out of ten and on the 10th day in six out of ten cultures at a maximum peak titer of 1:16, which subsequently decreased, becoming undetectable on the 18th day. However agglutination around the tissues continued, indicating that the agglutination around tissue is the more sensitive assay for demonstrating antibodies in this system.

Thus immune responses can be induced and maintained entirely in vitro. One might argue that this response is actually due to stimulation of preexisting natural antibodies that are undetectable. However, the fact that spleens from untreated normal mice did not respond to antigenic stimulation in vitro, the similarity of the kinetics of agglutinin formation in explants stimulated in vitro and those induced in vivo, and the observation that spleens from immunized mice respond to a secondary stimulation in vitro with no treatment of PHA or adjuvant (*11*) place this reaction with those described as primary immune responses. While treatment of donor-spleen tissue with PHA was essential for demonstrable agglutinin or hemolysin production no treatment was required to elicit the graft-versus-host reaction in vitro. The fact that PHA in itself is not involved in the immune response is suggested both by the specificity of the observed reaction and by the fact that adjuvant can elicit a similar reactivity of spleen explants. That no prior stimulation is necessary for evoking a graft-versus-host response is consistent with observations made on human lymphocytes in vitro (*12*). It may well be that spleen cells provide both antigenic stimulation and a stimulus to cell proliferation. Alternatively, this reaction may not require proliferation by lymphocytes at all, or it may simply represent a more sensitive assay of an immune process.

Why only some fragments from each spleen produce detectable antibodies is not clear. Whether the reason is technical or whether the explanation lies in a limited total number of competent cells present in an unimmunized spleen cannot be decided from our experiments. Since explants, once positive, remain positive for a period of up to 2 months, careful study of the period immediately after explantation is required.

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Brucite in Carbonate Secreted by the Red Alga Goniolithon sp.

Abstract. *X-ray diffraction studies, chemical analyses, infrared-absorption studies, and nuclear-magnetic-resonance spectrum analysis demonstrate the existence of the mineral brucite, $Mg(OH)_2$, in the skeletal carbonate secreted by the red marine alga* Goniolithon *sp. Electron microprobe examination of the carbonate shows that the brucite is concentrated in certain areas of the skeletal structure. These results explain the anomalously high solubility and large cell size of the* Goniolithon *"carbonate" observed by earlier investigators.*

Recent investigations of magnesian calcites secreted by marine organisms have directed particular attention to the skeletal carbonate secreted by the red alga *Goniolithon*. This interest is drawn in part by the exceptionally high magnesium content of the carbonate ($MgCO_3$, 24 to 29 mole percent) and also by its anomalous physical and chemical properties. First, the results of both saturometer and "equilibrium-*p*H" measurements indicate that the carbonate is considerably more soluble than extrapolation from the solubilities of solid solutions of calcite less rich in magnesium predicts (*1*). Second, the cell size of the carbonate, determined by x-ray powder diffraction, is much larger than its chemical composition would require, assuming that the magnesium is present in ideal solid solution in the calcite lattice (*2*). Noting that calcites less rich in magnesium show variations in cell size that closely approximate the ideal solid-solution pattern of Vegard's law, Goldsmith *et al.* (*3*) suggested that the skeletal material secreted by *Goniolithon* consists of a physical mixture of two phases: a solid-solution magnesian calcite and minor amounts of some phase richer in magnesium in amounts that are too small or too poorly crystallized to be detected by x-ray.

During the past 2 years, skeletal material deposited by *Goniolithon* has been carefully examined in three laboratories. These investigations were independently conceived and entirely different techniques were used. Although no single study provided sufficient evidence to justify a positive conclusion, the evidence provided by all three combined overwhelmingly supports only one conclusion. I will discuss these studies in logical sequence, with no suggestion of precedence (*4*).

In order to determine whether the skeletal material secreted by *Goniolithon* constitutes an homogeneous single phase, several specimens (*5*) were subjected to microprobe analysis (*6*). Partial wet-chemical analysis of the carbonate yielded an alkali-earth cation ratio that corresponded to a calcite of ideal solid solution containing nearly 29 mole percent of $MgCO_3$. X-ray diffraction studies, however, showed only a single phase (magnesian calcite), with a cell size that corresponded to an ideal solid solution containing approximately 18 mole percent of $MgCO_3$ (d_{10*4}, 2.975 Å) (*7*). Organic matter was removed from the samples with Clorox or H_2O_2, or both, and they were then mounted and polished for examination by electron microprobe (*8*); typical images are shown in Fig. 1.

Electron-back-scatter images (Fig. 1*a*) show the lattice-like structure of the skeletal material. The field of the photograph, showing a longitudinal section of a single algal "branch," is approximately 180 μ in diameter. The dark areas, depressions in the polished surface of the specimen, represent transected cellular openings in the structure. An image of the same field generated by electron-excited calcium-Kα fluorescence (Fig. 1*b*) indicates that the open cells are calcium-deficient areas sur-

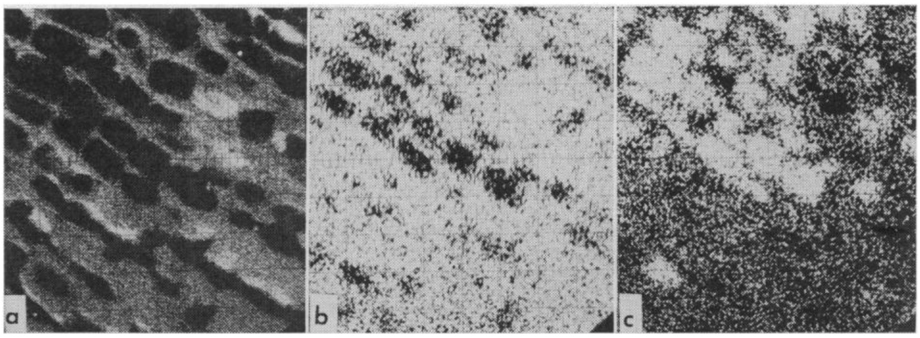


Fig. 1. Electron-microprobe images of *Goniolithon* skeletal material. *a*, Electron-backscatter image showing open cells (dark areas) in the transected skeletal structure; field of view, 180-μ diameter. *b*, Image of the same field by calcium-Kα fluorescence; note that the open pores shown in *a* appear deficient in calcium. *c*, Image of the same field by magnesium-Kα fluorescence showing high concentration of magnesium in the calcium-deficient pore spaces, and homogeneous distribution of lesser amounts of magnesium throughout the calcium-rich matrix.

rounded by a reasonably homogeneous matrix rich in calcium. Figure 1*c* shows the same field as it appears when electron-excited magnesium-Kα fluorescence is presented visually. Most of the calcium-deficient portions of the specimen (pores) appear to be lined with a highly magnesian phase, and magnesium is also uniformly distributed throughout the calcium-rich matrix. These results make it clear that the carbonate material secreted by *Goniolithon* is a mixture of two phases, as Goldsmith *et al.* suggested, and that these two phases appear to be systematically distributed within the skeletal structure.

Semiquantitative estimates of the calcium:magnesium ratios, based on measurements of fluorescence intensity, are 4.5 for the matrix and 0.05 or less for the pore lining. Assuming that these cations are present as carbonates, these intensity ratios suggest that the calcite solid solution is composed of $Ca_{0.82}Mg_{0.18}CO_3$, which is in good agreement with the estimated composition based on x-ray measurements of cell size. By difference, the unidentified phase must contain 13 mole percent of the magnesium determined by wet analysis. If present in amounts approaching even 10 percent by weight of the sample, however, the second phase should be easily identified by x-ray diffraction; its absence from diffraction patterns must be attributed to (i) extremely small particle size, (ii) very poor crystallinity, or (iii) very low molecular weight relative to that of the solid-solution phase. Three magnesium compounds of low molecular weight suggest themselves, MgO, $Mg(OH)_2$, and $MgCO_3$, but the second phase cannot be positively identified without additional data.

Table 1. Chemical composition of a specimen (from Rodriguez Key; 25°3′N, 80°30′W) of skeletal carbonate secreted by *Goniolithon*. TM, titrimetric; SC, spectrochemical; FP, flame photometric; TBM, turbidimetric; CM, colorimetric (supplied by Shell Development Co.). The data point to this empirical formula (sulfate-free, inorganic only):
$Ca_{0.76}Mg_{0.24}(CO_3)_{0.80}[(OH)_2]_{0.20}$

Component	Weight, dried at 105°C (%)	Mole	Sum of equivalent ions (mole)
	Inorganic		
CO_2 evolved by HCl, as CO_3^{--}	46.0	0.766	0.766(CO_3)
Ca (*TM*)	29.0	.724	
Mg (*TM*)	5.60	.230	
Ba (*SC*)	0.0009		
Sr (*SC*)	.32	.0037	
Na (*FP*)	.37		.958 (cations)
Cl (*TM*) (total halide)	.12		0.192 deficit
SO_4 (*TBM*) (total S)	.68		
P (*CM*)	.00		
Inorganic acid–insoluble	.33		
	Organic		
C	4.89		
H	0.70		
O (by difference)	5.61		
	Total		
	93.62		
	$(OH)_2$ to satisfy anion deficit (0.192 × 34.015)		
	6.53		
	Total, observed and calculated		
	100.15		

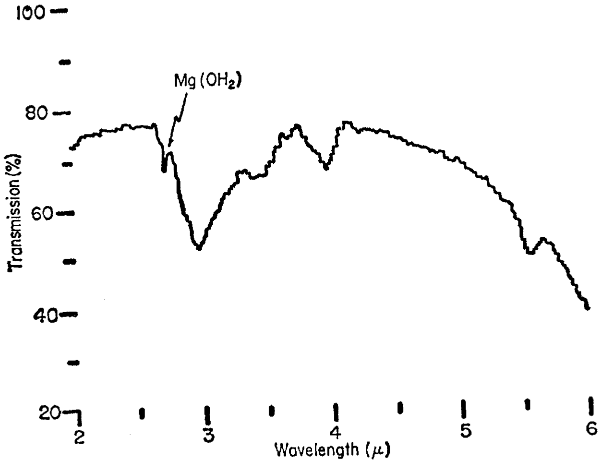
On the assumption that a second phase was present in the skeletal material secreted by *Goniolithon*, its identification was attempted by detailed chemical analysis (*9*); a brief description of the method follows. Standard inorganic analysis was made of the fraction of the sample soluble in 3*N* HCl, and combustion of a parallel sample yielded total CO_2; the difference between acid-evolved CO_2 and total CO_2 was taken to represent organic carbon present in the sample. From separate analyses of the residue of material leached with acetic acid, organic hydrogen and oxygen (by difference) were calculated. Analytical results of a specimen collected at Rodriguez Key appear in Table 1.

It is evident from the data in Table 1 that the number of moles of carbonate-forming cations substantially exceeds the number of moles of carbonate. In view of the exhaustiveness of the analysis it is most probable that this anion deficiency represents essential hydroxyl ions undetected in the analysis. By adding to the analytical results sufficient hydroxyl ion to make up the deficiency, the analysis is corrected to slightly greater than 100-percent recovery, and a tentative (sulfate-free) analytical formula for the material is obtained:

$$Ca_{0.76}Mg_{0.24}(CO_3)_{0.80}[(OH)_2]_{0.20}$$

These results are entirely consistent with those of analysis of other samples of *Goniolithon* skeletal secretions and support the conclusion that the carbonate material is a mixture of magnesian calcite in solid solution and a significant amount of brucite [$Mg(OH)_2$].

The presence of brucite in the carbonate was confirmed by infrared-absorption studies (*9*). Magnesium hydroxide has a sharp absorption band at a wavelength close to 2.7 μ; this band is strongly developed in infrared spectra of *Goniolithon* (Fig. 2) but is absent



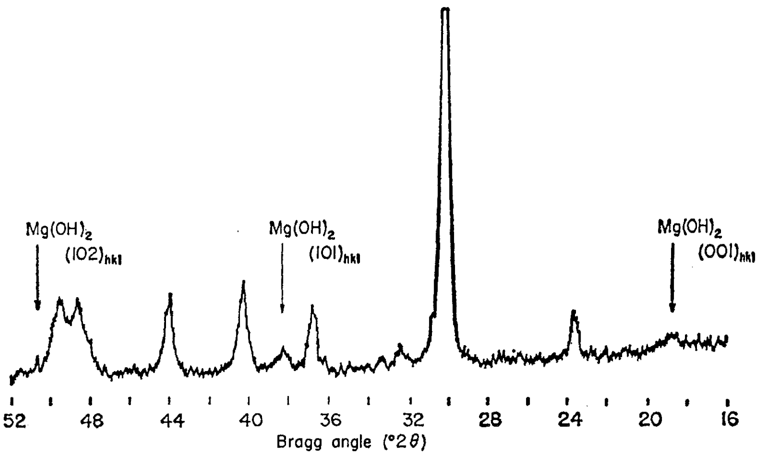


Fig. 2 (left). Infrared-absorption spectrum obtained from a sample of carbonate secreted by *Goniolithon*. The strong absorption band at 2.7 μ is characteristic of brucite, but was absent from other carbonate specimens. (Supplied by Shell Development Co.) Fig. 3 (right). X-ray powder-diffraction pattern (Cu-Kα) run on skeletal carbonate from *Goniolithon*. The strong maxima are those of a magnesian calcite containing approximately 22 mole percent $MgCO_3$. The low-intensity, diffuse peaks at 18.6°, 38.1°, and 50.8° 2θ correspond with the three strongest maxima of brucite. (Supplied by Marine Science Center, Lehigh University)

from the spectra of other calcites studied.

The presence of brucite in the *Goniolithon* secretion is further substantiated by the results of a broadline nuclear-magnetic-resonance (NMR) proton-spectrum study (*10*). The results of this study are necessarily somewhat generalized (the material examined was not a split of that subjected to chemical analysis) and their interpretation is less exact (broadline NMR cannot be used to establish the ionic form in which hydrogen ions are present in a compound). The study does show, however, that hydrogen is present in the material in approximately the amount postulated on the basis of the chemical analysis. The investigator wrote: "Line shape and second moment studies rule out the possibility that the hydrogen exists in the form of waters of hydration or in ions containing single hydrogen atoms which are randomly scattered throughout the lattice. These results are consistent with a structure in which two hydroxide ions replace a single carbonate ion in the lattice" (*11*). The investigator concludes that minor but significant amounts of magnesium hydroxite (brucite) are present in the *Goniolithon* secretion.

Despite failure in many laboratories to detect a second crystalline phase in calcite from *Goniolithon* by x-ray diffraction, a diffraction pattern was finally produced (Fig. 3) (*12*). The strong, well-defined peaks shown are those of a magnesian calcite. The position of the strongest peak, 30.1° 2θ (Bragg angle), corresponds to $d_{10 \cdot 4} = 2.968$ Å and reflects a solid-solution composition that is close to $Ca_{0.79}Mg_{0.21}CO_3$.

Three diffuse, low-intensity maxima, those at 18.6°, 38.1°, and 50.8° 2θ, cannot be attributed to the calcite lattice, however. Their low intensity and diffuse character suggest that the crystalline phase responsible for them is present in relatively small amounts and may be extremely fine-grained or very poorly crystalline, or both; their positions correspond closely to those of the three strongest maxima of brucite. It should be emphasized that these three low-intensity (brucite?) maxima do not appear in x-ray diffraction patterns of all *Goniolithon* samples examined. Moreover, although the primary aragonite maximum cannot be identified in the pattern shown, aragonite, which has been identified in some *Goniolithon* samples, has diffraction maxima close to 38.1° and 50.8° 2θ. Because of the consequent possibility of misinterpretation of adventitious aragonite peaks at 38.1° and 50.8° 2θ, the identification of brucite in the specimen must rest primarily upon the presence of the peak at 18.6° 2θ. These difficulties, together with the low peak intensity, incomplete pattern, and diffuse character of the peaks, make positive identification of brucite in the sample suspect on x-ray evidence alone, but when this is linked with the other independent evidence the x-ray pattern assumes considerable weight.

Assuming that the sample consists of but two crystalline phases, brucite and a magnesian calcite whose composition corresponds with that indicated by the cell size, one may calculate the weight percentage of brucite present. The resulting value, 5 percent, and the indications of very small grain size and poor crystallinity easily explain the difficulty and delay in identifying this second phase.

The skeletal material secreted by the red alga *Goniolithon* is not a homogeneous single phase, but rather a physical mixture of two crystalline phases. The dominant phase is an apparently homogeneous magnesian calcite solid solution, the composition of which is believed to be accurately reflected by the cell size. Excess magnesium determined by wet-chemical analysis is present as brucite, which is concentrated in the open pores of the skeletal structure. This conclusion is supported by the results of differential thermal analysis (*13*). The presence of brucite in the material explains the apparently anomalous cell size of the dominant phase observed by earlier investigators and the unexpected high solubility suggested by *p*H measurements. Although these results may clarify the interpretation of earlier studies, they pose new and interesting problems.

First, it would be of interest to determine whether other organisms secrete brucite and if so, whether the brucite is systematically distributed in the skeletal structure as it appears to be in *Goniolithon*. Second, it would be helpful in interpreting x-ray measurements of cell size to know at what composition, within the spectrum of organically secreted calcite, brucite first appears in significant amounts. Finally, it is apparent from the analyses described that the composition of the secretion varies widely; it would be in-widely; it would be interesting to determine the factors that control the composition of this phase—to determine for example whether composition is a characteristic of the secreting species or a reflection of the conditions (temperature, salinity, and so on) of deposition.

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24 May 1965

Brucite in the Calcareous Alga Goniolithon

Abstract. *Brucite, $Mg(OH)_2$, identified by differential thermal analysis, has been found in the high-magnesian calcite skeleton of* Goniolithon, *a marine calcareous alga. This mineral amounts to about 5 percent of the skeleton by weight, and can account for the discrepancy between total amount of magnesium determined by chemical analysis and the magnesium in metastable solid solution in calcite as determined by the size of the unit cell of calcite.*

Continued interest in the dolomite problem (*1*) has focused attention on the various ways in which magnesium and calcium are incorporated in the minerals of modern carbonate sediments. The frequent association of dolomite and organisms such as plants (*2*) has suggested the possibility of a causal relationship between the formation of dolomite and the metabolism of organisms. Sonnenfeld (*3*) concludes that it may be possible to produce dolomite on a calcite substrate with magnesium-rich sea water or ground water if a suitable bacterial, algal, or fungal symbiosis is present. A mechanism, however, to account for the low-temperature nucleation and growth of dolomite crystals has not yet been found, although much attention has been directed to organically derived calcites containing $MgCO_3$ in solid solution (up to 19 mole percent) (*4*). Such high-magnesian calcites are metastable under conditions of the earth's surface (*5*).

Two independent studies have provided evidence which suggests that not all of the magnesium of algal calcites is in solid solution. (i) The curve relating the size of the unit cell of calcite to the percentage of magnesium determined by chemical analysis is linear until the concentration of $MgCO_3$ is about 17 percent by weight (*4*). Above this value, higher magnesium concentrations have little apparent effect on the measurements of cell size, in contradiction to crystallochemical theory. (ii) Algal calcites heated at elevated temperatures (*5*) and CO_2 pressures where the calcite structure accommodates about 25 mole percent of $MgCO_3$ in stable solid solution were found by x-ray diffraction to contain about 21 moles, compared with about 19 moles before heating. Goldsmith *et al.* (*6*) attribute this result to finely divided or absorbed magnesium present in some form other than in the calcite structure. In this report, evidence is presented to show that brucite in small quantities is present in the calcareous skeleton of *Goniolithon.*

The differential thermal analysis apparatus (*7*) comprises two platinum capsules, 15 mm long and 2.5 mm in diameter, one containing about 80 mg of algal calcite crushed to pass a 325-mesh screen and the other containing approximately the same weight of Al_2O_3. The capsules are spot-welded to Platinel (*8*) thermocouple wires, and the sample containers themselves serve as the thermocouple in this arrangement. The differential thermocouple and temperature-measuring couple which can be incorporated in a stellite pressure vessel (outside diameter, 5 cm) or in a vacuum chamber permit differential thermal analyses in a range varying from very low water-vapor pressures to water-pressure values as high as 1400 atm. Signals from the differential couple are amplified with an Acromag solid-state, dc amplifier having a noise level and a drift of 1 and 5 microvolts, respectively, which permit high sensitivity and exceptional stability. Differential thermograms recorded with a heating rate of 10° per minute up to about 600°C contain a single endothermic peak (Fig. 1) at about 410°C at a water pressure of 1 atm. Samples of well-crystallized brucite from Wakefield, Quebec, yielded a peak temperature of 400°C at the same water pressure (*9*). The differential thermal analysis spectrum of brucite is readily distinguished from those of other possible magnesium minerals such as nesquehonite and hydromagnesite (*10*).

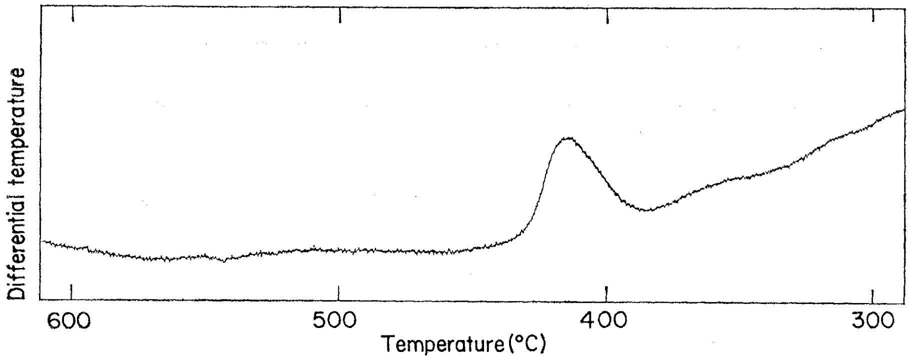

Fig. 1. Curve of differential thermal analysis of skeletal calcite of *Goniolithon* crushed to pass a 325-mesh screen; produced under vacuum conditions; temperature fom 300° to 600°C. Sample was untreated except for less than 1 minute of repeated percussion crushing and sieving.

An estimate of the abundance of brucite in two specimens of *Goniolithon* was made by means of a calibra-