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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14. Studies supported in part by grants from the Petroleum Research Fund of the American Chemical Society and from the Mineral Industries Experiment Station, Pennsylvania State University. N. D. Smith, Jr. (Shell Development Co.), kindly arranged the release of the Shell data. Contribution number 64–81 from the Mineral Industries Experiment Station, Pennsylvania State University.

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*.

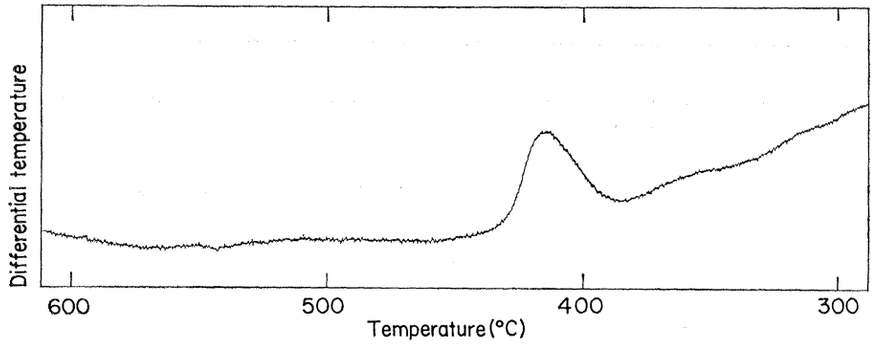

Fig. 1. Curve of differential thermal analysis of skeletal calcite of *Goniolithon* crushed to pass a 325-mesh screen; produced under vacuum conditions; temperature fom 300° to 600°C. Sample was untreated except for less than 1 minute of repeated percussion crushing and sieving.

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*).

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

tion curve relating peak area to the percentage of brucite in the sample. This curve was constructed from data obtained by the differential thermal analysis of artificial standards comprising mixtures of pure brucite and Iceland spar calcite. The results indicate between 4 and 5 percent of $Mg(OH)_2$ by weight. It appears to be the first recorded occurrence of this mineral in recent carbonate sediments. The distribution of brucite in natural, organic, high-magnesian calcites is currently under investigation with the use of the electron probe microanalyzer (*11*).

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References and Notes

1. Essentially, the dolomite problem involves the discrepancy between the existence of vast quantities of dolomite in ancient sedimentary carbonate rocks and the absence of any comparable sediments forming at the present time. This mineral has not been synthesized at temperatures and pressures of the marine environment, and the mechanism of dolomitization of pre-existing calcite sediments is very poorly understood. Ingerson maintains that the "problem of the origin of dolomite is one of the most fascinating as well as one of the most important in all of geochemistry or in sedimentary petrology." Reviews have been provided by P. Sonnenfeld, *Bull. Can. Petrol. Geol.* **12**, 101 (1964); E. Ingerson, *Geochim. Cosmochim. Acta* **26**, 815 (1962); and R. W. Fairbridge, *Soc. Econ. Paleontologists Mineralogists Spec. Publ. 5* (1957), p. 125.
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12. The investigations described in this report and in the report by R. F. Schmalz (*Science*, this issue, p. 993) were carried out independently. Our experiments were completed in July 1964, and the manuscript was prepared in August 1964. The results summarized by Schmalz were obtained by experiments conducted over a period of several years at the several laboratories mentioned in the text, and they were prepared for publication during the spring of 1965. Both manuscripts were submitted for publication at the same time.

24 May 1965

Inhibition of L1210 Tumor Growth by Thymus DNA

Abstract. *Growth of L1210 leukemia cells which had been previously incubated with thymus DNA was inhibited. Leukemia-cell DNA did not affect tumor growth under similar conditions. Pretreatment of the thymus DNA with deoxyribonuclease suppressed the DNA-induced inhibition. Both ribonuclease-treated DNA and untreated DNA inhibited tumor growth.*

Penetration of mammalian cells by DNA has been observed in a number of laboratories (*1*). The genetic transformation of mammalian cells by mammalian DNA has been reported to occur in tissue culture (*2*, *3*). This report describes work undertaken to determine any effects of DNA on the growth of tumor cells in vivo.

Thymus DNA was isolated by the method of Szybalska and Szybalski (*2*) from male $B6D2F_1$ mice weighing 20 to 25 g; DNA was isolated in the same way from L1210 leukemia cells which had been maintained in the ascitic form. Previously established methods were followed whenever DNA was treated with deoxyribonuclease (*4*) and ribonuclease (*2*). Leukemia cells were washed at 3°C with a phosphate-buffered saline (*2*) until free of red blood cells. Cell viability was determined by eosin staining. The cells were suspended at a concentration of 2 to 8 $\times$ 10^6 viable cells per milliliter in 4 ml of phosphate-buffered saline containing $5.5 \times 10^{-3}M$ glucose and $2 \times 10^{-4}M$ spermine. The cell suspension was then shaken very slowly for 1 hour at 37°C. When DNA was added to the incubation medium, it was present at a final concentration of 2 to 8 $\times$ 10^2 μg/ml, as determined spectrophotometrically (*5*). The cells were centrifuged after incubation and were then suspended in fresh phosphate-buffered saline. About 250,000 viable leukemia cells in 0.1 ml were injected subcutaneously into the lower left ventral regions of the $B6D2F_1$ mice.

Tumors were scored as positive when they were first detected by palpation as tiny nodules. They were often verified by a second observer and were rechecked each day. The following conventions were adopted in order to measure the degree of inhibition of tumor growth by DNA. The "reciprocal of the latent period" (RLP) was obtained by dividing 100 by the day on which the tumor first appeared (*6*). Similarly, the "reciprocal of the survival period" (RSP) was obtained by dividing 100 by the day on which the animal died. Thus, an animal that did not develop a tumor had an RLP of zero and an RSP of zero.

In the experiment illustrated in Fig. 1, 59 mice were injected with leukemia cells that had previously been incubated with thymus DNA; 50 control mice were injected with cells incubated without DNA. Mice injected with DNA-treated cells developed tumors later, and survived longer, than mice injected with saline-treated cells. Approximately 19 percent of the mice survived free of tumor growth after injections of the DNA-treated cells, whereas none of the control mice survived after injections of the saline-treated cells.

Other data (Table 1) demonstrate that thymus DNA significantly depressed both the mean RLP and the mean RSP and thus delayed both the growth of tumor and the onset of death of the animals. Pretreatment of the thymus DNA with deoxyribonuclease eliminated the DNA-induced effects. We have subsequently found that ribonuclease treatment of thymus DNA does not interfere with the tumor inhibitory action of the DNA. As shown in Table 1, leukemia DNA did not affect the growth of the tumor cells.

These results indicate that native DNA per se was the biologically active substance in the thymus DNA preparations, since treatment of these preparations with deoxyribonuclease

Table 1. The effect of DNA on L1210 tumor growth. The values reported are means ± the standard error of the mean. Percentage changes from the control values are given in parentheses.

Number of mice injected	Treatment of L1210 cells	Mean reciprocal of the latent period	Mean reciprocal of the survival period
40	Buffer-saline	14.8 ± 0.4	7.3 ± 0.2
40	Thymus DNA	9.7 ± 0.4 (− 34)*	5.6 ± 0.3 (− 23)*
20	DNase†	14.8 ± 0.9 (0)	6.9 ± 0.4 (− 6)
20	DNase-treated thymus DNA	15.0 ± 0.5 (+ 1)	7.0 ± 0.4 (− 4)
30	Leukemia DNA	14.7 ± 0.5 (− 1)	7.3 ± 0.2 (0)

* $p < 0.001$. † Deoxyribonuclease.

27 AUGUST 1965