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The Products of Photosynthesis by Zooxanthellae (*Symbiodinium microadriaticum*) of *Tridacna gigas* and Their Transfer to the Host

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Abstract

Juvenile clams of *Tridacna gigas* incubated in the light (500 μE $m^{-2}s^{-1}$) in the presence of $NaH^{14}CO_3$ accumulated radioactivity within the host tissue equivalent to 30% of the total fixed into organic compounds. For *in situ* and isolated zooxanthellae of *T. gigas*, most of the radioactivity following incubations of up to 10 min was associated with glucose and oligosaccharides, less with glutamate, alanine, aspartate, serine and succinate, very little with glycerol. Glucose accounts for over 40% of the total radioactive labelling in host tissues after 1 min ^{14}C-photosynthesis and, under pulse-chase conditions, loses its label as other compounds become increasingly labelled. Labelling located in glycerol accounted for approximately one-third of the total associated with extracellular products of ^{14}C-photosynthesis by isolated clam zooxanthellae incubated in the presence of host extracts. The low level of radioactivity associated with glycerol in *in vivo* incubations may be due to its rapid turnover or mineralisation.

Keywords: *Tridacna gigas*, zooxanthellae, photosynthetic products

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1. Introduction

The economic viability of giant clam farming in the tropical Indo-Pacific region is under intense investigation. Commercial and experimental hatcheries have been established to meet the demand for giant clam products and to re-stock regions of over-exploitation (Crawford et al., 1987).

It has been suggested (Yonge, 1980) that one factor contributing to the large size and relatively fast growth of these tridacnid bivalves is the presence of symbiotic algae (zooxanthellae, *Symbiodinium microadriaticum*) within the hemal sinuses of their hypertrophied siphon (mantle) (Fankboner, 1971; Trench et al., 1981a). The contribution of the zooxanthellae to the nutrition of the host clam is therefore an important consideration in maximizing the efficiency of the commercial cultivation of giant clams.

Evidence for the direct transfer of photosynthetic products from zooxanthellae to the host clam is surprisingly sparse and much of it is indirect since it is based largely on experiments with isolated zooxanthellae whose excretion of photosynthetic products can be stimulated by extracts of host tissue. Thus, in his studies with *Tridacna crocea*, Muscatine (1967) showed that in the presence of some component of host tissue, up to 40% of photosynthetically fixed carbon may be liberated from isolated clam zooxanthellae compared with 2% in the absence of tissue homogenate. Similarly Trench (Trench et al., 1981) reported release of between 39 and 45% of photosynthetically fixed carbon by zooxanthellae isolated from *T. maxima*.

Data from such experiments have been extrapolated to the *in vivo* situation and used to calculate the contribution of photosynthetically fixed carbon to the respiratory carbon requirement of the host (Trench et al., 1981a; Fisher et al., 1985; Fitt et al., 1986). Moreover, analyses of the major products excreted by isolated zooxanthellae have been cited as evidence of the nature of the metabolites transferred from zooxanthellae to host in the inact association. Thus, Muscatine (1967) reports that in short-term incubations (1–30 min), the major extracellular products of photosynthesis by zooxanthellae isolated from *T. crocea* was glycerol (more than 90% of the total) with glucose and an unidentified ninhydrin-positive substance accounting largely for the remainder. Muscatine concludes from this that glycerol is probably the means by which some of the free energy of photosynthesis by zooxanthellae is made available to the clam host.

The only direct evidence of transfer of metabolites from zooxanthellae to the clam host comes from Goreau et al. (1973) who used autoradiographic techniques to trance the movement of ^{14}C-labelled photosynthetic products

within the intact clam-zooxanthellae association of *T. maxima* f. *elongata*. The results, although inevitably qualitative, gave clear evidence of the incorporation of ^{14}C-labelled photosynthetic products into host tissue — most notably into the mucus-secreting glands. There was, however, no direct evidence of the nature of the major products transferred.

We have attempted, in this paper, to demonstrate the accumulation of photosynthetic products within the host tissue in intact juvenile clams of *T. gigas*. We have also tried to identify the major photosynthetic products that accumulate in the host and in *in situ* and isolated zooxanthellae.

2. Materials and Methods

Juvenile clams of *Tridacna gigas* were reared from larval stock at the Orpheus Island Research Station (18°37′S, 146°30′E) and transported to the University (wrapped in damp paper towels in an insulated container) where they were maintained for a few days in an open-air, circulating sea water aquarium under natural daylight conditions until required.

Photosynthesis by whole clams

Small clams (25–30 mm shell length) were scraped clean of attached material and rinsed in filtered sea water (FSW). The adductor muscle was carefully cut, the shell prized open to expose the mantle tissue an the clams incubated individually in small beakers containing 10 ml FSW at 25°C.

After a 10 min period of acclimation, $NaH^{14}CO_3$ was added to give a final activity of 37×10^4 Bq.ml^{-1} and the clams illuminated (500 μE m^{-2}s^{-1} from a water-screened tungsten filament lamp). Sample clams (3–5 per sample) were removed at intervals and fractionated individually as outlined in the flow diagram (Fig. 1). For pulse-chase experiments, juvenile clams, prepared as described above, were incubated in the light in the presence of $NaH^{14}CO_3$ for 1 min (pulse). They were then rapidly rinsed with FSW and returned to fresh FSW in the light, in the dark, or in the light in the presence of 10^{-5}M DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea) (chase). Sample clams were harvested at intervals, separated into the zooxanthellae and host components and prepared for later analysis as described in Fig. 1.

Photosynthesis by isolated zooxanthellae

Mantle tissue excised from juvenile clams was homogenised in FSW and the zooxanthellae isolated by filtration and centrifugation as described in Fig. 1. A suspension of the zooxanthellae in FSW was incubated in the light in the presence of $NaH^{14}CO_3$. The cells were harvested by centrifugation, digested and frozen for later analysis (Fig. 1).

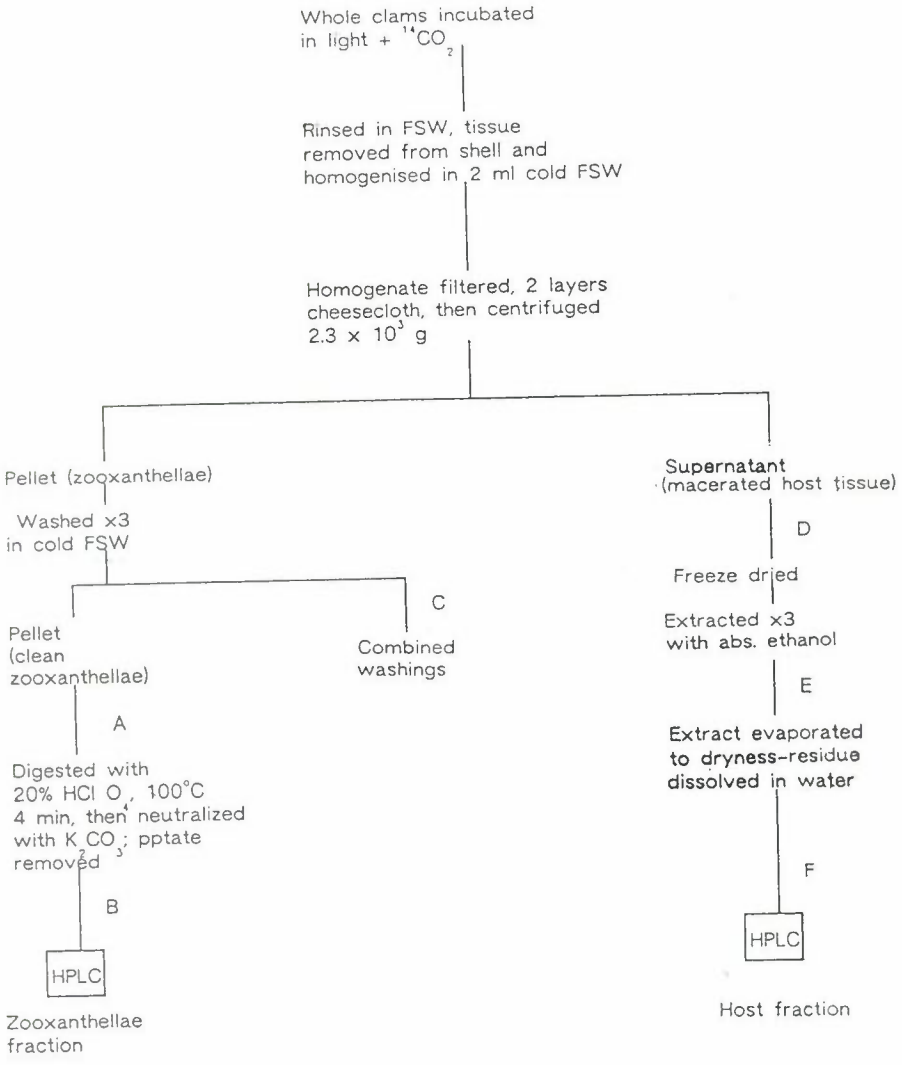

Figure 1. Flow diagram for preparation of host and zooxanthellae extracts of *Tridacna gigas* for analysis by HPLC following incubation in the presence of $^{14}CO_2$ in the light. (A–F indicate steps where aliquots were removed for measuring radioactivity and for chlorophyll estimation (Sample A only)).

The supernatant following extraction of zooxanthellae from mantle tissue was used as the aqueous host extract in experiments to test for effects upon photosynthesis and excretion of photosynthetic products by isolated zooxanthellae.

Extraction and analysis of the products of photosynthesis

Digestion and extraction of radioactive compounds from the pelleted zooxanthellae gave 95% recovery (sample B compared with Sample A, Fig. 1). Extraction from host tissue gave 60–70% recovery (sample F compared with sample D, Fig. 1) except for samples taken after 1 min incubation. After such short-term incubations, a significant portion of the fixed radioactivity remained in the residue following ethanol extraction. It could not be extracted with a chloroform: methanol (2:1) mixture but was extractable with cold water. The aqueous extract so obtained was subjected to electrophoresis on a thin layer cellulose plate (4 min at 200 V, with 4% formic acid as the electrolyte). This removed the salt and the radioactive compounds (which remained at the origin) were eluted with water. Addition of the radioactivity in this aqueous fraction to that in the original ethanol extract restored the expected 60–70% recovery level. The two extracts were bulked for later analysis.

Carbohydrates in the extracts were separated on a Waters Carbohydrate Analysis HPLC column using isocratic elution with either 65% or 85% acetonitrile in water. Fractions were collected with a Pharmacia FRAC-100 fraction collector and the radioactivity in each fraction determined by liquid scintillation spectrometry. Standards were detected with a Waters differential refractometer and the identity of monosaccharids confirmed by co-chromatography with authentic ^{14}C-labelled compounds.

Identify of oligosaccharides was determined by acid hydrolysis of extracts with 2 M (final concentration) CF_3CO_2 H followed by repeated washing and drying under a stream of nitrogen and final resuspension of the residue in water for analysis of the products of hydrolysis. Extract samples were also subjected to enzymic hydrolysis with α-glucosidase, β-glucosidase and invertase and the products analysed as described above.

Sugar phosphates were separated using a Partisil 10 μm SAX column (HPLC Technology), with 35 and 350 mM K_2HPO_4, pH 2.8 as eluting buffers (Giersch, 1979; Giersch et al., 1980). A Waters HPLC automated gradient controller was used to generate a concave gradient over a period of 10 min, followed by a further 20 min elution with 300 mM K_2HPO_4. The flow rate

was 1 ml min^{-1} and fractions (usually 0.33 ml) were collected as described above and counted for radioactivity. Standards were detected using a Waters model 400 variable wavelength detector at 210 nm.

The Partisil SAX 10 μm column was also used to separate aspartate and glutamate and to quantify succinate. These compounds were eluted with 6 mM K_2HPO_4, pH 2.8 at a flow rate of 1 ml min^{-1} and identified against CO_2-labelled standards.

Amino acids were separated and analysed using a Waters HPLC Amino Acid Analysis System. Amino acid standards were detected using a ninhydrin post-column reaction system and the identify of amino acids in the extracts confirmed by co-chromatography with authentic ^{14}C-labelled standard compounds.

Radioactivity in various extracts and in the identified early products of photosynthetic $^{14}CO_2$ fixation was normalised on a chlorophyll basis determined from acetone extracts of the clam-zooxanthellae association using the equation of Jeffrey and Humphrey (1975). The assimilation ratio for whole clams was expressed as μmol CO_2·mg chla^{-1} h^{-1} after estimating radioactivity in the supplied $NaH^{14}CO_3$ by scintillation counting of an aliquot and the total CO_2 in the sea water medium by the method of Strickland and Parsons (1968).

3. Results

Photosynthetic $^{14}CO_2$ fixation by whole clams and isolated zooxanthellae of Tridacna gigas

Light-dependent incorporation of radioactivity from supplied $^{14}CO_2$ into juvenile clams (fractions A+C+D, Fig. 1) was almost linear over a 10 min incubation period (Fig. 2a). The assimilation ratio for whole clams was 360± 140 μmol CO_2 mg chl$a^{-1}h^{-1}$ (n=4); that for isolated zooxanthellae was very variable (within the range 25–291 μmol CO_2·mg chl$a^{-1}h^{-1}$). After 10 min incubation, approximately one-third (32 ± 9.6%) of the radioactivity within the clam was associated with host tissue (fraction D, Fig. 1) (Fig. 2b). Most of the remainder stayed within the algal cells (fraction A, Fig. 1) (Fig. 2b) with a small proportion (less than 4% of the total) being recovered in the washings of the algal pellet (fraction C, Fig. 1). Since the *in vivo* location of the radioactivity in the latter fraction was in some doubt, it was not bulked with either of the two major fractions and not subjected to further analysis.

Since, to effect removal of the algae from the host tissue, all the soft parts of the clam were macerated after the incubation period, it is not possible to

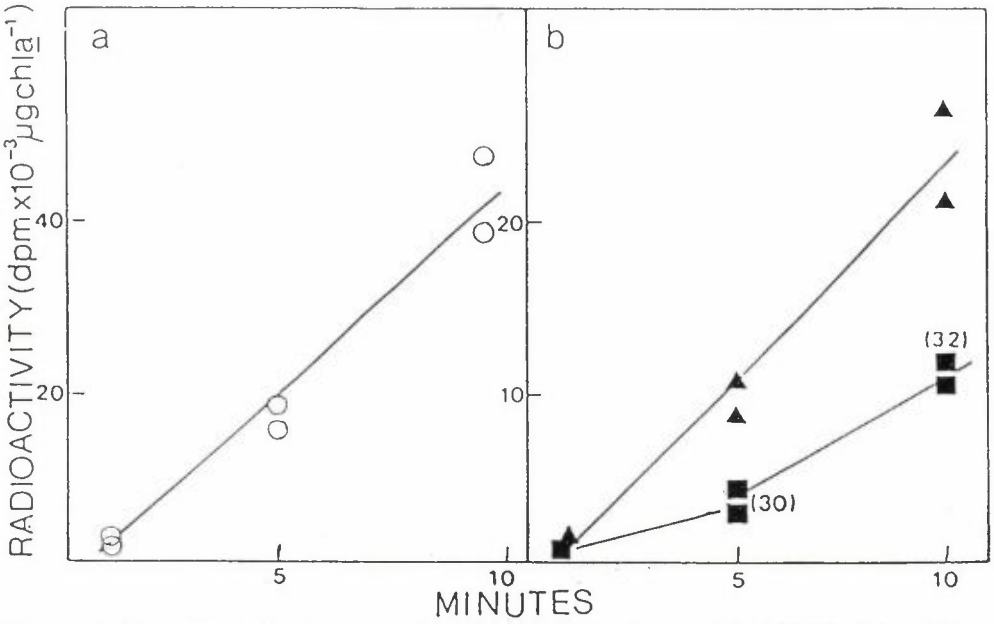

Figure 2a. Light-dependent incorporation of radioactivity from supplied $^{14}CO_2$ into whole clams of *Tridacna gigas*.

Figure 2b. Light-dependent incorporation of radioactivity from supplied $^{14}CO_2$ into the host (■) and zooxanthellae (▲) fraction of the *Tridacna gigas* association. Figures in brackets show mean values for radioactivity associated with host tissue as a percentage of the total fixed. Both figures show the results of duplicate experiments (25°C, 500 $\mu Em^{-2}s^{-1}$).

say whether radioactivity within the host was associated with mantle tissue only or was more widely distributed to other organs. Pieces of mantle tissue excised from larger clams and incubated in a similar manner showed a much lower level of overall incorporation of radioactivity (10–25% of that obtained with whole juvenile clams) but with a similar distribution of radioactivity between host-tissue and zooxanthellae.

In pulse-chase experiments with 1 min exposure to $^{14}CO_2$ in the light followed by 5 min chase there was continued fixation of radioactivity into clam tissue during the chase period in the light. This must be due to photosynthetic $^{14}CO_2$ fixation from a reservoir of [^{14}C]-bicarbonate not removed by the washing at the end of the pulse period. Radioactivity remaining in the zooxanthellae, expressed as a percentage of the total fixed, continued to increase during the chase period in the light (Fig. 3) suggesting that transfer of labelled products to the host was reduced during the chase period. In the absence of light, or in light + DCMU there was little or no redistribution

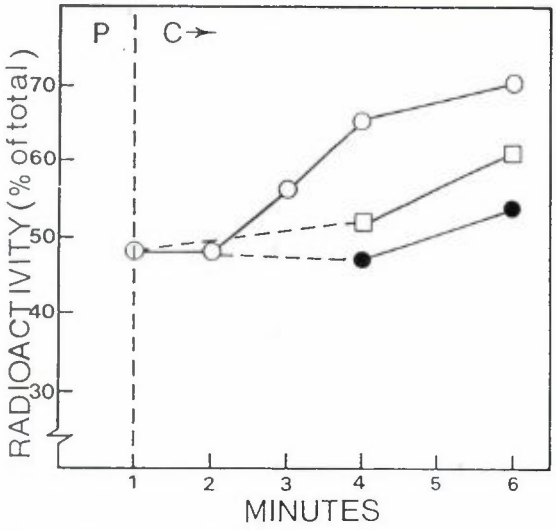

Figure 3. Proportion of the total radioactivity fixed that remains in the zooxanthellae of *Tridacna gigas* during a pulse-chase experiment with the chase period in light (○), darkness (●) or light + DCMU (□). Mean values for two experiments.

of radioactivity between zooxanthellae and host during the first 3 min of the chase period but some redistribution in favour of the zooxanthellae after 5 min (Fig. 3).

Labelling of the major products of $^{14}CO_2$ photosynthesis by isolated and in situ zooxanthellae associated with Tridacna gigas

Distribution of radioactivity between the major products of photosynthesis was similar for isolated and *in situ* zooxanthellae (Figs. 4a and b). Most of the radioactivity was, in each case, associated with glucose. There was also strong labelling of certain oligosaccharides having glucose as the major product of acid hydrolysis. Approximately 45% of the radioactivity associated with these oligosaccharides could be assigned to disaccharides, 19% to trisaccharides. The oligosaccharides were not identified further and did not respond to hydrolysis by invertase, α-glucosidase or β-glucosidase. Under the conditions of these experiments there was no significant labelling in phosphoglyceric acid, triose phosphate and sugar phosphates.

Changes with time in the proportion of fixed radioactivity in the products of $^{14}CO_2$ photosynthesis are shown in Figs. 4c and d. For isolated zooxanthellae, radioactivity in glucose, as a percentage of the total, was initially high but declined during the 10 min incubation period. The percentage radioactivity in the oligosaccharides also dropped during the latter part of this period. Percentage radioactivity in most of the other compounds increased

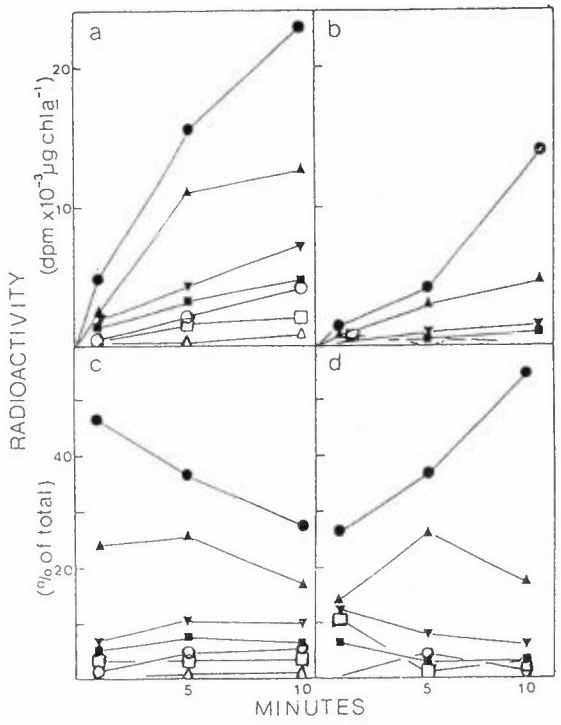

Figure 4. Radioactivity in the early products of photosynthetic $^{14}CO_2$ fixation by isolated (a) and *in situ* (b) zooxanthellae of *Tridacna gigas*; and radioactivity in the product expressed as a percentage of the total fixed by isolated (c) and *in situ* (d) zooxanthellae. (• – glucose, ▲ – oligosaccharides, ▼ – glutamate, ■ – aspartate, ○ – succinate, □ – alanine, △ – glycerol).

at first and then remained constant. Since total incorporation of radioactivity is linear over 10 min (see Fig. 2a), it appears that radioactivity in glucose and the oligosaccharides becomes redistributed to some other compounds not identified by the HPLC analysis used but accounting, according to our analysis of the non-aqueous fraction, for approximately 20% of the total labelling after 10 min incubation.

For *in situ* zooxanthellae, the proportion of radioactivity in glucose continued to increase during the 10 min period. Only in glutamate, alanine and aspartate was there a decrease in radioactivity, as a percentage of the total, and then only for the first 5 min or so. Radioactivity in the oligosaccharide fraction of *in situ* zooxanthellae was distributed between disacchardies, trisaccharides and higher molecular weight compounds in proportions similar to those in isolated zooxanthellae.

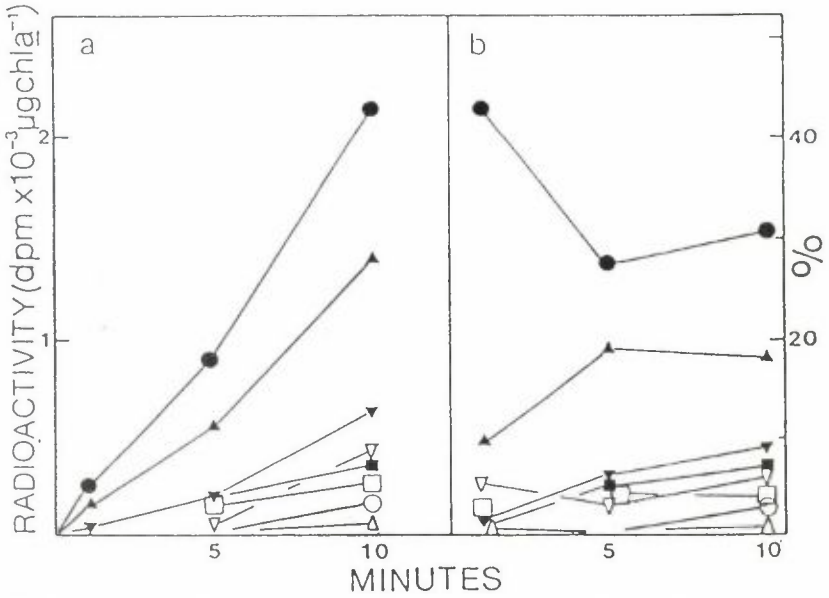

Figure 5a. Radioactivity in the early products of photosynthetic $^{14}CO_2$ fixation accumulating in the host tissue of *Tridacna gigas*.

Figure 5b. Percentage distribution of radioactivity in the early products of photosynthetic $^{14}CO_2$ fixation accumulating in the host tissue of *Tridacna gigas*. (25°C, 500 μE $m^{-2}s^{-1}$).
(• – glucose, ▲ – oligosaccharides, ▼ – glutamate, ▽ – unidentified monosaccharide, ■ – aspartate, □ – alanine, o – succinate, △ – serine).

Radioactively labelled products of $^{14}CO_2$ fixation accumulating in the Tridacna gigas host

Radioactively labelled products of $^{14}CO_2$ photosynthesis which could be extracted from host tissue are shown in Fig. 5. No significant labelling could be detected in glycerol, a compound previously reported to be the major excretory product of photosynthesis by isolated zooxanthellae in the presence of host (*Tridacna*) tissue homogenate (Muscatine, 1967). The initial high level of relative radioactivity in glucose and its subsequent decline during the incubation period (Fig. 5b) suggests that it may be a major product transferred from zooxanthellae to host.

Changes in the level of radioactivity in glucose and in the other labelled compounds (shown together for ease of presentation) accumulating in the host tissue during a pulse-chase experiment are shown in Fig. 6. Glucose was the only compound to show significant increase in radioactivity during the chase period, presumably as a result of continued transfer from photosyn-

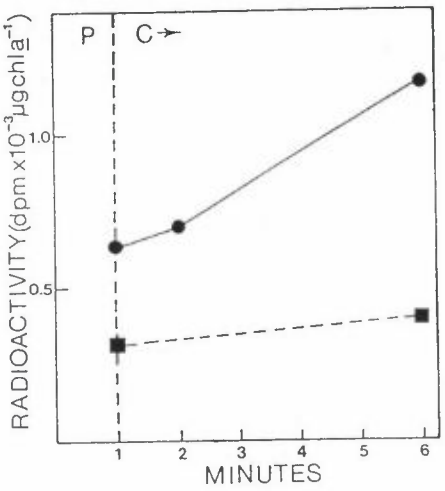

Figure 6. Redistribution of radioactivity between glucose (•) and the other labelled compounds (■) in the host tissue of *Tridacna gigas* in a pulse (light, $^{14}CO_2$)-chase (light $^{12}CO_2$) experiment (25°C, 500 µE $m^{-2}s^{-1}$).

thetic fixation of $^{14}CO_2$ from residual [^{14}C] bicarbonate carried over from the pulse period. Of the other compounds, only aspartic acid, glutamic acid and an unidentified sugar (sharing some of the chromatographic characteristics of fructose) showed small increases in radioactivity during the chase period.

Excretion of radioactively labelled products of $^{14}CO_2$ photosynthesis by isolated zooxanthelllae from Tridacna gigas

Isolated zooxanthellae undergoing photosynthetic $^{14}CO_2$ fixation release ^{14}C-labelled compounds to the outside medium at a very low rate (usually less than 1% of the total ^{14}C fixed during 45 min incubation). In the presence of an aqueous extract of host tissue, excretion of ^{14}C from isolated zooxanthellae varied in different experiments from as little as 2% to as much as 18% of the total fixed.

Distribution of labelling between the major intra- and extra-cellular photosynthetic products is shown in Table 1. Zooxanthellae photosynthesizing in a sea-water medium release radioactivity from supplied $^{14}CO_2$ primarily as glucose and oligosaccharides but with some succinate, aspartate and glycerol. Labelling associated with the oligosaccharides and glycerol in the extracellular medium was much higher than that associated with the same compounds within the algal cells. In the presence of host tissue extracts most of the extracellular radioactivity resided in succinate and glycerol, slightly less in glucose and the oligosaccharides. These findings were consistent between two such experiments performed.

Table 1. Percent distribution of radioactivity in intracellular and extracellular products of $^{14}CO_2$ photosynthesis by isolated zooxanthellae of *Tridacna gigas* in the presence or absence of host extract (45 min incubation, 25°C, 500 μE $m^{-2}s^{-1}$).

	Radioactivity (% of total fixed)			
	Filtered sea water (FSW)		FSW + host extract	
	Intracellular	Extracellular	Intracellular	Extracellular*
glucose	33	25	22	0
oligosaccharides	12	21	19	0
succinate	6	10	8	45
aspartate				1
glutamate	6	0	0	6
glycerol	2	8	3	33
other	41	36	48	15
Total radioactivity (dmp.μg chla^{-1})	370, 497	3, 994(1)**	328, 202	34, 554(10)

* Mean values from 2 experiments

** Figures in brackets — radioactivity in extracellular compounds as a percentage of the total fixed.

4. Discussion

Accumulation within the host tissue of radioactively labelled products of $^{14}CO_2$ photosynthesis by zooxanthellae has been confirmed here for juvenile clams of *T. gigas*. The extent to which the host tissue-related radioactivity underestimates overall translocation from the zooxanthellae cannot be gauged without more detailed knowledge of the fate of the translocated material, in particular its possible utilization in host cellular decarboxylations and consequent loss of radioactivity (Muscatine et al., 1984). Certainly the value reported here (c. 32% of the total radioactivity fixed into organic compounds) is slightly less than those reported by Muscatine (1967) for *T. crocea* and by Trench et al. (1981a) for *T. maxima* — both based on estimations of the release of fixed ^{14}C by isolated zooxanthellae in the presence of host tissue homogenate.

We have found isolated zooxanthellae of *T. gigas* to be very variable in their response to host tissue extracts and only in one experiment (out of a total of 12) did we record a reasonably high value (18.4% of the total carbon fixed over a 45 min period) for the release of photosynthetically fixed carbon in response to host tissue extracts. Wide variability in their release of photosynthetic products following isolation from the host has previously been reported for the zooxanthellae of *Cassiopeia xamachana* (Trench et al., 1981b).

We prefer, therefore, to base our estimations of transfer of photosynthetic products to the clam host upon *in vivo* ^{14}C incubations, recognising that they may be underestimations in comparison with, for example, estimations based on carbon mass balance which have been used for corals (Muscatine et al., 1984) but which have yet to be applied to clams.

Our pulse-chase experiments indicate that transfer of photosynthate between zooxanthellae and clam tissues is most active under conditions allowing high rates of photosynthesis suggesting that the major compounds transported are direct products of photosynthesis. In the complete absence of photosynthesis (i.e. during a chase period in the dark, or in light + DCMU) net movement of radioactivity is very much reduced or may even occur in the reverse direction, from host to zooxanthellae. A similar 'reverse transportation' has been reported form pulse-chase experiments with intact coral tips of *Acropora formosa* (Streamer et al., 1986).

Although there was a close similarity between isolated and *in situ* zooxanthellae in the distribution of radioactivity between the major products of photosynthetic $^{14}CO_2$ fixation, there were clear differences in the time course of such labelling. *In situ* zooxanthellae continued to accumulate radioactivity in glucose to a much greater extent than in any other compound while in isolated zooxanthellae radioactivity in glucose declined, with time, as a percentage of the total. This suggests that in isolated zooxanthellae, glucose may be a precursor of some other compounds, while for *in situ* zooxanthellae, glucose probably represents the end of a metabolic sequence, perhaps the major product exported to the host.

Glucose certainly accounts for much of the early ^{14}C-labelling in the host tissue. It represents over 40% of the total labelling after 1 min of photosynthesis but becomes less dominant thereafter in favour of increased relative labelling in the other compounds. Under pulse-chase conditions, glucose is the only compound in the host tissue to continue to gain radioactivity dur-

ing the chase period, again pointing to its probable key role in the transfer process.

In our system, glycerol retained significant ^{14}C-labelling only when it occurred as an extracellular product of photosynthesis by isolated zooxanthellae incubated in the presence of host tissue extract, agreeing with the findings of Muscatine (1967) with isolated zooxanthellae of *T. crocea*. Radioactively labelled glycerol did not figure prominently in any of our *in vivo* incubations.

In other associations, the low level of *in vivo* labelling of glycerol has been explained on the basis of its rapid turnover or mineralisation with consequent loss of radioactivity as $^{14}CO_2$. Thus, in the sea anemone *Condylactis gigantea*, significant *in vivo* accumulation of glycerol occurs only when sodium cyanide is used to induce partial uncoupling of respiration and photosynthesis (Battey and Patton, 1987). It is concluded from this that translocated glycerol does not normally accumulate in *Condylactis* (and probably other associations, as well) because it serves a special role as a respiratory substrate to support basal metabolism of the host.

Such studies involving inhibitors (or incubation in the presence of the presumed translocated compound — Lewis and Smith, 1971) have yet to be attempted for *T. gigas*. Until they are, it may be premature to nominate glycerol a the major translocated product in the giant clam association. Its appearance as a labelled extracellular product of photosynthetic $^{14}CO_2$ fixation by isolated zooxanthellae is not, on its own sufficient, in our opinion, to favour it for this role over, for example, glucose.

It may be significant that the autoradiographic studies of Goreau et al. (1973) showed that ^{14}C-labelled photosynthetic products accumulate primarily in glands associated with mucus production (that is, the pallial and ctenidial glands) and that Trench (reported in Goreau et al., 1973) has reported that algal photosynthetic products become chemically incorporated into the style of *T. crocea* as polysaccharide-protein complexes. It may be that in the tridacnids generally, photosynthetically fixed carbon becomes incorporated primarily into muco-polysaccharides for which, the glucose (and glucose-containing oligosaccharides) reported here to accumulate in the host tissue, might be a major precursor.

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