Reference: *Biol. Bull.*, 142: 236–242. (April, 1972)

BENEFIT TO SYMBIOTIC ZOOCHLORELLAE FROM FEEDING BY GREEN HYDRA [1]

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The study of various algal-invertebrate symbioses has largely concerned the effects of endosymbiotic algae on their invertebrate partners. The most conclusive of these studies have shown that the green algae (zoochlorellae) symbiotic with fresh-water invertebrates augment the survival of the host during periods of low food supply (Pringsheim, 1928; Karakashian, 1963; Muscatine and Lenhoff, 1965). This benefit is probably due to low molecular weight compounds of photosynthetic origin which the algae pass on to their hosts (Muscatine and Lenhoff, 1963; Muscatine, Karakashian and Karakashian, 1967).

The possibility that the algae also benefit from these associations is suggested by several reports in the literature. Yonge and Nicholls (1931) concluded that dinoflagellates (zooxanthellae) symbiotic with scleractinian corals removed waste products from coral tissue; presumably the algae could utilize these products in their own metabolism. Siegel (1960) reported that zoochlorellae survived in *Paramecium bursaria* after 100 generations of growth of the host in darkness. One implication of this finding is that the algae utilized heterotrophic carbon for growth in darkness, and that bacteria fed to the paramecia may have been the source of this carbon. Finally, uptake of ^{35}S by symbiotic algae from food ingested by animal hosts is indicated in green hydra (Muscatine and Lenhoff, 1965) and in a sea anemone (Cook, 1971).

The benefit implied by these examples is that food ingested by animal hosts is a nutrient source for algal endosymbionts, although rigorous experimental proof of this is lacking. The present paper presents evidence that feeding by green hydra promotes the growth of its symbiotic zoochlorellae under conditions which necessitate nutrient flow from the hydra to the algae.

General Materials and Methods

Green hydra were purchased from Carolina Biological Supply Co. (Burlington, North Carolina). The company identifies these animals as *Chlorohydra viridissima,* although the taxonomic status of these organisms appears to be in doubt (Oschman, 1967). All hydra used in my experiments were derived from these stocks. Stocks for all experiments were grown in M-solution (Muscatine, 1961) using the techniques of Loomis and Lenhoff (1956). All stock cultures were

[1] This paper is based on a portion of a dissertation submitted to the Graduate School of Arts and Sciences of Duke University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology.

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maintained at 22 ± 1° C under 24-hour fluorescent illumination (2000 lumens/m² at the surface of the cultures). In all experiments, "uniform hydra" were selected from stock cultures which had been fed brine shrimp nauplii daily for at least fourteen days prior to use. "Uniform hydra" are defined by Lenhoff and Bovaird (1961) as being hydra from logarithmically growing cultures which have one small bud and which have been starved for one day. These authors discuss why the animals may be considered uniform in size and in distribution of cellular components.

Results

Effect of feeding on growth of algal symbionts in light

Triplicate samples of five "uniform" hydra taken from constant light stocks were fed an excess of freshly hatched brine shrimp nauplii at intervals ranging from once daily to once every four days for thirteen days; illumination and temperature conditions were as described for the maintenance of stock cultures. All culture solutions were changed one hour after feeding and again five hours later; during the second change the hydra were removed from their dishes and the dishes wiped clear of accumulated debris. The hydra were returned to their dishes with clean culture solution.

On the fourteenth day after the start of the experiment, the hydra in each dish were counted and then homogenized with a tissue grinder. I separated algal cells from the suspension by centrifugation (275 *g* for ten minutes). After three washes with deionized water the algae were collected on membrane filters (0.45 μ^2 mesh); these filters were mounted on microscope slides and the algae counted with the aid of a phase contrast microscope.

The final number of algal cells in each culture fed in the light increased with increased feeding frequency (Fig. 1, white histograms). The number of algal cells per hydranth appeared to remain constant (Fig. 2, white histograms).

These results imply that the growth rates of green hydra and its symbiotic algae are similar. Since the doubling time of these hydra when fed daily in constant light is 2.0 days (Cook, 1970), this presumably is the doubling time of the algae grown under these conditions. This is a slower growth rate than that of autotrophic *Chlorella* strains in culture (Samajima and Myers, 1958) and may indicate some "limitation" of the growth of the symbionts. It is also possible that "excess" algae leave the hydra. Taylor (1969) reports that the endosymbiotic zooxanthellae of a sea anemone are occasionally released from the animal; I made no attempts to quantify any such losses of algal cells to the medium.

Effect of feeding on growth of algal symbionts in darkness

To quantify possible heterotrophic growth of the algae the above experiment was repeated in darkness. Triplicate samples of five "uniform" hydra taken from stocks grown under constant illumination were fed and cleaned as described in the previous section. These experimental cultures were maintained in a closed box painted black on the inside; the boxes were kept in a light-tight drawer. Feeding and cleaning of these cultures occurred under safelight illumination (Kodak Wratten OA, 20-watt bulb at twenty feet). Total safelight exposure did not ex-

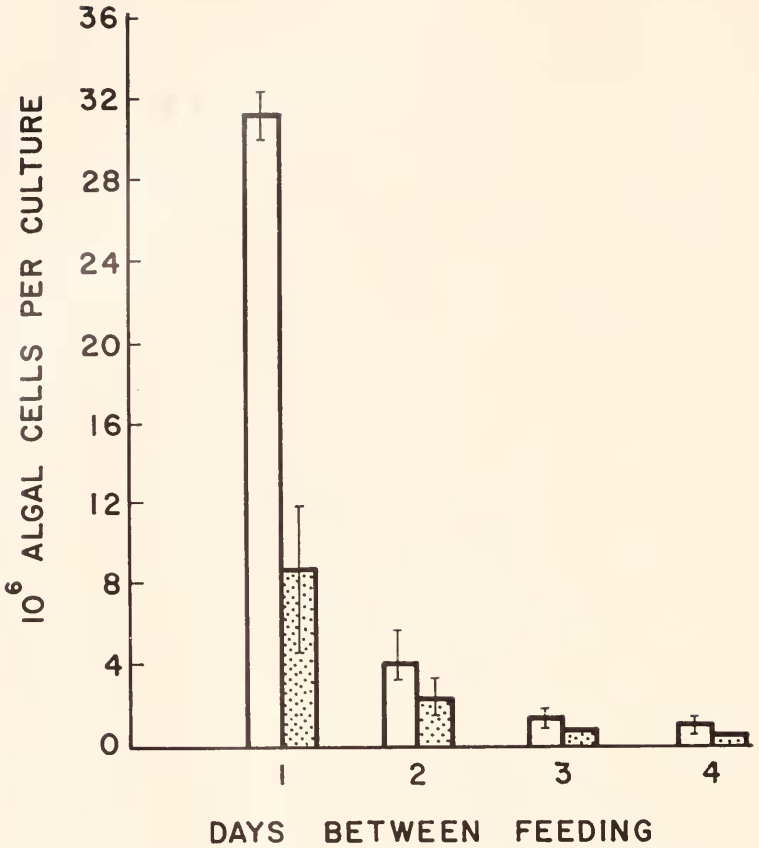

FIGURE 1. The algal cell content of hydra cultures after 13 days of feeding at various frequencies. The data represent the mean number of algal cells in each culture dish at the termination of the experiment. The white histograms represent the algal cells from cultures fed in constant light; the stippled histograms represent the number of algal cells from hydra fed in darkness. The vertical bars represent the range; N = 3 for all samples.

ceed one hour per day. Counter tops and solution changes were examined for animals which may have been poured off; these animals were returned to the proper dish. After fourteen days the hydra and the algal cells were counted as described for the light experiment.

In darkness as well as in light increased feeding of the hydra resulted in increased algal growth (Fig. 1, stippled histograms), although the absolute number of algal cells at any feeding frequency in the dark was always less than that of cultures fed in the light at the same rate. Comparisons of growth rates of hydra and algae in darkness (Fig. 2, stippled histograms) imply that, as in the light, bud production by the hydra and increase in algal cell numbers occurred at similar rates.

It follows from the above results that growth rates of hydra in darkness were lower than growth rates of hydra fed at corresponding rates in the light. This lower growth rate in darkness is also suggested by the data of Stiven (1965),

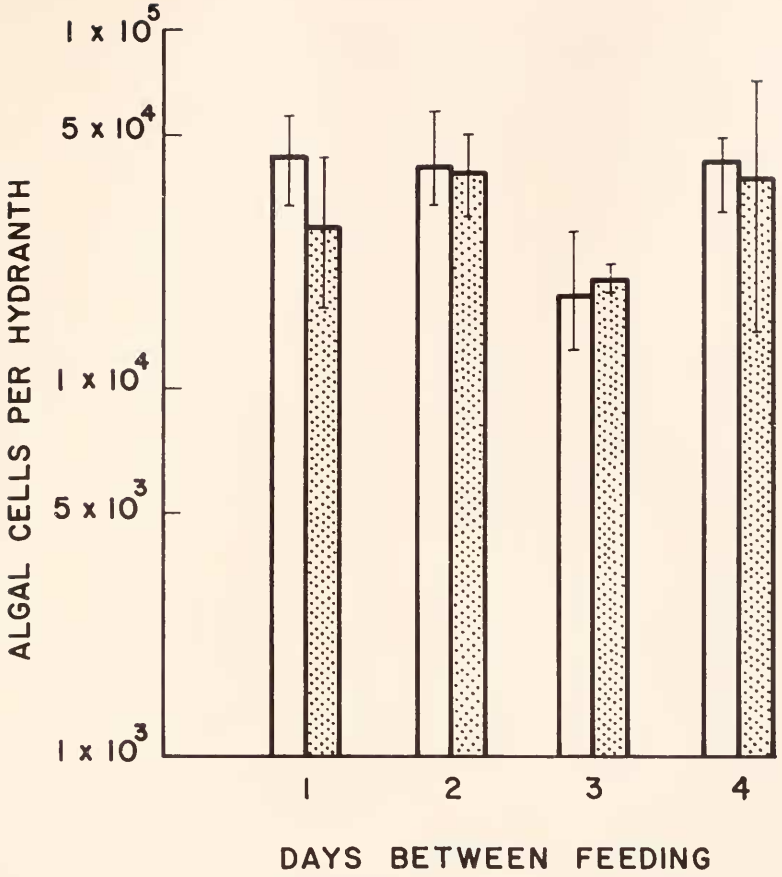

FIGURE 2. The number of algal cells per hydranth in hydra cultures after 13 days of feeding at various frequencies. A hydranth is defined as any bud. The white histograms represent cultures fed in continuous light; stippled histograms represent cultures fed in darkness. The vertical bars represent the range; N = 3 for all samples.

particularly when hydra were fed less often than once every 24 hours. However, in my studies I cannot exclude the possibility that hydra were lost during the feeding and cleaning periods; such losses would imply that the growth rates of both hydra and algae in darkness were greater than I found.

Carbon fixation by green hydra in light and darkness

The results of the previous section suggested that the algae growing in darkness utilized heterotrophic carbon sources for growth; however, it is possible that photosynthesis may have occurred in the dark cultures, particularly during the periods of feeding and cleaning when the animals were exposed to the safelight. To assay the relative amounts of photosynthesis in the constant light and dark conditions I exposed "uniform" green hydra in "M-solution" containing $NaH^{14}CO_3$

(0.27 μc/mg, 0.25 μc/ml) to either 24 hours of constant light or to one hour of safelight followed by 23 hours of darkness. Radioactivity of the hydra was assayed by the method of Muscatine and Lenhoff (1963).

Hydra in the dark fixed less than 1½% of the carbon fixed by hydra in the light. Since hydra tissue is capable of heterotrophic CO_2 fixation (Lenhoff, 1959), I take this result to indicate that photosynthesis by the dark cultures was negligible compared to that in the light so that the algae may be using exogenous reduced carbon sources for growth in darkness.

Uptake of ^{14}C by algae from food ingested by green hydra

One source of the reduced carbon used by the algae for growth in darkness could be food ingested by the hydra. I investigated the possible uptake by the algae of carbon from ingested food in preliminary studies by feeding ^{14}C-labelled brine shrimp nauplii to green hydra. Yeast cells were labelled with D-glucose-^{14}C (uniformly labelled; New England Nuclear) and were fed to freshly hatched brine shrimp nauplii; the larvae became radioactive within 2 days. The labelled nauplii were then fed to hydra in both continuous light and continuous darkness. Forty-eight hours after feeding, pooled samples of 5–10 hydra were homogenized, and the algae separated by centrifugation (270 *g*). Washed algal pellets were collected on Millipore filters (0.45 μ^2 mesh) and the filters glued onto planchets. Aliquots of algae-free hydra supernatants were also placed on planchets.

Analysis of the distribution of label between hydra supernatants and algal pellets in two trials in the light showed that 22% and 26% of the total recovered radioactivity was in the algal fraction, while two samples kept in continuous darkness yielded 25% and 34% of the total radioactivity in the algal fraction. Estimates of contamination showed that no more than 10% of the radioactive hydra tissue is recovered in the algal fraction by this technique (Cook, 1970); thus it appears that carbon from ingested food is taken up by zoochlorellae in both light and darkness.

Discussion

"M-solution" contains no phosphorus, sulfur, or trace elements, and no nitrogen except that found in "Tris" buffer (Muscatine, 1961); therefore these inorganic nutrients must be supplied to both hydra and algae by the food of the hydra in both light and darkness. This is borne out in tracer experiments in which ^{35}S has been traced from ingested food to the zoochlorellae of green hydra cultured in "M-solution" (Muscatine and Lenhoff, 1965). Since photosynthesis by green hydra in the dark experiments described in this paper is negligible, and since ^{14}C is taken up by the algae from ingested food, it follows that the food of the hydra is the source of some of the reduced carbon used by the algae for growth in darkness. It is also possible that the algae use the "Tris" present in "M-solution" as a source of organic carbon for this purpose.

Three lines of evidence suggest that organic nutrients may also flow from coelenterates to algal endosymbionts in the light, and that these nutrients are metabolically used by the algae. First, zoochlorellae take up ^{14}C from food ingested by hydra in the light (this paper), although this may represent photo-

synthetic incorporation of CO_2 produced by the hydra; the resolution of this question will depend on the analysis of labelled compounds in the algal and animal fractions. Secondly, several independent reports indicate that the symbionts of green hydra have not been grown successfully in culture outside the hydra (Loefer, 1936; Park, Greenblatt, Mattern and Merril, 1965; Muscatine *et al.,* 1967). This is not the case for zoochlorellae isolated from *Paramecium bursaria* (Loefer, 1936) and *Spongilla lacustris* (Muscatine *et al.,* 1967), and perhaps indicates the loss of metabolic pathways in the hydra symbionts. Presumably these pathways would have normally synthesized metabolites which can be supplied by the hydra so that the algae have become dependent upon the hydra to supply certain "essential" nutrients.

Finally, the possibility of a metabolic loss in algal endosymbionts is indicated by the work of von Holt (1968). He found that the dinoflagellates (zooxanthellae) symbiotic with a West Indian zoanthid contained no free glycine labelled with ^{14}C after a 3 hour incubation with $^{14}CO_2$, even though labelled glycine was detectable in animal tissue. This result suggests that the algae are unable to synthesize glycine from photosynthetic precursors, at least within the 3-hour limit of the experiment, so that the algae may have to depend upon the animal to supply this amino acid.

The selective value of such metabolic "defects" has been experimentally demonstrated in bacteria (Zamenhof and Eichhorn, 1967). These workers found that "defective" bacteria grew faster than "wild-type" bacteria when both are grown on complete media and suggest that such defective bacteria may benefit because they do not have to manufacture the DNA and synthetic machinery required for deleted pathways. It is conceivable that a similar situation may exist in the green hydra symbionts; metabolic blocks in carbon metabolism should therefore be looked for in these algae.

I thank S. A. Wainwright and my wife Susan for their criticisms throughout this study. This work was supported by the Cocos Foundation and by USPHS Grant FR-07-070 to Duke University.

Summary

1. The symbiotic zoochlorellae of green hydra grow faster in both light and darkness with increased feeding by the hydra, although growth of algae and of hydra fed at similar frequencies is always less in darkness.

2. The algal cell content of green hydra is similar under all conditions of feeding in both light and darkness.

3. Photosynthesis by green hydra in darkness is negligible when compared to that by hydra in the light.

4. The algae take up ^{14}C from brine shrimp ingested by the hydra in both light and darkness.

5. It is suggested that food ingested by the hydra serves as a source of inorganic nutrients for the algae, as well as a pool of organic nutrients which the algae utilize for heterotrophic growth in darkness and which the algae may require for growth in the light.

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