179. *An Electron Microscopic Proof for a Path
of Nutritive Substances from Zooxanthellae
to the Reef Coral Tissue*^{*)}

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There are two different opinions concerning the meaning of zooxanthellae in corals. One is represented by Yonge⁷⁾ who concluded that zooxanthellae are functional, serving as consumers of waste products of the corals and as donors of oxygen in return, and that they are not used as food for the corals. The other is represented by Boschma²⁾ who considered that zooxanthellae are used as nutrition for corals. In regard to a symbiotic relation of zooxanthella with other coelenterates, Gohar³⁾ believed that *Xenia* used zooxanthellae as a nutritive source. Bayer¹⁾ extended this to other octocorals from histological differences in the distribution of nematocysts and zooxanthellae in them. Muscatine and Hand⁶⁾ have recognized autoradiographic patterns of labelled carbon in zooxanthellae and in host cells after the immersion of a sea-anemone in a medium containing CO₂ with the carbon isotope C¹⁴. They concluded that the labelled CO₂ is taken into zooxanthellae and transmitted by them to the host tissue. That is, they considered that the zooxanthellae served as a nutritive source for the sea-anemone.

However, the true relation between zooxanthellae and their host cells in corals and sea-anemones remained ambiguous until my previous work⁵⁾ revealed the former to be intercellular symbionts. But, this being so, how are the nutritive substances of zooxanthellae forwarded to the coral cells?

Some colonies of *Oulastrea crispata* have been cultured in a small bottle on my laboratory table at room temperature without any supply of food but only window light. These corals have survived for a long period; the longest exceeded fifteen months. They are healthy and have increased in their polyp number. Similar experiments with *Acropora*, *Goniopora*, *Hydnophora*, and *Cyphastrea* have been going on successfully for three months at about 20°C under constant illumination. These observations suggest that the symbiotic zooxanthellae with their autotrophic life contribute to the nutrition of the host coral. However, there are no degenerating zooxanthellae

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in the tissues of such coral as has been observed by Yonge and Nichols.⁸⁾ This is true even under electron microscopic observations.

During the examination of electron micrographs of the zooxanthellae in corals and sea-anemones it is found that the outer surface of the plasma membrane of the zooxanthellae is covered with a thick layer of scale-like substances as was mentioned briefly in the previous paper.⁵⁾ The present paper deals with a further study of such scale-like substances and their fate in the coral tissues. There is some evidence that these scale-like substances are derived from the plasma membrane of the zooxanthellae and that they afterwards decompose and are absorbed into the surrounding coral cells. That is, the coral tissues receive nutritive substances from the zooxanthellae without sacrificing these symbionts.

The materials and method used in this experiment were the same as those of the previous papers on the nerve plexus⁴⁾ and on zooxanthellae in a reef coral.⁵⁾ Additional observations were carried out

Fig. 1. An enlarged view of parts of zooxanthellae in the endoderm of *Oulastrea crispata* and their neighboring coral cells. A, protoplasmic membrane; B, scale-like substances; C, chloroplast; D, desmosome between coral cells; G, glycogen granules. Scale indicates 1 micron. $\times 15000$

Fig. 2. Higher magnification of a part of the surface region of the zooxanthella showing detailed structures of the protoplasmic membrane and the scale-like substances. E, part of coral cell; F, decomposed portion of scale-like substance; H, homogeneous layer. $\times 57000$

on samples from several corals such as *Goniopora*, *Lobophyllia*, and *Hydnophora* and also on some zooxanthellae-carrying sea-anemones.

Fig. 1 shows an enlarged view of parts of the surface regions of zooxanthellae in the endoderm of *Oulastrea crispata*. The outer surface of the zooxanthella is covered by a protoplasmic membrane which is composed of a thin double membrane, and by an underlying layer of rather homogeneous structure measuring about 500–700Å in thickness. In this layer, however, there is no sign of the presence of cellulose or fibrous structures such as have been reported in plant cell walls.

Covering the protoplasmic membrane there is a thick accumulation of scale-like substances. A closer observation of these substances reveals that they are composed of fragments of double membranes whose structure is quite identical with that of the protoplasmic membrane. In order to demonstrate this more clearly, Fig. 2 gives a further enlargement of a similar region from another picture. This fact suggests that the scale-like substances may be derived from the protoplasmic membrane or from decomposed fragments of it. The scale-like substances are observed in the form of narrow stripes which swell somewhat especially at the outer region. Then, they disappear, probably scattering into fine particles. In the coral cells facing the

Fig. 3. A zooxanthella in the endoderm of *Lobophyllia* which had been kept in darkness for 12 hours showing absence of scale-like substances and glycogen granules. Scale indicates 1 micron. $\times 8000$

place where they disappear there are many granular substances of various dimensions, each of which is composed of fine particles. These granules may be glycogen granules which have been absorbed as fine particles and stored in the cell.

The density of accumulation of scale-like substances varies greatly from one to another or even from region to region in one zooxanthella. This is probably caused by differences in physiological condi-

tions of both the zooxanthellae and the surrounding coral cells. When the coral is kept in darkness for a long period, say 12 hours, the scale-like substances show a tendency to disappear as has been shown

in Fig. 3.

A preliminary experiment to detect carbohydrates released by zooxanthellae has been carried out by employing suspensions of zooxanthellae which have extruded from *Acropora*. One half of the suspension was exposed to direct sunshine for 3 hours and the other half was kept in darkness as a control. The experimental medium, after being freed from the zooxanthella by a centrifuge, showed reliable differences from that of the control in reactions to Fehling's solution.

These facts suggest that there is a pathway of carbohydrates from the zooxanthella to the coral cells.

References

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