomae of C. xamachana. a. Algal cells (s) adjacent to host endodermal cells 2 min after injection of algae into host coelenteron. ×1200. b. Contact of algal cell (s) with host endodermal cell 5 min after injection of algae. Note host-cell flagellum adjacent to alga (arrows). ×6700. c. Pseudopods (arrows) of host endodermal cell extending over algal cell 5 min after injection of algae. ×3000.
Colley & Fitt, 1981; Colley & Trench, 1983). In order to distinguish between these two alternatives, some symbiotic scyphistomae were treated with DCMU for 3 days while control animals were maintained without DCMU. Animals in DCMU for 3 days had degenerate-appearing algae in loose-fitting vacuoles (Fig. 9A), usually containing...
DISCUSSION

The question whether algal symbionts are digested by their invertebrate hosts has been debated for over 50 years (Boschma, 1924, 1925; Yonge & Nichols, 1931; Yonge, 1936; Mansour, 1946a,b,c). Observations of disintegrating algae in the host (Oschman, 1968; Karakashian, Karakashian & Rudzinska, 1968; Weiss, 1976; Steele & Goreau, 1977), including evidence of acid-phosphatase activity associated with degenerating algae (Fankboner, 1971; Karakashian & Karakashian, 1973), have been interpreted as evidence of host digestion of their symbionts. However, results of these types of experiments do not rule out algal autodegradation (Muscatine, 1973; Trench, 1974, 1979), especially in the light of the recent discovery that *S. microadriaticum* has
Table 1. Percentage of S. microadriaticum containing acid phosphatase label before (outside scyphistomae) and after (inside scyphistomae) phagocytosis by endodermal cells of scyphistomae of C. xamachana

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Outside scyphistomae</th>
<th>Inside scyphistomae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live algae</td>
<td>0·25</td>
<td>21·0 (505)</td>
</tr>
<tr>
<td></td>
<td>0·5</td>
<td>35·4 (225)</td>
</tr>
<tr>
<td></td>
<td>2·0</td>
<td>24·3 (169)</td>
</tr>
<tr>
<td></td>
<td>1440·0</td>
<td>–</td>
</tr>
<tr>
<td>Heat-killed algae</td>
<td>2·0</td>
<td>0* (50)</td>
</tr>
</tbody>
</table>

* Algal enzymes denatured.

Time = h after injection into scyphistomae or after isolation. Numbers of algae observed are given in parentheses.

Table 2. Percentage of vacuoles in endodermal cells of scyphistomae of C. xamachana containing both S. microadriaticum and ferritin 1 h and 2 h after being injected with live freshly isolated or heat-killed freshly isolated algae (A) and in previously infected scyphistomae maintained in DCMU or sea-water for 3 days before being injected with ferritin (B)

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Freshly isolated</td>
<td>8·3 (72)</td>
</tr>
<tr>
<td>Heat-killed</td>
<td>85·7 (35)</td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Sea-water</td>
<td>–</td>
</tr>
<tr>
<td>DCMU</td>
<td>–</td>
</tr>
</tbody>
</table>

See the text for details. Numbers of algae observed are given in parentheses.

its own intrinsic acid phosphatases (Trench et al. 1981). Our results show only a slight increase in the proportion of live algal cells labelled with acid phosphatase after phagocytosis into digestive cells, suggesting that most of the enzyme found originated from the alga and not the host.

Two different views of 'digestion' of S. microadriaticum may be derived from observations of 'dead' and 'live' algae in the host cell. If one assumes that the host is 'farming' its algae (cf. Yonge, 1936; Steele & Goreau, 1977), then there must be a means of selecting which algae are digested and which are left to reproduce. Another possibility is that a live healthy alga inhibits normal host digestion by inhibiting
phago-lysosome fusion and possibly by resisting enzymic attack, while a dead or dying alga is unable to inhibit the normal digestive process. From this point of view, the host treats the symbiosome like any other phagosome after the alga dies or fails to function properly; lysosomes bind to the vacuoles and the hydrolytic enzymes released help to break down the contents. Our data on binding of labelled primary (acid phosphatase) and secondary (ferritin) lysosomes to vacuoles containing algae are consistent with this latter hypothesis. Algal hydrolytic enzymes may initiate and/or facilitate this process, thereby adding an autodegradative component.

The actual molecular mechanism of inhibition of lysosomal fusion has not been determined in any intracellular system. Present research has focussed on secretion of an enzyme that blocks fusion (cf. Lowrie, Jackett & Ratcliffe, 1975) or changes the properties of the phagosome membrane to inhibit lysosome fusion. In some systems anionic substances inhibit phago-lysosome formation, possibly through charge repulsion (Hart & Young, 1979). Polycationic polyanions can overcome this inhibition. Hohman et al. (1982) found that Hydra lysosomes bind to phagosomes containing live Chlorella pretreated with polylysine, whereas few instances of lysosome-binding to vacuoles containing untreated Chlorella were found. This is consistent with the theory that a change in the membrane charge or chemistry, possibly induced by a live symbiont, may alter lysosome fusion with the algal vacuole. Phagosome membranes in Amoeba (Bowers, 1980), Paramecium (Allen, 1976), and Tetrahymena (Kitajima
Endocytosis and persistence of zooxanthellae

209 & Thompson, 1977) have been shown to differ in particle densities and chemical composition (higher protein to phospholipid ratios and glycosphingolipid content) from the plasma membrane from which they were derived. The change apparently takes place within 15 min of ingestion and is not evident during the early stages of phagocytosis when the phagosome membrane is still part of the plasmalemma. Freeze-fracture evidence (Meier, Reisser & Wiessner, 1980) of differences between membranes of perialgal and digestive vacuoles in Paramecium bursaria suggests that live algae do in fact change the chemical properties of the phagosome.

Hypotheses of the method of algal uptake by marine coelenterates (Muscatine et al. 1975b; Taylor, 1973; Kinzie, 1974; Trench, 1979) have been based largely on electron microscopy of Hydra digestive cells (Cook et al. 1978; McNeil, 1981). We present here direct evidence showing that the method of entry of S. microadriaticum into a coelenterate host is by phagocytosis by endodermal cells (confirmed by Colley & Trench, 1983). Phagocytosis of live freshly isolated algae occurs in only a fraction of the endodermal cells of scyphistomae. The average number of endodermal cells in a 1 mm diameter scyphistoma was calculated from SEM and TEM pictures to be about 160 000. Therefore, between 1 and 7 % of these cells take up algae, depending on the concentration injected. This is in contrast to endocytosis of Chlorella by 95 % of the endoderm cells of Hydra viridis (P. McNeil, personal communication). There was apparently no difference in the modes of uptake of live or dead S. microadriaticum as judged by profiles seen in transmission electron microscopy. S. microadriaticum reside in individual vacuoles after engulfment. Live algae were found in tight-fitting vacuoles at the base of the host cell, away from apical areas of maximum lysosomal activity 24 h after injection, whereas dead algae were found in loose-fitting vacuoles and are not seen in macerations of scyphistomae 48 h after injection.

Transmission and scanning EM showed that the digestive cells of scyphistomae of C. xamachana phagocytose Artemia in a fashion similar to other coelenterates using 'pseudopods', 'overlapping folds' and 'ruffles' (Gauthier, 1963; Afzelius & Rosen, 1965; McNeil, 1981). There have been no reports documenting morphological differences in phagocytosis of food particles by coelenterate digestive cells.

Endocytosis of ferritin occurs in the classical pinocytotic fashion, including membrane invagination, fusion of microvesicles into larger vesicles (Lentz, 1966) and subsequent fusion to lysosomes. Ferritin is also found between some cells (Fig. 1A; cf. Lentz, 1966). Levels of acid phosphatase/ferritin double-labelling of digestive vacuoles after 2 h (Fig. 2) are probably minimal estimates, since heavy acid-phosphatase labelling (i.e. see Fig. 1n) tends to obliterate the ferritin label and TEM photographs may not show both heterogeneous labels in the same vacuole in the same EM section.

In summary, the symbiotic dinoflagellate S. microadriaticum is phagocytosed by endodermal cells of the coelenterate C. xamachana. The algae may use three mechanisms to help escape host digestive attack. First, live healthy symbionts are transported to the base of the host cell, away from most but not all lysosomal activity. Secondly, live algae in vacuoles inhibit lysosomal fusion. Thirdly, our data
do not exclude the possibility that *S. microadriaticum* is resistant to lysosomal enzymes. So far we have not seen ferritin inside degenerate algal cells. None of these mechanisms of resisting host digestion is found with dead symbionts, food particles or ferritin.

We thank R. Gill and D. Pierce for technical assistance on the electron microscope and Drs N. Colley, S. Fisher, A. Gibor, L. Muscatine and F. Wilkerson for comments on the manuscript. This work was supported in part by NSF grant PCM 78-15209 and U.S. AID grant DPE-5542-G-SS-1085 (to R.K.T.).

REFERENCES


Endocytosis and persistence of zooxanthellae


W. K. Fitt and R. K. Trench


(Received 11 April 1983–Accepted 9 May 1983)