

Incorporation of Metabolic CO₂ into Coral Skeleton

Two possible sources for the carbonate in the calcium carbonate coral skeleton are (a) soluble carbonates from seawater and (b) carbon dioxide produced in metabolism by the living coral tissue. Although radioisotopic tracer experiments have established that carbonate from seawater is incorporated into the skeleton by many corals¹⁻³, evidence for incorporation of metabolic CO₂ into the skeleton is much less direct. Goreau⁴ found that calcification rates measured by incorporation of radioactive carbonate from seawater were usually lower than those measured by using radioactive calcium; he proposed that the labelled carbonate might be diluted by unlabelled carbonate in coral tissue. In addition, findings that the ratios of ¹³C to ¹²C and of ¹⁸O to ¹⁶O in seawater differ from those in coral skeleton suggest that not all of the skeletal carbonate originates directly from carbonate in seawater⁴⁻⁷. To examine further the possibility that some skeletal carbonate originates from metabolic CO₂, I fed ¹⁴C-labelled mouse tissue to small individuals of the coral *Fungia scutaria*, a solitary polyp containing symbiotic algae.

Table 1. ¹⁴C ACTIVITY IN ORGANIC AND CARBONATE FRACTIONS OF CORAL

Days fasted after ¹⁴ C feeding	Per cent activity in organic carbon	Per cent activity in carbonate
4-6 (3)*	95.7	4.3
13 (4)*	91.1	8.9

* No. of corals.

To obtain labelled mouse tissue, we injected a laboratory mouse intraperitoneally with 0.5 mCi of ¹⁴C-labelled protein hydrolysate in 0.1 ml. physiological saline. The mouse was killed 24 h later. Pieces of liver, kidney and intestine were fed to small corals (90-240 mm diameter, 150-1,650 mg dry weight), over a period of several days. The animals were then fasted, one group for 4-6 days, another for 13 days. At the end of the period of fasting, the tissue and skeletal components were separated as follows: each coral was placed in a small beaker and covered with concentrated ammonium hydroxide (58 per cent). The beakers were heated in a water-bath at 60°-70° C for about 1 h. The loosened tissue was easily washed off the skeleton with jets of ammonium hydroxide solution. The tissue in ammonium hydroxide was homogenized with a 'Teflon' pestle and brought to known volume. Aliquots were removed to planchets, acidified with HCl, dried, and counted on a Nuclear-Chicago gas-flow detector model 470. The skeletons were washed under a strong jet of water to remove any remaining fragments of tissue, dried in an oven at 100° C, and weighed. The clean skeletons were dissolved in 6 M HCl in evacuated flasks, and the CO₂ evolved was recovered in a small beaker containing 5 M KOH on the bottom of each flask⁸. After the skeletons had dissolved, 1 h was allowed for the CO₂ to become more completely absorbed; the flasks were then opened, and aliquots of the KOH solution were removed for counting. Approximately 95 per cent of the ¹⁴C from skeletal carbonate was recovered. The acid-insoluble residue, which included the organic matrix of the skeleton, was collected on a 'Millipore' filter, dried and counted. The acid filtrate was also counted, but did not contain measurable amounts of ¹⁴C. Separate control experiments showed that no measurable amount of ¹⁴CO₂ was released from tissue treated with acid. The data are presented in Table 1. Values for tissue and organic matrix were combined to give a single value for total organic ¹⁴C; the matrix value was never more than 2 per cent of the total.

A portion of the activity was consistently found in the carbonate of the skeleton, offering direct evidence that metabolic CO₂ was incorporated into the skeletal carbonate. Also, the distribution of the labelled carbon in the

two groups of corals was significantly different ($P < 0.05$). Among the corals fasted for a longer period (13 days), the percentage of radioactivity in skeletal carbonate was twice that of the first group (fasted 4 days), while the percentage of activity in the tissue decreased. For this difference to reflect only metabolic loss of labelled organic carbon would require that the total ¹⁴C of the second group be reduced almost by half, while in fact there is no significant difference between the means for total ¹⁴C in the two groups ($P = 0.33$). Alternatively, the apparent shift in distribution of the labelled carbon may be real, that is, the animals may have continued to calcify during the fasting period and, in doing so, fixed some of their metabolic ¹⁴CO₂ into skeletal carbonate. Also, some of the metabolic CO₂ may have been photosynthetically fixed by the symbiotic algae in the tissues of the coral. These two mechanisms could account for the negligible turnover of total ¹⁴C in this experiment and may make possible very efficient conservation of organic carbon taken in food by the corals.

My evidence that metabolic CO₂ is deposited in coral skeleton as carbonate supports Goreau's interpretation of his experiments in which he found that calcification rates calculated from ¹⁴C in skeletal carbonate were lower than those calculated from skeletal ⁴⁵Ca. He interpreted the lower carbonate values as being the result of isotopic dilution of the exogenous carbonate pool with unlabelled CO₂ in the coral tissue. It is important to note, however, that the relative contributions of carbonate from seawater and from metabolism are still unknown. The sources of carbonate for mineral deposition at any one instant might be expected to vary with such factors as the degree of illumination and the nutritional state of the animal.

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Skeletons of *Lumbricus terrestris* L. and *Arenicola marina* (L.)

FOR the theoretical investigation of their locomotion, worm-like animals have conveniently been treated as thin walled, liquid-filled cylinders¹⁻³. Observation of living animals, however, suggests that rigidity of the body wall, both inherent and developed by contraction of the muscular layers, represents a more or less important component of the total force exerted on the environment.

Some references to the rigidity and elasticity of the body wall in worms appear in the literature⁴⁻⁹, and Clark¹⁰ discussed radial forces exerted by worms in relation to the presence and absence of septa; but no quantitative