

## Monohydrocalcite in a guinea pig bladder stone, a novel occurrence

H. CATHERINE W. SKINNER, GEORGE W. OSBALDISTON AND ANDREW N. WILNER

Yale University School of Medicine  
Department of Surgery (Orthopaedics) and Section of Comparative Medicine  
New Haven, Connecticut 06510

### Abstract

The rare mineral, monohydrocalcite,  $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ , has been identified in a guinea pig bladder stone. Three other natural occurrences have been described, the most recent from lacustrine deposits in the southeast of South Australia. Our unique pathological specimen, a concretion of alternating white and tan bands, one centimeter in diameter, contains 7 percent  $\text{MgO}$ , 8 percent  $\text{PO}_4$ , less than 1 percent  $\text{FeO}$  and no  $\text{Na}_2\text{O}$  or  $\text{K}_2\text{O}$ . Optical characteristics ( $\epsilon = 1.548$ ,  $\omega = 1.594$ ), X-ray diffraction data ( $P3$ ,  $a = 10.602$ ,  $c = 7.548\text{A}$ ) and IR spectral data compare well with those of previous descriptions. The three most intense diffraction maxima are  $d_{100} = 4.34$ ,  $d_{411} = 1.935$ , and  $d_{112} = 3.08\text{A}$ .

The occurrences of monohydrocalcite in nature are probably the result of biological activity. Preservation of this ephemeral phase may be enhanced in confined biologically-maintained environments. The monohydrocalcite of the guinea pig stone becomes magnesian calcite on exposure to the atmosphere. The magnesian calcite of some invertebrate calcified tissues may have a monohydrocalcite precursor. Similar mechanisms of biologic control of the depositional environment may also apply to the South Australian lacustrine occurrence of monohydrocalcite/magnesian calcite and protodolomite/dolomite described from adjacent lagoons. We suggest that formation of the mineral may be determined by distinct biological species with unique specificities and reactions in the aqueous environment.

### Introduction

The rare mineral monohydrocalcite,  $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ , first described as a compound by Kraus and Schrieber (1930), was one of several calcium carbonate hydrates found during investigations on reactions of Portland cement. Documented natural occurrences include the type material in Lake Issyk-Kul, Kirgiziya (Sapozhaikov and Tsevetkow, 1959), in speleothems in a West German cave (Fishbeck and Muller, 1971), in otoliths of the tiger shark, *Galeocerdo*, (Carlstrom, 1963), and monohydrocalcite-organic material aggregates covered by a mucilaginous layer of blue green algae from Lake Fellmongery, in the southeast of South Australia (Taylor, 1975). We report on a guinea pig bladder stone which is composed of monohydrocalcite. In chemical composition this pathological deposit is similar to the aggregates described by Taylor (1975), but more closely resembles monohydrocalcite deposits identified in air-conditioning equipment by Marschner (1969). A second guinea pig bladder calculus was analyzed and showed

only magnesian calcite. Bladder calculi from guinea pigs have not previously been reported.

### Physical and chemical properties

The guinea pig stone is a whitish-tan concretion, 1 cm in diameter. The surface is composed of an extremely fine radiating mixture of white crystallites arranged in clusters, producing a faintly botryoidal outline. On sectioning, alternating white and tan shells of remarkably uniform thickness were observed (Fig. 1). The stone parted along one of the concentric shells, most likely a zone of differential compaction. The inner surface also appeared botryoidal with fine crystallites.

Samples from the center, several interior sites and the surface of the stone gave identical X-ray diffraction patterns, characteristic of monohydrocalcite. No other phase was detected during X-ray or optical examination. The crystals are optically negative with  $\epsilon = 1.548$ ,  $\omega = 1.594$ . These values, obtained by observing the Becke line using blue-filtered white

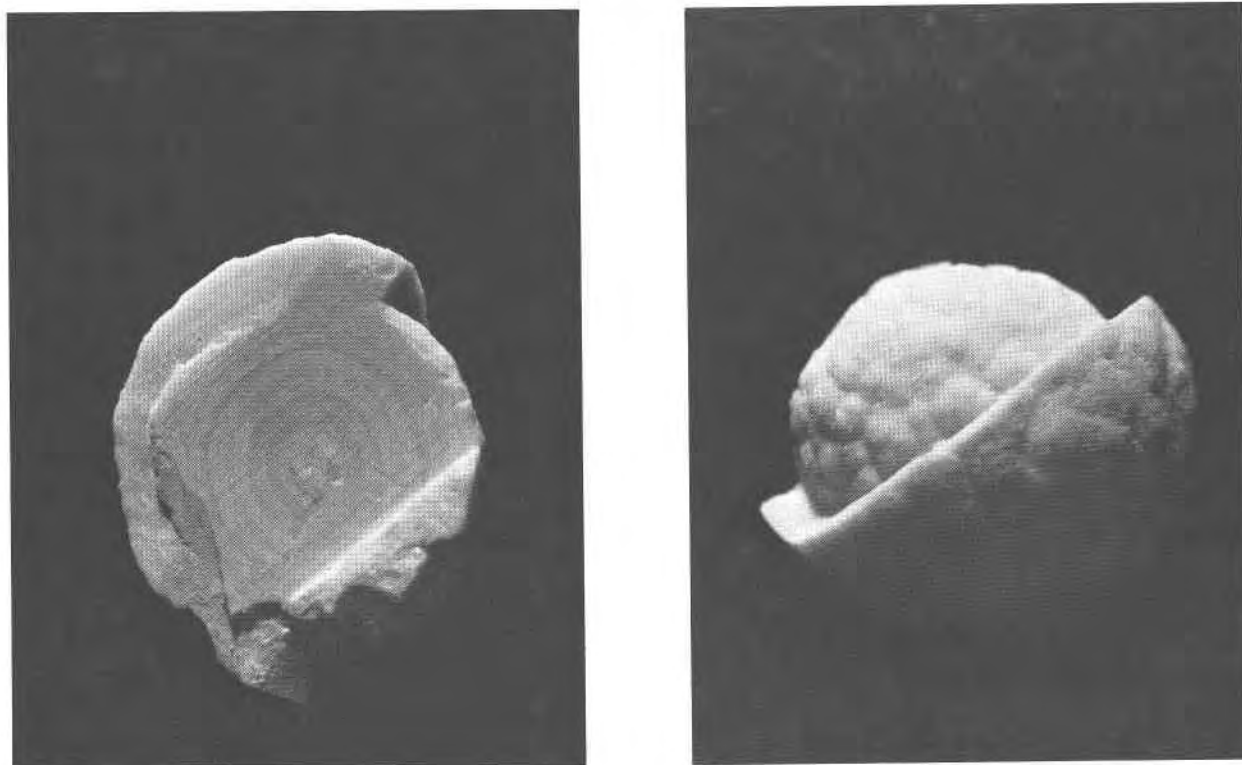


FIG. 1. Monohydrocalcite guinea pig stone, approximately 300 mg; concentric alternating tan and white banding can be seen in section.

light, are somewhat higher than those reported by Lippman (1959) on synthetic material (1.542, 1.590).

A portion of the stone was dissolved overnight in 3N HCl over a waterbath at 56°C. Calcium was determined using a modified EDTA titration (Bett and Fraser, 1959), which minimizes interference from magnesium and phosphate. Magnesium (Spare, 1962), iron (Goodwin *et al.*, 1966), and phosphate (Crouch and Malmstadt, 1967) assays were performed, utilizing standard colorimetric methods. Carbonate was determined manometrically as CO<sub>2</sub> (Van Slyke and Folch, 1940). Table 1 shows that the MgO content of the calculus is several times that of the geological occurrence described by Taylor (1975) and is similar to monohydrocalcite identified from air-conditioning equipment by Marschner (1969). She identified monohydrocalcite and nesquehonite (MgCO<sub>3</sub>·3H<sub>2</sub>O) in one sample, while monohydrocalcite was associated with anhydrous calcium carbonates in other samples. She related the formation of distinct magnesium phases to high levels of magnesium in the waters supplied to the air-conditioning units. The guinea pig calculus is richer in Mg and Fe [compared to the monohydrocalcite of Lake Fellmongery] and contains no sodium. The high

phosphate content (8.5%) which distinguishes the stone sample might be anticipated in light of the biological origin. Unfortunately no chemical analytical data are available on the tiger shark otolith. The 10 percent insoluble fraction reported in air-conditioner samples by Marschner (1969) might be organic debris. In any case, the air-conditioner occurrence could result from bacterial activity, either directly or indirectly. The relative increase in the amount precipitated with time certainly does not preclude biological contribution or association.

X-ray data on the calculus (Table 2) are similar to those for otolith material (Carlstrom, 1963). Both gave fewer diffraction maxima than the Lake Fellmongery sample. We used a Guinier powder diffraction camera and CuK $\alpha$  radiation. The film (Fig. 2) was scanned using a computer-controlled optical scanner system, programmed to record position and breadth of the diffraction maxima. The maxima observed for the guinea pig monohydrocalcite (Fig. 2A) were 0.5 mm in breadth compared to 0.18 mm for standard NaCl examined simultaneously for the same  $2\theta$  and intensity range. Unit-cell parameters were calculated using the trigonal space group suggested by Kohatsu and McCauley (1973).

TABLE 1. Chemical analyses of monohydrocalcite.

	Guinea Pig Stone	Taylor (1975)	Marschner (1969)
CaO	37.74	42.5	25.34*
MgO	7.29	2.49	10.96
FeO	0.80	0.13	0.05
SrO	---	0.40	---
Na <sub>2</sub> O	0.00	0.69	1.22
K <sub>2</sub> O	0.00	0.07	---
CO <sub>2</sub>	34.50	33.60	31.68
PO <sub>4</sub>	8.46	---	tr
SO <sub>4</sub>	---	---	tr
H <sub>2</sub> O(100)		2.1	21.9
H <sub>2</sub> O+		15.9	
Insoluble	5.0	5.0	10.0

\*recalculated as oxides from original data, Science 165, 119-1121 (1969), nesquehonite also present in this sample.

The stone material dehydrated on exposure to air to magnesian calcite, which gave broader diffraction maxima (0.8–1.0mm). Both carbonate phases were observed on X-ray examination of the stone after drying at 100°C under vacuum (1.4kg/cm<sup>2</sup>) for 24 hours. The X-ray pattern of a second guinea pig stone gave only magnesian calcite diffraction maxima and is shown in Figure 2C.

IR analysis showed that the carbonate ion of the stone material exhibited lower site symmetry than the carbonate ion in optical calcite. The spectrum of our material (Fig. 3) is somewhat diffuse but virtually identical to the spectra of synthetic monohydrocalcite produced by McCauley (personal communication, 1976) using silica gel techniques (McCauley and Roy, 1974). Active modes include  $\gamma_1$ ,  $\gamma_3$ , and  $\gamma_4$ . The latter two show double peaks indicating removal of the degeneracy for these modes. The broad area of spectral absorbance at 3300–3600 and the sharp peak at 2930cm<sup>-1</sup> are ascribed to OH stretching frequency bands, while the 1630–1660 absorbance is probably due to H<sub>2</sub>O bending motions. The frequencies and assignments are compatible with the spectral analysis on nesquehonite and hydro-magnesite presented by White (1971).

### Discussion

We have presented a brief description of an unique occurrence of a rare mineral species, monohydrocalcite. This biological occurrence may have implications for the mode of formation of magnesian

calcite in the exoskeleton of many invertebrates and in lagoonal sediments such as those found in the southeast of South Australia.

The guinea pig is strictly herbivorous and produces a highly alkaline urine. Under normal physiologic conditions the bladder may be incompletely voided with retention of small volumes of urine. The nucleation and formation of a bladder stone could result from supersaturation during periods of low fluid level. Cellular debris could act as nucleating material. Alternatively, it is possible that stones form as a sequel to bacterial infection. Bacterial extra-cellular products might alter the chemical characteristics of the urine, such as a decreased pH, as well as provide debris to act as a nidus for stone formation. Little high-molecular weight organic material is associated with either calculus; therefore, it is unlikely that infection is associated with calculus formation in these cases. Because we know of no reports of calculus disease in this common laboratory animal, it would seem that guinea pigs are not prone to bladder stone formation. However, no stone would form or grow if the urine were not saturated with respect to the phase. The entire stone is a single mineral species, which implies uniformity of depositional environment and maintenance of saturated conditions over a reasonable time period.

Both bladder and lagoon environments are characterized by fluctuating fluid levels, salinity variations, and microflora (and organic molecule) proliferation. They are both distinctive for their class of deposition, exhibiting abnormally high values of Mg/Ca and pH. The average temperature in the South Australian la-

TABLE 2. X-ray powder diffraction data for guinea pig monohydrocalcite.

hkl*	d <sub>(obs)</sub>	I <sub>(rel)</sub>
110	5.30	35
111	4.34	100
112	3.08	95
301	2.838	43
221	2.501	10
302	2.380	27
222	2.170	54
410	2.004	11
411	1.935	98
223	1.824	3
114	1.779	6
333	1.440	5

\*Indexed assuming Lippman's pseudo-cell to enable direct comparison with Table 3, Taylor (1975) p. 694.

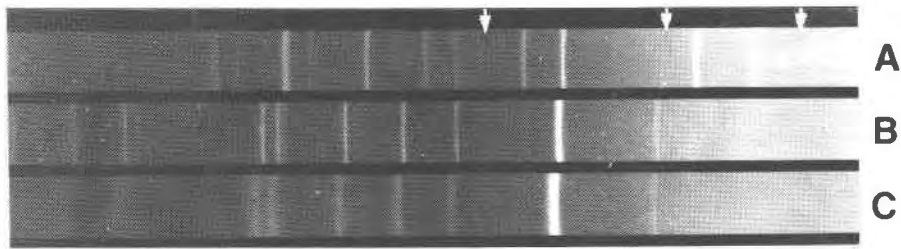


FIG. 2. Portion of X-ray powder diffraction patterns; Guinier (multiple sample) camera,  $\text{CuK}\alpha$  radiation. (A) Guinea pig stone #1 monohydrocalcite; arrows indicate positions of three strongest maxima of nesquehonite. (B) Optical calcite. (C) Guinea pig stone #2—magnesian calcite; note broadness and shift of all maxima relative to calcite pattern.

goons during precipitation is probably not much different from that in the animal, although the fluctuations in lagoons would be greater. No details on temperature of formation for Lake Fellmongery material have been published. In the guinea pig stone all the carbon has an organic origin. There will be some organic compounds, but the major portion, the inorganic carbonate ( $\text{CO}_3$ ), must result from the metabolism of the bladder cells, blood, or other cells and/or bacteria. Therefore biological activity is totally and directly responsible for the precipitation of monohydrocalcite in the case of the guinea pig stone. The Lake Fellmongery sample has close association with algae, which may enhance precipitation of monohydrocalcite by increasing the pH and  $\text{CO}_2$  in the local environment. However, the material shows a positive  $^{13}\text{C}$ , implying carbon contribution from another source, most probably atmospheric  $\text{CO}_2$ . We do not have sufficient sample to make accurate isotope determinations, but we anticipate that the guinea pig stone would show large negative  $^{13}\text{C}$ , similar to values for algae from Lake Fellmongery or biogenic carbonates.

Taylor (1975) identified isolated pellets of mono-

hydrocalcite from the upper 25–30 cm of the sediment pile in Lake Fellmongery, Australia. However, the pelletal sands of the beach rock contained high-magnesium calcite (33 mol %  $\text{MgCO}_3$ ). The monohydrocalcite from this locality was stable in air, but transformed to magnesian calcite in the presence of water (Taylor, 1975). The transition was retarded by magnesium. The monohydrocalcite of the urinary stone transformed in the atmosphere in a few months to magnesian calcite with approximately 10 mol percent  $\text{MgCO}_3$ . We anticipated this reaction when, after heating a portion of the stone under vacuum for 24 hours, both monohydrocalcite and magnesian calcite were observed on X-ray analysis. A second guinea pig stone, examined after being exposed to the atmosphere for an unknown length of time, consisted of magnesian calcite only (Fig. 2C). Monohydrocalcite appears to be the precursor to magnesian calcite in the stones. Perhaps it is also precursor of some of the material in the lacustrine deposits of the southeast of South Australia. We speculate that monohydrocalcite may be an earlier mineral phase in the calcification of invertebrates which show magnesian calcite exoskeletons. Identification of the phase mono-

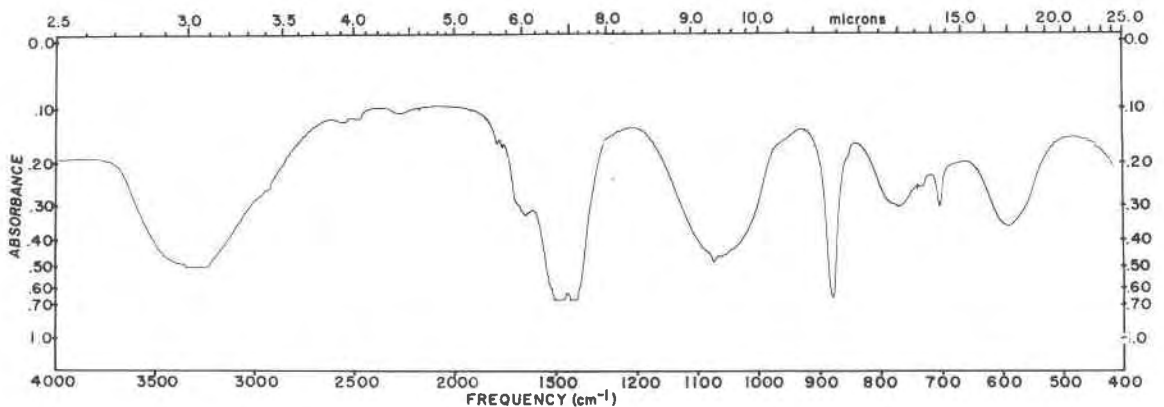


FIG. 3. Infrared spectrum of guinea pig stone #1 identified as monohydrocalcite. Perkin Elmer 337, 2 mg sample/300 mg KBr.

hydrocalcite probably depends on its preservation: for the urinary stone, it is enclosed within the bladder; for lacustrine/cave environments, it is covered by the mucia of green algae; for calcifying invertebrates, it could be deposition within the epithelial mantle.

The question whether a metabolizing cell or a living species is required for the precipitation of monohydrocalcite is a matter of semantics. Monohydrocalcite is an extracellular product in both the guinea pig calculus and in the lake deposit. The role of specific organisms and precise biochemical reactions must be delineated before we understand the occurrence of the mineral.

Several intriguing areas for future investigations in basic mineralization problems are triggered by this occurrence of the mineral species and its mechanism of precipitation. (1) Is it possible that monohydrocalcite is a precursor to the formation of magnesian calcites in normal calcification processes of some invertebrates? (2) From the Lake Fellmongery occurrence it appears that magnesian monohydrocalcite exists in close proximity to magnesian calcite pelletal material: is it a precursor in this geologic locality? (3) Could the formation of sedimentary dolomite originally described from the same area (Alderman and Skinner, 1959; Skinner, 1963) be analogous? That is, are there some bacterial/algal species that create and maintain local and unique physicochemical conditions in the lagoonal waters which are conducive to the formation of 'protodolomite' and eventually well-ordered dolomite?

#### Acknowledgments

We are grateful to Ms. Deborah Gilbert for the chemical analyses and Robert Biltz for the IR and carbonate analyses of the guinea pig calculus.

#### References

Alderman, A. R. and H. C. W. Skinner (1959) Dolomite sedimentation in the south east of South Australia. *Am. J. Sci.*, 255, 561-567.

- Bett, I. M. and G. P. Fraser (1959) A rapid macro-method for determining serum calcium. *Clin. Chem. Acta.*, 4, 346-356.
- Carlstrom, D. (1963) A crystallographic study of vertebrate otoliths. *Biol. Bull.*, 125, 441-462.
- Crough, S. R. and H. V. Malmstadt (1967) A mechanistic investigation of molybdenum blue method for determination of phosphate. *Anal. Chem.*, 39, 1084-1089.
- Fishbeck, R. and G. Muller (1971) Monohydrocalcite, hydro-magnesian nesquehonite, dolomite, aragonite and calcite in speleothems of the Frankische Schweiz, W. Germany. *Contrib. Mineral. Petrol.*, 33, 87-92.
- Goodwin, J. F., B. Murphy and M. Guillemette (1966) Direct measurement of serum iron and binding capacity. *Clin. Chem.*, 12, 47-57.
- Kohatsu, I. and J. W. McCauley (1973) Evidence of carbonate disorder in  $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ . *Am. Mineral.*, 58, 1102 (Abstr.).
- Kraus, F. and W. Schrieber (1930) Die Hydrates des Calciumcarbonats. *Z. anorg. allgem. Chem.*, 188, 259-273.
- Lippmann, F. (1959) Darstellung und Kristallographische Daten von  $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ . *Naturwissenschaften*, 46, 533.
- Marschner, H. (1969) Hydrocalcite ( $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ ) and nesquehonite ( $\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$ ) in carbonate scales. *Science*, 165, 1119-1121.
- McCauley, J. W. and R. Roy (1974) Controlled nucleation and crystal growth of various  $\text{CaCO}_3$  phases by silica gel technique. *Am. Mineral.*, 59, 947-963.
- Sapozhaikov, D. and A. Tsevetkov (1959) Precipitation of hydrous calcium carbonate on the bottom of Lake Issyk-Kul. *Dokl. Akad. Nauk. SSSR*, 124, 131-133.
- Skinner, H. C. W. (1963) Precipitation of calcium dolomites and magnesian calcites in the south east of South Australia. *Am. J. Sci.*, 261, 449-472.
- Spare, P. D. (1962) A study of titan yellow lake methods for the estimation of serum magnesium. *Am. J. Clin. Pathol.*, 37, 232-236.
- Taylor, G. F. (1975) The occurrence of monohydrocalcite in two small lakes in the southeast of South Australia. *Am. Mineral.*, 60, 690-697.
- Van Slyke, D. D. and J. Folch (1940) Manometric carbon determination. *J. Biol. Chem.*, 136, 509.
- White, W. B. (1971) Infra-red characterization of water and hydroxyl ion in the basic magnesium carbonate minerals. *Am. Mineral.*, 56, 46-53.

Manuscript received, February 26, 1976; accepted for publication, October 26, 1976.