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THE PHYSIOLOGY OF SKELETON FORMATION IN CORALS.
I. A METHOD FOR MEASURING THE RATE OF CALCIUM DEPOSITION BY CORALS UNDER DIFFERENT CONDITIONS

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The purpose of this study is to examine the rate of growth of reef-building corals by measuring the calcium deposition in the skeleton with the aid of a new method using radioactive calcium-45 as tracer. With this procedure it was possible to determine calcification rates in the different parts of coral colonies, and to estimate quantitatively the effect of light and darkness, zooxanthellae and carbonic anhydrase inhibitors on skeletogenesis.

Numerous attempts have been made in the past to estimate the growth rates of reef-building corals, mostly by letting weighed and measured coral colonies grow in their natural habitat for periods of months to years (Agassiz, 1890; Abe, 1940; Boschma, 1936; Edmondson, 1929; Kawaguti, 1941; Ma, 1937; Mayor, 1924; Motoda, 1940; Stephenson and Stephenson, 1933; Tamura and Hada, 1932; Vaughan, 1919). Recently, Kawaguti and Sakumoto (1948) tried, by a chemical method, to determine the rate of calcium uptake of corals in light and darkness.

Using calcium-45 as tracer, we have developed a rapid and precise method for measuring the rate of incorporation of calcium into the coral skeleton under controlled laboratory conditions (Goreau, 1957). The preliminary experiments, described here, were carried out on the following coral species: *Manicina areolata* (Linné), *Cladocora arbuscula* (Lesueur), *Porites divaricata* (Lesueur), *Acropora prolifera* (Lamarck), *Madracis decactis* (Lyman) and *Oculina diffusa* (Lamarck) from Jamaica, B. W. I.; *Acropora conferta* (Quelch) from Eniwetok Atoll; and *Montipora verrucosa* (Lamarck), *Porites compressa* (Quelch), *Pocillopora damicornis* (Linné) and *Porolithon* sp., a coralline alga, from Hawaii.

All the madreporarian corals used in these experiments are shallow-water forms which contain zooxanthellae. Among these, *Oculina diffusa* is the only species which has not been collected from reefs, but it is common in Kingston Harbour where it grows on rocks on a muddy bottom (Goreau, 1958). The Hawaiian *Porolithon* listed above is a calcareous alga of the family *Corallinaceae*, representatives of which are important reef builders in the Central Pacific (Emery, Tracey and Ladd, 1954).

PROCEDURE

Freshly collected coral colonies in good condition were put into glass vessels containing filtered sea water and fitted with tight covers. Aeration, circulation and pH were maintained by bubbling a slow stream of air through the water. The

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temperature was kept to within 1° C. during the experiments (about 25° C. in Jamaica and Hawaii, 28.5° C. in Eniwetok) by keeping the vessels partly immersed in a water bath. After allowing the coral to acclimatize for twenty-four hours, neutralized $\text{Ca}^{45}\text{Cl}_2$ was added to give about 20,000 c.p.m./ml. of sea water. The amount of calcium thus added was less than five per cent of the total dissolved Ca^{++} already present. The initial activity was determined by counting 60- μl aliquots taken from each vessel after one hour, to allow for complete mixing of the isotope.

In addition to the living corals, pieces of clean dead corallum from the same species were included in each vessel to act as controls for measuring the inorganic isotopic exchange rate of the coral skeleton during the experiments.

Samples of coral and water were repeatedly taken, starting with three hours from the beginning of the experiment, by the following method: a coral colony, together with its control, was removed from the vessel and small pieces were cut off with scissors or cutting pliers. From five to fifteen replicate samples of about one hundred milligrams each were taken at a time. Samples were collected only from homologous parts of the colonies. This was particularly important in branching corals such as *Acropora* and *Porites* where there were shown to be strong differences in the rate of calcium uptake between the apical and lateral branch polyps.

The coral pieces were placed on filter paper to remove excess radioactive sea water, then washed in five two-minute changes of slightly alkaline distilled water. After this, each sample was dissolved in a separate tube containing two milliliters dilute HCl, and heated to boiling. The coral suspension was homogenized to disperse the organic matter. The contents of each tube were made up to five milliliters with distilled water, and a 500- μl aliquot was taken for Kjeldahl nitrogen determination.

The calcium in each tube was precipitated as the oxalate by the method of Vogel (1943), and filtered out on pre-weighed Whatman No. 42 filter paper planchets, using a cone to spread the precipitate in circles of uniform diameter. The dried and weighed samples were counted with an end window G-M tube, and the observed activity corrected for self-absorption.

In the early stages of these investigations, the question arose of choosing a suitable parameter on the basis of which the calcium uptake could be expressed. For example, Mayor (1924) measured coral respiration in terms of tissue weight after the corallum had been dissolved with nitric acid; Odum and Odum (1955) determined biomass by loss on ignition at 600° C.; and Kawaguti and Sakumoto (1948) measured calcification rates per gram coral. None of these methods was considered satisfactory. The writer had previously used organic nitrogen as a measure of total cellular matter in corals (Goreau, 1956). The relationship of organic nitrogen to tissue weight was determined for the polyps of *Mussa angulosa*, a coral from which fairly large skeleton-free pieces of tissue could be readily obtained. In this species nitrogen constituted 2 per cent of the wet weight and 11.2 per cent of the dry weight. All results, save those of the exchange controls which lacked tissue, were expressed in terms of calcium deposited per milligram of nitrogen, on the assumption that the nitrogen is a measure of the total coral (plus zooxanthellae) protein present. Nitrogen was determined by the micro-Kjeldahl method of Ma and Zuazaga (1942).

The amount of calcium taken up by the coral was calculated from the specific activity of the sea water in the vessels. This was determined by counting 60- μl

water aliquots spread to a constant diameter in lens paper circles mounted on microscope coverslips and dried under a lamp. The observed count was corrected for self-absorption and the specific activity of the water calculated from its calcium content.

THE CALCIUM EXCHANGE IN THE SKELETON CONTROLS

Equilibrium exchanges of calcium between the skeleton and sea water were determined on samples of dead coral devoid of tissue, and run at the same time as the living experimental colonies. Isotopic equilibrium appeared to be established

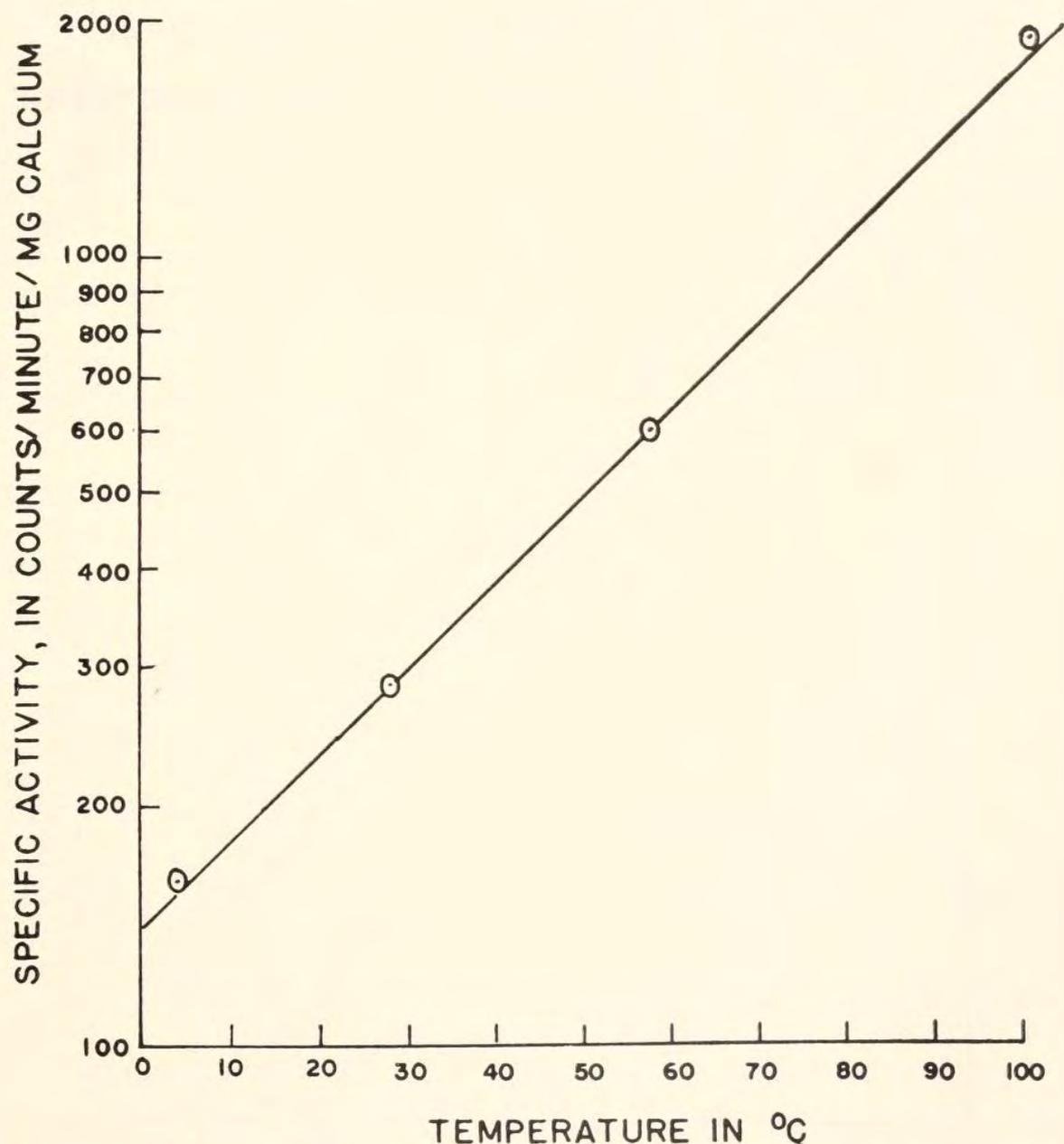


FIGURE 1. Calcium-45 exchange of small pieces of corallum from *Manicina areolata* with sea water at 4° C., 28° C., 58° C. and 100° C. The coral was carefully cleaned to remove all organic matter, and the experiments ran for twenty-four hours. The ordinate is the specific activity plotted on a logarithmic scale.

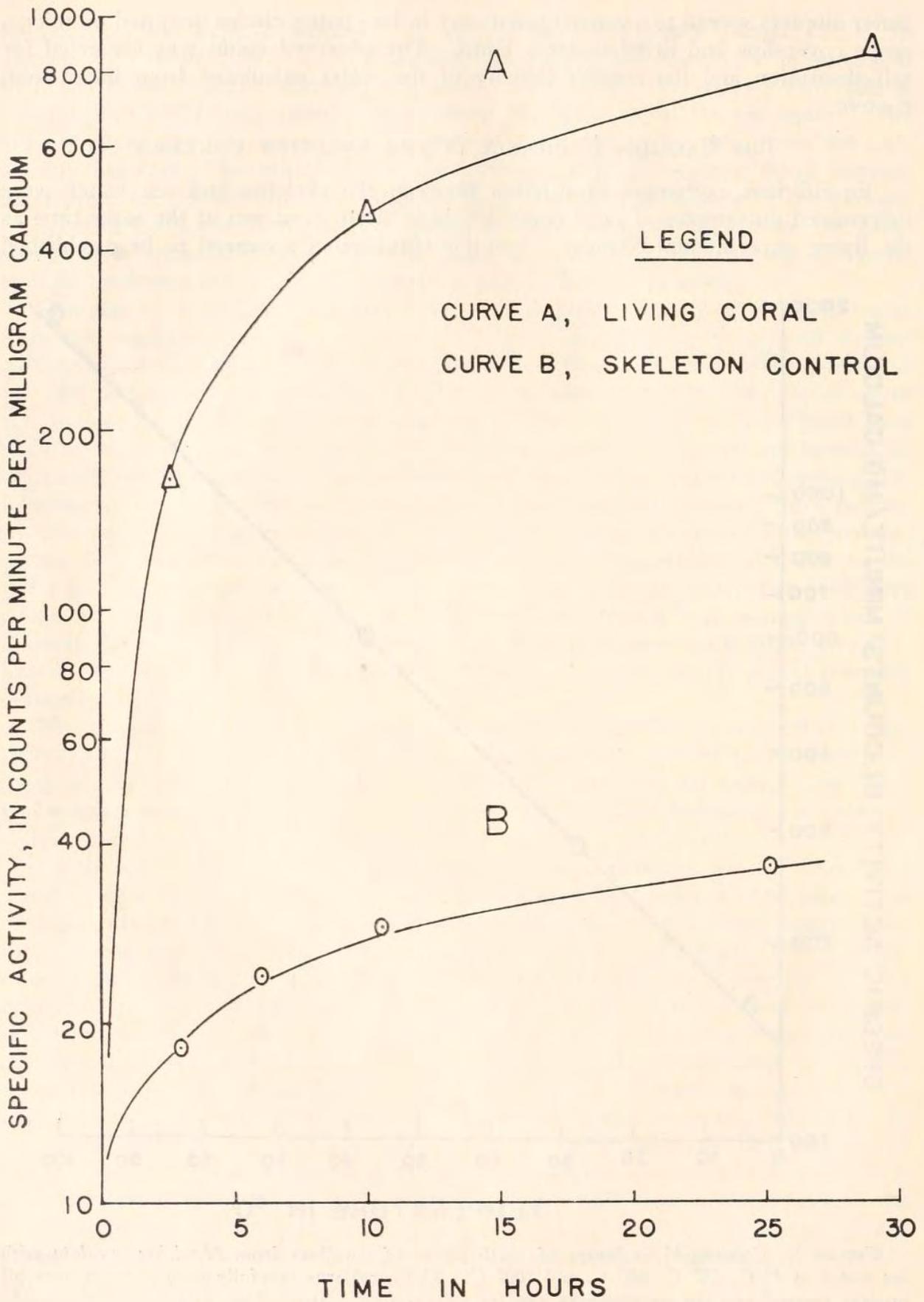


FIGURE 2.

rather slowly, but in most species tested, the process was sixty to eighty per cent complete at the end of twenty-four hours. As expected, the rate of exchange with sea water was strongly temperature-dependent. This is demonstrated in Figure 1, which shows the specific activity of small pieces of *Manicina areolata* which have been allowed to equilibrate at different temperatures in sea water containing calcium-45. In most species tested, the rate of calcium-45 deposition in the living coral was much faster than in the skeleton controls. This is shown in Figure 2 for *Acropora prolifera*, in which the specific activity of the dead corallum is about five per cent that of the living coral at the end of twenty-nine hours. In water of a given specific activity the equilibration rate appears to be much slower in imperforate corals such as *Oculina* or *Phyllangia* than in perforate species such as *Acropora* or *Porites*. The effect of the total skeletal surface on the exchange rate is being studied.

There is some evidence that the living coenosarc forms a barrier which restricts calcium exchange of the skeleton with the sea water. In a number of experiments in which the calcium rate of the experimental colonies was very low, it was noted that the specific activity of the skeleton controls was higher than that of the living coral. It has been previously demonstrated by Goreau and Bowen (1955) that the exchangeable calcium in the tissues of the cold water coral *Astrangia danae* is maintained at only about eighty-eight per cent of the calcium concentration in the sea, *i.e.*, calcium tends to be excluded from the tissues of coral. Until more evidence is available, it is difficult to state precisely the extent to which coral tissues can restrict the calcium exchange of the underlying skeleton with sea water. This problem is now under investigation.

THE EFFECT OF LIGHT ON CALCIUM DEPOSITION IN CORALS AND OTHER HERMATYPES

Light has long been recognized as an essential environmental factor in the growth of tropical reef building corals (Vaughan, 1919; Edmondson, 1928; Verwey, 1930; Kawaguti, 1937a, 1937b; Yonge, 1940; Vaughan and Wells, 1943) and other hermatypes such as *Lithothamnion* and *Millepora*. Yonge and Nicholls (1931a), Yonge (1940) and Kawaguti (1944) stated that this was due to photosynthesis by unicellular zooxanthellae contained within the cells of the gastrodermis. Kawaguti and Sakumoto (1948) claimed that in five species of reef corals the uptake of calcium was greater in light than in darkness. Their observations were based on changes in the calcium content of small volumes of sea water when corals were put in, the results being expressed in terms of milligrams of calcium taken up per hour per gram of coral.

In our experiments, the effect of illumination on deposition of calcium-45 was determined by exposing one series of coral colonies to a standard light source while keeping a control series in darkness under otherwise equal conditions. The light source was a twin bank of 20-watt fluorescent tubes in a reflector housing located about one foot above the experimental vessels.

FIGURE 2. Comparison of the calcium-45 deposition and exchange in living and dead colonies of *Acropora prolifera*. The results from the living coral have been re-calculated in terms of the specific activity to permit direct comparison with the exchange controls which were devoid of organic matter. Both controls and experimentals were run under identical conditions at the same time. The specific activity is plotted on a logarithmic ordinate.

The results of our preliminary experiments are given in Table I which shows calcium uptake in nine species of coral, and a coralline alga (*Porolithon*). In two of these species, dark experiments were not run; only the results of light experiments are shown. In most species, there was a significant increase in the calcification rate on exposure of the coral to a light. The course of a typical experiment is seen in Figure 3 which shows the progressive incorporation of calcium-45 into the skeleton of the Caribbean staghorn coral *Acropora prolifera* in light and darkness.

The pH in both light and dark vessels was measured every six hours with a Beckman Model G pH meter. This showed that the observed differences in the calcification rate in the light and dark experiments were not due to a decrease in the pH of the water of the dark experiments, as such changes were prevented by continuous aeration with a stream of air. It is probable that the negative calcium balance found by Kawaguti and Sakumoto (1948) in some corals in darkness was caused by a lowering of the pH, due to the failure of these workers to aerate or stir the water in their experimental vessels.

TABLE I

Calcification rates in the apical polyps of branching coral species, in μg calcium $\text{mg. N}^{-1} \text{hr.}^{-1}$
Number of samples in brackets

| Species | Calcification in light | Calcification in dark | P |
|-------------------------------|------------------------|-----------------------|--------|
| <i>Cladocora arbuscula</i> | 6.3±1.58 (9) | 6.1±0.20* (10) | 0.7 |
| <i>Porites divaricata</i> | 9.8±0.54 (10) | 5.0±1.00 (8) | 0.01 |
| <i>Porites compressa</i> | 7.8±1.70 (11) | 7.4±2.10 (7) | <0.7 |
| <i>Acropora prolifera</i> | 12.4±6.50 (12) | 7.2±5.00* (11) | <0.05 |
| <i>Acropora conferta</i> | 8.2±3.76 (10)* | — — | — |
| <i>Montipora verrucosa</i> | 11.9±5.60 (9) | 9.7±3.4 (10) | 0.3 |
| <i>Pocillopora damicornis</i> | 10.3±3.90 (11) | 6.8±2.1 (10) | <0.03 |
| <i>Madracis decactis</i> | 1.0±0.49 (12) | — — | — |
| <i>Oculina diffusa</i> | 1.6±0.38 (7) | 0.8±0.15* (9) | 0.01 |
| <i>Porolithon</i> sp. | 8.8±0.58 (11) | 3.3±0.55 (13) | <0.001 |

* Measurements made on individual polyps.

As seen by the standard deviations of the results, there were usually large variations in the calcium uptake rates of individual samples even if these were taken from adjacent morphologically comparable regions of the same colony. This was never true of the exchange controls. The scatter was not attributable to injury, as all damaged corals were discarded, and the error in counting, weighing and nitrogen determinations was kept below three per cent. In regard to this, our tentative interpretation is that the calcification rates of individual polyps fluctuate, and that some are in a resting stage while others are more or less vigorously growing.

THE EFFECT OF THE REMOVAL OF ZOOXANTHELLAE ON THE CALCIFICATION RATE OF SOME REEF CORALS

All tropical reef-building corals contain zooxanthellae. Their presence as intracellular symbionts in the tissue of the coelenterate host has resulted in a great deal of controversy as to their possible role in the biological economy of the coral reef and its component animals. Boschma (1924, 1925a, 1925b, 1925c, 1926, 1929)

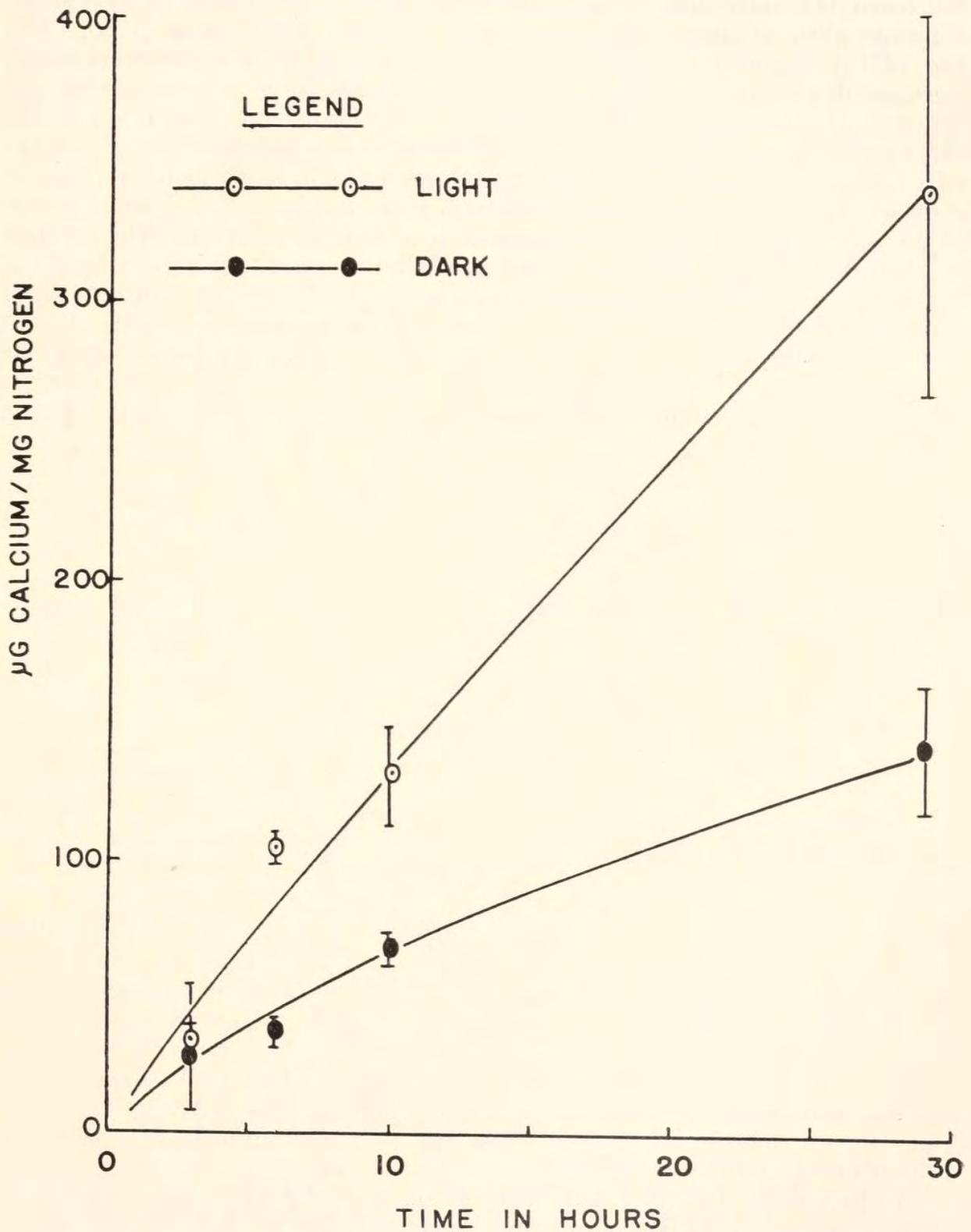


FIGURE 3. The progressive incorporation of calcium into the skeleton of *Acropora prolifera* in light and dark. The results are expressed as μg calcium taken up per milligram nitrogen. The vertical lines drawn through the points represent the standard deviation of the means.

concluded that corals could digest zooxanthellae in the lateral lobes of the mesenterial filaments when no animal food was available. Yonge and Nicholls (1930, 1931a and 1931b) demonstrated that, under the conditions of their experiments, corals were unable to derive enough food from the zooxanthellae to prevent starvation if deprived of their normal animal food supply. They also showed that zooxanthellae could not be digested by corals due to the absence of carbohydrate-splitting digestive enzymes and that these algae were extruded intact and in large numbers when the coral was kept in darkness for long periods of time, or whenever the metabolic rate of the coral was depressed, *i.e.*, by starvation or high temperature. The question of whether or not the reef-building corals are at least in part herbivorous, *i.e.*, feeding on their zooxanthellae, has recently been revived by Sargent and Austin (1954) and Odum and Odum (1955) who concluded from their productivity studies that at least some of the organic matter produced by zooxanthellae and boring algae may be utilized by the coral host. Unfortunately, these authors were unable to verify the existence of such an internal food cycle by experimental means. At the present

TABLE II
Calcium uptake by colonies of Oculina diffusa and Manicina areolata
in presence and absence of zooxanthellae
Number of samples in brackets

| Species | Light | | Dark | |
|-----------------------|---------------------|-----------------------|--------------------|-----------------------|
| | With zooxanthellae | Without zooxanthellae | With zooxanthellae | Without zooxanthellae |
| <i>O. diffusa</i> * | 1.63 ± 0.38 (7) | 0.37 ± 0.01 (6) | 0.81 ± 0.15 (9) | 0.26 ± 0.01 (5) |
| <i>M. areolata</i> ** | 462.00 ± 63.20 (11) | 28.40 ± 7.80 (9) | 71.70 ± 14.90 (8) | 30.20 ± 6.20 (10) |

* Measurements made on individual polyps, in $\mu\text{g Ca mg. N}^{-1} \text{hr.}^{-1}$.

** Individual samples taken from different colonies, calcium uptake expressed in counts per minute per milligram skeletal calcium at eighty hours.

time, it is still necessary to agree with the conclusions of Yonge and Nicholls (1930, 1931b) that reef corals are specialized carnivores, the exceptional proliferative powers of which are probably due to an increased metabolic efficiency made possible by the ability of the zooxanthellae to assimilate many of the metabolic waste products of the animal host.

The zooxanthellae *per se* are not necessary to individual coral polyps, nor do they appear to be directly linked with the calcification process since they are absent from deep sea and cold water corals, while they are present in many non-calcareous tropical shallow water coelenterates.

We have determined the effect of the presence or absence of the zooxanthellae on reef coral calcification in *Manicina areolata* and *Oculina diffusa*. Colonies of these corals, which are normally yellowish or greenish brown in colour, were kept in circulating sea water in darkened tanks for periods of about six weeks, to cause gradual extrusion of the zooxanthellae. The experiments were run only when the coenosarc of the corals became completely colourless and transparent, and when small pieces failed to give the chlorophyll test on extraction with eighty per cent

TABLE III

Calcification rates in different parts of branching coral colonies, in $\mu\text{g Ca mg. N}^{-1} \text{hr.}^{-1}$
Number of samples in brackets

| Species | Apical polyps of primary branches | Lateral polyps |
|----------------------|-----------------------------------|-----------------|
| <i>M. verrucosa</i> | 11.8±3.90 (9) | 1.38±0.50 (6)* |
| <i>P. compressa</i> | 7.8±1.70 (11) | 1.55±0.20 (5)* |
| <i>P. damicornis</i> | 6.8±2.65 (11) | 1.31±0.72 (6)* |
| <i>A. conferta</i> | 8.2±3.76 (10) | 1.87±0.92 (8)** |

* Lateral polyps taken from base of branch.

** Apical polyps of secondary branches.

acetone. These decolorized corals were at all times fully expanded and appeared to be normal, except for the lack of zooxanthellae.

The experiments were conducted in both light and darkness, as described in the foregoing section. In the two species observed so far, loss of the zooxanthellae caused the rate of calcium deposition to fall to very low levels as shown in Table II. The results for *Manicina areolata* are expressed in terms of the specific activity owing to the accidental loss of the nitrogen samples. The experiment on *Oculina diffusa* ran for eight days and the results are given in terms of the nitrogen content. It is significant that removal of the zooxanthellae almost abolishes the response of the calcification reaction to light which is seen in the normal controls containing zooxanthellae.

Although the zooxanthellae seem to play an important role in determining calcification rates in reef-building corals, certain, as yet unknown, physiological factors operate to control the basic mineralization process in a manner which bears no obvious relationship to the number of algae present in a given species. This is illustrated by the fact that large apical polyps of some of the branching acroporid corals contain few zooxanthellae but calcify several times faster per unit of tissue nitrogen than the yellowish brown lateral polyps which are literally stuffed with algae.

TABLE IV

Calcium-45 uptake of coral treated with 10^{-3} M Diamox
in light and darkness, in $\mu\text{g Ca mg. N}^{-1} \text{hr.}^{-1}$
Number of samples in brackets

| Species | Light control | Light with Diamox | Dark control | Dark with Diamox |
|----------------------|----------------|-----------------------|----------------|-----------------------|
| <i>M. decactis</i> | 0.98±0.49 (12) | 0.56±0.05 (10) | | |
| <i>P. divaricata</i> | 9.80±0.54 (10) | 4.80±0.55 (8) | 5.00±1.00 (8) | 3.3±0.20 (11) |
| <i>C. arbuscula</i> | 6.30±1.58 (9) | 3.40±0.95 (10) | 6.10±0.20 (10) | 3.6±0.55 (7) |
| <i>O. diffusa</i> | | | | |
| Zooxanthellae | 1.63±0.38 (7) | 0.30±0.02 (8) | 0.81±0.15 (9) | Not measurable (6) |
| No zooxanthellae | 0.37±0.01 (6) | Not measurable (6) | 0.26±0.01 (5) | Not measurable (6) |

CALCIFICATION RATES IN DIFFERENT PARTS OF A CORAL COLONY

A glance at any living coral will show that there must be large variations in the growth rates of different parts of the same colony, especially in branching species. A field analysis of the differential growth pattern of reef corals was published by Stephenson and Stephenson (1933). With our method, the growth rates in different parts of the same colony were quantitatively measured. Studies on four species of branching corals, summarized in Table III, show that the calcification rates of the apical parts of such corals are from four to eight times faster than growth in the lateral and basal regions. Well developed calcification gradients are found in corals which have a strongly oriented growth pattern. An example of this is seen

Calcification rates in different parts of a colony
of Acropora conferta

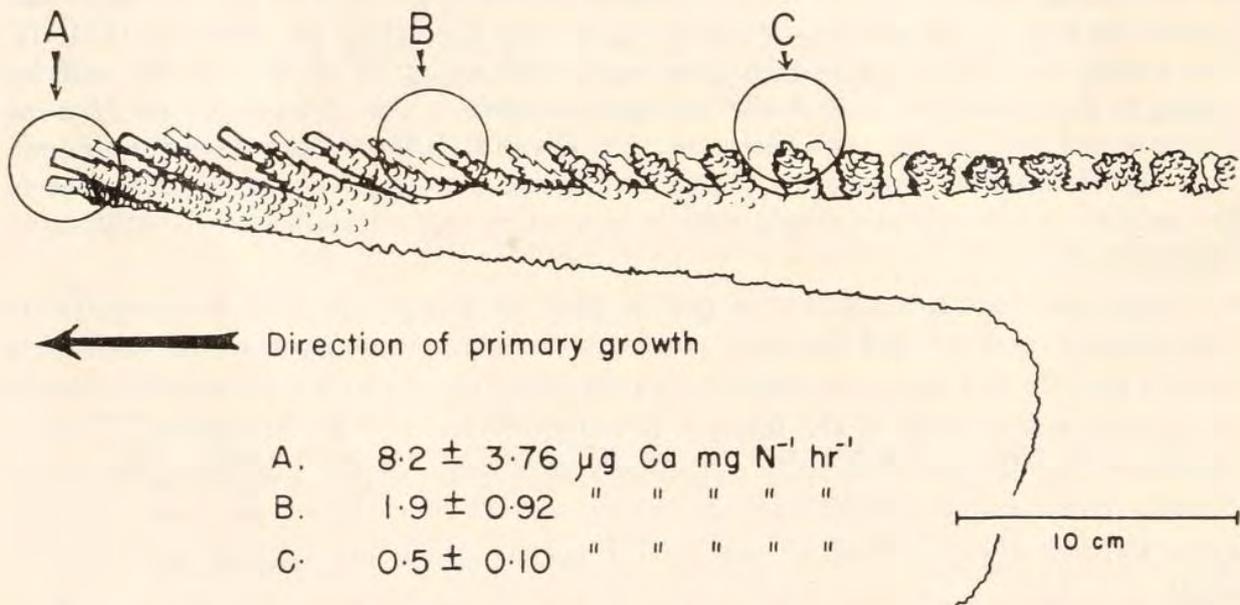


FIGURE 4. Calcification rates in three different parts of a colony of *Acropora conferta*. Only the apical polyps were sampled, their relative positions being indicated by the circles on the diagram. The calcium deposition rate is highest in the large pale apical polyps which are oriented in the direction of primary growth, and marked by circle A. At positions B and C, progressively further away from the growing edge of the colony, the calcification rate becomes greatly reduced.

in the important Pacific reef-building coral *Acropora conferta* in which the primary direction of growth is horizontally outward from a center, resulting in the formation of large tabular colonies. The main growth occurs in the tips of numerous radially outgrowing branches, the apical polyps of which are colored a pale pastel mauve. The apical polyps of the secondary branches are still pale but smaller, whereas those of the tertiary branches are almost indistinguishable from the yellowish brown lateral polyps. The results of a typical experiment are summarized in Figure 4, the location of the different branches being shown in the diagram.

THE EFFECT OF A CARBONIC ANHYDRASE INHIBITOR ON CALCIUM DEPOSITION IN CORALS

Wilbur and Jodrey (1955) demonstrated that shell formation in the oyster *Crassostrea virginica* was greatly reduced in the presence of small concentrations of certain heterocyclic sulfonamides which are powerful specific inhibitors of the enzyme anhydrase. In a series of unpublished experiments we found this enzyme present in all of the twenty-three coral species that were tested. Although carbonic anhydrase was also found in several species of sea anemones and zoanthidea, none of which are calcareous, it was of some interest to determine whether the inhibition of this enzyme had any effect on the calcification rates of corals. The inhibitor used in these preliminary experiments was 2 acetyl-amino 1,3,4,diathiazole-5-sulfonamide, or Diamox. This compound was supplied through the kindness of the Lederle Laboratories Division of the American Cyanamid Division. The experiments were carried out by placing healthy coral colonies into a 10^{-3} M (approx. 1:20,000) solution of Diamox in sea water and adding calcium-45 twelve hours later. The experiments were run in light and dark, each having a control without Diamox. All corals used in these experiments could survive 1:20,000 Diamox for at least two weeks, provided they were kept in the light. In darkness, survival time was reduced to about five or six days.

In *Porites divaricata*, a fast growing shallow-water coral that tolerates strong light, treatment with 10^{-3} M Diamox in the light caused a fifty-one per cent fall in the calcification rate. Exclusion of light caused the calcium uptake to fall a further thirty-four per cent in the presence of Diamox, as shown in Table IV. It is interesting to note that, in this species, the inhibitor had about the same effect as exclusion of light, both causing a fall of about fifty per cent in the calcification rate. This seems to indicate that, as far as their potentiating effect on the calcification rate is concerned, the action of carbonic anhydrase and that of photosynthesizing zooxanthellae are similar and probably synergistic.

In *Cladocora arbuscula*, a coral which grows best in a somewhat deeper and shadier environment, exclusion of light appears to have relatively little effect on the calcification rate, as shown in Table IV, and the per cent inhibition of the calcium uptake produced by Diamox is about the same in light as in darkness. Thus, in this coral, the zooxanthellae appear to play a much less important part in the calcification process than carbonic anhydrase.

In *Oculina diffusa*, the relative effects of carbonic anhydrase and zooxanthellae could be studied in more detail since it was possible to grow this coral without its algae. In the presence of zooxanthellae, there was a fifty-nine per cent decrease in calcium uptake on exclusion of light, whereas Diamox in the light caused approximately eighty per cent inhibition. In darkness, with zooxanthellae and in the presence of Diamox, calcification could not be measured under the conditions of our experiment. Similar results were found in light and darkness in zooxanthellae-less colonies, where there appeared to be practically complete cessation of measurable calcification in the presence of Diamox. These results indicate that in this species carbonic anhydrase exerts a somewhat greater effect on the calcification rate than do the zooxanthellae.

In all four species of reef corals so far tested, 10^{-3} M Diamox caused a forty per cent to fifty per cent decrease of the calcification rate. This concentration of Diamox

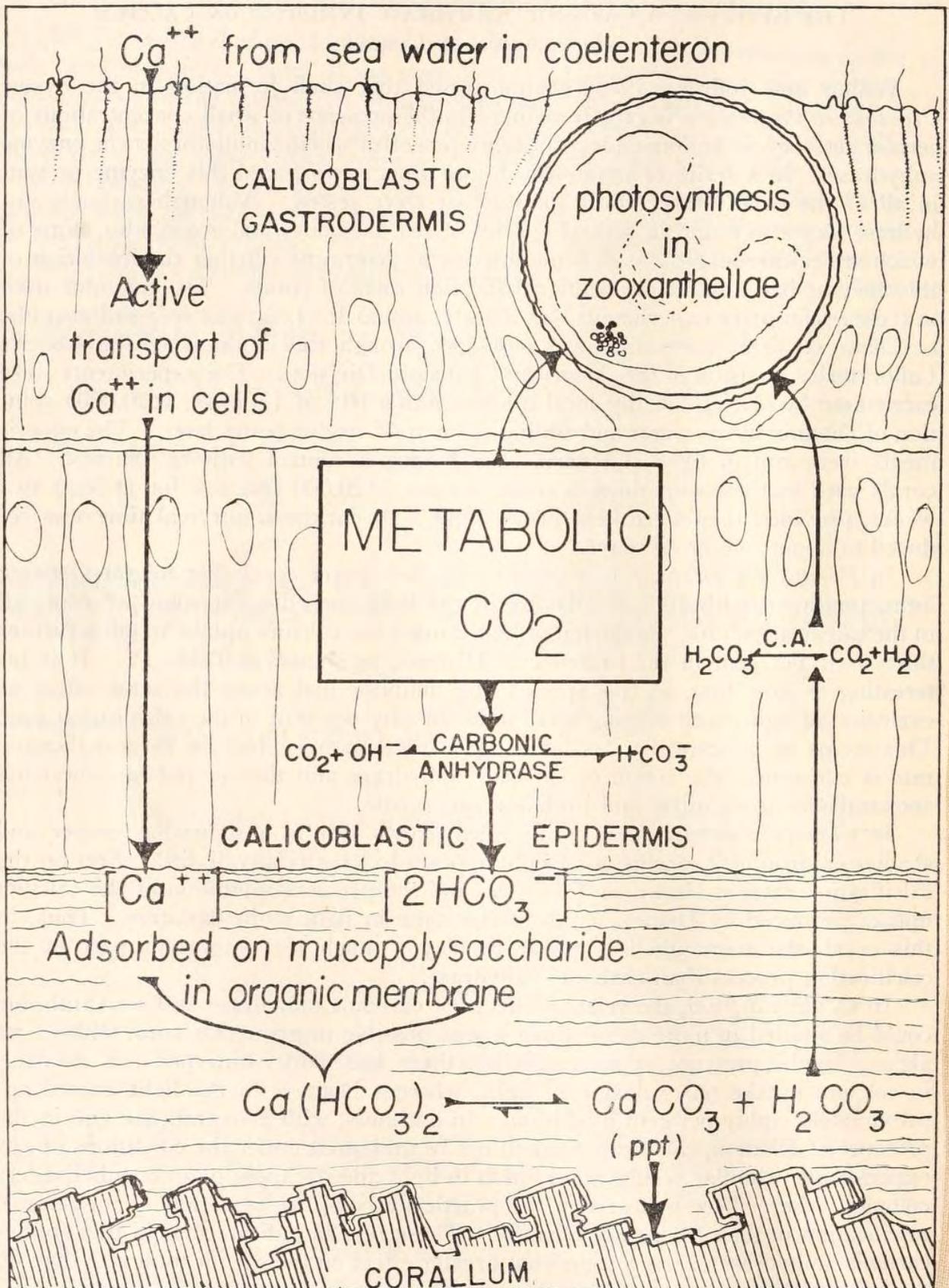


FIGURE 5. Diagram to show possible pathways of calcium and carbonate during calcification in a reef-building coral. A diagrammatic cross-section of the calicoblastic body wall at the base of the polyp is shown but the parts are not drawn to scale. The coelenteron and the

was sufficient to cause complete inhibition of carbonic anhydrase activity in coral homogenates as measured by the method of Meldrum and Roughton (1933). It is obvious that neither zooxanthellae nor carbonic anhydrase in themselves are essential to the calcification process, since this still goes on in the absence of one or both, though at a greatly reduced rate.

DISCUSSION

The experiments described in this paper show that calcium deposition by madreporarian corals and other calcareous reef-builders can be determined under a variety of controlled conditions. The methods used here constitute a first step in the development of an accurate procedure for the rapid measurement of calcification rates applicable to further experimental studies of the physiology of skeletogenesis in corals.

The question arises as to whether coral growth rates determined under laboratory conditions can be compared to those found on the open reef under natural conditions. Since the experiments described above were not designed to test this, we are now conducting field studies, using a modified technic which will be described in a subsequent paper. Preliminary results show that calcification rates of coral are somewhat higher on the open reef than reported here, and that our standard light source was too bright for optimal coral growth. We have evidence that this latter factor accounts for the small and sometimes insignificant dark-light growth differences observed in some coral species as shown in Table I; *i.e.*, high light intensities could partially inhibit coral growth. The quantitative relationship of light intensity and other factors with coral growth is now under investigation.

A working hypothesis has been developed to help to interpret some of our results and to delineate the role played by the zooxanthellae and carbonic anhydrase in skeletogenesis of the reef-building madreporarian corals. To be satisfactory, such a hypothesis must account for: 1) the species-specific morphology of the skeleton; 2) its formation external to the body proper; 3) its chemical composition which is over ninety-nine per cent CaCO_3 and less than one per cent MgCO_3 (Vinogradov, 1953); and 4) the crystalline nature of the mineral matter which is nearly pure aragonite, according to Meigen (1903) and Chave (1954).

The calcification process is considered as a reaction in which Ca^{++} and CO_3^{--} are brought to the calcification centers by separate pathways. The weight of histological evidence now indicates that the mineralization process occurs outside the calicoblastic epidermis (Matthai, 1918; Hayashi, 1937; Goreau, 1956) which secretes an organic matrix that may act as a template on which the final stages of skeletogenesis take place. It is of interest that this organic matrix contains an acid mucopolysaccharide-like substance (Goreau, 1956). This gives rise to the possibility that Ca^{++} , taken up from sea water and transported across the body wall to the external surface of the calicoblast, is adsorbed by ion exchange on an acidic space lattice provided by the mucopolysaccharide in the organic matrix. Here

flagellated gastrodermis containing a zooxanthella are shown at the top of the figure, the calicoblastic epidermis is in the middle and the organic membrane with crystals of calcareous matter are at the bottom. The boring algae, the effects of which are problematical, have been omitted for simplicity. The direction of growth is upward, *i.e.*, calcium deposition is in a downward direction.

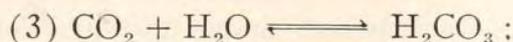
the Ca^{++} combines with HCO_3^- by the following reaction:



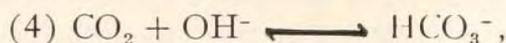
The unstable product of this reaction then breaks down:



with the formation of calcium carbonate and carbonic acid. As long as calcium is not a limiting factor, the rate of formation of calcium carbonate will depend on the rate with which the carbonic acid is removed from the site of calcification. This can be accomplished through the fixation of CO_2 by photosynthesizing zooxanthellae and/or the action of carbonic anhydrase. The proposed scheme is summarized in Figure 5. It is expected, therefore, that if the zooxanthellae are prevented from photosynthesizing by keeping the coral in darkness, or if the algae are completely removed, the velocity of calcification will decrease, due to slowing down of reaction (2). Since carbonic anhydrase has an action which is, in this respect, physiologically equivalent to that of the zooxanthellae, the inhibition of the enzyme will also result in a slowing down of the calcification rate. The greatest decrease occurs when the corals are kept in darkness in the presence of a carbonic anhydrase inhibitor. The fact that calcification still goes on under these conditions simply shows that neither the enzyme nor the algae determine the basic calcification reaction, but that they can exert a strong influence on its over-all rate. This is in agreement with the work of Wilbur and Jodrey (1955) who showed that carbonic anhydrase does not affect shell calcification in the oyster unless the rate is limited by one of the following reactions:



or



hence the enzyme cannot be a primary factor in calcification as was previously assumed by Stolkowsky (1950) for mollusk shells.

An interesting problem arises from our data on calcification rates of reef corals from which the zooxanthellae had been removed. The second part of Table II shows that in darkness normal corals calcify from two to three times faster than corals which have lost their zooxanthellae. This suggests that the presence of these algal symbionts, even when not photosynthesizing, may have a potentiating effect on the calcification rate of the coral host. It is thus considered possible that the zooxanthellae can exert a general stimulant effect on the host's metabolism, mediated through a vitamin or hormone-like factor. This function of the zooxanthellae would to some extent be independent of the photosynthetically controlled "janitorial" activities of these algae which result in the assimilation of the animal host's metabolic waste products. It is hoped that work now in progress will provide more evidence for this interesting possibility.

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SUMMARY

1. A method is described for the accurate measurement of calcification rates in reef-building corals under various controlled conditions, using calcium-45 as tracer.

2. At the temperatures of the experiments, there was a slow but appreciable isotopic exchange between the coral skeleton and sea water. There are indications that this is considerably less in living coral where the tissue forms a barrier against such exchange.

3. In many of the reef-building corals tested so far, the calcification rate was significantly lowered by the exclusion of light.

4. The calcification rate of reef corals grown in darkness for prolonged periods of time to remove the zooxanthellae is considerably reduced and seems independent of the light intensity.

5. Variations in the growth rates of different parts of coral colonies were measured. The existence of growth gradients was demonstrated in a number of species.

6. Calcium uptake was greatly reduced on the addition of Diamox, a specific carbonic anhydrase inhibitor. In those species tested, the effect of carbonic anhydrase inhibition and exclusion of light was in the same direction. In the presence of complete inhibition of carbonic anhydrase there was still an uptake, even in darkness.

7. It was concluded that the effect of light on reef coral growth is in part mediated through the zooxanthellae. The decreased calcification rates of reef corals in darkness, in the absence of zooxanthellae or in the presence of a carbonic anhydrase inhibitor suggest that the rapid calcification of these corals may be dependent on efficient removal of H_2CO_3 .

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