

## Carbon Fixation & Excretion in Symbiotic Algae (Zooxanthellae) in the Presence of Host Homogenates

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**Rates of carbon fixation and excretion in zooxanthellae, isolated from the massive coral *Favia pallida*, were found to decrease with time. In the presence of host homogenate, the two rates were enhanced. Zooxanthellae responded to tissue homogenate of other species of corals also, but the responses were of different degrees. Excretion of carbon continued both in the light and darkness. Tissue homogenate from *F. pallida* stimulated carbon fixation in zooxanthellae even after 4 hr which is longer than the reported duration of 3 hr regarding the effectiveness of the homogenate. Probably the property of zooxanthellae to excrete photo-synthetically fixed material in the presence of host homogenate is to provide a source of nutrition to corals.**

IN an earlier communication it has been pointed out that zooplankton organisms: found in the vicinity of coral reefs are unable to meet the nutritional requirements of the reef, and that the soluble material produced by zooxanthellae present in the gastrodermal cells of corals is of possible nutritional value to the corals<sup>1</sup>. The present study has been undertaken to demonstrate *in vitro* the influence of tissue homogenates obtained from the host (massive coral, *Favia pallida*), and other different hosts on the carbon fixation and excretion of zooxanthellae. It has been shown that corals possessing zooxanthellae show calcification rates many times greater in the light than in the dark, and in those corals which have lost their zooxanthellae, calcification is slow and remains unaffected by light<sup>2-4</sup>. It has also been shown that soluble material produced by zooxanthellae as a result of photosynthesis, is released by them and this product finds its way into the host tissue<sup>5-9</sup>.

### Materials and Methods

Specimens of *Favia pallida*, a reef-building coral, were collected from the fringing reef of Palk Bay, near Mandapam, at a depth of 1-2 m. These were transferred to the laboratory in plastic buckets containing sea water and kept in aquarium tanks with running sea water for 1-2 days. Before the extraction of zooxanthellae from the corals and the preparation of tissue homogenate, all adhering organisms (epiphytic) were removed from the corals. Specimens which were found to be infected with boring organisms were discarded. From the healthy corals, small pieces were cut and each fragment was crushed with a pestle and mortar. The crushed material was transferred to 250 ml conical flask and agitated vigorously with 25 ml sea water until a brown suspension of zooxanthellae began to appear at the top. This suspension was first passed through folds of fine cloth so as to remove coral pieces and tissues and then centrifuged at 3000 rpm for 2 min

to allow zooxanthellae to settle. The supernatant (homogenate) was drawn off with a pipette and kept separately. Zooxanthellae cells were washed several times with millipore-filtered sea water and resuspended in 15 ml filtered sea water. The concentration of the resuspended zooxanthellae was determined by a haemocytometer and a suspension of approximately  $1.5 \times 10^6$  cells/ml was prepared. Aliquots of these were then incubated with 5 ml filtered sea water and 1 ml of  $5 \mu\text{Ci NaH}^{14}\text{CO}_3$ . Incubations were either done without any tissue homogenate or with 1 ml tissue homogenate obtained from *Favia pallida* or from other corals. Incubations were carried out for 1, 2, 3 hr in test tubes placed about 10 cm apart in front of a panel of cool fluorescent light of 10 klux intensity. The test tubes were shaken at regular intervals to keep zooxanthellae, which have a tendency to form clumps, free.

Carbon fixation by zooxanthellae with or without tissue homogenate was determined after filtering the samples and counting the dried filters. Carbon excretion by zooxanthellae was measured by taking 0.2 ml of the incubated filtrate on metal planchets, acidifying it with dilute HCl to remove excess carbonate, drying it under an infrared lamp and counting the planchets.

All counts were corrected for background but not for self absorption, and the results of carbon fixation and excretion were expressed as counts/100 sec. A single experiment was carried out under each head.

### Results

**Influence of host homogenate on fixation and excretion** — In controls, carbon fixation and excretion by zooxanthellae decreased with time (Table 1). With host homogenate the rate of carbon fixation increased during the first 2 hr and the rate of excretion during the 1st hr only. The fixed carbon began to be excreted rapidly during the 2nd hr, and thereafter, both the rates declined and became almost similar to those of the control.

*Influence of different homogenates* — Zooxanthellae extracted from *F. pallida* were incubated with fresh tissue homogenates of 4 different species of corals including the host. All the reef-building corals are known to harbour zooxanthellae in their cells. The duration of the incubation with <sup>14</sup>C was 1 hr. Controls only had sea water. Rates of carbon fixation and excretion are given in Table 2. Zooxanthellae responded to tissue homogenate of other species of corals just as they do in the presence of their own host-tissue homogenate (*F. pallida*). Both carbon fixation and excretion increased with different tissue homogenate, but the increase was of varying degrees. It is, however, not clear from the experiment to what extent the response of zooxanthellae is specific to their own host. The consistency of each set of values of fixation and excretion between controls and experimental samples containing homogenates of different host species (Table 2) probably indicates that there is no host-specificity.

*Excretion. in darkness* — Four samples of zooxanthellae suspensions were incubated with host homogenate for 1 hr in light and then kept in darkness for 3 hr. Rates of excretion were determined after each hour and these are shown in Fig. 1. Excretion at the end of the 1st hr, i.e. immediately before the exposure to darkness, was 9.57 counts/100 sec. In darkness, at the end of the 1st hr, it increased to 1044 counts/100 sec and then declined

to 754 and 94 counts at the end of 2nd and 3rd hr respectively (Fig. 1).

*Effect of homogenate on the behaviour of zooxanthellae* — Eight samples of the suspension of zooxanthellae in sea water were taken in test tubes. All the samples were from the same batch of zooxanthellae. Of these, 4 samples (1-4) were kept as control. They were incubated with <sup>14</sup>C for 1-4 hr, i.e. after each hour of incubation, one test tube was withdrawn. In the 5th sample, fresh host-tissue homogenate was added at the very start of the experiment while in the other three (6th, 7th and 8th samples), the tissue homogenate was added at the end of 1, 2 and 3 hr respectively. Thus the experimental samples, like the controls, also had an incubation of 1-4 hr, but these were with homogenates. At the end of each hour, carbon fixation and excretion were determined in both sets of samples. The results are presented in Fig. 2 (a, b). In the controls (Fig. 2a), zooxanthellae showed a progressive decline in carbon fixation with time. With the addition of homogenate after each hour, the rate of carbon fixation became significantly higher than those of the controls, although the decline was evident from the first hour itself. After 4 hr, there was no marked difference between experimental and control samples.

No significant difference was found in the rates of excretion between the experimental and control except that at the end of the 1st hr, the rate of excretion in the experimental sample was greater than that of the control (Fig. 2b).

From the above experiment it is clear that the zooxanthellae get activated with the tissue homogenate only for the first 3 hr after isolation. It may also mean that the host-tissue homogenate loses its effectiveness after 3 hr. The latter point was investigated more precisely in the following experiment :

*Stability of homogenate* — Ten samples of zooxanthellae suspension in sea water were taken in test tubes. Of these, 5 were kept as controls. To study the stability of the homogenate on the behaviour of zooxanthellae, 1 ml of fresh host homogenate was added to the 6th sample at the begin-

TABLE 1 — CARBON FIXATION AND EXCRETION BY ZOOXANTHELLAE EXTRACTED FROM *F. pallida* WITH AND WITHOUT HOST HOMOGENATE

(<sup>14</sup>C activity is expressed as counts/100 sec)

Duration of experiment (hr)	Carbon fixation		Carbon excretion	
	Without host homogenate (control)	With host homogenate	Without host homogenate (control)	With host homogenate
1				
2	4797	11729	648	2023
3	2956	3600	228	200

TABLE 2 — RESPONSE OF ZOOXANTHELLAE TO DIFFERENT HOST HOMOGENATES

(Duration of experiment: 1 hr, and <sup>14</sup>C activity is expressed as counts/100 sec)

Host homogenate (species)	Carbon fixation		Carbon excretion	
	Without host homogenate (control)	With host homogenate	Without host homogenate (control)	With host homogenate
	3052			
<i>Favia pallida</i>	2174	3575	936	1276
<i>F. valencienneci</i>		4695	593	668
<i>Platygyra</i> sp.	3070	4580	595	950
<i>Turbinaria</i> sp.	1405	2841	1176	1296

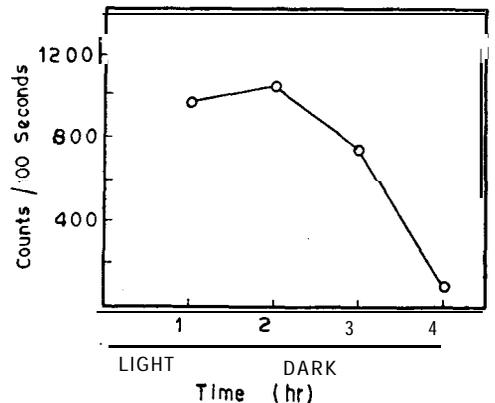


Fig. 1 — Excretion in zooxanthellae after the 1st hr in light and 3 hr in darkness

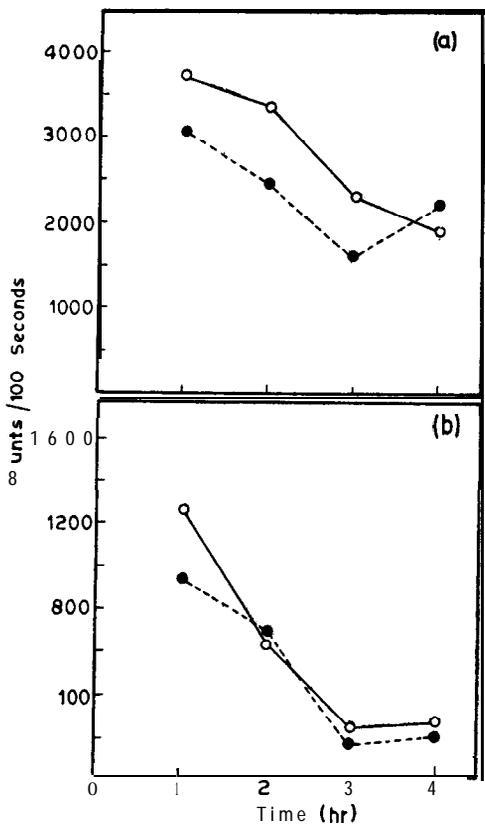


Fig. 2—Influence of fresh host homogenate on carbon fixation and excretion of zooxanthellae [(a) Carbon fixation, (b) carbon excretion. Broken lines: control (without homogenate) ; solid lines: experimental (with homogenate of *F. pallida*). Addition of homogenate was done at the end of every hour]

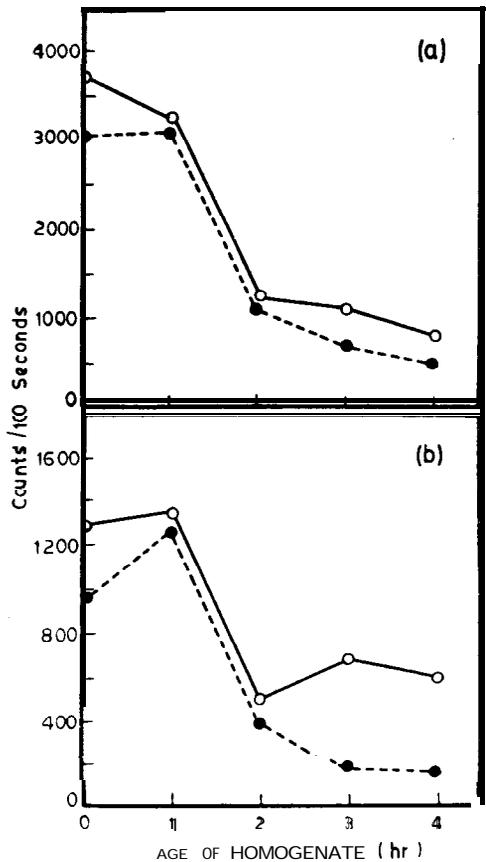


Fig. 3 — Influence of stored homogenate on carbon fixation and excretion of zooxanthellae [(a) Carbon fixation; (b) carbon excretion. Broken lines: control (without homogenate); solid lines: experimental (with homogenate of *F. pallida*). Homogenate was added every hour after it was stored for 1-4 hr]

ning of the experiment. The remaining quantity of the homogenate was stored in darkness at room temperature with a range of 26-28°C. To the remaining 4 samples (7th, 8th, 9th and 10th), the same quantity (1 ml) of homogenate was added at the end of 1st, 2nd, 3rd and 4th hr of the experiment respectively, so that the effect of 1-4 hr old homogenate could be determined.

From the results (Fig. 3) it is evident that the homogenate even after 4 hr of storage can stimulate carbon fixation but its effectiveness decreases as time passes (Fig. 3a). The effect on excretion, however, remained well marked even after 4 hr (Fig. 3b). Muscatine *et al.*<sup>10</sup> showed that the host homogenates of *Agaricia agaricites* and *Pocillopora damicornis* lose their ability to influence the excretion by zooxanthellae after 3 hr. The homogenate of *F. pallida*, on the other hand, seems to remain active up to 4 hr. The reasons for this prolonged effectiveness are not known and can perhaps be determined by a comparative study of the homogenate fractions of different species.

**Effect of dilution of homogenate**—To study the effect of diluted homogenate on the carbon fixation of zooxanthellae, the following experiment was conducted: Homogenate obtained from another coral, *Acropora* sp., was diluted to 75, 50 and 25% of its original concentration with filtered sea water. Diluted

homogenates were then added in equal volumes to 4 test tubes containing equal quantities of zooxanthellae suspension obtained from *Acropora*. Rates of carbon fixation in test tubes containing 100, 75, 50 and 25% concentrations of host homogenate were 7889, 7393, 9870 and 7085 counts/100 sec respectively. Maximum fixation occurred at 50% concentration. This confirms the findings of Muscatine *et al.*<sup>10</sup> that half-strength homogenate gives the maximum response of carbon fixation and excretion in zooxanthellae.

### Discussion

These experiments indicate that zooxanthellae after isolation show a progressive decline in their rates of fixation and excretion. In the presence of host homogenate, these rates are enhanced and attain a two-fold increase. In earlier studies, zooxanthellae isolated from anemone and zoanthids<sup>11</sup> have been found to show, within 6 hr, a progressive decline in the excretion of their fixed labelled carbon. This feature of zooxanthellae has been attributed to their reversion to free living conditions within a short time after isolation. Zooxanthellae are basically dinoflagellates and the properties of their *in vitro* cultures are similar to those of other

dinoflagellates<sup>11</sup>. However, when these dinoflagellates begin to live inside the coral tissues, i.e. when they become zooxanthellae, their physiological properties change, and they develop a symbiotic relationship with the host.

Excretion in zooxanthellae also increases in the presence of host homogenate. It continues both in the light and darkness. However, the rate is higher during the first hour of darkness than during a similar period in light. It decreases thereafter. Similar behaviour of zooxanthellae, isolated from corals, sea anemones and zoanthids<sup>10,11</sup>, has been observed earlier. This property of zooxanthellae has some interesting ecological implications and suggests that the photosynthetic material fixed by the zooxanthellae in *vivo* is continuously translocated into the coral tissues. The process of excretion is not necessarily restricted to daylight hours but occurs at night also<sup>10</sup>. Possibly, the excreted material provides a source of nutrition to corals.

Stimulation of both carbon fixation and excretion by the host homogenate undoubtedly suggests the presence of some such factors in the coral tissue which control the metabolism of zooxanthellae. Zooxanthellae seem to respond to different homogenates to varying degrees. This response is not necessarily confined to coral homogenates alone, but can occur even when homogenates from such animals which are phylogenetically removed, are used. For example, the homogenate of the bivalve, *Tridacna*, which is also known to possess zooxanthellae, has been found to stimulate the zooxanthellae extracted from the branching coral *Pocillopora*<sup>12</sup>. Similarly, it has been shown that the homogenate from the aposymbiotic anemone, *Anthopleura*, had no stimulatory effect, but when the anemones were infected with the zooxanthellae, the stimulatory effect was restored. Thus the key to the stimulatory effect seems to lie in the zooxanthellae themselves, because no matter in what

animals these occur as a symbiont, because of their presence, the host tissue seems to develop stimulatory effect<sup>11</sup>.

However, varying degrees of responses obtained in the present study with different host homogenates, suggest that the stimulatory effect on zooxanthellae is possibly a relative phenomenon, and whatever factor is responsible for such an effect, though common to all the hosts, has some minor differences which presumably determine its effectiveness both in strength and time. Further studies are needed to demonstrate whether it is a single factor or a combination of several factors, contributed by both host and symbiont, which gives rise to this relationship.

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### References

1. QASIM, S. Z., RHATTATHIRI, P. M. A. & REDDY, C. V. G., *Int. Revue ges. Hydrobiol.*, 57 (1972), 207.
2. GOREAU, T. F. & GOREAU, N. I., *Biol. Bull. mar. biol. Lab. Woods Hole*, 117 (1959), 239.
3. KAWAGUTI, S. & SAKUMOTO, D., *Bull. oceanogr. Inst. Taiwan*, 4 (1948), 65.
4. PEARSE, V. B. & MUSCATINE, L., *Biol. Bull. mar. biol. Lab. Woods Hole*, 141 (1971), 350.
5. LEWIS, D. H. & SMITH, D. C., *Proc. R. Soc.*, 178(B) (1971), 111.
6. GOREAU, T. F. & GOREAU, N. I., *Science*, N.Y., 131 (1960), 668.
7. VON HOLT, C. & VON HOLT, M., *Comp. Biochem. Physiol.*, 24 (1968), 83.
8. MUSCATINE, L. & CERNICHIARI, E., *Biol. Bull. mar. biol. Lab. Woods Hole*, 137 (1969), 506.
9. SMITH, D. C., MUSCATINE, L. & LEWIS, D. H., *Biol. Rev.*, 44 (1969), 17.
10. MUSCATINE, L., POOL, R. R. & CERNICHIARI, E., *Mar. Biol.*, 13 (1972), 298.
11. TRENCH, R. K., *Proc. R. Soc.*, 177(B) (1971), 251.
12. MUSCATINE, L., *Science*, N.Y., 156 (1967), 516.