the auxins indoleacetic acid, naphthaleneacetic acid, or 2,4-dichlorophenoxyacetic acid. The amount of reducing sugar in the incubation medium was assayed (Fig. 1) by the method of Somogyi (*8*).

Amylolytic activity was enhanced by concentrations of gibberellic acid in excess of 0.001 μg/ml; the greatest effect was obtained at 0.1 μg/ml. Indoleacetic acid, at concentrations less than 10 μg/ml, had no effect on this process in either the presence or absence of gibberellic acid; higher concentrations inhibited the induction of amylolytic activity. These results agree with those of Paleg (*7*) and show that our system is comparable to those studied previously.

The anti-auxin *p*-chlorophenoxyisobutyric acid had no effect on the induction of amylolytic activity at concentrations less than 10 μg/ml, and at higher concentrations it was inhibitory (Fig. 1). Since the amounts of anti-auxin required for inhibition were greater than those required for inhibition of auxin action in other tissues (*6*), the possibility had to be considered that this inhibition might be due to a toxic effect on the cells rather than to a competitive effect on endogenous auxin. These two types of inhibition can be differentiated on the basis of the response of anti-auxin treated tissues to exogenous auxin. If the inhibition is due to competitive interaction between endogenous auxin and anti-auxin, additional auxin will partially reverse the inhibition; a nonspecific inhibition will not be affected by additional auxin.

Addition of auxin to endosperms that had been treated with anti-auxin was without effect whether the auxin was indoleacetic acid (Fig. 2), naphthaleneacetic acid, or 2,4-dichlorophenoxyacetic acid (Table 1). In no case was any reversal of the inhibition obtained. Hence the effects of high concentrations of *p*-chlorophenoxyisobutyric acid are not due to antagonism between the anti-auxin and endogenous auxin. It appears that endogenous auxin is not involved in the increase in amylolytic activity induced by gibberellic acid in barley endosperm. In this system the action of gibberellic acid is independent of the presence or absence of auxin.

Table 1. Inability of the auxins naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) to reverse inhibition induced by *p*-chlorophenoxyisobutyric acid (PCIB). Endosperms were incubated in basic medium with 100 μg of PCIB per milliliter and concentrations of auxin as indicated. Reducing sugar content of medium, in milligrams of glucose equivalents per gram of endosperm, was determined after 22 hours at 30°C. The control, without PCIB, had a reducing sugar content of 60.2 mg/g.

Auxin concn. (μg/ml)	Reducing sugar (mg/g)	
	2,4-D	NAA
0	23.5	20.2
0.1	23.2	21.2
1	25.3	21.8
10	20.0	20.8
100	17.7	10.0

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Systematic Relationships between Carbon and Oxygen Isotopes in Carbonates Deposited by Modern Corals and Algae

Abstract. *Analyses of organic carbonates from Jamaican coral reefs show a positive correlation between the $O^{18}:O^{16}$ ratio and the $C^{13}:C^{12}$ ratio in some taxonomic groups of corals and algae, but essentially no correlation (nearly constant O^{18}) in one suborder of reef-building corals. The strontium and magnesium contents apparently are controlled mainly by skeletal mineralogy and show no correlation with carbon or oxygen isotopic composition. The observed positive correlations between C^{13} and O^{18} content may be due to calcification processes utilizing carbon-oxygen compounds from two isotopically different sources or utilizing selected portions of a wide spectrum of carbon-oxygen compounds in which there is a positive correlation between $C^{13}:C^{12}$ and $O^{18}:O^{16}$ ratios. Coral and algal carbonates from Jamaican waters, with an annual temperature range of only about 4°C, exhibit a total δC^{13} range of more than 13 per mil and a δO^{18} range of more than 4 per mil. The wide isotopic variability resulting from vital effects of calcifying organisms must be taken into account in applying isotopic analysis to the study of sedimentary carbonate rocks which may include reef-derived carbonates.*

The isotopic and elemental composition of organic marine carbonates is of interest in both biological and geological studies; compositional variations may provide indirect evidence regarding calcification processes and various geological problems, including the origin of limestones (*1*), ocean paleotemperatures, and some aspects of paleogeography, particularly regarding proximity to ancient shorelines (*2*) or coral reefs (*3*). Variations of carbonate isotopic composition due mainly to differences in external environment, from one marine biologic community to another, may be obscured by relatively large differences due to food selectivity and to metabolic isotope fractionation by various calcifying organisms living together in the same environment (see *4*).

Organic carbonates which appear to be deposited out of oxygen isotopic equilibrium with sea water, and whose compositions presumably are strongly affected by vital effects of the organisms, include those precipitated by coelenterates, echinoderms, and some algae (*5*, *6*); they also exhibit wide variability of carbon isotope ratios in contrast with the isotopic composition of mollusk shells, for example, which is relatively constant within one biologic community (*7*).

The object of this investigation was to discover whether the isotopic and chemical compositions of organic reef carbonates exhibit any systematic relationship within and between taxonomic groups of calcifying organisms from the same general environment. Reef sites in Jamaica were selected for study because they have a wide variety of Caribbean corals and algae and because the shal-

low-water Jamaican reefs have relatively stable conditions of temperature and salinity. During 1962 and 1963, surface water at a station near Jamaica showed a temperature range from 26.5° to 29.6°C and a salinity range from 35.2 to 36.2 parts per thousand (*8*).

Most of the Jamaican specimens were collected and identified by T. F. Goreau (University of the West Indies); they are mainly from north shore reefs between Falmouth and Port Antonio. Some (*9*) are from south shore reefs near Port Royal, and a few specimens from non-Jamaican sites are included for comparison. No detailed temperature or salinity records are available for individual reef sites.

Specimens were cleaned in commercial Clorox (5-percent solution of sodium hypochlorite), then washed, air dried, crushed to pass an 80-mesh screen, and heated for 20 minutes at 420°C in flowing helium to remove or pyrolize organic material. Carbon dioxide samples were extracted by treatment of the prepared specimens with 100-percent phosphoric acid, and the isotopic composition was measured by mass spectrometer; standard comparison techniques (*10*) were used. Isotopic compositions, the means of duplicate measurements made at different times, are expressed in the conventional δ notation as parts-per-thousand differences of O^{18} or C^{13} content relative to the Chicago PDB standard carbon dioxide.

Results of carbon and oxygen isotopic analyses of the reef carbonates are reported elsewhere in detail (*11*), along with a consideration of the effects of depth, water temperature, and calcification rate. Data for the carbonates of shallow-water corals and red algae from Jamaican reefs are plotted in Figs. 1 and 2 and compared with the ranges of δC^{13} and δO^{18} of carbonates precipitated by green algae and mollusks in the same water, that is, δC^{13}, -0.1 to $+4.7$ per mil, and δO^{18}, -1.3 to -4.4 per mil. Similar ranges of isotopic composition of algal aragonites have previously been reported (*6*, *12*).

Carbonate isotopic compositions of shoal-water reef-building corals (*13*) from Jamaica are plotted in two separate taxonomic groups (Figs. 1 and 2). Those of suborder Faviina (Fig. 1), as well as the reef-building corals of suborders Astrocoeniina and Fungiina (triangles, Fig. 2), are relatively deficient in both C^{13} and O^{18}, in comparison with carbonates deposited by green algae and mollusks.

Carbonates deposited by non-reef-building corals (circles, Fig. 2) and by red algae (squares, Fig. 2) exhibit wide ranges of isotopic composition; some overlap the indicated compositional range of green algae and mollusk shell.

A principal result of this study is the observation that there are systematic relationships between δC^{13} and δO^{18} of carbonates precipitated by some taxonomic groups of reef organisms from Jamaica. Corals of suborder Faviina (Fig. 1) have nearly constant δO^{18}; the trend line through points representing δC^{13} and δO^{18} of shoal-water specimens has a slight negative slope.

In contrast with the Faviina, reef-building corals of suborders Astrocoeniina and Fungiina (triangles, Fig. 2) exhibit a positive correlation between δC^{13} and δO^{18}. No explanation can be offered at present, although it seems noteworthy that the Astrocoeniina and Fungiina are more closely related to one another than to the Faviina (see *14*).

The analyzed non-reef-building corals (circles, Fig. 2) show a similar positive correlation between δC^{13} and δO^{18}; the trend line is nearly parallel to that for the reef-building Astrocoeniina and Fungiina, but is offset toward relative enrichment in O^{18}.

Carbonates deposited by the red algae (squares, Fig. 2) also show a general positive correlation between carbon and oxygen isotopic composition. In this case there may be less justification for drawing a trend line through all of the diagram points, because a difference of mineralogy is involved; the two lower points, near $\delta C^{13} = +4$ per mil, represent aragonite deposited by *Galaxaura*, whereas the remaining four points represent magnesian calcite deposited by *Amphiroa* and *Goniolithon*. Among the octocorallia as well as the red algae there are some calcite-forming species and some aragonite-forming species. In both groups, calcite is relatively deficient in C^{13} and aragonite is relatively enriched (*11*). A similar difference in C^{13} content between algal aragonites and an algal calcite (from *Corallina*) was recorded by Craig (*4*), although the polymorphic form of the carbonates was not mentioned. It is not implied here that the polymorphic form of calcium carbonate has an effect on δC^{13}; rather, it seems likely that both the mineralogy and the carbon isotopic

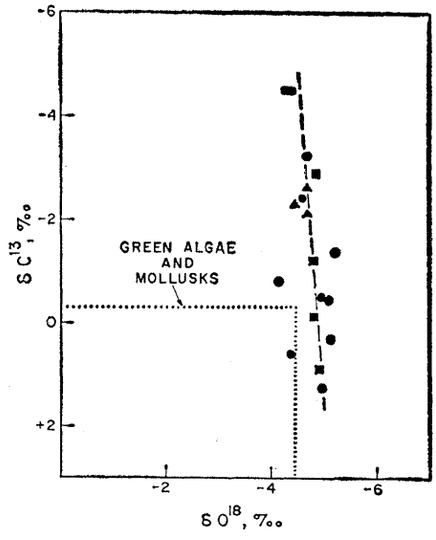

Fig. 1. Isotopic composition of carbonate skeletons of reef-building corals, suborder Faviina, from shoal-water Jamaican reefs. Families included are Faviidae (●), Oculinidae (■), Meandrinidae (▲), and Mussidae (■). The dotted line shows the compositional limits of carbonates deposited by green algae and mollusks in the same waters ($^0/_{00}$ denotes per mil).

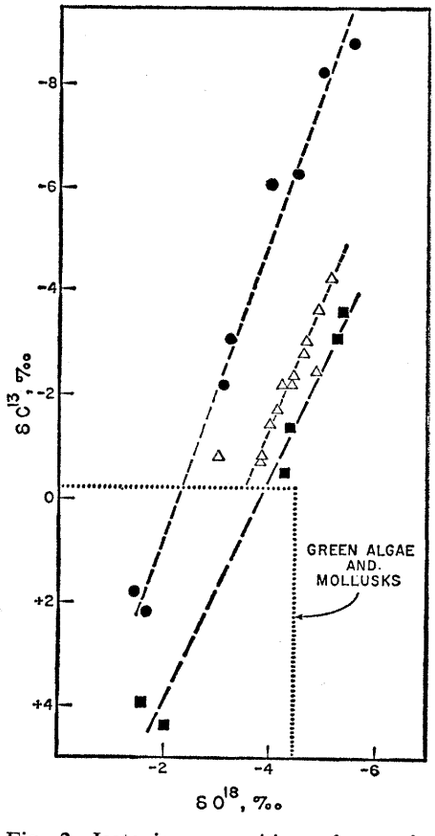

Fig. 2. Isotopic composition of organic carbonates from shoal-water Jamaican reefs, including carbonates deposited by non-reef-building scleractinian corals (●), by reef-building (hermatypic) corals (△) except the Faviina, and by red algae (■).

composition are controlled by metabolic processes.

In order to test for possible relationships between isotopic and elemental composition, 5 calcite samples and 23 aragonite samples were analyzed spectrographically for strontium and magnesium. The specimens, part of a large group which had previously been analyzed for carbon and oxygen isotope ratios (*11*), were selected to cover the full range of carbon isotopic composition for each taxonomic group. For that purpose, specimen selection was not restricted to those from Jamaican reefs.

Isotopic and chemical data are shown in Table 1. There is no apparent relationship between isotopic composition and the strontium or magnesium content. The aragonite samples average 0.86 percent for strontium and 0.15 percent for magnesium, whereas the calcite specimens average 0.25 percent Sr, 5.9 percent Mg. The results thus confirm previous observations (for example, *15*, *16*) to the effect that skeletal mineralogy exerts a primary control on the strontium and magnesium content. There are no significant differences in strontium content among the aragonites of the various taxonomic groups represented. Calcite deposited by the red algae has a somewhat higher magnesium content (Table 1) than that deposited by the Octocorallia, in accord with observations of Chave (*16*).

The observed relationships between taxonomy and the carbon and oxygen isotope ratios are in sharp contrast to the general absence of such a relationship between taxonomy and the Sr : Ca ratio of the analyzed organic carbonates. It seems likely that calcium and strontium follow a more direct pathway to the calcification site, as suggested by Goreau (*17*), and that the Sr : Ca ratio is not appreciably changed by metabolic processes. This is in accord with the observations of Lowenstam (*15*) on the carbonate tests of most marine invertebrates.

The above-noted positive correlations between C^{13} and O^{18} content of some corals (Fig. 2) may be due to mixing and utilization, at the calcification site, of both carbon and oxygen from different sources or metabolic pathways. In that connection, Revelle and Fairbridge (*18*) quote Craig to the effect that marine invertebrate tests appear to contain a mixture of carbon derived from metabolic activity and carbon derived from sea water bicarbonate. The present results lead one to suspect two sources of carbon-oxygen compound in which both the oxygen and the carbon are isotopically different. Two possible alternatives are suggested as working hypotheses to be tested by specific tracer experiments: (i) the carbon and oxygen that are relatively enriched in C^{13} and O^{18} may be derived from marine bicarbonate and mixed at the calcification site with metabolic carbon-oxygen compounds relatively deficient in C^{13} and O^{18} and derived from selected components of the suspended or dissolved food web, or (ii) the carbon and oxygen supplied to the calcification site come from a wide spectrum of inorganic and organic compounds, such as those analyzed by Parker (*19*), which may vary in oxygen isotopic composition in parallel with the observed variation in carbon isotopes. It would appear desirable to determine oxygen isotope fractionation as well as carbon isotope fractionation in experiments such as those of Abelson and Hoering (*20*) on amino acids. It has been demonstrated (*21*) that many marine invertebrates are capable of removing and utilizing amino acids as well as carbohydrates from dilute solutions.

The observed differences among taxonomic groups, in their C^{13} and O^{18} content, may be due partly to differences in their selectivity for suspended and dissolved food web components. It appears that pronounced difference in selectivity or in calcification mechanism are to be expected between corals

Table 1. Isotopic composition and strontium and magnesium content of organic carbonates.

Sample No.	Genus	Locality	δC^{13} (per mil)	δO^{18} (per mil)	Sr (%)	Mg (%)
		Scleractinian corals (aragonite)				
	Suborder Fungiina					
14	*Agaricia*	Jamaica	−1.92	−3.78	0.80	0.27
18	*Siderastrea*	Jamaica	−0.69	−3.78	.78	.06
19	*Siderastrea*	Jamaica	−2.34	−4.44	.80	.29
21	*Porites*	Jamaica	−3.00	−4.68	.84	.09
24	*Porites*	Jamaica	−4.24	−5.14	.87	.18
	Suborder Faviina					
29	*Diploria*	Jamaica	+1.26	−4.96	.79	.21
30	*Diploria*	Andros Island, B.W.I.	+0.30	−4.87	.90	.21
(37)*	*Astrangia*	Jamaica	−2.22	−3.10	.69	.43
(39)	*Astrangia*	Woods Hole, Mass.	−4.93	−3.94	1.04	.13
(41)	*Phyllangia*	Jamaica	−6.10	−3.99	0.88	.10
(42)	*Phyllangia*	Jamaica	−8.26	−4.97	.82	.09
	Suborder Caryophylliina					
(64)	*Desmophyllum*	Jamaica	+1.79	−1.45	.96	.11
(65)	*Desmophyllum*	Jamaica	+2.18	−1.64	.97	.06
	Suborder Dendrophylliina					
(77)	*Dendrophyllia*	Philippines	−9.28	−5.74	.85	.08
		Octocorallia (aragonite)				
83	*Heliopora*	Bikini	+3.76	−3.22	0.74	0.30
		Hydrozoa (aragonite)				
88	*Millepora*	Jamaica	−0.36	−2.14	0.81	0.10
90	*Millepora*	S. Atlantic	−1.16	−3.29	.85	.18
91	*Stylaster*	Jamaica	+0.53	−3.00	.78	.08
93	*Stylaster*	Jamaica	+1.25	−2.11	.84	.06
96	*Allopora*	Aleutian Islands	−6.40	−1.14	.98	.04
		Green algae (aragonite)				
102	*Halimeda*	Jamaica	+2.59	−1.91	0.97	0.13
103	*Halimeda*	Jamaica	+0.06	−4.01	.97	.08
106	*Udotea*	Jamaica	+4.48	−2.25	.86	.22
			Mean, 23 aragonite specimens:		0.86	0.15
		Octocorallia (calcite)				
80	*Tubipora*	Philippines	−0.36	−4.78	0.27	5.0
81	*Telesto*	S. Atlantic	−3.00	−4.49	.22	4.5
		Red algae (calcite)				
112	*Goniolithon*	Jamaica	−1.38	−4.38	0.28	6.4†
113	*Goniolithon*	Jamaica	−3.61	−5.38	.26	6.7†
114	*Amphiroa*	Jamaica	−0.55	−4.26	.27	7.0†
			Mean, 5 calcite specimens:		0.25	5.9

* Ahermatypic (non-reef-building) corals are indicated by parentheses around sample number. † X-ray diffraction gives lower magnesium content (5.5%) in the calcite structure. Excess Mg may be in brucite.

of the suborder Faviina (Fig. 1) and the corals that exhibit a positive correlation between δC^{13} and δO^{18} (Fig. 2).

The isotopic differences between the ahermatypic corals (circles, Fig. 2) and the other corals may be due to the absence of associated zooxanthellae in the former. The present results do not, however, provide direct evidence bearing on the controversy as to whether zooxanthellae are used as food by their coral hosts or whether they merely stimulate coral metabolism and calcification by producing oxygen and absorbing carbon dioxide and waste products (*17*).

The results appear to have a bearing on the study of ancient reefs and reef-derived sedimentary rocks. It is confirmed that the isotopic composition of reef-derived carbonate sediments will be mainly determined by the relative proportions of carbonates supplied by different organisms; effects of depth, water temperature, and calcification rate (*11*) are relatively minor in comparison with the wide range of isotopic variability that can be attributed to vital effects of the various calcifying organisms. It is possible that the variability of isotopic composition of carbonate sediments recorded by Gross (*12*) and Friedman (*22*) is partly due to primary differences, as well as to the introduction of secondary calcite during diagenesis and lithification. A fossil reef or a reef-derived carbonate rock consisting dominantly of coral fragments should be deficient in both C^{13} and O^{18}, in comparison with carbonate sediments remote from a reef (*3*) where green algae and mollusks are dominant carbonate contributors.

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Histones and Basic Polyamino Acids Stimulate the Uptake of Albumin by Tumor Cells in Culture

Abstract. *Basic proteins and polyamino acids are taken up by mammalian cells at rates up to 3000 times greater than serum albumin. When given together with serum albumin they increase the albumin uptake by a factor that correlates with their own rate of uptake and can reach more than 50-fold. The lowest threshold of activity detected (10^{-10}M) is comparable to the activities of the most potent membrane-active agents.*

A number of electron microscope and histochemical investigations have demonstrated the penetration of intact proteins into mammalian cells. Little is known, however, about the order of magnitude, the mechanism, and the significance of this process. In a previous study (*1*) we reported that monolayers of sarcoma-180 cells, grown and tested in Eagle's medium, bound I^{131}-labeled albumin at a low rate approximating 10^5 molecules per cell per hour. We have since found that the addition of low concentrations of histones or of basic polyamino acids increases this albumin uptake 10- to 50-fold. Now we want to describe this effect, to comment on its possible mechanism, and to discuss its biological implications.

Monolayers of sarcoma-180 cells were exposed for periods of 30 seconds to 2 hours to tissue-culture medium containing human serum albumin labeled with I^{131} (*1*). After rinsing, detachment with trypsin, and washing, the cells were treated with 5 percent trichloroacetic acid and the specific radioactivity of the total cell protein was determined (*1*).

As shown in Fig. 1, calf thymus histone (*2*) stimulated the uptake of albumin up to 15-fold. Crude histone preparations of different commercial origin had comparable effects. However, different histone fractions from a single tissue gave strikingly different results. Thus, arginine-rich histones were markedly more active than crude histones, whereas the lysine-rich histone fraction was essentially inactive (*2*) (Fig. 2). Significant stimulation ($P < .005$) was produced by 1.0 μg/ml of crude histone and 0.3 μg/ml of arginine-rich histone. In comparable concentrations, protamine sulfate and poly-L-lysine stimulated albumin uptake to a lesser extent, but their threshold concentrations and the time curves of their effects were similar. All the basic polyamino acids tested (*2*) stimulated albumin uptake, the most active being poly-L-ornithine, which increased the uptake about 45-fold at a concentration of 10 μg/ml (Fig. 2). Its threshold for activity was well below 0.1 μg/ml, that is, considerably less than $5 \times 10^{-10} M$, a concentration which, under the conditions of our experiments, corresponds to about 10^4 molecules per cell. The D-lysine, DL-lysine, and L-histidine polymers and an L-lysine : tyrosine copolymer (19 : 1)