quantum mechanical resonances when they are strongly coupled. The amount of this renormalization depends on the strength of scattering; the larger the scattering, the larger the adjustment. Moreover, it is physically plausible that as the concentration of scatterers increases, the material properties of the renormalized effective embedding medium approach those of the scatterers themselves. Consequently, the individual scattering resonances inevitably become leaky and weakened, as the effective contrast between a scatterer and the embedding medium diminishes. In fact, in the limit of the embedding medium having the same properties as the scatterers, the resonances must vanish entirely. It is precisely this effective renormalization of a strongly scattering medium that is sensed by the coherent group velocity. In our calculation, the coated sphere also possesses these scattering resonances, and these are modified by the coupling to the embedding medium. Our procedure effectively identifies the frequencies and wave vectors of the minima in these coupled scattering resonances; these correspond to the dispersion curve. This approach indicates directly how a propagating wave of frequency ω is forced by the renormalization of the embedding medium to select the wave vector k that allows it to propagate through the medium with the least scattering. Hence, the physical origin of the remarkably low velocities of ballistic propagation lies in the renormalization of the effective medium by strong resonant scattering, as correctly described by our theoretical model. Finally we note that, although our experimental and theoretical approach has focused on acoustic waves, we expect the same features to be observed for any form of classical wave propagating in disordered materials; thus, electromagnetic radiation, such as light or microwaves, should show similar behavior, and confirmation of this would provide an important generalization of our results.

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Calcification in Hermatypic and Ahermatypic Corals

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The evolutionary development of tropical coral reefs is presently ascribed to the association of corals with symbiotic algae (zooxanthellae) and to the enhancement of calcification by light. Contrary to this idea, the calcification rate in a non–reef-building tropical coral (*Tubastrea faulkneri*) without symbiotic algae was the same as the light-enhanced rate in a zooxanthellate reef-building coral (*Galaxea fascicularis*). The mechanisms of calcification, however, differed between the two species. Instead of being "light-enhanced," calcification in corals with algae was "dark-repressed." The evolutionary development of coral reefs may therefore not be related to light-enhanced calcification resulting from the association of corals with symbiotic algae.

It is generally thought that the formation of coral reefs in shallow tropical seas has been possible because the association of symbiotic algae (zooxanthellae) with hermatypic (reef-building) corals facilitates rapid calcification. The calcification rate of zooxanthellate hermatypic corals has been considered to be greater than that of azooxanthellate corals [categorization according to Schuhmacher and Zibrowius (*1*)]. Calcification in the former is said to be light-enhanced (*2*). It is indisputable that light profoundly affects calcification rates in these corals, the phenomenon first being noted by Kawaguti and Sakumoto (*3*) and subsequently confirmed by Goreau (*4*) and many other authors (*2*). The experimental evidence, however, supporting the assertion that calcification in zooxanthellate hermatypic corals proceeds at a higher rate than in azooxanthellate corals appears to be very limited. The evidence is largely based on the finding that the calcification rate in an ahermatypic Atlantic coral *Astrangea danae* was so low at 8° to 10°C as to be undetectable (*5*). Although *Astrangea* is an ahermatypic coral, it appears to have a facultative symbiotic relation with zooxanthellae. Jacques *et al.* (*6*) showed that colonies of *Astrangea* that contained zooxanthellae did exhibit light-enhanced calcification at 15° to 27°C, with calcium incorporation rates that were comparable to those of reef corals. These experiments do not show that tropical azooxanthellate corals, which never contain zooxanthellae, calcify at lower rates than do tropical zooxanthellate hermatypic corals. The supposition that the calcification rate is lower in tropical azooxanthellate corals then rests primarily on observations showing that rates are lower in hermatypic corals that have been experimentally deprived of zooxanthellae (*4*); there appear to be no measurements of calcification rates in tropical azooxanthellate corals and therefore no direct comparisons. The present paper seeks to rectify this omission.

Experiments were carried out at Heron Island Research Station on the Great Barrier Reef of Australia. Two species of coral were selected that have large descrete polyps that can be readily separated. The hermatypic zooxanthellate coral *Galaxea fascicularis* has polyps of similar size and form to those of the ahermatypic azooxanthellate coral *Tubastrea faulkneri*, and care was taken to use polyps from the same colonies and match the sizes of polyps from each species as closely as possible.

Two experiments designed to measure ^{45}Ca incorporation into the coral skeleton were carried out on different days. Polyps were incubated in containers of aerated filtered seawater placed in large outdoor aquariums under the same conditions of time (4 hours) and temperature (25°C) (*7*). *Tubastrea* was incubated in shade (5 μmol s^{-1} m^{-2}) because it is normally found subtidally on coral reefs in caves or under overhangs. *Galaxea* was obtained intertidally and was incubated in full sunlight (540 to 1080 μmol s^{-1} m^{-2}). The rates of calcium incorporation per mass of skeleton (*8*) were shown to be the same in the two species, being 0.65 ± 0.18 μmol g^{-1} $hour^{-1}$ ($n = 5$) for *Tubastrea* and 0.60 ± 0.09 μmol g^{-1} $hour^{-1}$ ($n = 5$) for *Galaxea* ($P > 0.05$, t test).

A further experiment was carried out under similar conditions but for a longer period of time (6.5 hours) and at higher temperature (27° to 28°C). Polyps of *Tubastrea* and *Galaxea* were incubated in ^{45}Ca

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as before, with and without the addition of the photosynthetic inhibitor DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] (*9*, *10*). In the absence of DCMU, the rate of calcium incorporation into the skeleton by *Galaxea* was 0.54 ± 0.04 μmol g^{-1} $hour^{-1}$ (n = 6) and was not significantly different [$P > 0.05$, analysis of variance (ANOVA)] from incorporation by *Tubastrea*, which was 0.61 ± 0.08 μmol g^{-1} $hour^{-1}$ (n = 6). In the presence of DCMU, the rate of calcium incorporation was drastically reduced in *Galaxea*, as anticipated, to 0.17 ± 0.03 μmol g^{-1} $hour^{-1}$ (n = 6) (P = 0.0001, ANOVA) but remained undiminished in *Tubastrea* at 0.56 ± 0.06 μmol g^{-1} $hour^{-1}$ (n = 6) ($P > 0.05$, ANOVA).

The rates of calcium incorporation by the skeleton can also be expressed in terms of tissue mass (*11*). For the previous experiment, control values were 0.97 ± 0.06 μmol g^{-1} of tissue per hour for *Galaxea* and 0.44 ± 0.03 μmol g^{-1} of tissue per hour for *Tubastrea*. This could indicate that calcium transport rates by the tissues are higher in *Galaxea* than in *Tubastrea*; however, the lower rate in *Tubastrea* is attributable to the fact that the tissue/skeleton mass ratio in this coral is almost 2.5 times that of *Galaxea*. The greater tissue mass in *Tubastrea* is primarily associated with its considerably larger tentacles and oral disc, which are noncalcifying regions of the polyp and are most unlikely to be involved in calcium transport.

The foregoing experiments indicate that calcification rates in *Galaxea* and *Tubastrea* were similar. The use of various inhibitors and agonists revealed differences in the processes of calcification between the two species (Figs. 1 to 3) (*12*). Calcium uptake was inhibited in both species by the calcium adenosine triphosphatase (ATPase) inhibitor Ruthenium red and by the Na/K ATPase inhibitor ouabain (Fig. 1). This suggests that active calcium transport (*13*–*15*) and possibly Na^+/Ca^{2+} exchange (*16*, *17*) are involved in calcification in both species. However, the calcium ionophore A23187 (Fig. 2) and verapamil, a potent blocker of L-type voltage-dependent calcium channels (*17*, *18*) (Fig. 3), depressed calcium uptake in *Galaxea* but had no effect on calcium uptake by *Tubastrea*. Conversely, cyclic AMP (cAMP) had no effect on calcium uptake by *Galaxea* (Fig. 1A) but depressed uptake in *Tubastrea* (Fig. 1B). Thapsigargin, which raises intracellular calcium levels in a variety of cells by inhibiting Ca ATPase associated with intracellular membranes (the endoplasmic reticulum) (*19*), had no effect on calcium uptake by either species (Fig. 2). When polyps were exposed to A23187 and cAMP together (Fig. 2), the cAMP appeared to moderate the effect of A23187 in *Galaxea* and potentiated the effect in *Tubastrea*, but the differences were not statistically significant.

The rate of ^{45}Ca uptake in *Tubastrea* was shown to be the same in light (0.48 ± 0.03 μmol g^{-1} $hour^{-1}$, n = 6) or dark (0.58 ± 0.05 μmol g^{-1} $hour^{-1}$, n = 6) (P = 0.08, t test) (*20*). In *Galaxea* this was not the case, and the differences are similar to those seen with DCMU. Calcium uptake in the dark in *Galaxea* has been shown by autoradiography to be focally localized (*21*). It is possible, therefore, that the rates of dark calcification, at the sites where calcification occurs, could be similar to light calcification rates. Thus it was of interest to determine whether dark calcification was affected by inhibitors and agonists in a similar way as was light calcification. Polyps were incubated as before, but in containers shrouded by black plastic and at night for 4 hours. Uptake of ^{45}Ca in dead controls was not significantly different from uptake in live controls. Uptake was not affected by the metabolic inhibitor CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) (*22*), ouabain, or cAMP. However, as in the light, ^{45}Ca uptake was reduced by verapamil, A23187, and Ruthenium red (Fig. 4). The failure of CCCP to depress ^{45}Ca uptake confirms observations (*13*, *23*) that CCCP does not affect dark calcification rates in *Acropora*; but it is not consistent with the finding of Barnes (*24*), who showed, using the alkalinity anomaly method, that CCCP did depress dark calcification. The uptake of ^{45}Ca in dead polyps is presumed to be due to isotopic exchange and occurred at a rate that was indistinguishable from that of the live control polyps. This is in agreement with the observations of Chalker (*13*).

The only difference between light and dark calcification in *Galaxea* with respect to the effects of inhibitors and agonists was seen in the response to ouabain. Ouabain depressed light calcification, but had no effect on dark calcification. Autoradiography has shown that ^{45}Ca is deposited principally on the outside of the corallite wall in the light (*21*). The finding that active transport of Ca^{2+} occurs across the oral epithelia (*14*) is consistent with this observation. In most corals, polyps in the light have their mouths closed, and therefore the entry of Ca^{2+} into the coelenteron may be across the oral epithelia, which may be the site of action of ouabain. At night the polyps expand and their mouths open, allowing water to enter the coelenteron and extrathecal coelenteron. The aboral epithelia may thus be the site of action of Ruthenium red, verapamil, and A23187. An alternative, but not necessarily exclusive, explanation is that ouabain in some way affects the photosynthetic relation of the zooxanthellae with calcification. However, because ouabain also dramatically depresses calcification in the azooxanthellate *Tubastrea*, this seems an unlikely explanation.

Dark calcification in *Galaxea* differs markedly from calcification in *Tubastrea* in that ouabain and cAMP do not affect dark calcification, whereas these agents significantly depress calcification in *Tubastrea*.

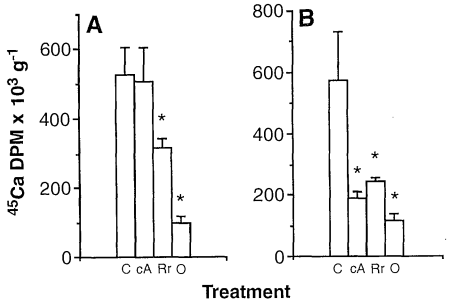

Fig. 1. Uptake of ^{45}Ca by polyps of (**A**) *G. fascicularis* and (**B**) *T. faulkneri*, untreated (C) and in the presence of cAMP (cA), Ruthenium red (Rr), and ouabain (O). Values shown are means ± SE (n = 6); asterisks indicate a significant difference from the control value ($P < 0.05$, one-way ANOVA).

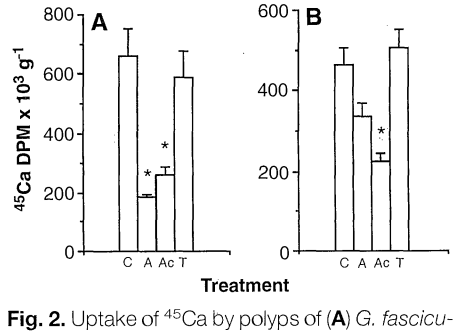

Fig. 2. Uptake of ^{45}Ca by polyps of (**A**) *G. fascicularis* and (**B**) *T. faulkneri*, untreated (C) and in the presence of A23187 (A), A23187 + cAMP (Ac), and thapsigargin (T). Values shown are means ± SE (n = 6); asterisks indicate a significant difference from the control value ($P < 0.05$, one-way ANOVA).

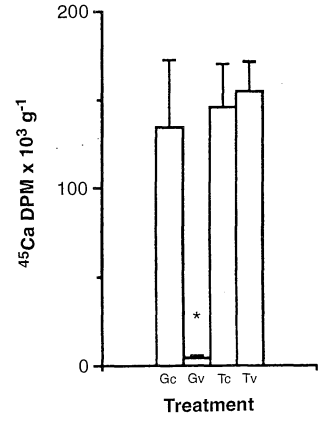

Fig. 3. Uptake of ^{45}Ca by polyps of *G. fascicularis* (G) and *T. faulkneri* (T), untreated (Gc, Tc) and in the presence of verapamil (Gv, Tv). Values shown are means ± SE (n = 6); the asterisk indicates a significant difference from the control value ($P < 0.05$, t test).

Verapamil greatly decreases dark calcification in *Galaxea* but has no effect on calcification in *Tubastrea*. There thus appear to be at least three different mechanisms of calcification in scleractinian corals, including light calcification and dark calcification in zooxanthellate corals and calcification in azooxanthellate corals.

Although the cellular sites of action of the various compounds affecting calcium uptake are unknown at present, the effects do serve to demonstrate that there are substantial differences between the calcification processes in the zooxanthellate *Galaxea* and the azooxanthellate *Tubastrea*. It appears that in both species, calcium ATPase is involved and that either the ionic integrity of the cells is essential or that a Na^+/Ca^{2+} exchange mechanism is also involved. Verapamil-sensitive L-type calcium channels may be implicated in calcification in *Galaxea*, but they do not appear to have a role in *Tubastrea*. Cyclic AMP may act in a regulatory pathway for calcification in *Tubastrea* but not in *Galaxea*. Possibly calcium ions themselves are involved in this role in *Galaxea*, as indicated by the effect of the calcium ionophore A23187.

A high-affinity calcium ATPase has been previously demonstrated biochemically in *Galaxea* (*15*), and calcium ATPase is central to a model of calcification proposed by McConnaughey (*25*). In essence, this model conceives of calcification as being an enhancer of photosynthesis. This is achieved by a calcium-proton exchanging ATPase supplying protons for the conversion of extracellular HCO_3^- into CO_2, which is used for photosynthesis. In this model, calcium carbonate precipitation is seen as being incidental to a process that functions primarily as a source of protons. The results presented here are not inconsistent with this view. It may be conjectured that zooxanthellae control calcification in some way in order to enhance CO_2 availability in the light and conserve the metabolites of the host in the dark when photosynthesis does not occur. This is consistent with the observation that dark calcification in *Galaxea* is focal (*21*) and particularly occurs in regions such as the exert septa where zooxanthellae are absent (*26*). It is also known that the axial polyps, in which zooxanthellae are absent, of a number of *Acropora* species calcify at high rates in light and dark (*27*). Thus, zooxanthellae may repress calcification in skeletal areas only in their immediate proximity. It is therefore not surprising that azooxanthellate corals calcify at similar rates in light and dark and have different mechanisms of calcification and control of calcification. The success of zooxanthellate corals as reef builders may be more attributable to their autotrophic capabilities than to enhanced calcification rates.

The results presented here suggest that (in comparison to calcification in *Tubastrea*) calcification in *Galaxea*, rather than being enhanced in the presence of light and when photosynthesis occurs, may be repressed in the dark and in the absence of photosynthesis. This finding implies that light-enhanced calcification may not be a major reason for the evolutionary development of tropical coral reefs.

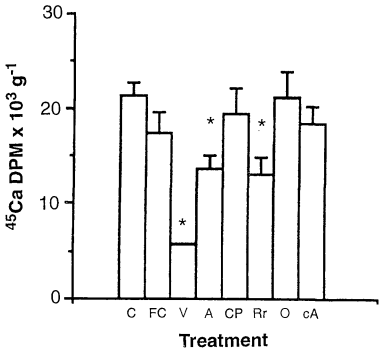

Fig. 4. Uptake of ^{45}Ca by polyps of *G. fascicularis* in the dark, untreated (C) and in the presence of verapamil (V), A23187 (A), CCCP (CP), Ruthenium red (Rr), ouabain (O), and cAMP (cA). A control series of polyps fixed in 100% ethanol (FC) was included to assess isotopic exchange. Values shown are means ± SE (n = 6); asterisks indicate a significant difference from the control value (P < 0.05, one-way ANOVA).

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7. Polyps were separated with minimum damage to tissue and allowed to recover for 2 days in running seawater. They were incubated in glass jars of filtered (0.2 μm) seawater (five polyps per 100 ml) containing ^{45}Ca as $CaCl_2$ at an activity of 1 μCi ml^{-1}. The incubation medium was stirred by aeration, and the jars were maintained at constant temperature by immersion in a large aquarium with flow-through seawater at the same temperature as that of reef seawater.
8. Rates of ^{45}Ca incorporation were obtained by scintillation counting (with a Beckman LS6800 with H number quench correction) of aliquots (200 μl in 5 ml of Beckman Ready-Solv GP scintillation fluid) of solutions of skeleton digested with concentrated HCl after rapid removal of tissue (10 min at 58°C) with 12% sodium hypochlorite solution (bleach). Before this treatment, polyps were vigorously rinsed in seawater for 5 min. They were cut into pieces, and any part of the corallite that was not covered by epithelium was discarded, thereby eliminating skeleton in which isotopic exchange may have taken place.
9. DCMU was added to seawater to make a saturated solution, which was then filtered. The final concentration was $<5.10^{-4}$ mol $liter^{-1}$.
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11. In order to obtain tissue mass after incubation in ^{45}Ca (*7*), polyps were removed from the rinse water and shaken until no more fluid dripped from them. The polyps were then weighed, treated with bleach, and reweighed after overnight drying at 60°C. This procedure allowed approximate wet tissue mass to be calculated in addition to skeletal mass. Wet tissue mass was compared with tissue protein concentration measured by Peterson's modification of the Lowery method [G. L. Peterson, *Anal. Biochem.* **83**, 346 (1977)] after digestion of the tissues of *Galaxea* polyps in 5N sodium hydroxide. A linear relation between wet mass and protein concentration was obtained (r = 0.8, n = 18). Protein concentration in *Tubastrea* tissues could not be measured because the tissue digest was strongly colored.
12. Incubations were carried out as in the first experiments. The light conditions, water temperature, and time of day were similar, but not identical, for all experiments. Polyps were preincubated for 30 min before ^{45}Ca was added. The following concentrations were used: dibutyryl cAMP, 1 mmol $liter^{-1}$; Ruthenium red, 0.02 mmol $liter^{-1}$; ouabain, 1 mmol $liter^{-1}$; verafamil in DMSO (dimethyl sulfoxide), 100 nmol $liter^{-1}$; A23187 in DMSO, 10 μmol $liter^{-1}$; and thapsigargin in ethanol, 100 nmol $liter^{-1}$. All chemicals were obtained from Sigma Chemical Company. The concentration of DMSO in seawater did not exceed 0.1% and that of ethanol did not exceed 0.004%. Preliminary experiments showed that these concentrations had no effect, compared with controls, on ^{45}Ca uptake. The data for the experiments represented in Figs. 1 to 3 have been normalized with respect to differences in ^{45}Ca activity in the incubation seawater but have not been normalized with respect to time. The variation in incubation times (3 to 4 hours in Figs. 1 and 2, 1 hour in Fig. 3) largely accounts for the differences in control values, although these are not intended to be compared because experimental conditions were not identical.
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16. Inhibition of Na/K ATPase by ouabain would disrupt the Na^+ gradient across cell membranes, which would lead to changes in the rate or direction (or both) of Na^+/Ca^{2+} exchange.
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20. The experimental procedure was the same as in the first experiment. *Tubastrea* polyps from two colonies were incubated in ^{45}Ca for 6.5 hours at 23°C. The light polyps were under shade cloth and were exposed to sunlight at 800 μmol s^{-1} m^{-2}. Dark polyps were in a jar shrouded by black plastic.
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28. I thank I. Lawn for facilities at Heron Island Research Station. Corals were collected under permit no. G91/353 from the Great Barrier Reef Marine Parks Authority.

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