- 21. E. W. Sexton, J. Mar. Biol. Assoc. U.K. 13, 340
- and J. S. Huxley, *ibid.* **12**, 506 (1919–1922). 22.
- E. W. Sexton, *ibid.* 18, 355 (1932-1933).
 H. P. Wolvekamp and T. H. Waterman, in *The Physiology of Crustacea*, T. H. Waterman, Ed. (Academic Press, New York, 1960), vol. 1, pp. 5-100
- 25. The taxonomic description of the amphipods caught has not been completed but will be a part of the studies in progress by other participants on the expedition.
- 26. I am greatly indebted to R. V. Boxtel, B. Minor, and R. Wilson for untiring assistance and helpful discussions; to Dr. K. L. Smith, Jr., for his help as chief scientist, for helpful discussions aboard the scientist, for helpful discussions aboard the R.V. *Thomas Washington*, and for his criticisms of the manuscript; and to M. E. Horn, the ship's first officer, for the speedy recovery of the free vehicles. Supported under National Science Foundation OCE 76-12017 and OCE 76-80874 and under Energy Research and Development Administration contract EY-76-03-0034-241-142.

26 October 1977; revised 15 February 1978

Mineralization Kinetics: A Constant Composition Approach

Abstract. A new method is described for studying, reproducibly, the kinetics of crystallization of minerals under conditions of constant solution composition even at very low supersaturations. For calcium phosphates the method provides direct evidence for octacalcium phosphate as the precursor to hydroxyapatite precipitation at physiological pH.

The precipitation of sparingly soluble salts from their supersaturated solutions finds application in a wide variety of scientific fields. The carbonates of calcium and magnesium are of great interest in limnology (1), oceanography (2), and sedimentology (3). Important manifestations of nucleation and crystallization of calcium phosphates are the transformations in soil following phosphate fertilization, removal of phosphate from wastewater by the addition of lime, formation of teeth and bones, and pathological stone formation in the urinary tract. Precipitation of the sulfates of calcium and barium creates serious problems in desalination and in oil-field scale formation. In this report we describe a new and highly reproducible method for studying rates of mineralization, even at very low supersaturation, with a precision hitherto unobtainable.

Although numerous spontaneous precipitation studies of minerals have been made, they suffer from the disadvantage that the size and size distribution of the solid particles change during the course of the reaction. Also, the usual assumption of homogeneous nucleation (4) is open to question, since it is doubtful if any medium is sufficiently free from foreign nucleating sites to preclude heterogeneous nucleation (5). The problems associated with the irreproducibility of the results of such studies were overcome with the development of seeded growth techniques (6, 7), which enabled the effects of factors such as temperature, supersaturation, and ionic strength to be studied quantitatively (8-10).

The precipitation of calcium phosphate phases is of particular interest. Under solution conditions normally encountered biologically, hydroxyapatite $[Ca_5(PO_4)_3OH, hereafter HAP]$ is the thermodynamically stable phase. How-2 JUNE 1978

ever, most calcium phosphate solutions in crystal growth experiments are initially supersaturated with respect to four additional phases; in order of increasing solubility at physiological pH these are tricalcium phosphate [Ca₃(PO₄)₂, hereafter TCP], octacalcium phosphate $[Ca_4H(PO_4)_3 \cdot 2\frac{1}{2}H_2O, hereafter OCP],$ anhydrous dicalcium phosphate (CaH-PO₄, hereafter DCPA), and dicalcium phosphate dihydrate (CaHPO₄ · 2H₂O, hereafter DCPD). Hydroxyapatite does not normally precipitate directly from solution, although it has been shown to grow on HAP seed crystals at very low supersaturations (10). From experiments involving the mineralization and x-ray analysis of epiphyseal rat cartilage, Posner (11) identified an x-ray amorphous material of approximately TCP composition. This phase has also been proposed as a precursor to HAP in the formation of teeth and bone (12). In addition, both OCP (13) and DCPD (14) have been proposed as possible precursor phases.

The development of a *p*H-stat method

(15) enabled studies to be made of calcium phosphate crystal growth on wellcharacterized synthetic and natural seed material under conditions of constant hydrogen ion activity. These methods, however, still suffered from the disadvantage that the calcium and phosphate ionic concentrations varied appreciably during the reactions. At each stage, therefore, the supersaturated solutions were metastable with respect to different calcium phosphate phases, which could form and subsequently dissolve as the concentrations in the supersaturated solutions decreased. Since the concentration changes became very small as the reaction proceeded, a relative analytical error of only a few percent in the total calcium or total phosphate concentration could preclude differentiation between the possible crystalline phases.

To overcome the problems associated with the changing solution composition during precipitation, a new method is reported here in which the chemical potentials of the solution species are maintained constant during the reaction. After the addition of well-characterized seed material to a stirred, stable supersaturated solution of calcium phosphate at the required pH, the concentrations of lattice ions were maintained constant by the simultaneous addition of reagent solutions containing calcium and phosphate ions, controlled by a glass electrode probe. The compositions of the reagent solutions were calculated from the results of exploratory measurements. The method is illustrated by three experiments at 37° C and the physiological *p*H of 7.40, in which the solutions were supersaturated with respect to all phases other than DCPD and DCPA. These are summarized in Table 1. In experiment 1377, the solution Ca/P molar ratio, R, initially 1.39, decreased by 4 percent

Table 1. Constant composition seeded growth of HAP crystals at pH 7.40, 37°C. Symbols:

T_{Ca} , total calcium concentration; R, molar Ca/P ratio in solution at each time.								
Experiment 1377*			Experiment 1777 [†]			Experiment 2577‡		
Time (min)	Т _{са} (mM)	R	Time (min)	T _{Ca} (mM)	R	Time (min)	Т _{са} (mM)	R
0	0.800	1.39	0	0.800	1.450	0	1.200	1.33
5	0.788	1.39	20	0.797	1.457	1	1.197	1.32
13	0.770	1.38	34	0.796	1.457	3	1.208	1.32
30	0.755	1.35	48	0.797	1.450	5	1.214	1.32
40	0.752	1.35	60	0.794	1.457	7	1.234	1.31
			75	0.800	1.462	10	1.248	1.30
			90	0.797	1.457			
Mean					1.456			1.32
Standa	ard deviatio	n			0.004			0.01

*Initial solutions: 150 ml of 0.800 mM CaCl₂, 0.575 mM KH₂PO₄, 0.854 mM KOH, and 5.0 mg of HAP seed. Titrant solutions: 10.00 mM CaCl₂ and 7.19 mM KH₂PO₄ with 12.81 mM KOH. 1.000 mM CaCl₂, 0.552 mM KH₂PO₄, 0.457 mM KOH, 8.40 mM KCl, and 5.0 mg of HAP seed. Titrant solutions: 10.00 mM CaCl₂ and 6.90 mM with KH₂PO₄ with 11.91 mM KOH. 1.200 mM CaCl₂, 0.900 mM KH₂PO₄, 0.715 mM KOH, 24.27 mM KCl, and 5.0 mg of HAP seed. Titrant solutions: 26.67 mM CaCl₂ and 20.00 mM KH₂PO₄ with 34.10 mM KOH.

during the precipitation, indicating formation of a solid phase with an average Ca/P ratio greater than 1.39. Readjustment of the reagent solution concentrations to a Ca/P ratio of 1.45 in a subsequent experiment (experiment 1777) resulted in crystal growth in which all solution parameters remained constant to within the experimental error in the analytical determinations, ± 0.3 percent. In addition, it was found that the rate of reaction was directly proportional to the inoculating seed concentration, confirming that growth of the crystals occurred without interference from secondary nucleation. To have obtained a kinetic precipitation stoichiometry to this precision by techniques previously used would have required concentration analyses to at least ± 0.03 percent. It is significant that in previous crystal growth experiments at physiological pH, using both synthetic and natural enamel and dentin seed, the molar Ca/P ratio actually precipitating on the seed material was normally in the range of 1.45 ± 0.05 (16). Although it has been assumed (11)that this ratio is close to that of β -TCP, and thus TCP has been invoked as the precursor, it is now clear that the ratio is significantly lower than the 1.50 required for TCP. Hydrolysis would be expected only to increase the molar Ca/P ratio, which further rules out TCP as the precursor phase.

In preliminary experiments it was observed that as the rate of precipitation exceeded a critical value of about 3 \times 10⁻³ g of precipitate per liter per minute, the apparent Ca/P ratio of the solid phase became less than 1.45. To examine this phenomenon further, in experiment 2577 the initial supersaturation was increased, but to a value still below that for DCPD. The results shown in Table 1 clearly indicate a Ca/P ratio corresponding to OCP in the early stages of the reaction, and this phase was confirmed by x-ray analysis. At longer times (15 to 20 minutes) hydrolysis to a more basic phase took place with a Ca/P ratio of approximately 1.45, the value observed in so many previous calcium phosphate precipitation studies. It is highly significant that, using the constant composition method, more than twice the original seed material could be grown as OCP in the early stages of the reaction. The results support a model for calcium phosphate precipitation in which OCP, formed as a precursor phase, hydrolyzes either partially or completely to HAP, depending on the relative rates of the hydrolysis and precipitation reactions. The observed Ca/P values that are normally greater than 1.33 can be accounted for by assuming that one in every three molecules of OCP transforms into HAP, leading to a Ca/P ratio of 1.44, within 0.01 of the observed value in experiment 1777. The hydrolysis probably takes place one layer at a time, as Brown et al. (13) proposed on the basis of a unit-cell x-ray analysis of the phases.

To our knowledge, the results presented here provide the first direct experimental evidence for OCP as the kinetically favored precursor in calcium phosphate precipitation at physiological pHfrom solutions supersaturated with respect to OCP, TCP, and HAP but not supersaturated with respect to DCPD or DCPA. The new method will enable studies to be made at very low supersaturations, and the influence of factors such as temperature, ionic strength, the nature and concentration of the seed, trace inhibitors, and fluid dynamics can be investigated unambiguously since these parameters do not change the supersaturation significantly. In our laboratory the method is being successfully used to study not only calcium phosphate mineralization (17) but also carbonates (18), sulfates (19), and oxalates (20) of calcium, as well as other important reactants such as magnesium hydroxide (21).

M. B. TOMSON*, G. H. NANCOLLAS Chemistry Department, State

University of New York, Buffalo 14214

References and Notes

- 1. W. Stumm and J. J. Morgan, Aquatic Chemistry (Wiley-Interscience, New York, 1970), pp. 161-195
- 2. P. E. Cloud, in Chemical Oceanography, J. P.
- P. E. Cloud, In *Chemical Oceanography*, J. F. Riley and G. Skirrow, Eds. (Academic Press, New York, 1965), vol. 2, pp. 127–158.
 R. A. Berner, *Principles of Chemical Sedi-mentology* (McGraw-Hill, New York, 1971).
 A. G. Walton, *Science* 148, 601 (1965).
- A. G. Walton, Science 148, 601 (1965).
 _____, The Formation and Properties of Precipitates (Interscience, New York, 1967).
 C. W. Davies and A. L. Jones, Faraday Discuss. Chem. Soc. 5, 1903 (1949).
 G. H. Nancollas and N. Purdie, Q. Rev. Chem. Science 141 (1064).
- G. H. 1 (1964).
 G. H. Nancollas and M. M. Reddy, Soc. Pet. Eng. J. 1974, 117 (1974). 8.
- Eng. J. 1974, 117 (1974).
 9. _____, J. Colloid Interface Sci. 37, 824 (1971).
 10. G. H. Nancollas and B. Tomazic, J. Phys. Chem. 78, 2218 (1974).
 11. A. S. Posner, Physiol. Rev. 49, 760 (1969).
- A. S. Posner, Physiol. Rev. 49, 760 (1969).
 M. J. Dallemagne and L. J. Richelle, in Biological Mineralization, I. Zipkin, Ed. (Wiley, New York, 1973), pp. 23-42.
 W. E. Brown, J. P. Smith, J. R. Lehr, W. A. Frazier, Nature (London) 196, 1050 (1962).
 M. D. Francis and N. C. Webb, Calcif. Tissue Res. 6, 355 (1959).
 G. H. Nancollas and M. S. Mohan, Arch. Oral Biol. 18, 731 (1970).

- G. H. Nancollas and M. S. Mohan, Arch. Oral Biol. 15, 731 (1970).
 G. H. Nancollas and M. B. Tomson, Faraday Discuss. Chem. Soc. 61, 177 (1976).
 Z. Amjad, M. B. Tomson, G. H. Nancollas, D. Koutsoukos, J. Dental Res., in press.
 T. Koutsoukos, G. H. Nancollas, M. B. Tom-con in properties.
- son, in preparation. 19. A. Eralp, B. Tomazic, G. H. Nancollas, in preparation
- 20. M. Sheehan and G. H. Nancollas, in preparation.
- 21. C. C. Chieng and G. H. Nancollas, in preparation.
- 22. Supported by NIH grants DE 03223 and AM 19048, NSF grant ENG 74-15486, and the Petro-leum Research Fund of the American Chemical ociety
- Present address: Department of Environmental Science and Engineering, Rice University, Houston, Texas 77001.

21 December 1977; revised 28 February 1978

Land Floras: The Major Late Phanerozoic Atmospheric Carbon Dioxide/Oxygen Control

Abstract. Since at least the late Mesozoic, the abundance of terrestrial vegetation has been the major factor in atmospheric carbon dioxide/oxygen fluctuations. Of modern ecosystem types occupying more than 1 percent of the earth's surface, productivity/area ratios of terrestrial ecosystems (excepting tundra and alpine meadow, desert scrub, and rock, ice, and sand) exceed those of marine ecosystems and probably have done so for much of late Phanerozoic time. Reduction of terrestrial ecosystems during marine transgression would decrease the world primary productivity, thus increasing the atmospheric carbon dioxide concentration and decreasing the oxygen concentration. Regression would produce opposite effects.

It is now widely accepted that the varying abundance of photosynthesizing phytoplankton in the oceans has had major impact on controlling atmospheric CO_2/O_2 ratios through geologic time. Elaborate models have been constructed that relate the terminal Paleozoic and Mesozoic worldwide faunal extinctions to phytoplankton failure (1-3). Of the factors that control phytoplankton abundance, for example, continental physiography, sea-level changes, upwelling, climatic fluctuation, and nutrient abundance, all relate to or are affected by relative land/sea ratios. Because it was long believed that the productivity of marine phytoplankton exceeded that of terrestrial plants, it was believed that flooding of continental areas by epeiric seas would increase the total world primary productivity and that regression of epeiric seas to the ocean basins would reduce it. Thus, in the geographical areas that have been alternately flooded and drained by transgressions and regressions of epeiric seas, little attention has been focused upon the net primary productivity of terrestrial ecosystems that would have replaced marine ecosystems during a regression. Evaluation of mod-