

REVIEWS IN MINERALOGY AND GEOCHEMISTRY

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BIOMINERALIZATION

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FRONT COVER: Coccolith calcite. Colorized high resolution scanning electron micrograph of *Calcidiscus leptoporus* ssp. *quadriperforatus* with heterococcoliths on left (blue) showing interlocking crystals with rhombic faces and holococcoliths on the right (violet) formed of minute rhombohedra (from Young and Henriksen, this volume; image by Markus Geisen).

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DEDICATION

Dr. William C. Luth has had a long and distinguished career in research, education and in the government. He was a leader in experimental petrology and in training graduate students at Stanford University. His efforts at Sandia National Laboratory and at the Department of Energy's headquarters resulted in the initiation and long-term support of many of the cutting edge research projects whose results form the foundations of these short courses. Bill's broad interest in understanding fundamental geochemical processes and their applications to national problems is a continuous thread through both his university and government career. He retired in 1996, but his efforts to foster excellent basic research, and to promote the development of advanced analytical capabilities gave a unique focus to the basic research portfolio in Geosciences at the Department of Energy. He has been, and continues to be, a friend and mentor to many of us. It is appropriate to celebrate his career in education and government service with this series of courses in cutting-edge geochemistry that have particular focus on Department of Energy-related science, at a time when he can still enjoy the recognition of his contributions.

BIOMINERALIZATION

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Reviews in Mineralogy and Geochemistry

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FOREWORD

In this volume, the editors Patricia Dove, James De Yoreo, and Steve Weiner have integrated a diverse group of contributors from the earth, biological and materials disciplines who introduce us to the concepts of biological mineralization, examine the major biomineralization processes, and describe their impact on earth history. This volume offers an excellent opportunity for both specialists and non-specialists to understand the basic principles of biomineralization. It also gives us a view into the future of this growing field of research. It was prepared in advance of a two-day short course (December 6–7, 2003) on Biomineralization, jointly sponsored by GS and MSA, and held at the Silverado Resort and Conference Center in Napa Valley, California prior to the fall AGU meeting in San Francisco.

Trish deserves a long vacation after the supreme effort she put into assembling this volume (and *on time* for the short course!). The many hours she spent reviewing, editing, revising, as well as her ability to keep things on-track made this volume possible. Steve's reviews also significantly improved many of the chapters. The authors are commended for their hard work. Thank you. And, as always, I thank my infinitely patient and supportive family, Kevin, Ethan and Natalie.

Jodi J. Rosso, Series Editor
West Richland, Washington
October 25, 2003

PREFACE AND ACKNOWLEDGMENTS

Since the dawn of life on earth, organisms have played roles in mineral formation in processes broadly known as biomineralization. This biologically-mediated organization of aqueous ions into amorphous and crystalline materials results in materials that are as simple as adventitious precipitates or as complex as exquisitely fabricated structures that meet specialized functionalities. The purpose of this volume of *Reviews in Mineralogy and Geochemistry* is to provide students and professionals in the earth sciences with a review that focuses upon the various processes by which organisms direct the formation of minerals. Our framework of examining biominerals from the viewpoints of major mineralization strategies distinguishes this volume from most previous reviews. The review begins by introducing the reader to over-arching principles that are needed to investigate biomineralization phenomena and shows the current state of knowledge regarding the major approaches to mineralization that organisms have developed over the course of Earth history. By exploring the complexities that underlie the “synthesis” of biogenic materials, and therefore the basis for how compositions and structures of biominerals are mediated (or not), we believe this volume will be instrumental in propelling studies of biomineralization to a new level of research questions that are grounded in an understanding of the underlying biological phenomena. To make this happen, the volume contains contributions from a special group of authors whose areas of expertise are as varied as the biominerals themselves. Special thanks are due to these scientists for meeting the deadlines with their comprehensive contributions.

Foreword, Preface & Acknowledgments

We also thank the individuals and organizations who have made possible the timely publication of this review. The Series editor, Jodi Rosso, cheerfully handled the production of the volume through our tight publication schedule. Alex Speer was helpful throughout the process and we thank him for sharing his experience regarding how to ensure a successful review and short course. Many other people have contributed to seeing the success of this volume and we thank Drs. Selim Elhadj, Nizhou Han, and Laura Wasylenki for carefully proofreading several chapters and helping with the many reference searches. Ellen Mathena and David Rugh helped to collect materials and to work with draft manuscripts. PMD also thanks the Department of Energy, Division of Chemical Sciences, Geosciences and Biosciences (DE-FG02-00ER15112) and the National Science Foundation (NSF-OCE-0083173) for supporting her time on this project.

We are very grateful to the U.S. Department of Energy, Office of Basic Energy Sciences, Chemical Sciences, Geosciences and Biosciences Division, for special funding in honor of Bill Luth (see dedication). We especially thank Dr. Nick Woodward for making this support possible. Additional funding was also provided by Lawrence Livermore National Laboratory. We also thank the College of Science and the Department of Geosciences at Virginia Tech for providing supplementary funds that allowed us to award registration fee waivers to 20 of the student participants. While we are all students of this fascinating field, it is a delight to see the high level of interest in the next generation of scientists.

Patricia M. Dove
Steve Weiner
James J. De Yoreo

Blacksburg, Virginia
September 2003

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1 **An Overview of Biomineralization Processes and the Problem of the Vital Effect**

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"Biomineralization links soft organic tissues, which are compositionally akin to the atmosphere and oceans, with the hard materials of the solid Earth. It provides organisms with skeletons and shells while they are alive, and when they die these are deposited as sediment in environments from river plains to the deep ocean floor. It is also these hard, resistant products of life which are mainly responsible for the Earth's fossil record. Consequently, biomineralization involves biologists, chemists, and geologists in interdisciplinary studies at one of the interfaces between Earth and life."

(Leadbeater and Riding 1986)

INTRODUCTION

Biomineralization refers to the processes by which organisms form minerals. The control exerted by many organisms over mineral formation distinguishes these processes from abiotic mineralization. The latter was the primary focus of earth scientists over the last century, but the emergence of biogeochemistry and the urgency of understanding the past and future evolution of the Earth are moving biological mineralization to the forefront of various fields of science, including the earth sciences.

The growth in biogeochemistry has led to a number of new exciting research areas where the distinctions between the biological, chemical, and earth sciences disciplines melt away. Of the wonderful topics that are receiving renewed attention, the study of biomineral formation is perhaps the most fascinating. Truly at the interface of earth and life, biomineralization is a discipline that is certain to see major advancements as a new generation of scientists brings cross-disciplinary training and new experimental and computational methods to the most daunting problems. It is, however, by no means a new field. The first book on biomineralization was published in 1924 in German by W.J. Schmidt (Schmidt 1924), and the subject has continued to intrigue a dedicated community of scientists for many years. Until the early 1980s the field was known as "calcification," reflecting the predominance of biologically formed calcium-containing minerals. As more and more biogenic minerals were discovered that contained other cations, the field became known as "biomineralization." An invaluable knowledge base has been established in a literature that is found at virtually every call number in the scientific library. With styles as varied as the biominerals themselves, the initiate will find that a number of authors have extensively assessed the state of knowledge in texts (e.g., Lowenstam and Weiner 1989; Simkiss and Wilbur 1989; Mann 2001) and specialized reviews (e.g., Westbroek 1983; Leadbeater and Riding 1986; Crick 1989; Bäuerlein 2000).

With this RiMG review, the foremost goal of the authors is to create a volume focused upon topics essential for scientists, and in particular earth scientists, entering the field. With this objective in mind, our approach is first to establish relevant aspects of molecular biology and protein chemistry (e.g., Evans 2003) and then the thermodynamic principles necessary for mineralization to occur (e.g., De Yoreo and Vekilov 2003). Equipped with this toolbox of essentials and Nature as our guide, we examine in some detail six major biomineralization processes. As much as possible, we attempt to de-emphasize specifics unique to some organisms and, instead focus on the major mineralizing strategies. Fundamental to this approach is an evolutionary perspective of the field. It is noteworthy that underlying mechanisms for controlling the biomineralization processes appear to be used again and again by members of many phyla.

The last part of this volume examines views of how biomineralization processes have been employed by organisms over Earth history and their intertwined relations to earth environments. By considering the impacts of these relations upon global biogeochemical cycles, studying the temporal and geochemical results of mineral-sequestering activities, and asking critical questions about the topic, we can arrive at a deeper understanding of biological mineralization and of life on earth.

This chapter

This introductory chapter is divided into two main sections: an overview of basic biomineralization strategies and processes and a discussion of how understanding more about biomineralization mechanisms may shed light on the manner in which environmental signals may or may not be embedded in the minerals produced by organisms.

Despite the fact that the hallmark of biomineralization is the control that organisms exert over the mineralization process, it has been noted by earth scientists for the last 50 years that biologically produced minerals often contain embedded within their compositions, signatures that reflect the external environment in which the animal lived. Thus many geochemists have focused on extracting the signal for past seawater temperatures, salinities, productivities, extent of sea water saturation, and more. The task has not been an easy one! In many cases, the control processes either completely eliminate the signals or shift them. Sorting out this so-called vital effect (Urey 1951) or physiological effect (Epstein et al. 1951) from the environmental signal remains a problem yet to be solved. In the second part of this chapter, we will discuss some of the principles of mineralization in terms of the vital effect—a subject of much current interest to the earth sciences community.

OVERVIEW OF BIOMINERALS

Over the last 3500 Myr or so, first prokaryotes and then eukaryotes developed the ability to form minerals. At the end of the Precambrian, and in particular at the base of the Cambrian some 540 Myr ago, organisms from many different phyla evolved the ability to form many of the 64 different minerals known to date (e.g., Knoll 2003). While the names and corresponding chemical compositions of minerals produced by organisms are given in Table 1 (Weiner and Addadi 2002), this list is unlikely to be complete, as new biologically produced minerals continue to be discovered.

What is a biomineral?

The term biomineral refers not only to a mineral produced by organisms, but also to the fact that almost all of these mineralized products are composite materials comprised of both mineral and organic components. Furthermore, having formed under controlled conditions, biomineral phases often have properties such as shape, size, crystallinity, isotopic and trace

element compositions quite unlike its inorganically formed counterpart. The term “biomineral” reflects all this complexity. Figure 1 illustrates this by comparing part of a single calcite crystal formed by an echinoderm to synthetic single crystals of calcite.

Some comments on the major groups of biominerals

As indicated by Table 1, calcium is the cation of choice for most organisms. The calcium-bearing minerals comprise about 50% of known biominerals (Lowenstam and Weiner 1989). This comes as no surprise because calcium fulfills many fundamental functions in cellular metabolism (Lowenstam and Margulis 1980; Simkiss and Wilbur 1989; Berridge et al. 1998). This dominance of calcium-bearing minerals is what led to the widespread usage of the term calcification. However, reader beware! The term refers to the formation of calcium-containing phosphate, carbonate, oxalate and other mineral types. Table 1 also shows that about 25% of the biominerals are amorphous in that they do not diffract X-rays. Amorphous silica is commonly formed by organisms and has been investigated extensively (see Perry 2003). Another well studied biogenic amorphous mineral, is the granules of amorphous hydrous iron phosphate deposited as granules in the skin of the holothurian, *Molpadia* (Lowenstam and Rossman 1975), shown in Figure 2. Among these amorphous minerals are those that have the same chemical composition, but differ by degree of short range order (Addadi et al. 2003). Many of these have been discovered only in the last few years; hence they present a fascinating new area of research.

The calcium carbonate minerals are the most abundant biogenic minerals, both in terms of the quantities produced and their widespread distribution among many different taxa (Lowenstam and Weiner 1989). Of the eight known polymorphs of calcium carbonate, seven are crystalline and one is amorphous. Three of the polymorphs—calcite, aragonite and vaterite—are pure calcium carbonate, while two—monohydrocalcite and the stable forms of amorphous calcium carbonate—contain one water molecule per calcium carbonate (Addadi et al. 2003). Surprisingly, the transient forms of amorphous calcium carbonate do not contain water (Addadi et al. 2003). One of the major challenges in the field of biomineralization is to understand the mechanism(s) by which biological systems determine which polymorph will precipitate. This is genetically controlled and is almost always achieved with 100% fidelity.

Phosphates comprise about 25% of the biogenic mineral types. Except for struvite and brushite, most phosphate minerals are produced by controlled mineralization (see subsequent section). The most abundantly produced phosphate mineral is carbonated hydroxyapatite, also called dahllite (Lowenstam and Weiner 1989). It is the mineral present in vertebrate bones and teeth, as well as in the shells of inarticulate brachiopods. Note that the non-carbonated member of this family, hydroxyapatite, is not known to be formed biologically. Biogenic carbonate apatite crystals are usually plate-shaped and are exceedingly small (2–4 nm thick and some tens of nanometers long and wide; Weiner and Price 1986). It is interesting to note that synthetic carbonated hydroxyapatites precipitated under conditions similar to those found in vertebrate physiology are also plate-shaped and small (Moradian-Oldak et al. 1990). Therefore it is not the biological environment in which they form that gives them the plate shape which is most unusual for a mineral that crystallizes in the hexagonal crystallographic system. The fact that the biologically formed crystals are so small generally indicates that they are also rather unstable; hence they are typically more soluble than hydroxyapatite (Stumm 1992). They are also very difficult to characterize structurally because their high surface/volume results in many atoms being perturbed. With new spectroscopic applications designed to overcome the problems of working with small particles and the difficulties associated with hydrous phases (Waychunas 2001), current views of phosphate biomineral compositions and structures could still undergo significant revision.

Table 1. The names and chemical compositions of minerals produced by biologically induced and controlled mineralization processes

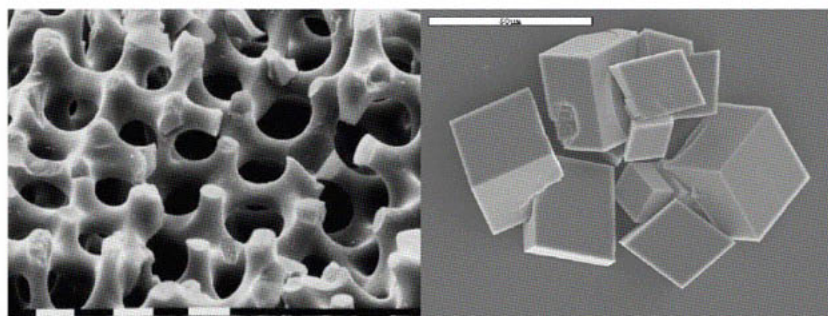
<i>Name</i>	<i>Formula</i>
<u>Carbonates</u>	
Calcite	CaCO_3
Mg-calcite	$(\text{Mg}_x\text{Ca}_{1-x})\text{CO}_3$
Aragonite	CaCO_3
Vaterite	CaCO_3
Monohydrocalcite	$\text{CaCO}_3 \cdot \text{H}_2\text{O}$
Protodolomite	$\text{CaMg}(\text{CO}_3)_2$
Hydrocerussite	$\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$
Amorphous Calcium Carbonate (at least 5 forms)	$\text{CaCO}_3 \cdot \text{H}_2\text{O}$ or CaCO_3
<u>Phosphates</u>	
Octacalcium phosphate	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6$
Brushite	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$
Franconite	$\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$
Carbonated-hydroxylapatite (dahllite)	$\text{Ca}_5(\text{PO}_4)_3(\text{CO}_3)_2(\text{OH})$
Whitlockite	$\text{Ca}_{18}\text{H}_2(\text{Mg,Fe})_2^{+2}(\text{PO}_4)_{14}$
Struvite	$\text{Mg}(\text{NH}_4)(\text{PO}_4) \cdot 6\text{H}_2\text{O}$
Vivianite	$\text{Fe}_3^{+2}(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$
Amorphous Calcium Phosphate (at least 6 forms)	variable
Amorphous Calcium Pyrophosphate	$\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$
<u>Sulfates</u>	
Gypsum	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
Barite	BaSO_4
Celestite	SrSO_4
Jarosite	$\text{KFe}_3^{+3}(\text{SO}_4)_2(\text{OH})_6$
<u>Sulfides</u>	
Pyrite	FeS_2
Hydrotroilite	$\text{FeS} \cdot n\text{H}_2\text{O}$
Sphalerite	ZnS
Wurtzite	ZnS
Galena	PbS
Greigite	Fe_3S_4
Mackinawite	$(\text{Fe,Ni})_9\text{S}_8$
Amorphous Pyrrhotite	Fe_{1-x}S ($x = 0-0.17$)
Acanthite	Ag_2S
<u>Arsenates</u>	
Orpiment	As_2S_3
<u>Hydrated Silica</u>	
Amorphous Silica	$\text{SiO}_2 \cdot n\text{H}_2\text{O}$
<u>Chlorides</u>	
Atacamite	$\text{Cu}_2\text{Cl}(\text{OH})_3$
<u>Fluorides</u>	
Fluorite	CaF_2
Hieratite	K_2SiF_6
<u>Metals</u>	
Sulfur	S

Table 1 continued.

<i>Name</i>	<i>Formula</i>
<u>Oxides</u>	
Magnetite	Fe_3O_4
Amorphous Ilmenite	$\text{Fe}^{+2}\text{TiO}_3$
Amorphous Iron Oxide	Fe_2O_3
Amorphous Manganese Oxide	Mn_3O_4
<u>Hydroxides & Hydrous Oxides</u>	
Goethite	$\alpha\text{-FeOOH}$
Lepidocrocite	$\gamma\text{-FeOOH}$
Ferrihydrite	$5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$
Todorokite	$(\text{Mn}^{+2}\text{CaMg})\text{Mn}_3^{+4}\text{O}_7 \cdot \text{H}_2\text{O}$
Birnessite	$\text{Na}_4\text{Mn}_{14}\text{O}_{27} \cdot 9\text{H}_2\text{O}$
<u>Organic Crystals*</u>	
Earlandite	$\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$
Whewellite	$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$
Weddelite	$\text{CaC}_2\text{O}_4 \cdot (2+X)\text{H}_2\text{O}$ ($X < 0.5$)
Glushinskite	$\text{MgC}_2\text{O}_4 \cdot 4\text{H}_2\text{O}$
Manganese Oxalate (unnamed)	$\text{Mn}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
Sodium urate	$\text{C}_5\text{H}_3\text{N}_4\text{NaO}_3$
Uric Acid	$\text{C}_5\text{H}_4\text{N}_4\text{O}_3$
Ca tartrate	$\text{C}_4\text{H}_4\text{CaO}_6$
Ca malate	$\text{C}_4\text{H}_4\text{CaO}_5$
Paraffin Hydrocarbon	
Guanine	$\text{C}_5\text{H}_7(\text{NH}_2)\text{N}_4\text{O}$

* by the convention of Lowenstam & Weiner (1989)

References: Lowenstam & Weiner (1989), Simkiss & Wilbur (1989), Mann (2001), Weiner and Addadi (2002)

**Figure 1.** Comparison of calcite single crystals: (left) stereom of echinoderm and (right) synthetically produced rhombohedral forms.

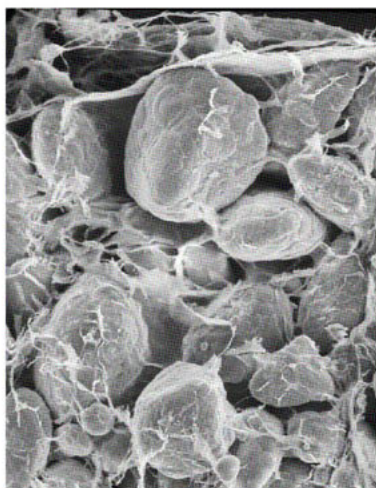


Figure 2. Granules of amorphous hydrous iron phosphate deposited in the skin of the holothurian, *Molpadia*. (Micrograph from the collection of the late H.A. Lowenstam). Diameter of largest granule about 200 microns.

It is noteworthy that each of the mineral classes includes one or more phases that contain water and/or hydroxyl groups (Table 1). These hydrated forms comprise about 60% of the biogenic minerals. In particular, all of the silica biominerals reported to date are hydrated (e.g., Lowenstam and Weiner 1989), and there is mounting evidence that many crystalline carbonate biominerals first form as hydrated phases (Beniash et al. 1997, 1999; Weiss et al. 2002; Addadi et al. 2003). The abundance of hydrated biominerals is no accident. Hydrated phases are favored over anhydrous counterparts by significantly lowering energetic barriers to nucleation and growth from aqueous solution (e.g., Stumm 1992). Organisms are metabolic misers; thus they use the Ostwald-Lussac rule to their advantage by favoring the precipitation of the lowest energy phases (Nancollas 1982).

The iron biominerals are not readily evaluated by mineral class because they have significant occurrences as oxides, hydroxides, and sulfides (Bazylinski and Moskovitz 1997; Konhauser 1997). Some iron sulfate and phosphate minerals are also reported (Konhauser 1998). When one considers the abundance of iron in the biosphere (particularly in the early earth) versus the very low solubility of most iron-bearing minerals, one expects this diversity and abundance. The iron biominerals are of particular significance because they comprise approximately 40% of all minerals formed by organisms (Lowenstam 1986; Bazylinski and Frankel 2003), and magnetite formation is believed to be the most ancient matrix-mediated biomineralizing system. As such, Kirschvink and Hagadorn (2000) have suggested that magnetic biominerals, and iron minerals in general, could contain clues to other aspects of controlled mineralization.

Table 1 also contains a group of so-called “organic” minerals. Despite the contradiction in terms, these are crystalline phases formed by organisms probably by the same underlying strategies used for “normal” mineral formation. We suspect that many of these minerals remain to be discovered, and exploring the functions they perform will be fascinating. Even DNA is known to transform into crystalline phases in bacteria subjected to stress (Minsky et al. 2002). The dense packing of the crystal provides protection from damage during periods of dormancy when much of the metabolic processes are nearly shut down.

Unique character of minerals formed by biological systems

Biominerals meet the criteria for being true minerals, but they can also possess other characteristics that distinguish them from their inorganically produced counterparts. The most obvious trait is that biogenic minerals have unusual external morphologies. It is perhaps the intricacy and diversity of bio-originated structures (e.g., Figure 3) that first attracts mineralogically-inclined persons into the field of biomineralization. For example, beautiful single crystals that express an unusual set of faces are found in magnetite (see Bazylinski and Frankel 2003). The SEM images presented throughout this volume capture the imagination by illustrating the astonishing ability of Nature to grow minerals of a complexity that is (for now) an impossible task for humankind to replicate! Implicit in this complexity is the intriguing ability of organisms to impose a "handedness" upon the external morphology of biominerals. Good examples are found from the microscopic (magnetosomes and coccoliths) to macroscopic (*Nautilus*) organisms. The question of how organisms use molecules to transfer chiral information to crystalline surfaces and thus induce asymmetrical biomineral structures is an on going one (e.g., Addadi and Weiner 2001; Orme 2001; De Yoreo and Vekilov 2003). Remarkably, genetic regulation runs the controlled-mineralization "program" to form biominerals with unusual morphologies using great fidelity again and again.

A second characteristic of biominerals is that many are actually composites or agglomerations of crystals separated by organic material. In many organisms, they exist as small bodies distributed within a complex framework of macromolecular frameworks such as collagen or chitin (Addadi et al. 2003). X-ray diffraction studies have led to an acceptance that the crystalline biominerals are typically single crystals. There are certainly examples of this, but higher resolution studies are showing this is not always the case (Wilt 1999). Rather, some "single crystal" biominerals are actually a mosaic of domains delimited by organic layers or they may contain significant occlusions of proteinaceous material (Wilt 2002). Yet, they exhibit many of the same diffraction properties as those of a single crystal (Simkiss 1986). As biominerals

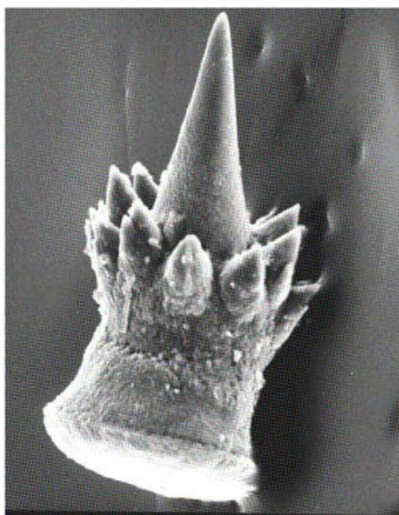


Figure 3. Calcitic spicule formed by the ascidian *Bathypera ovoidea*. Length about 120 microns. (Micrograph from the collection of the late H.A. Lowenstam).

become increasingly characterized, a better understanding of both short-range and long-range structures and assembly of biominerals could change our interpretations of mineralization mechanisms.

BIOMINERALIZATION CONCEPTS

For nucleation and growth to occur, biomineral formation requires a localized zone that achieves and maintains a sufficient supersaturation. In most biological systems, the site of mineral deposition is isolated from the environment by a physical delimiting geometry. The actual size of that site or volume is sometimes ambiguous, but it is generally agreed that this region must limit diffusion into/out of the system or utilize a type of compartment. The extent of this isolation can be passive and minimal such as observed when bacteria cluster to form an intercellular zone that is diffusion-limited. At the other extreme, intracellular vesicles create compartmentalized environments where compositions can be precisely regulated. This compartment must be capable of modifying the activity of at least one biomineral constituent (usually the cation) as well as protons and possibly other ions. Any fluxes in ion chemistry must meet one constraint: the fluid must maintain electroneutrality. Ion supply (or removal) occurs by two means: Active pumping associated with organelles near the sites of mineralization or passive diffusion gradients. As will be shown throughout this volume, organisms use a great variety of anatomical arrangements to facilitate ion movement.

The chemical compositions of *in vivo* fluids at and adjacent to sites of biomineral formation have direct relevance to our understanding of the mineralization processes and of the consequent degree of control exerted by the organism on its internal environment. Later in this volume, we will be reminded that these fluid compositions reflect, to variable degrees, the marine roots of organisms in evolutionary history (e.g., Knoll 2003). Surprisingly, however, little information has been published concerning fluids for most organisms. Table 2 compares the major solute compositions of seawater and an average freshwater to the typical compositions of several biological fluids. One sees that the major constituents in the extrapallial fluid of marine mollusks reflect the higher salinity of its saltwater environment. (Extrapallial fluids are found in the space between the secretory epithelium of the mantle and the growth surface of the nacreous aragonite biomineral. See illustration in Zaremba et al. 1996). In contrast, extrapallial fluids of freshwater mollusks have much higher solute concentrations than their low salinity environment. A comparison of the two organisms reveals very different fluid compositions, yet both form aragonitic shells!

Table 2 reminds us that organisms possess fluids with significant ionic strengths. This means that mineralization studies must address the solution chemistry in terms of ion activity, not concentration. Careful use of activity coefficient models is necessary to estimate the supersaturation of growth environments. For example, calcium ion in a blood plasma with the physiological ionic strength of 0.15 molal (see Table 2) has an activity coefficient of approximately 0.3, compared to a value of 1.0 in an infinitely dilute solution (e.g., Langmuir 1997). In high salinity fluids associated with marine environments, the correction to activity becomes greater (and sometimes more ambiguous). In addition to affecting supersaturation, ionic strength mediates the charges of precursor molecules, thus affecting the stabilization of colloids and amorphous gels (e.g., Iler 1979; Perry 2003).

Investigations of cellular processes have led to general insights regarding cation concentrations in the tissues of higher organisms (da Silva and Williams 1991). In biology calcium is very highly controlled at levels in the range of 10^{-8} to 10^{-6} M, owing

Table 2. Summary of ion compositions in natural waters and biological fluids.

	Concentration (mmol/kg)						Concentration (μmol/kg)						pH	I.S.		
	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	Si ²⁺	Li ⁺	Cu ²⁺	Zn ²⁺	Fe _T			Mn	(PO ₄) _T
Seawater ¹	479	10.4	10.5	54.3	2.0	558	28.9	88-92	26-27	na	na	na	na	<0.1-3 ¹	8.1	700
Marine Algae ³ (<i>Valonia</i>)	80	400	1.5	50	(10)?			na	na	na	na	na	na	5 (HPO ₄ ²⁻)	na	na
Marine mollusk ⁴ (<i>Pinctada fucata</i>)	431	12.6	9.7	50.7	3.6	524	27.9	89	29	2.9	176	22.4	4.4	502	7.4	664
Freshwater ²	0.31	0.04	0.37	0.15	0.87	0.22	0.12	na	na	na	na	na	na	na	4-7	
Freshwater mollusk ⁴ (<i>Hyriopsis schlegelii</i>)	22.1	0.57	4.1	0.63	10.5	15.0	5.2	4.3	na	5.2	22.3	56.4	54.2	41.1	8.15	44
Red blood cells ³	11	92	10 ⁻⁴	2.5	(10)?	50		na	na	na	na	na	na	3 (HPO ₄ ²⁻)	na	94-106
Blood plasma ³	160	10	2	2	30	100		na	na	na	na	na	na	3 (HPO ₄ ²⁻)	7.4	158

Notes:

- 1 For a salinity of 35 parts per thousand, 25°C, P = 1 atm (Berner and Berner 1996)
- 2 Average "actual" world river composition. (Berner and Berner 1996)
- 3 da Silva and Williams (1991)
- 4 Wada and Fujinuki (1974)

to its key roles in signaling and metabolic processes. Similarly, free magnesium ion is reported to be approximately 10^{-3} M in all compartments except some vesicles. Incidentally, Simkiss (1986) noted the essential role of magnesium ion in mineralization processes because of its role in stabilizing carbonate and phosphate crystal formation. Other elements such as zinc are found at concentrations as low as 10^{-9} in the cytoplasm, while the free ion has concentrations as high as 10^{-3} M in some vesicles. In contrast, manganese has concentrations of approximately 10^{-8} M almost everywhere, whether in or out of the cell, but may be $\ll 10^{-8}$ M in eukaryotic cells.

BASIC PROCESSES OF BIOMINERALIZATION

Biomineralization processes are divided into two fundamentally different groups based upon their degree of biological control. Lowenstam (1981) introduced these as “biologically induced” and “organic matrix-mediated,” with the latter generalized by Mann (1983) to “biologically controlled” mineralization. Recognizing that the detailed processes of biomineralization within this convention are as varied as the organisms themselves, this section outlines basic mineralization strategies to help the reader place the information presented in subsequent chapters into a mechanistic framework. We emphasize “location, location” to show: 1) how/where biomineral constituents can be concentrated as ions or solid phases; 2) types of translocation that can occur; and 3) resting places and transformation of the end-products. Using this approach, we can further define the variable degrees of biological control. It is the specific nature and degree of control that is central to understanding the extent of biological control of the elemental compositions of biominerals. Likewise, transport mechanisms and hydration environments are certain to also affect minor element chemistry. These are the roots of deciphering the vital effect.

Biologically induced mineralization

The secondary precipitation of minerals that occurs as a result of interactions between biological activity and the environment is termed “biologically induced” mineralization. In this situation, cell surfaces often act as causative agents for nucleation and subsequent mineral growth. The biological system has little control over the type and habit of minerals deposited, although the metabolic processes employed by the organism within its particular redox environment mediate pH, $p\text{CO}_2$ and the compositions of secretion products (e.g., McConnaughey 1989a; Fortin et al. 1997; Tebo et al. 1997; Frankel and Bazylnski and Frankel 2003). These chemical conditions favor particular mineral types in an indirect way (Figure 4). In some cases, biological surfaces are important in the induction stage because nucleation often occurs directly on the cell wall, and the resulting biominerals can remain firmly attached. In open waters, this epicellular mineralization can lead to encrustation so complete that gravitation overcomes buoyancy, and they settle through the water column. The sediment record attests to the extensive occurrence of this phenomenon (e.g., Knoll 2003; Van Cappellen 2003).

Heterogeneity is the hallmark of biologically induced minerals. Frankel and Bazylnski (2003) show that the compositions of minerals resulting from induced processes vary as greatly as the environments in which they form. This heterogeneity includes variable external morphology (typically poorly defined), water content, trace/minor element compositions, structure and particle size. Since these characteristics also typify inorganically precipitated minerals, unambiguous interpretations of the sediment and rock record continue to confound interpretations of earth environments.

Biologically controlled mineralization

In “biologically controlled” mineralization, the organism uses cellular activities to

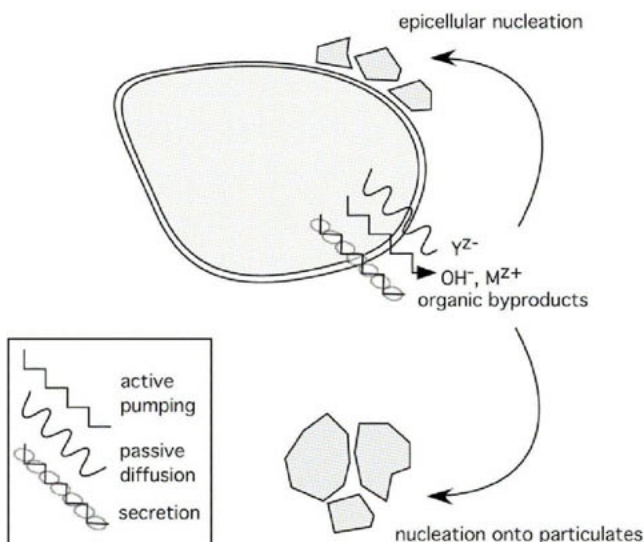


Figure 4. Schematic of biologically induced mineralization. Mineral precipitates form as a result of metabolic activities that affect pH, $p\text{CO}_2$, and secretion products. The cell is a causative agent only, without control over mineral type or habit.

direct the nucleation, growth, morphology and final location of the mineral that is deposited. While the degree of control varies across species, almost all controlled mineralization processes occur in an isolated environment. The results can be remarkably sophisticated, species-specific products that give the organism specialized biological functions.

Biologically controlled mineralization processes can be described as occurring extra-, inter- or intracellularly. These distinctions refer to the locations of the mineralization site with reference to the cells responsible for mineralization. However, not all mineralization processes can be classified in this simple manner. In some cases, mineral formation begins within the cell and then proceeds outside the cell. Identifying what are in essence, end members at least helps us understand the overall complexity. We will therefore discuss extra-, inter- and intracellular biomineralization separately.

Biologically controlled extracellular mineralization. In extracellular mineralization, the cell produces a macromolecular matrix outside the cell in an area that will become the site of mineralization. The term matrix refers to a group of macromolecules comprised of proteins, polysaccharides or glycoproteins that assemble to form a three-dimensional framework. The matrix composition is unique in that many of its proteins contain a high proportion of acidic amino acids (especially aspartate) and phosphorylated groups (Veis and Perry 1967; Weiner 1979; Weiner et al. 1983a,b; Swift and Wheeler 1992). The structures and compositions of these organic frameworks are genetically programmed to perform essential regulating and/or organizing functions that will result in the formation of composite biominerals.

There are two means by which the cell can transfer constituents to the matrix. In the first, the cell may actively pump cations through the membrane and into the surrounding region (e.g., Simkiss 1986). Once out of the cell, the supersaturation level of the fluid is established and maintained by ion diffusion over relatively large distances to the organic matrix (Figure 5a). In the second approach, the cations may be concentrated within the cell into cation-loaded vesicles, exported through the membrane and later broken down by precursor compounds at the organic matrix (Figure 5b). The latter mechanism is used for cartilage mineralization in the epiphyseal growth plate (Ali 1983). It is also probably used by sea urchin larvae to introduce amorphous calcium carbonate into spicule-forming vesicles, where it subsequently crystallizes into calcite (Beniash et al. 1999). Anion movement is typically the result of passive diffusion in response to the electroneutrality requirement and is ultimately driven by pH gradients created during cation transport (Simkiss 1976; McConnaughey 1989b). In both approaches, the cell works actively to supply cations to an external organic matrix for "on-site" nucleation and growth. This is distinguished from the epicellular nucleation and growth that occur during biologically induced mineralization.

Almost all structures that form by extracellular processes develop upon a pre-formed matrix derived from secretory products of multicellular epithelial tissues. Watabe and Kingsley (1989) suggest that these tissues have additional significance as extensive

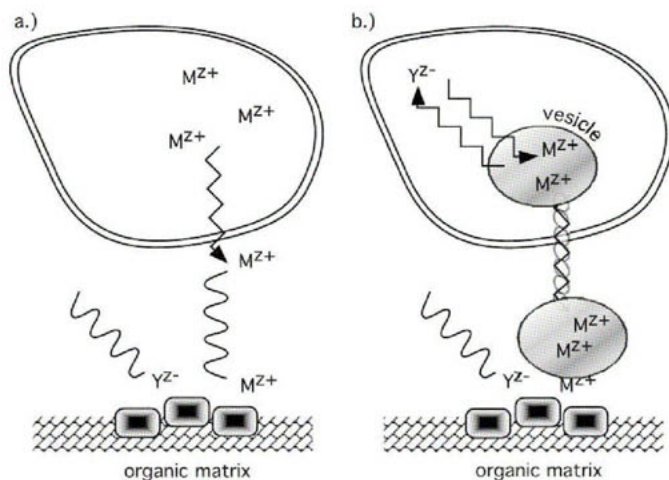
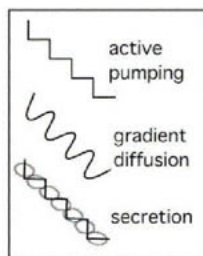


Figure 5. Illustrations of biologically controlled extracellular mineralization showing that this process is distinguished by nucleation outside of the cell. a.) Cations are pumped across the cell membrane and move by passive diffusion through extracellular fluids to the site of mineralization. b.) Cations are concentrated intracellularly as aqueous ions into a vesicle that is subsequently secreted. Compartment breakdown at site of mineralization releases cations for biomineral formation.



substrates that facilitate the formation of massive skeletons. Examples of organisms believed to mineralize primarily by extracellular biologically controlled processes include the external tests of certain foraminifera (Towe and Cifelli 1967; Zeebe and Sanyal 2002; Erez 2003), cephalopod statoliths (Bettencourt and Guerra 2000), shells of mollusks (Gregoire et al 1955; Crenshaw 1980; Weiner and Traub 1980, 1984; Falini et al. 1996; Pereira-Mouries et al. 2002; Weiss et al. 2002; Gotliv et al. 2003); exoskeletons of bryozoans (Rucker and Carver 1969), scleractinian corals (Constantz 1986; Constantz and Meike 1989), bones and teeth (e.g., discussion in Lowenstam and Weiner 1989; Veis 2003).

To understand mineralization mechanisms, one must possess knowledge regarding the structures of the organic matrix components and of the entire framework. One of the most thoroughly investigated matrices in this respect is the mollusk shell nacreous layer. X-ray and electron diffraction reveal that the most ordered component is β -chitin, and the most abundant component is silk fibroin, but it shows little evidence of order based on diffraction (Weiner and Traub 1984). It has also been shown that there is a well defined spatial relation between the chitin of the framework and the associated aragonite mineral. This implies that the nucleation mechanism is most likely epitaxial (Weiner and Traub 1984). The acidic components impart a chemical activity and template structure that direct the resulting mineralization process. Not much is known about their secondary structures. Mollusk acidic matrix proteins are thought to mainly adopt the β -sheet structure (Worms and Weiner 1986). More recent structural information on the nacreous layer raises the interesting possibilities that the silk fibroin component of the matrix is a gel (Levi-Kalishman et al. 2001) (Figure 6) and that it does not form layers on either side of the chitin, as was proposed by Weiner and Traub (1984). Several mollusk shell matrix proteins have been sequenced. Most have been purified by gel electrophoresis and are not highly acidic.

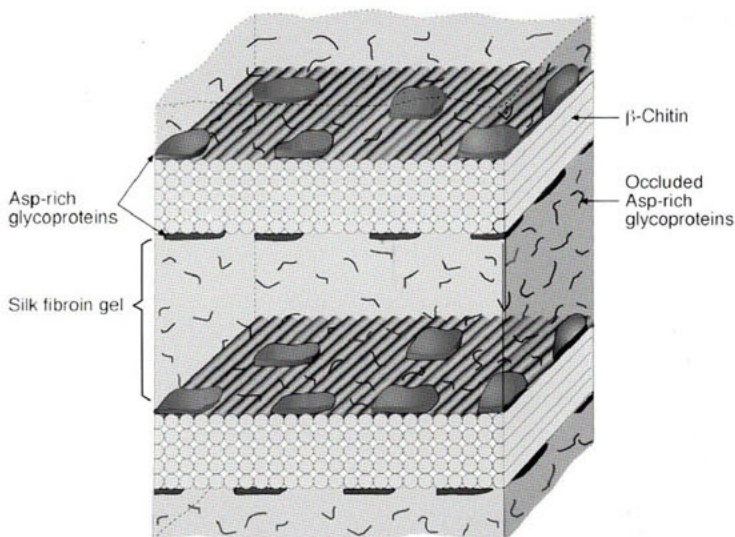


Figure 6. Model of the nacreous layer organic matrix, proposing that chitin and acidic macromolecules constitute the major framework constituents, and that the silk fibroin component forms a gel between the layers (Levi-Kalishman et al. 2001).

This contrasts with the overall amino acid composition of the soluble components in the matrix. This dichotomy may be due to the fact that only with massive and rapid fixation of the proteins in a gel after separation by electrophoresis can the highly acidic proteins be visualized (Gotliv et al. 2003). The one highly acidic protein that has been sequenced from mollusk shells has a fascinating domain-like structure that repeats itself many times (Sarashina and Endo 1998). It will be most interesting to discover its function.

Determining the functions of matrix proteins is the real bottleneck in understanding extracellular matrix mineralization. Several assays are used for demonstrating a protein's ability to modulate mineral nucleation and growth in various ways (Wheeler et al. 1981; Belcher et al. 1996). One that is reproducible and fairly specific was developed by Falini et al. (1996). It assays for aragonite-specific nucleation. It has been used to show that in mollusk nacreous layer matrices, some of the proteins are indeed able to specifically nucleate aragonite (Gotliv et al. 2003). Interestingly, in this *in vitro* assay, the aragonite is nucleated via a transient amorphous calcium carbonate (ACC) phase that also does not contain tightly-associated water molecules. It has been shown that mollusk larvae also produce their shells via an ACC transient phase (Weiss et al. 2002).

Biologically controlled intercellular mineralization. This type of mineralization is not widespread. It typically occurs in single-celled organisms that exist as a community. At first glance, intercellular mineral formation appears to be a variant of extracellular mineralization. In this case, however, the epidermis of the individual organisms serves as the primary means of isolating the site of mineralization (Mann 2001). As shown in Figure 7, the epithelial substrate reproducibly directs the nucleation and growth of

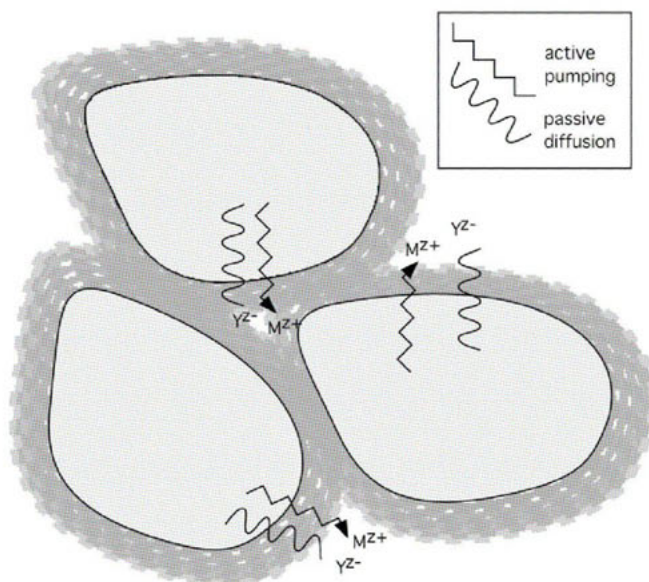


Figure 7. In biologically controlled intercellular mineralization, the epithelial surfaces of cells are used as the organic substrate for nucleation and growth with a preferred orientation. Cations are pumped out of the cell and fluid compositions are regulated to maintain control over the biomineral type and extent of growth.

specific biomineral phases over large areas of cell surfaces. Mineralization between cells can become so extensive as to completely fill the intercellular spaces, thus forming a type of exoskeleton. At first glance, this might appear to be a type of biologically induced mineralization, but studies have shown that the epidermis of individual organisms directs the polymorph and shape of the biomineral that forms. An example is found in calcareous algae that nucleate and grow calcite with a *c*-axis orientation that is perpendicular to the cell surface (Borowitzka et al. 1974; Borowitzka 1982).

Biologically controlled intracellular mineralization. Controlled mineralization can also occur also within specialized vesicles or vacuoles that direct the nucleation of biominerals *within* the cell. This is a widespread strategy. These compartmentalized crystallization environments govern the resulting biomineral composition and morphology. In this situation, the cell has a high degree of control upon the concentrations of cation and anion biomineral constituents in an environment where an organic matrix may also be active as a nucleating template. The compartment membrane also regulates the pH, $p\text{CO}_2$ and—at least to some extent—minor and trace element compositions. Indeed, the mineralized structures that develop from intracellular processes often exhibit highly intricate species-specific morphologies.

The schematics in Figure 8 show that the designation “intracellular mineralization”

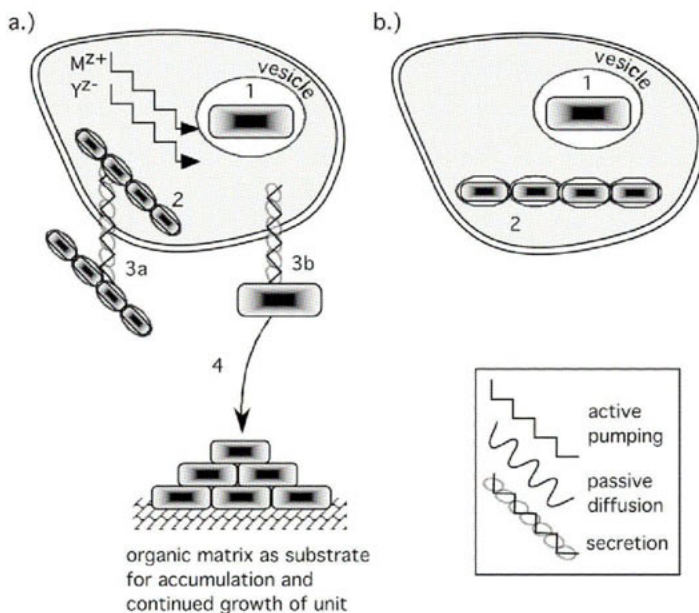


Figure 8. Schematics of biologically controlled intracellular mineralization shows that nucleation occurs within the cell in a specialized vesicle. a.) Biomineral is nucleated (1) within a compartment in the intracellular environment. These growth units may be assembled intracellularly (2) for subsequent secretion (3a) or secreted as an individual unit (3b) for subsequent organization into a higher order growth structures as a massive or organic-mineral composite. b.) In a less frequently used type of mineralization, the biomineral unit remains within the cell (1) as a single growth unit or is organized intracellularly (2) into a higher order structure.

is a broad concept that encompasses a number of fates for the initial compartment-based precipitate. Figure 8a illustrates the general scenario whereby biominerals form intracellularly before eventually becoming extracellular. These structures may leave the cell as individual units or be preassembled prior to extrusion through the membrane. An example of the latter is used by the Haptophyte algae. These organisms preassemble the single crystal segments of calcite intracellularly into a coccolith structure before passing the mature sheath through the cell membrane. Biomineral is transported through the cell membrane by two general means. First, the vesicle(s) or vacuole(s) may migrate to the membrane where the mature biomineral as an individual or pre-assembled structure is extruded by exocytosis. In an alternative process, the compartment membrane fuses with the plasma membrane, and a "premature" biomineral becomes exposed through a membrane breach (Watabe and Kingsley 1989).

Species-specific fates await the secreted crystals in the extracellular environment (Figure 8a). In a relatively straightforward usage, biominerals can be immediately employed without further growth or modification. For example, the mature coccolith structures extruded by Haptophyte algae are immediately used to enclose the cell (Brownlee et al. 1994; de Vrind-de Jong et al. 1994; de Vrind-de Jong and de Vrind 1997; Young and Henriksen 2003). Similarly, the silica mineralizing algae possess vesicles that appear to mold the amorphous silica into pre-fabricated scales prior to release (Watabe and Kingsley 1989).

The release of vesicular biominerals can also mark the beginning of a secondary assembly process whereby the biominerals interact with an extracellular organic matrix (Figure 8a) to become an ordered extracellular structure. Some miliolid foraminifera form bundles composed of an array of oriented crystals. Each crystal is enveloped by organic material, then bundles are passed through the cell membrane by exocytosis. Other miliolids accrete pre-formed crystals and matrix materials onto extracellular surfaces to form a loosely packed wall structure in a "stack-of-bricks" process (e.g., Berthold 1976; Hemleben, et al. 1986).

Echinoderms are perhaps the most surprising exploiters of an intracellular strategy to form huge mineralized products, some of which are centimeters long. These form within a vesicle that is the product of many cells fusing their membranes. The mineral is exposed to the environment only if and when the membrane is degraded (Markel 1986). Although the spicular structures of sea urchin larvae begin as amorphous calcium carbonate, the final products are typically single crystals. These biominerals often express a preferred crystallographic orientation and have smooth curved surfaces (Beniash et al. 1997; Beniash 1999; Wilt 2002).

Not all mineralized structures of intracellular origin become extracellular. Figure 8b shows that the biomineral-containing vesicles may form intracellularly and subsequently stay within the cell. While this mineralization process is not widely used by many phyla, for some organisms, it is a unique and important strategy. Perhaps the best example is exhibited by the magnetosome-producing bacteria. These structures are membrane-bound, euhedral crystals of magnetite or greigite that assemble via their magnetic fields to produce biomineral "chains" (Bazylinski 1996; Schüler and Frankel 1999; Bazylinski and Frankel 2003). Intracellular mineralization is also found in the radiolarians. These organisms form siliceous spicules within cytoplasmic extensions via numerous small vesicles that stream up to the cytoplasmic compartment (Simpson and Volcani 1981; Simkiss 1986). Plants also use intracellular mineralization processes to deposit calcium as oxalate crystals (Raven et al. 1982; Webb 1999; Skinner and Jahren 2003). Figure 9 illustrates an example of these unusually-shaped crystals of calcium oxalate monohydrate (whewellite) produced by the plant *Psychotria*.



Figure 9. Calcium oxalate monohydrate (whewellite) crystals produced by the plant *Psychotria*. Scale bar 10 microns. (From the collection of the late H.A. Lowenstam).

The walls, or frustules, of diatoms are also the result of intracellular mineralization. Using a combination of two intracellular processes, diatoms first polymerize silica into specialized vesicles on an organic matrix of polypeptides (Swift and Wheeler 1991, 1992; Kroger et al. 1999, 2002). Silica is subsequently moved to the interior surface of the cell membrane to form an intracellular frame (Garrone et al. 1981). Matrix controls on frustule patterning are discussed elsewhere (Noll et al. 2002; Pohnert 2002; Sumper 2002; Vrieling et al. 2002). For more details, see Perry (2003).

Comments on biomineralization processes

The framework of processes outlined above is useful to represent end members, but one must realize that as we examine the evolution and biochemistry of mineralization processes in this volume, these simple representations will be developed into a near-continuum of different strategies with considerable complexity. Part of this complexity lies in how organisms control their local environment to make a morphologically and functionally diverse group of minerals through changes in local chemistry and different shaping strategies. However, an additional question for earth scientists involves how biological mineralization processes determine the compositional signatures contained in biogenic minerals. With the increasing reliance upon compositional proxies as indicators of past environmental conditions, this is a problem that needs to be addressed. It is discussed in some detail below.

VITAL EFFECTS

The preservation of biogenic minerals in the fossil record offers wonderful opportunities for reconstructing the ancient environments in which organisms lived, and also for learning more about the evolution of their metabolism and physiology (reviewed in Chapter 11 of Lowenstam and Weiner 1989). What exactly can be learned, however, also depends upon how much we understand about the biomineralization processes

involved in skeleton formation and the states of preservation of the fossil material being analyzed. Much attention has been paid to the latter when it comes to fossils extracted from strata now on the continent, but surprisingly little attention seems to be paid to the preservation states of shells of marine organisms in deep-sea sediments. We will focus, however, on the many open questions that concern the signals embedded in biogenic minerals and in particular questions related to the biological processes overriding the environmental signals—the so-called vital effect (Urey et al. 1951).

The first quantitative use of biogenic minerals for extracting information about the environment of formation was the demonstration that the stable oxygen isotopic composition of some taxa (mollusks in particular) reflects in part the temperature of the water in which the mollusks lived (Urey et al. 1951). Epstein et al. (1951) noted however that not all biogenic minerals are deposited in isotopic equilibrium and that “the presence of a physiological effect in the case of certain groups of animals such as the echinoderms and corals, and plants such as a coralline algae, has seemed probable” (p. 424). This so-called “physiological effect” in time came to be referred to by the geochemical community as a “vital effect.” Furthermore, because most geochemists investigated biogenic materials to reconstruct the paleoenvironments in which animals lived, the vital effect, just like post-burial diagenetic effects, represented a severe hindrance to their goals.

To this day, the most common approach used to resolve the problem involves identifying the taxa that faithfully record environmental parameters while avoiding the others. This empirical approach has the obvious advantage that while one uses these apparently “reliable” organisms, there is no need to worry about the other organisms with their associated complications. The problem is that in the absence of a deep understanding of the vital effects, it is well nigh impossible to know when the recording is really faithful, particularly when differentiating between equilibrium and small non-equilibrium effects. This problem has become acute in the last decade with the realization that the climate is changing and that one of our most promising means of assessing the significance of this change is to understand the fine details of past climatic changes. Understanding vital effects is therefore of considerable current importance.

At this point, we do not have the answers, and a perusal of this book describing the current state of the art in the field of biomineralization will probably not provide many answers. This is not because the subject matter is too difficult to resolve, but because the interests of most of the scientists investigating the field of biomineralization are elsewhere. Very few members of the geochemical community have made this their major interest, but those who have made very significant contributions (e.g., Erez 1978; McConnaughey 1989). A much more concerted effort, however, is needed.

Two basic categories of vital effects

Kinetic effects. McCrea (1950) investigated disequilibrium effects in an *in vitro* system in which calcium carbonate was precipitated under varying conditions. The greatest departures from equilibrium were found when the precipitates formed rapidly at temperatures below 15°C or above 60°C. Epstein et al. (1953) identified this potential problem in biogenic calcium carbonate when they noted that the calcium carbonate deposited by abalones, in an attempt to fill up holes that had been drilled in their shells, was not in oxygen isotopic equilibrium with the water in which the abalones lived (see also Epstein and Lowenstam 1954). Although the effect was small, it was clearly identifiable. They attributed it to the rapidity with which the material was laid down. Since then many different observations of diverse organisms have been reported in which mineral laid down rapidly appears to be out of isotopic equilibrium (Weber and Woodhead 1970; Land et al. 1975; Erez 1978; McConnaughey 1989a; Ziveri et al. 2003).

The mechanisms responsible for this may be due to varying kinetics of the uptake, transport and/or deposition stages of mineral formation. Clearly the issue is complex.

Taxonomic effects. Urey et al. (1951) also recognized that certain phyla may not deposit their skeletal material in isotopic equilibrium with the environment, and within a few years it was clearly demonstrated that some phyla completely control their isotopic compositions (Craig 1953), as well as their Mg and Sr skeletal contents (Chave 1954; Blackmon and Todd 1959; Lowenstam 1963). Perhaps the best example is the Echinodermata (Weber and Raup 1966), although it has been noted in the echinoderms as well as other taxa that superimposed on the vital effect are variations that do reflect environmental changes (Weber and Raup 1966). At the same time other phyla were declared equilibrium depositors and hence useful for paleoenvironmental reconstructions. These were mainly the mollusks, the brachiopods and the planktonic foraminifera. It has subsequently been found in these phyla as well, that the situation is more complicated, with some members of the phylum being faithful recorders, and others not (Duplessy et al. 1970; Carpenter and Lohmann 1995; Letizia et al. 1997). Thus the concept of good and bad species was introduced. This designation, however, only applies to a given proxy. Many marine mollusks and planktonic foraminifera deposit their shells in isotopic equilibrium with the environment (Epstein et al. 1953; Erez and Luz 1983), but most do not have Sr or Mg contents that are in equilibrium with the environmental water in which they live (Elderfield et al. 1996). The mechanisms involved are therefore obviously different for different shell properties, and obtaining an in-depth understanding of this complicated phenomenon is by no means simple. In fact at this juncture, the key question to be addressed is probably how equilibrium is obtained, rather than how is non-equilibrium obtained? The latter is the rule, and the former is the exception to the rule.

Equilibrium with the environment—the real puzzle

It has been widely noted that in almost all organisms that control mineral formation, and the mollusks are no exception, the mineralization process takes place in isolated compartments within cells or between cells (Lowenstam and Weiner 1989; Simkiss and Wilbur 1989). Furthermore, all the different cells responsible for mineralization have to transport the raw materials to the site of deposition, which may be remote from the ultimate source of the ions, namely the environment. Often the ions are stored temporarily in membrane-bound vesicles and then re-dissolved. From this perspective, it is not surprising that equilibrium can actually be maintained between the shell and the environment. If we could understand how this is achieved, we would probably understand much more about the reasons for non-equilibrium.

In this section, we will try to analyze this issue, focusing more on the formation of the mineral phase itself rather than on the uptake, transport and temporary storage processes. See Erez (2003) for details on these important aspects, as well as for a discussion of the vital effect in this context.

Over the last two decades, the problem has become more compelling as more and more properties of biogenic minerals are being used as proxies for different aspects of the paleoenvironment. In the 1950s, the pioneering work was done on oxygen and carbon stable isotopic compositions, and soon afterwards the variations in minor elements (particularly strontium and magnesium contents) were also measured (Chave 1954; Lowenstam 1964). Today more than 10 proxies are known for biogenic minerals that are purported to monitor many different environmental parameters in addition to temperature. Each proxy may have its own vital effect story. We will approach the subject by discussing aspects of the biomineralization process in relation to possible vital effect mechanisms for different proxies.

Carbonic anhydrase. This enzyme essentially catalyses the hydration and dehydration of $\text{CO}_2(\text{aq})$, which in the absence of the enzyme is a rather slow reaction (Simkiss and Wilbur 1989). If it is present at the site of deposition and is working efficiently, it should not only overcome the slow hydration of aqueous CO_2 , but also eliminate any kinetic isotopic fractionation at least with respect to oxygen (McConnaughey 1989a). Carbonic anhydrase is widely distributed (e.g., it is present in corals; Ip et al. 1991) and in the gland responsible for eggshell formation in birds (Gay and Mueller 1973). It is known indirectly to be involved in mineralization as an inhibitor of the enzyme to reduce the rate of mineralization (Goreau 1959). It has been identified in the membrane of the spicule forming vacuole in the gorgonian, *Leptogorgia* (Kingsley and Watabe 1987). In fact, a special form of carbonic anhydrase has been identified as a matrix component in mollusk shells (Miyamoto et al. 1996). This form is special in the sense that the carbonic anhydrase sequence is spliced into a shell matrix protein. So this enzyme really is at the site of mineral formation in mollusks. Is this the secret of equilibrium deposition of oxygen isotopes in mollusk shells?

The medium from which mineral forms (see also Erez 2003). There is little information on the chemistry of the medium from which minerals form in biological environments. Some studies involving mollusks analyzed the fluid between the organ that forms the shell (the mantle) and the shell itself and showed that it has a chemical composition close to seawater (Crenshaw 1972). It is not clear, however, whether this extrapallial fluid really is the medium from which mineral forms. There is much indirect evidence based on analyses of the trace elements in carbonate-containing biogenic minerals that the composition of the medium from which mineral forms does not generally reflect seawater composition. A survey of Mg and Sr contents in mollusk shells showed that members of almost all the 5 major classes control the amounts of these two elements that enter the shell (e.g., Table 2). Only the chitons have Mg and Sr contents that correspond to equilibrium deposition from seawater (Lowenstam 1963). Recently, Cohen et al. (2002) proposed that the reef corals changed the composition of the medium over the diurnal cycle. At night, this fluid has a composition that is approximately sea water but becomes significantly out of equilibrium during the day when symbiotic photosynthesis is active (see Cohen and McConnaughey 2003).

It is always tacitly assumed that mineral deposition must occur from a saturated solution. Saturated it must be, although it is conceivable that a strategy for controlling mineral deposition at the appropriate site may be to localize saturation only at that site, and maintain under-saturated conditions elsewhere. There is no evidence for this, however. In fact a recent study indicates quite the opposite; there is no solution as such from which mineral forms! A cryo-TEM study of the forming spicule of sea urchin larvae showed that the membrane of the vesicle in which the spicule grows is juxtaposed to the forming spicule. Thus there is little or no space for a liquid phase (Beniash et al. 1999). An alternative is that the crystalline mineral forms from a transient amorphous precursor phase, and in echinoderm larvae at least, the latter appears to be formed in vesicles within cells adjacent to the location of spicule formation (Beniash et al. 1999). The vesicles presumably transfer their mineral content as a colloidal solid phase into the site where the spicule forms. Even though this is by no means proven to be a general strategy in biology, the possible implications it presents for understanding vital effects could be most significant.

Transient mineral precursor phases. It has been shown that both mollusk and echinoderm larvae form their aragonitic shells and calcitic spicules, respectively, via a transient amorphous calcium carbonate (ACC) precursor phase (Beniash et al. 1997; Weiss et al. 2002; Addadi et al. 2003). It has not as yet been demonstrated that adults also form their skeletons in this manner. Note that these phyla are on two completely different

branches of the animal phylogenetic tree, and, as both have opted for the same strategy, the phenomenon may be widespread. Thus, even though it is premature to invoke this strategy as an important factor in explaining vital effects, it is worth examining briefly.

The formation of ACC occurs from a highly supersaturated solution. This can only be achieved if additives are present that prevent deposition of the crystalline phases. One such additive is Mg, and the concentrations required for ACC to form are similar to those found in sea water (Raz et al. 2000). It has also been shown that certain proteins are able to induce the formation and stabilization of ACC in the absence of Mg, which would otherwise almost instantaneously transform into a crystalline polymorph (Aizenberg et al. 1996). So at least in bivalve and echinoderm larval shells, Mg and proteins could play important roles in setting up the initial medium from which crystalline calcium carbonate forms. In fact *in vitro* experiments with proteins extracted from sea urchin larval spicules show that Mg has to be present for these proteins to induce ACC formation, although in lower amounts than is present in seawater (Raz et al. 2003). So in this case, a cooperative effect seems to exist between these specialized proteins and Mg.

If indeed the ACC precursor strategy turns out to be widespread in biology, this discovery will have many possible implications for vital effect mechanisms. Under what conditions can isotopic equilibrium be achieved during the transformation of ACC to aragonite or calcite, and what would be the differences in concentration of say, Mg, in the precursor versus mature phases? Interestingly, it has been noted that the transient forms of ACC are not hydrated in the two cases that have been studied (Addadi et al. 2003), as opposed to the stable forms of biogenic ACC. This lack of hydration would reduce the possibility of oxygen isotopic fractionation during the transformation process, as the major factor would be whether or not all of the ACC is converted into the mature crystalline form. If water is present and has to be removed during the transformation, then the efficiency of the process and the extent to which this water is in equilibrium with the environment, become important in terms of oxygen isotope fractionation. See Aizenberg et al. (2003a) for interesting observations and a discussion of this subject.

McCrea (1950) did show that when calcium carbonate forms very rapidly from seawater at temperatures below 15°C or above 60°C, variations in the oxygen isotopic composition of the precipitates occur of up to 5 ppm. He described these precipitates as gelatinous, as opposed to the coagulating precipitates formed within that temperature range. It would be interesting to repeat these type of experiments, while characterizing the atomic structure of the precipitates particularly in terms of atomic order. Could ACC be involved?

Adkins et al. (2003) studied the variations in oxygen and carbon isotopic compositions in deep sea corals. They noted that the most negative values for both oxygen and carbon isotopic compositions are at the trabecular centers and proposed an interesting mechanism to explain the vital effect related to the presence of a pH gradient. The assumption that they clearly state is that the mechanism of mineralization is the same for all parts of the trabecula. Is this really true? There has been considerable discussion about the precise mineral phase at these centers. An electron diffraction study indicates that calcite is present at the trabecular centers (Constantz and Meike 1989). To our knowledge, this observation has not been confirmed. Despite the real risk of speculating about speculations, it would be interesting to consider the implications if ACC functioned as a transient precursor phase of aragonite at the trabecular centers. Proving or disproving this will be a challenge because of the transient nature of ACC.

Biogenic mineral phase. Surprisingly, many open questions still remain regarding the detailed nature of the mineral phases formed by organisms. We submit that a better

understanding of some of these questions will shed light on some vital effect mechanisms.

1. The mineral phase itself: The crystalline mineral phases produced by organisms are generally well known. If, however, a skeletal tissue contains both a crystalline phase and an amorphous phase, the latter may easily be overlooked, especially if only X-ray diffraction is used to identify the bulk mineral phase. Such juxtapositioning of crystalline and amorphous carbonate minerals has recently been reported in sponge and ascidian spicules (Aizenberg et al. 2003b). It is well known, for example, that within foraminiferal shells variations in Mg contents can differ markedly from one location to another (Bender et al. 1975; Duckworth 1977; Brown and Elderfield 1996). The structural properties of the high Mg calcite phase may be quite different from those of the low Mg phase. It is conceivable that even the relative proportions of the different phases may have a direct bearing on temperature-related effects, as they do for the proportions of calcite and aragonite in some mollusk shells (Lowenstam 1954).
2. Surface to bulk ratio of individual crystals: The surface of an individual crystal is a prime location for adsorption of trace metals. In biology the crystals are often enveloped in an organic layer (the matrix), which may or may not interfere with the adsorption of the metals. The surface areas of large crystals in relation to their bulk (volume) are small, and thus complications due to trace element adsorption on the crystal surfaces are less likely. This is not the case for small crystals. The extreme example is bone. Bone crystals have the shape of extremely thin plates, just a few nanometers thick (Weiner and Price 1986). Thus mature bone crystals are only some 15-20 atomic layers thick, and a major proportion of the atoms are disordered just because they are at, or close, to the surface. These are therefore preferred sites for metal adsorption. Biogenic aragonite crystals are almost always much smaller than biogenic calcite crystals, with cross-sectional dimensions of the usually needle-shaped crystals in the micron range (Treves et al. 2003). The one major exception is the aragonite of the nacreous layer of mollusk shells, where the crystals are much larger. Thus knowledge of the surface to bulk ratio of the biogenic tissue being used for proxy analysis may shed light on whether or not surface adsorption of trace metals is significant compared with incorporation within the bulk. Such variations may differ subtly between taxa.
3. Extent of crystalline order and disorder within the bulk: A single crystal contains defects over and above the defects present at or close to the surface of the crystal. For the trace elements that are included in the bulk of the crystal, it is of interest to differentiate between those that are located at grain boundaries and/or dislocations and those that substitute for a major ion in a lattice location. This has been checked for Mg in several biogenic calcites by analyzing the total Mg contents and the Mg content based on the peak shift in X-ray diffraction. The latter is only due to Mg present in lattice positions. It was found that in all cases the Mg is almost entirely in the lattice position (Raz et al. 2000). This need not always be the case. Knowing that for, say, foraminiferal calcite, it also is only in the lattice, will provide greater assurance that the trace element distribution can be characterized by a unique fractionation factor. Alternatively, the relative distributions of a specific trace element between the two sites may explain species variations in part.
4. Occluded macromolecules: Echinoderms, mollusks, brachiopods, calcareous sponges and ascidians are all known to occlude macromolecules within their

crystals (Berman et al. 1993). These are usually glycoproteins, and it has been shown by single-crystal, high-resolution X-ray diffraction analyses that they are often located along specific crystal planes. Despite the fact that they are very large compared to the atomic order within the crystal, the glycoproteins do not cause major dislocations. Some disorder does result, as can be detected by line width broadening. These sites (probably indistinguishable from grain boundaries) within the crystal bulk are also possible locations for trace metals. The presence of occluded macromolecules and ions influences the solubility of the mineral phase, and this may directly bear on the mechanisms of differential dissolution of different genera of foraminifera and coccolithophoridae observed in deep-sea sediments.

Crystal shape. The shape of a crystal reflects the nature of the environment in which it grows. In biology this environment, and hence crystal shapes, are generally very well controlled. Detailed analyses of crystal shapes and, in particular, the exact crystal faces expressed can shed much light on the mechanisms of growth. If a very unstable face is expressed, then some process must be responsible for its stabilization. The unusual face may be a nucleating plane, as has been suggested for the formation of certain types of calcium oxalate monohydrate crystals in plants (Bouropoulos et al. 2001) and calcareous sponge spicules (Aizenberg et al. 1995). Another example of unstable faces being expressed occurs in the coccoliths (Young et al. 1999; Young and Henriksen 2003). These faces could in part explain different solubilities of coccolith calcite between species, as unstable faces are more soluble than stable ones. Curved surfaces of calcite are quite common, especially in cases where the crystal grows in a vesicle. These surfaces may be curved down to the atomic level. How such a curved surface is stabilized is not known, nor are its solubility characteristics. In general, detailed characterizations of crystal surfaces may be most helpful in understanding modes of formation and, in turn, vital effects, as well as solubility properties.

Ontogenetic variations. Variations in mineralization are known to occur in many different genera, including foraminifera. These too need to be understood in order to determine whether or not they can contribute to the complexities of equilibrium and non-equilibrium deposition. It may also be possible that the change in mineralization mechanism during growth is not a switch from one mechanism to another, but the addition of another mechanism in the juvenile and adult stages. Lowenstam and Weiner (1989) did raise this possibility for the scleractinian corals vis-a-vis the trabeculae centers of calcification.

Crystal maturation. Once formed, crystals may continue to change over long periods of time, especially if their surface to volume ratios are very high. This is the case in bone, where a sintering process occurs even within the lifetime of the animal and continues after death (Legeros et al. 1987). Thus diagenetic processes, even in the deep sea may not only involve dissolution, but also sintering. Obviously if in a closed system, this should have little effect on proxies. It can, however, complicate efforts to understand the basic mechanisms of deposition vis a vis proxies, as the mature mineral may not faithfully reflect the mineral phase formed initially. Chemical change may also occur during diagenesis. It has been shown, for example, that aragonite formed secondarily in coral skeletons even while the coral was still submerged (Spiro 1971).

Concluding comment

Given our present state of knowledge, the prospects for finding simple, robust explanations for different vital effects in terms of biomineralization mechanisms may seem hopeless at worst, and challenging at best. This, in our opinion, is most likely not

the case. More than anything else, the apparent magnitude of the problem is due to so few studies having been carried out with this purpose in mind. We believe that once a critical mass of research gets underway, pieces of this puzzle will begin to fall into place. We concur with the opinions of some (Schrage and Linsley 2002) that as the importance of paleoceanography continues to increase because of global change effects, and with it the wide use of proxies for reconstructing the paleo-ocean environment, the need for understanding the basic mechanisms of mineralization that pertain to these problems is paramount. We hope that as our understanding of "vital effects" improves, this term which has so often been used as "a façade hiding our ignorance" (Lowenstam and Weiner 1989), will become instead a vehicle for understanding important aspects of the biomineralization process itself.

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