8.04 Biomineralization

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8.04.1 INTRODUCTION

Biomineralization is the process by which living forms influence the precipitation of mineral materials. The process creates heterogeneous accumulations, composites composed of biologic (or organic) and inorganic compounds, with inhomogeneous distributions that reflect the environment in which they form. The products are, however, disequilibrium assemblages, created and maintained during life by dynamic metabolism and which, on death, may retain some of the original characteristics. The living forms discussed in this chapter produce carbonate, phosphate, oxalate, silica, iron, or sulfur-containing minerals illustrating the remarkable range of biomineralization chemistries and mechanisms. Biomineralization, in the broadest use of the term, has played a role in Earth cycles since water appeared on the surface. The general perception that the onset of biomineralization coincides with the appearance of fossils that left "hard parts" amenable to analysis is marked geologically as the dawn of the Cambrian. At least for some of us, the origins of life, and possibly biomineralization, go back 3.8 Gyr. The startling chemical and biological range encompassed by the term biomineralization implies that life forms have adapted and altered geoenvironments from the beginning, and will continue to do so, an essential ingredient for establishing and maintaining Earth's environment in the future as in the past (Figure 1).

8.04.1.1 Outline of the Chapter

It is only with the advent of more sensitive, higher-resolution techniques that we can identify the exact mineral components, and appreciate the precision control exercised by the life forms on their mineralized structures, and the potential that their formation and evolution are responses to the environment and climatic, or any other pervasive geochemical changes. We first present the crystal chemistry, or mineralogy, of some of the common minerals that are encountered in biomineralization (Section 8.04.2) and then provide examples from the range of life forms. We start with the Archea and Bacteria although we are just beginning to investigate their mineralization processes. However, these primitive forms were probably the first to generate the mechanisms that led to the accumulation of elements, the precursor to the formation of biominerals. The details of the mechanisms and the ranges of possible creatures in these classes remain to be fully defined and understood. However, we consider them an essential base to

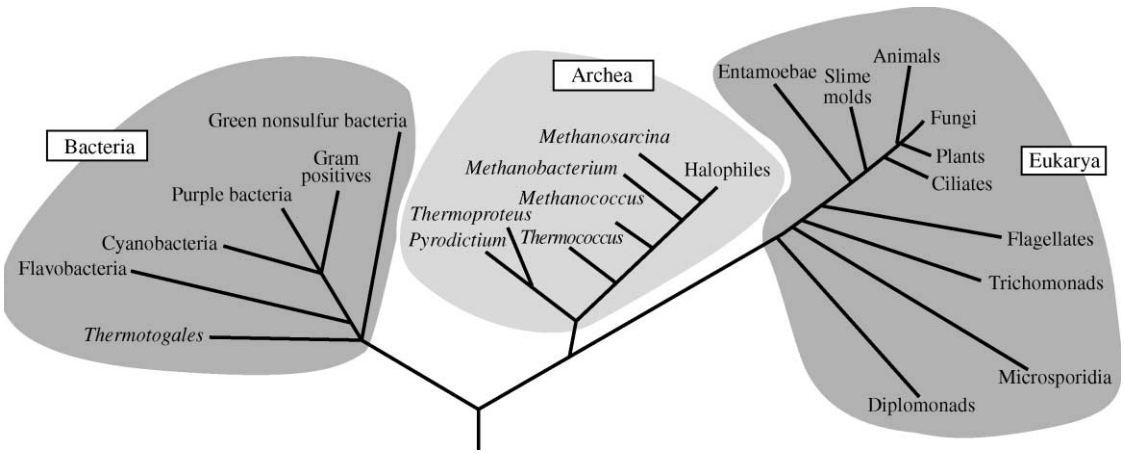


Figure 1 The "tree of life" (source Raven *et al.*, 1999, figure 13.8, p. 270).

any biomineralization discussions. Further, and perhaps most intriguing, many other biomineralizing forms incorporate them as symbionts. Following these opening sections, we move onto $CaCO_3$ deposition associated with cyanophytes (photosynthetic cyanobacteria) that marks the end of the Precambrian period, ~0.6 Gyr ago (Riding, 1982). At the start of the Cambrian period (~570 Myr ago), calcareous skeletons appear; rapidly and dramatically (within 40–50 Myr) some form of biomineralized structures appear in all existing phyla. Since that time only corals, some algae, and the vertebrates have developed new skeletons in the marine habitat (Simkiss and Wilbur, 1989); thus, the obvious developments of biomineralization are concentrated into ~1% of Earth's history, along with all other major diversifications of life. We present information, and include references to works we think will be most helpful to geochemists, on selected invertebrate and vertebrate skeletons many of which have a vast literature available. We select a few structures, such as teeth (in chitons and humans), because they are examples of different mineralizing systems whose tissue textures, and mechanisms, are unique, and because they may be, or have been, important to geochemical studies. In looking to future opportunities we include brief introductions to otoliths and antlers, because they offer novel sampling sites to test geochemical variations in the present environment.

The importance of the survival of land-based communities on plants suggested that our purview must include plant biomineralization, the materials, mechanisms, and strategies. For our summary we ask "why biomineralize?" and offer a few suggestions based predominantly on plant researches. The reasons for biomineralization we have outlined are appropriate to other mineral-producing life forms, and have often been discussed. However, in the process of reviewing the evolutionary development, some novel approaches, if not answers, to this basic question are preferred.

8.04.1.2 Definitions and General Background on Biomineralization

We classify biomineralization in our examples either as extracellular or as intracellular (Pentecost, 1985a) and include the specific cell types if known. We follow the standard definitions of Borowitzka (1982) that extracellular biomineralization involves inorganic, often crystalline, materials forming on the outer wall of the cell, within the cell wall, or in the immediate surrounding tissue areas, and is the usual type of biomineralization. Intracellular biomineralization is mineral formation within the cell such as the calcite formation for the group of algae the coccolithophoridae (Section 8.04.3.4.3). Another illustration of the diversity of intracellular biomineralization is found in the freshwater green alga *Spirogyra hatillensis* T. that contains calcium oxalate inclusions. The inclusions are not associated with the central vacuole, but instead are in cytoplasmic strands (Pueschel, 2001).

With the advent of eukaryotes, subdivisions within the cell, or compartments, were created. Within these subcellular compartments, or specialized anatomical sites, mineralization may be facilitated, with the result that biomineralization became more extensive and diverse. By creating lipid membranes, the eukaryotes could selectively "pump" ions and bioaccumulate them in a small volume. In plants, this subcellular compartment is usually a vacuole (Matile, 1978), whose membrane may serve both as a pre-existing surface for nucleation and as the ultimate determinant of mineral shape, as the crystal(s) grow to fill the vacuole (Simkiss and Wilbur, 1989). Most ion pumps translocate ions against electrochemical gradients (Carapoli and Scarpa, 1982) accomplished either by attaching the ion to a carrier molecule that is moving with an electrochemical gradient, or by directly using ATP as an energy source for the translocation. Often described for Ca^{2+} transport, similar transport mechanisms have been suggested to supply the anions that control the onset of mineral deposition in cells (Simkiss and Wilbur, 1989). Like cations, anions are involved in a wide variety of cell activities. Primary among them is the ability of anionic complexes, e.g., carbonate and phosphate, to act as inorganic pH buffers. In support of the anion-supply hypothesis, it has long been recognized that there is a positive relationship between photosynthetic rate (acquisition of CO_2) and rate of calcite biomineralization in algae. Specific studies have shown that when *Corallina officianalis* algae achieve a certain level of photosynthesis, the relationship between calcite biomineralization rate and CO_2 acquisition is roughly linear (Pentecost, 1978).

An organic matrix or pre-existing nucleation surface is usually considered to be the determining feature in many systems, especially the higher biomineralizing systems, such as the vertebrates (Section 8.04.3.7). Organic matrices within biomineralizing plant vacuoles (Webb *et al.*, 1995) provide the sites where "seed" cations bind as loose chelates (Tyler and Simkiss, 1958), and can be alternately soluble and insoluble (Wheeler *et al.*, 1981), or a combination of the two (Degens, 1976). Several nucleation centers may be present within a matrix, and each may grow independently, and perhaps produce similar crystallographically oriented biominerals (Simkiss and Wilbur, 1989). Although animal cell membranes are becoming

well known, a limited number of studies have investigated the protein and lipid compositions of membranes within plant vacuoles. The crystal-associated organics in most biomineralizing systems contain a complex assortment of polypeptides (Miller, 1984; Webb *et al*., 1995) and fatty acids (Jahren *et al*., 1998) that could aid nucleation. In the invertebrates and plants the vacuole effectively separates the crystal from the sap, allowing for chemical control over the environment of crystallization (Webb, 1999). As growing crystals fill the vacuole, the matrix is compressed between neighboring crystallites giving rise to the mosaic or "honeycomb" structure of many biominerals.

Within multicellular organisms, biomineralization is determined by specific tissues and cells, which we describe for the calcium phosphate biomineralization in human teeth (Section 8.04.3.7.4). Within *Thalassia testudinum* (Turtlegrass) silica exists only as isolated deposits within leaves (Brack-Hanes and Greco, 1988), while within *Rheum officinale* M. (Chinese rhubarb), calcium oxalate is confined to the roots (Duke, 1992). Some biomineralized structures, such as the silica "hairs" found on *Phalaris canariensis* (Annual canarygrass) (Perry *et al*., 1984), are associated exclusively with the defense structures of the plant. Similarly, some plants only biomineralize around reproductive tissues, such as the silica-aragonite endocarp surrounding the endosperm within the *Celtis* spp. (Hackberry) fruit (Cowan *et al*., 1997). It has been shown that in sugar cane, different cell types accumulate silicon at very different rates (Sakai and Sanford, 1984).

The favored hypothesis regarding why cells within the same organism biomineralize with different minerals, e.g., calcite and aragonite in bivalves, is that there are different organic moieties which lead to changed nucleation opportunities. For example, permeability of a vacuole membrane in plants may lead to different concentrations of the ions and elements within, or to the inclusion of inhibitors that prevent nucleation of a specific mineral forms (Goodwin and Mercer, 1982).

The biomineralization mechanisms are far from fully investigated and understood for most species, but come close for the coccolithophorids. What is certainly true is that there is not a single or grand scheme or mechanism that is established throughout living forms. What can be taken from this brief overview is that biomineralization entails cooperative efforts involving cells, producing and controlling organic and inorganic molecules that combine, in structurally distinct ways, the ultimate and unique expression of a species. Biomineralization which added inorganic to the usual organic moieties necessary for life increased the possibility for survival in new environments and extended the range of potential ecological niches available on Earth.

8.04.2 BIOMINERALS

8.04.2.1 Calcium Carbonates

8.04.2.1.1 Calcite

The most widely known biomineral is calcite, which forms the "hard part" of many common invertebrates such as corals and mollusks. The anhydrous calcium carbonate $CaCO_3$ is one of the eight mineral end-members with identical crystal structure but different divalent cations, that form the calcite group (Gaines *et al*., 1997, p. 427). These minerals, many almost as common as calcite, all crystallize in the hexagonal/rhombohedral space group, R3c. Figure 2(A), a projection of the three-dimensional crystal structure down the unique "*c*" axis shows the array of the planar and trigonal carbonate ions (CO_3^{2-}). The trigonal species (CO_3^{2-}) is composed of a carbon atom at the center and coplanar with three equidistant oxygen ions. Carbonate ions form layers along the *c*-axis and alternate with the cations in the calcite sequence along the *c*-axis $-Ca-CO_3-Ca-CO_3-$ and every other layer of CO_3 ions points in the opposite direction. Calcium is in octahedral, or sixfold, coordination with six oxygen ions from six different carbonate groups, i.e., the divalent calcium ion has hexagonal distribution. However, the superposition of the alternating directions of the trigonal ions in the third dimension is a distinguishing feature of the structure, and influences the morphology of the crystalline form. Rhombs are the dominant morphology expressed during inorganic crystallization of calcite, and the shape often encountered when the crystals are fractured, or cleaved (Figure 2(B)).

Perfect, clear calcite crystals that showed either hexagonal, or rhombohedral, characteristics fascinated seventeenth-century scientists, and were instrumental in advancing physics and materials science. Cleavage fragments were used in the discovery of double refraction, and the polarization of light in the mid-seventeenth to early nineteenth centuries. The X-ray diffraction studies by Bragg in the twentieth century confirmed the relationship between the optical character and the morphology. Calcite shows amazingly high birefringence, a property directly related to the anisotropy of the structure. The ability to generate plane polarized light from the insertion of an oriented calcite crystal in the beam led to the polarizing microscope, a tool still providing the most useful technique for an initial discrimination of minerals and other crystalline materials, and a quick way to identify calcite. The properties of anisotropy in crystals and the use of polarizing microscopy for

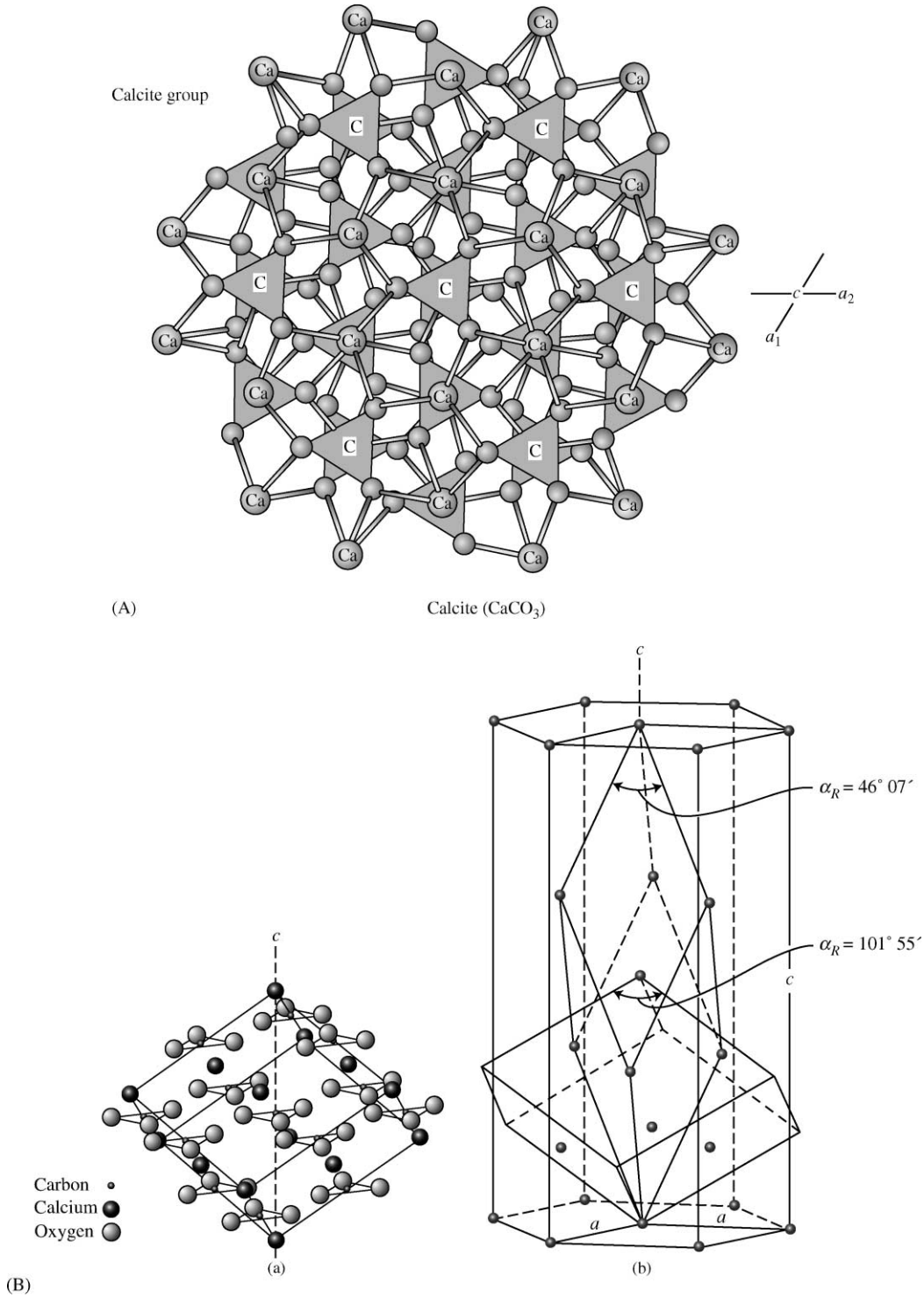


Figure 2 (A) Calcite. The crystal structure projected down the unique *c*-axis showing the hexagonal disposition of Ca and CO_3 ions (Gaines *et al*., 1997, p. 427). (B) Morphological relationships: (a) the arrangement of Ca and CO_3 groups relative to the calcite cleavage rhomb; (b) the true rhombohedral unit cell (steep rhomb) to the cleavage rhomb and the hexagonal cell where $c/a = 3.42$ (Gaines *et al*., 1997, p. 429). (C) Plot of the possible "best fit," 15%, and next best fit, 30%, of cations that could take the place of Ca in sixfold coordination in the calcite structure (courtesy of Stefan Nicolescu, Department of Geology and Geophysics, Yale University).

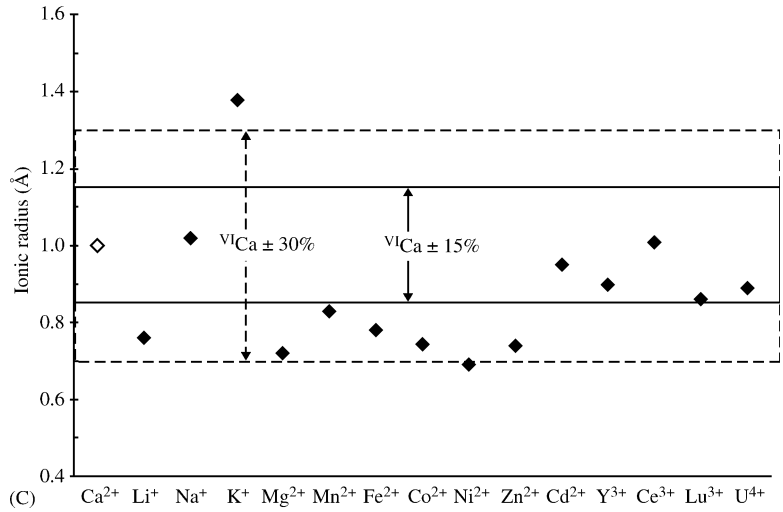


Figure 2 (continued).

complete identification of any crystalline solid is reviewed in Klein and Hurlbut (2000) and in most optical mineralogy texts.

The isostructural calcite group consists of several minerals with the following elements in place of calcium: magnesium, iron, manganese, cobalt, zinc, cadmium, and nickel. All these mineral carbonates have different names and are considered end-members in the series with an ideal composition expressed, e.g., as $MgCO_3$, for the mineral magnesite. These are the elements most likely to be incorporated in calcite whether formed strictly inorganically or precipitated when the elements are bioaccumulated. It should be pointed out that there is virtually no substitution for the carbonate group in calcite group structures (Reeder, 1983).

Figure 2(C) is the result of a calculation that illustrates which cations might fit into sixfold coordination position in the calcite group structures. It is an interesting insight as both light and heavy rare earth elements are possible substitutes for calcium in the calcium carbonate structure, i.e., they plot within ±15% of the calcium ionic size. However, some of the end-members incorporate elements into this crystal structure and are outside this deviation but within ±30%, an expression of the potential physical expansion for this layered crystal structure. These are ionic charge differences important in whether a stable crystalline structure can be produced. Trace amounts of all these ions can be incorporated in calcite and may dictate the morphology of the crystallites. Therefore, the presence and amount of any ions in the environment in which carbonate crystallization occurs may possibly be recorded. However, in spite of the predominance of sodium and potassium in the solutions where biomineralization takes place, neither element is found in calcite to any extent. These single charged species more readily associate with Cl^-, and together with their size (larger than calcium ionic size), mean that they are not usually accommodated in the rhombohedral calcite structure. The formation of any biomineral indicates the composition of the ions available in the surrounding media and habitat with the specific mineral species and form dictated by an input of energy, to overcome the nucleation barrier. The continued availability of ions is essential for growth of the mineral species consistent with the biological needs.

Calcite can easily be identified without a microscope by its ready dissolution in 1 N HCl, often fizzing or forming bubbles indicating the liberation of CO_2. The other isostructural members of the calcite group are less soluble, but since all of these minerals are usually well crystallized, X-ray diffraction and analysis can identify them as a group member. In addition, the diffraction maxima positions can be used to estimate the quantities of different elements incorporated. The diffraction analysis relies on a physical shift in the crystal structure parameters based on the size of the cation, and is not a chemical analysis.

8.04.2.1.2 Aragonite

An equally important carbonate mineral is aragonite, the common polymorph of calcite. Polymorphism of minerals implies the same chemical composition but distinct crystal structure. The aragonite structure also has alternate layers of carbonate groups and cations with the

triangular CO_3 pointing in opposite directions along the *c*-axis, but the cations are in ninefold coordination catering to larger size elements such as strontium, barium, and lead. Aragonite crystallizes in the orthorhombic space group Pmcn and is one of the members of an isostructural group that includes these other cations (Gaines *et al*., 1997, pp. 440–448). Figure 3(a) projected down the crystallographic *c*-axis allows comparison with the calcite structure and the different polygonal arrangement of calcium. The carbon of the carbonate groups in aragonite is very slightly out of the plane (0.026 Å), somewhat displaced along the *b*-axis (0.20 Å), and the plane defined by the three oxygens is tilted ~2.5°. In addition, the cations are puckered, alternating ~0.05 Å above and below the cation plane. Six bonds are made between the calcium with two oxygens in each of three carbonate groups and three shorter bonds with the corner oxygens of three other carbonate groups. The pseudohexagonal array has stacking order along "*c*" of $A(CO_3)_1-B(CO_3)_2-A(CO_3)_1-B(CO_3)_2$, which results in orthorhombic symmetry. Crystals have a prismatic habit, elongated and flattened along the *c*-axis appearing acicular. Most aragonite that appears to be in the form of single crystals is usually twinned, a mosaic composed of discrete differently oriented

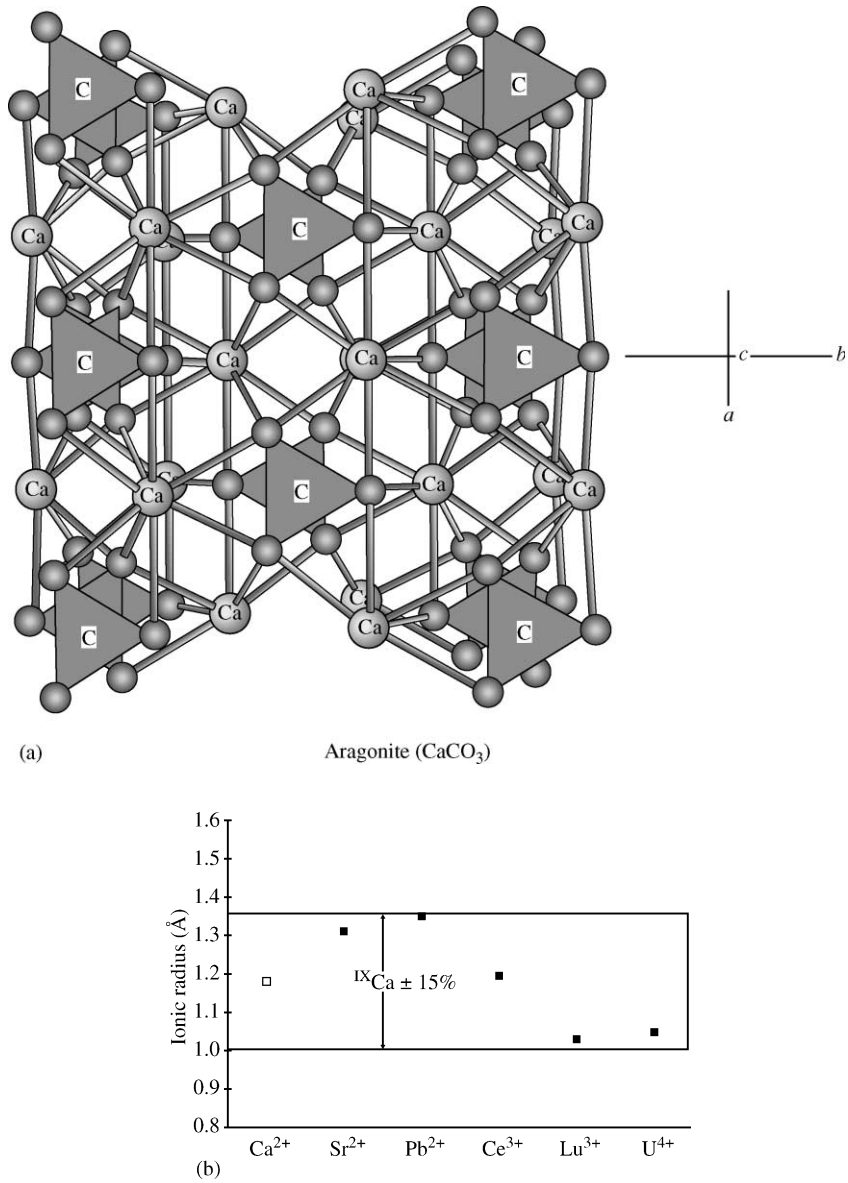


Figure 3 (a) Aragonite. The crystal structure projected down the *c*-axis (Gaines *et al*., 1997, p. 441). (b) Plot of the "best fit," 15%, of cations that could take the place of Ca in eightfold coordination in the aragonite structure (courtesy of Stefan Nicolescu, Department of Geology and Geophysics, Yale University).

portions of the same mineral (with identical crystallographic parameters) and related by strict geometric laws. These polysynthetically twinned aragonites produce lamellae oriented parallel to the *c*-axis that can be detected as fine striations in large crystals.

Some substitution of strontium (up to 14 mol.%), of lead (2 mol.% reported) but no barium has been reported in aragonite, although investigations at elevated temperatures and pressures show almost complete miscibility of these elements in the structure (Gaines *et al*., 1997, p. 442), and $SrCO_3$ (strontionite), $BaCO_3$ (witherite), and $PbCO_3$ (cerussite) are common minerals. A calculated plot (Figure 3(b)) for cations in ninefold coordination shows that this coordination theoretically allows trivalent rare earth elements and quadravalent U^{4+}, and many other elements to be substituents in the structure. Ytterbium, europium, samarium, and radium carbonates with aragonite structure have been synthesized (Spear, 1983).

8.04.2.1.3 Vaterite

A third polymorph of $CaCO_3$, vaterite, may form as an inorganic precipitate, but is most likely to be encountered as the mineral forming in the nacre of mollusks (Section 8.04.3.4.6). The mineral crystallizes in the hexagonal/rhombohedral space group $P6_3/mmc$, and the calcium is in sixfold coordination (Gaines *et al*., 1997, p. 440). It is unstable and often reverts to calcite over time. Elevated temperatures or water high in NaCl will accelerate the transition; in dry environments, vaterite will slowly revert to calcite.

8.04.2.2 Silica

Anhydrous SiO_2, silica, is one of the most common rock-forming minerals, quartz (Frondel, 1960). Large (inches to feet), transparent, and clear, hexagonal crystals (rock crystal) are found in some locations, but the mineral also occurs in a variety of crystalline forms, and may be clear to milky, transparent or opaque, and from white to black, with all shades of colors in between (Rossman, 1994). Quartz is the dominant mineral phase in some rocks, as it is in sands, and in soils, and many of the colored varieties have been used as decorations, and in jewelry, for as long as humans have existed. The earliest tools were made from flint and chert, microcrystalline varieties of quartz, that had all the qualities desired: a hardness of 7 and sharp edges on fracture. Mineralogists have studied all these aspects, and have assiduously determined variations in crystal structure brought about under different temperature and pressure conditions (Graetsch, 1994; Gaines *et al*., 1997, pp.1568–1586). However, it is the low-temperature hydrated variety of silica, opal ($SiO_2{\cdot}n\,H_2O$) which is a biomineral.

8.04.2.2.1 Opal

Opal is composed of differing amounts and arrangements of structural units of amorphous SiO_2, water, and the crystalline polymorphs of quartz, crystobalite, and tridymite (Gaines *et al*., 1997, pp. 1587–1592). A disordered solid, the mineral may display colors due to patchy areas of short-term crystalline order (Figures 4(A) and (B)). Close-packed silica spheres, typically 0.25 μm in diameter, about half the wavelength of visible light, randomly associate producing vacancies and stacking faults containing variable amounts of water in the aggregated solid (Levin and Ott, 1933). Jewelry made from opaline material is cut *en cabachon* to maximize the play of colors.

When totally random, without regularized crystal structural order, a substance is called amorphous. The material will have no discernable X-ray diffraction pattern, an indication of the lack of crystalline order. The biological material has very limited order. A variety known as opal-A with only one diffraction band is identified in samples from deep-sea deposits which are the remains of diatoms and radiolarians (there are also freshwater "diatomites"). Another variety is opal-CT, a discontinuous combination of the crystalline polymorphs of SiO_2, cristobalite, and/ or tridymite, and water (Jones and Segnit, 1971; Jones *et al*., 1966; Graetsch, 1994). Crystobalite and tridymite have multiple modifications of their crystal structures known as high- and low-temperature forms (see Gaines *et al*., 1997, pp. 1568–1586) for more details). Many names have been applied to the different varieties of "amorphous hydrated silica" as the solid responds to the source of the silica and the environment, and takes up locally dictated morphologies. Some silica deposits will change over time with compaction or diagenesis. For example, diatomaceous earth is soft and fine grained with individual diatoms visible with some magnification, while "tripolite" is a variety of diatomaceous earth showing little evidence of diatom remains.

Opalline materials may not show any texture at the level of a polarizing microscope but may appear platy, or fibrous, at the higher resolution of transmission or scanning electron microscopy (De Jong *et al*., 1987). Using magic angle spinning of ^{29}Si, a nuclear magnetic resonance technique, opal-CT was shown to be effectively amorphous compared to the anhydrous silica species, crystobalite and trydimite. However, opal-CT is birefringent with an index of refraction that varies

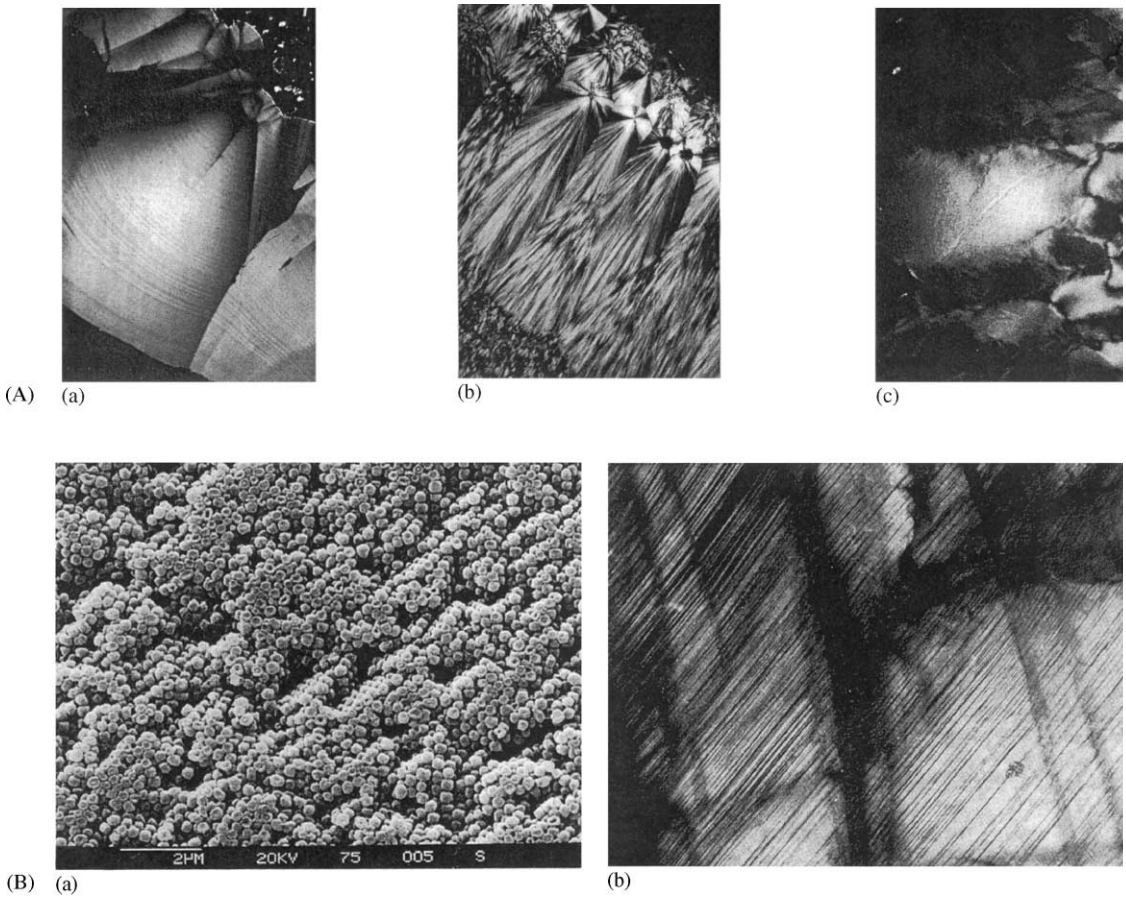


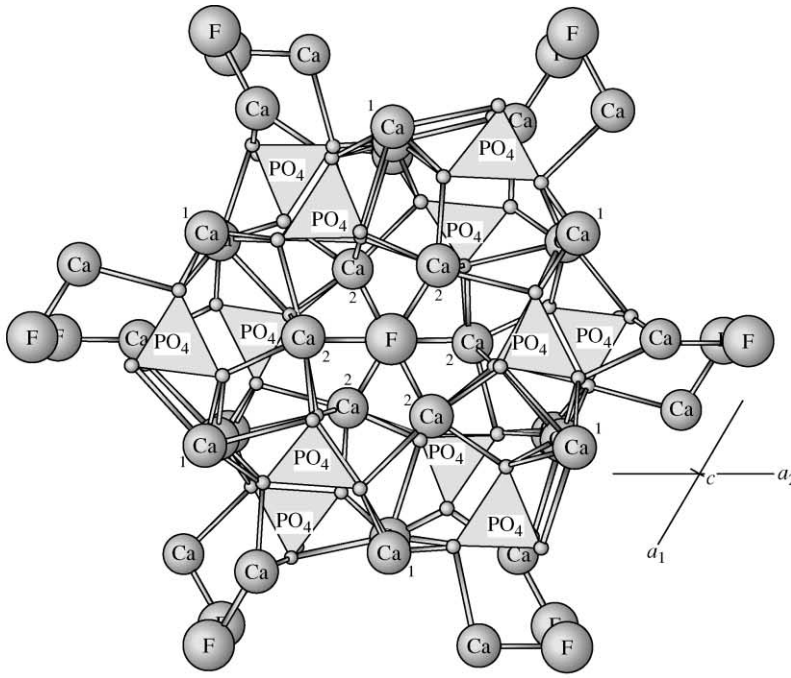
Figure 4 (A) Microradiographs using crossed polars of three forms of opal: (a) opal-C, (b) opal-CT, (c) opal-AN. The long edge of the micrographs is 1.11 mm. (B) Precious opal: (a) scanning electron micrograph and (b) petrographic thin section micrograph, crossed polars. The long edge of the micrograph is 1.11 mm.

from $N = 1.43$ to 1.46. The X-ray diffraction pattern, which shows broadened maxima due to very fine grain size as well as packing variations, will reflect the amount of SiO_2 polymorphs relative to water content. Opal-CT may also be formed in volcanic rocks.

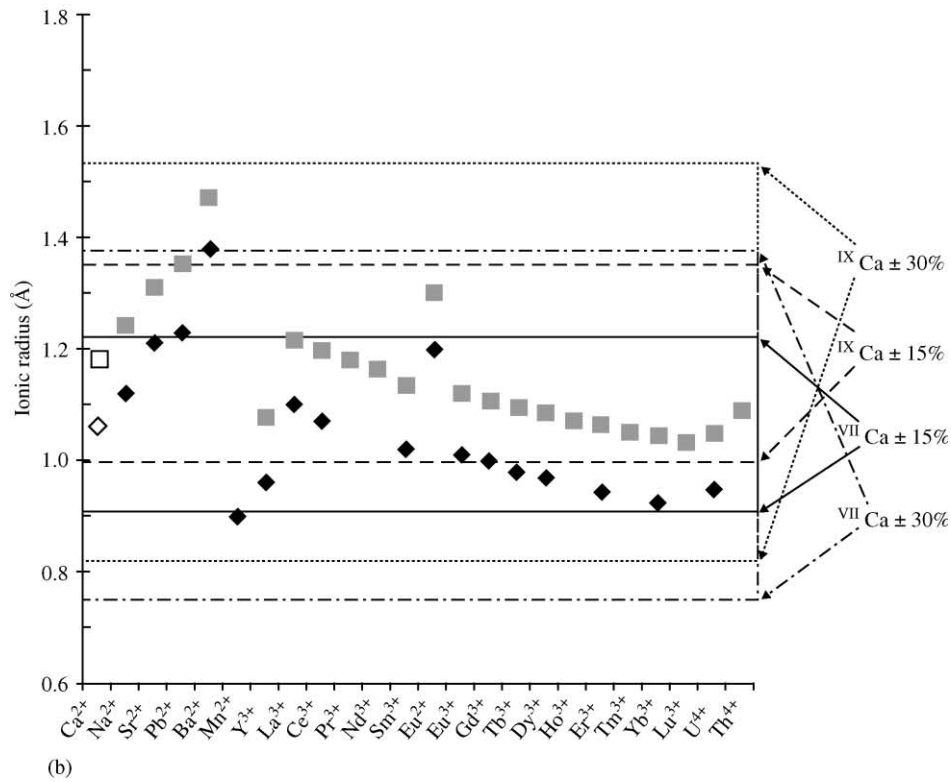
A combination of several different opaline materials may be found juxtaposed. For example, in fossilized wood both tridymite and crystobalite may be determined in a sample while adjacent portions of the sample may be composed of opal-A. The silicified wood may be transparent or translucent, clear and colorless, or white, yellow, red, brown, and black, indicating inclusions of other, usually iron-containing, complexes during precipitation of the colloid or gel. The faithfully preserved structures of fossilized wood suggest that the replacement phenomena are molecule-by-molecule processes that take place under low temperatures and pressures, and require concomitant removal of nonsiliceous compounds but do not disrupt the cellularity of the woody tissues. Alternatively, primary biomineralization by living forms of hydrated silica can produce amazingly delicate structures. The morphology and mechanisms in diatoms, radiolarians, and the phytoliths that occur in grasses and plants are described below.

8.04.2.3 Bioapatite

Apatite is the name for a group of minerals with the general formula $A_5(TO_4)_3Z$ (Gaines *et al.*, 1997, pp. 854–868) in which A is usually calcium, but may be strontium, lead, or barium; T is usually P, but As and V forms crystallize with the same hexagonal or pseudohexagonal crystal structural symmetry, and Z may be OH or one of the halogens. Fluorapatite, $Ca_5(PO_4)_3F$ (Figure 5(a)), is the most common form occurring in trace quantities in igneous, metamorphic, and sedimentary rocks. Often selected for analyses because of the wide range of other elements, specifically the rare earths, uranium, and thorium, that may be incorporated (Skinner *et al.*, 2003).



(a) Fluorapatite ($Ca_5(PO_4)_3$ F)



(b)

Figure 5 (a) Fluorapatite. The crystal structure projected down the unique *c*-axis showing the hexagonal disposition of the Ca ions and PO_4 groups around the F ion. Ca_1 in sevenfold coordination, Ca_2 in ninefold coordination (Gaines *et al*., 1997, p. 855). (b) Plot of the "best fit," 15%, and next best fit, 30%, of cations that could take the place of Ca in sevenfold (◆) and in ninefold (□) coordination in fluorapatite structure (source Skinner *et al*., 2003).

It is easily identified by its optical or X-ray diffraction characteristics as it is always well crystallized. The apatite formed at low temperatures, however, is not well crystallized. For example, the phosphate species in the Phosphoria Formation of western US, though a calcium apatite containing a wide range of other elements, is very fine grained and associated with many other fine-grained or poorly crystalline materials, i.e., clays (Gulbrandson, 1966). It is this low-temperature, poorly crystalline form of calcium apatite that is found in biomineralized tissues. The bioapatite, predominantly a hydroxylapatite, has a formula that can be written as $Ca_5(PO_4,CO_3)_3$ (OH, F, Cl, CO_3).

In this formula CO_3 is shown as a substituent in the PO_4 structural site and in the OH or halogen site. Biological apatites may contain up to 6 wt.% CO_2. For a particular bioapatite sample, it is difficult to determine the actual quantity of CO_2 or how it is incorporated. Bioapatites are so fine grained that spectroscopic and wide and small angle X-ray techniques have been applied in efforts to interpret the possible crystallographic location (Elliott, 2002). In addition, the mineral is usually intimately mixed with bioorganic molecules, and extraction is required before analysis (Skinner *et al.*, 1972). The exact location of carbonate in bioapatites remains a question. If carbonate substitutes for even a small portion of the phosphate in the structure, there must be some lattice disruption as the planar CO_3 ion has a double negative charge compared to the tetrahedral phosphate group that has a triple negative charge. Further, it is the phosphate framework and the associated calcium ions that are responsible for the characteristic hexagonal structure and morphology of the crystals. The spatial and charge differences may give rise to disruptions and vacancies in the structure if CO_3 is incorporated. However, the usual suggestion is that the carbonate is balanced by concomitant substitution of other elements for calcium. Calcium occurs in two different lattice sites: Ca_1 is in sevenfold coordination with the oxygens on the phosphate groups, while Ca_2 is in ninefold coordination with phosphate oxygens and the OH or F. Figure 5(b) compares the possible cationic substitutions for calcium. It shows that virtually all cations whether single, double, or triply charged are of appropriate size to fit into the metal ion sites associated with the PO_4 oxygens. Triply charged species could compensate for carbonate inclusion maintaining local charge balance in the structure. If the carbonate inserts in the OH or halogen site in the structure, similar disruptions, vacancies, or substitutions would be necessary to maintain a balanced apatite lattice.

What is clear is that bioapatites are poorly crystalline, and carbonate may be a major factor affecting crystallinity. Whether the carbonate ion is actually within the lattice in small amounts interfering with crystal structure formation, or adsorbed on the very large surface area of the tiny crystallites, it will affect the stability of the phase and the crystallite size. The crystallites in bone are less than 1,000 Å along the *c*-axis or the long dimension of apatite, and only a few unit cells (<50 Å) in the *a*-dimensions (Elliott, 2002). These very small sizes are typical of biological apatites. Such size and morphology may be an advantage when the mineral is deposited intra-cellularly, or inter-biomolecularly, as it is in bone (Section 8.05.3.7.2). Since mineral resorption is an essential part of normal tissue metabolism maintaining the stability of bone, this mineral/chemical system for biomineralization has been well chosen (Skinner, 2002).

Although essential and predominant in bones and teeth, bioapatites have been found in all living forms including bacteria, invertebrates, and plants (e.g., Macintyre *et al.*, 2000). Bioapatite is also the mineral that occurs in some aberrant or pathological deposits (Skinner, 2000).

8.04.2.4 Iron Oxides and Hydroxides

The most visible iron-containing minerals are the ubiquitous subaerial rusts on rocks. They are ferric oxide or oxyhydroxide flocculants of exceedingly fine grain size that change over time. They may be considered abiotic, but more likely their localization and formation are expressions of the presence of microorganisms whose metabolism depends on iron (Section 8.04.3.1). All organisms from microbes to humans require iron with one exception: fermenting lactic streptoccoci (Ehrlich, 2002, p. 347). Iron is one of the elements utilized for the transfer of electrons in oxidation and reduction reactions. Special proteins, the cytochromes, which have iron-bearing heme groups, or the ferrodoxins, which are Fe–S organic complexes, are prominent cell constituents in aerobic or anaerobic microorganisms and are detected in creatures and plants on up the phylogenetic tree. We will not address the potentially biologically mediated rust mineral formation; instead, we focus on the mineral magnetite, because its formation and properties play important roles in several life forms.

8.04.2.4.1 Magnetite

Magnetite, ideal formula Fe_3O_4, is an intriguing mineral, and not only because it is magnetic. The formula could be written $Fe^{2+}Fe_2^{3+}O_4$, which more precisely designates one of its peculiarities. It contains both ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions implying synthesis, growth, and stability

within an environment where oxidized and reduced states of iron are present and maintained. Magnetite is a member of the spinel mineral group that crystallizes in the isometric space group Fd3m. In the basic unit (unit cell) that completely describes the chemistry and crystal structure, there are 32 oxygen ions and 24 cations with eight of the cations in tetrahedral (fourfold or A site) coordination and the remaining 16 in octahedral (sixfold or B site) coordination with the oxygen ions. A view of the structure (Figure 6) oriented with the vertical [111] crystallographic direction shows layers of oxygen atoms alternating with layers of cations in this projection of the three-dimensional array. Layers of octahedrally coordinated iron alternate with layers in which the cations are distributed among two possible metal sites, A and B positions in the ratio A/B = 2/1. There are two types of spinel structures, normal and inverse, depending on the trivalent and divalent metal ion distribution. The *normal* structure would have eight ferrous (Fe^{2+}) ions in the A site and 16 ferric (Fe^{3+}) ions in the B site. *Inverse* would have eight ferric (Fe^{3+}) ions in the A site and for the remaining 16 cations, one-half or eight Fe^{2+} and eight Fe^{3+} are in the B site. Magnetite has the inverse spinel form with predominance of Fe^{3+}. In natural materials there may be slight excess or a deficiency of total cations relative to oxygen (Flint, 1984). Another mineral species, maghemite, also magnetic, formula γ-$Fe_{2.67}O_4$, indicates lower amounts of iron relative to oxygen, and is also known to be biologically formed (Gaines *et al*., 1997, p. 229)

The predominance of ferric cations in magnetite is mandatory, but magnesium, manganese, zinc, nickel, copper, and germanium may substitute for some of the iron within the spinel structure, occurring in solid solution in a magnetite, or maghemite. When these elements are dominant cations, they are the end-members in the spinel group of minerals with separate names (Gaines *et al*., 1997, pp. 293–294). As might be suspected, these elements in addition to iron that are likely to be detected when magnetites are chemically analyzed.

Magnetite is widely distributed usually with octahedral, or spherical, morphology, in igneous and metamorphic rocks. Magnetite is also found in limestones, in fumerolic deposits, and in living forms. For the latter the mineral plays some very important role, which we highlight in Sections 8.04.3.3.2 and 8.04.4.6.3. Black magnetite, with a specific gravity greater than 5, is easily detected and separated from sediments and tissues by its magnetic properties and the identification can be confirmed by X-ray diffraction.

Studies using high-resolution transmission electron microscopy coupled with Mossbauer spectroscopy on experimental systems (Tamaura *et al*., 1981, 1983) and on flocculants, the precipitates associated with bacterial activity (Schwertmann and Fitzpatrick, 1992), often detect the presence of other iron minerals such as the more hydrated iron

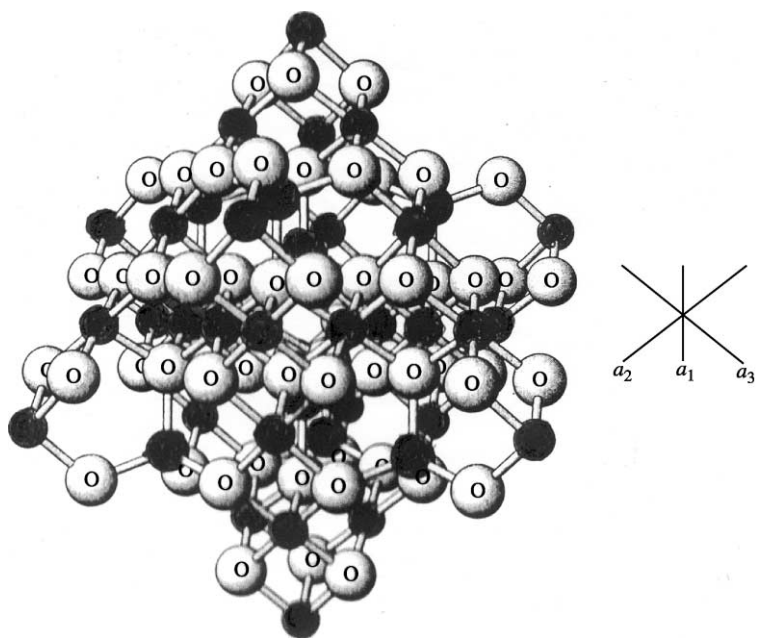


Figure 6 Magnetite. The crystal structure of magnetite, a member of the spinel group of minerals. The [111] crystallographic direction is vertical to show the horizontal appearance of cubic-close-packed oxygen (O) atoms. Fe^{2+} and Fe^{3+} are in tetrahedral and in octahedral interstices (both dark colored) (after Gaines *et al*., 1997, p. 293).

oxide phases—ferrihydrite, $Fe_5O_8H{\cdot}4H_2O$, lepidocrocite, γ-FeO(OH), and goethite, α-FeO(OH), as well as hematite, Fe_2O_3. A calculated Eh–pH diagram indicates that there is a much larger field for biologically mediated magnetite formation from the metastable ferrihydrite phase compared with the inorganic formation of common goethite (Skinner and Fitzpatrick, 1992). The relationships between oxidized iron mineral phases and iron sulfides (e.g., pyrite, FeS_2), in natural environments and in mine waste sites, so visibly obvious in the environment, have sparked interest in the roles of microbes, and their biomineralization utilizing iron and the deposition of many different iron minerals (Rabenhorst and James, 1992; Bigham *et al*., 1992).

8.04.3 EXAMPLES OF BIOMINERALIZATION

8.04.3.1 Introduction

Archea and Bacteria, two of the three domains that make up the Tree of Life as presently understood (see Figure 1), are grouped together as prokaryotes. The classes under the Archea and Bacteria are relatively simple. They are unicellular organisms that are only beginning to be explored. Their genetic information is not packaged within a membrane, and therefore the cells do not have a true nucleus. Archea and Bacteria lack mitochondria and chloroplasts, organelles that are prominent in the third domain, the Eukarya, and assist in respiration or photosynthesis. These functions are carried out in, or on, the membranes of the cell walls, or in some prokaryotes with the aid of symbionts. Archea are distinguished from Bacteria by the composition and structure of the cell wall, and by the ribosomes and the enzymes produced (Brock and Madigan, 1988; Ehrlich, 2002).

Within the Archea are methanogens and extremophiles (hypersaline and/or thermotolerant species), whereas Bacteria include species that can metabolize hydrogen in aerobic and anaerobic environments, can nitrify or denitrify, reduce sulfate or oxidize sulfur, or utilize iron and manganese in either their oxidized or reduced forms to gain energy. The number and diversity of bacterial species is enormous and still growing. A relatively new arena, characterizing the range of Archea, has captured scientific interest (Woese *et al*., 1990). In future, the largest collection of living forms with the greatest diversity may well be Archea.

Both Archea and Bacteria include heterotrophs, i.e., creatures that can derive energy and carbon needed for the construction of their essential bioorganic molecules through the assimilation and oxidation of organic compounds. We know of aerobic and anaerobic species and facultative organisms that use oxygen as a terminal electron acceptor when it is available, and when not, they may use reducible inorganic (e.g., nitrate or ferric ions) or organic compounds in the metabolic activities. There are fermentors, species that enzymatically control the breakdown of energy-rich organic compounds, such as carbohydrates, to carbon dioxide and water, in the process of securing other nutrients, and chemolithotrophs, microorganisms that derive energy from oxygenation of inorganic compounds. These latter assimilate carbon from CO_2, HCO_3^-, or CO_3^{2-} without the benefit of light, and therefore survive in deep-sea hydrothermal vent sites. The discovery of this vast range of "bugs" has caused us to rethink some very basic questions on alternate sources of energy for creating and maintaining living creatures and the origin of life.

These "simple" forms accumulate, actively or passively, elements from their environment to construct metabolically essential components: nucleic acids, proteins, lipids, including a range of cations. Such anabolic activities require catabolic machinery and the ability to adapt to the surroundings. In the process of producing, maintaining, and reproducing, they maximize the selection and transport of inorganic and organic complexes, and are some of the most important geological agents and catalysts (Ehrlich, 2002, pp.119–151; Krumbein, 1986).

While they are about sustaining themselves, they change their environment. Some accumulations can rightfully be labeled biomineralization in that these creatures are involved with the precipitation of a variety of minerals. The mineralization may take place adjacent to the metabolizing form, associated with, but appearing nonessential to, their life cycle. Since eukaryotes incorporate a wide range of these living forms as symbionts to facilitate the same functions, a significant portion of future research in biomineralization will probably be devoted to investigating their range of activities, determining the details of the processes involved, and integrating that information into geoenvironments and monitoring ecological changes. They are some of the most important, if incompletely charted, agents in the geochemistry of the Earth and we would be remiss if we did not include these most primitive organisms in this chapter on biomineralization.

8.04.3.2 Sulfur Biomineralization

There are two large groups of prokaryotic organisms: those that oxidize and those that reduce sulfur compounds, sulfur being an element with one of the widest possible ranges of oxidation

states (−II to +VI). There are chemolithotrophic bacteria that are able to disproportionate sulfur all the way from H_2S to SO_4^{2-} but only in the presence of sulfide scavengers such as FeOOH, $FeCO_3$, or MnO_2 (Janssen *et al*., 1996), but most organisms are either sulfate reducers or sulfur and hydrogen sulfide oxidizers. There are aerobic, anaerobic, and facultative forms that perform these reactions, from the Bacteria and Archea kingdoms that occur in marine or freshwaters, on land, in soils, and in sediments (Barns and Nierzwicki-Bauer, 1997), and perhaps most importantly, the reducers and oxidizers are often juxtaposed, occur together and act in consort. Morphologically these creatures can be cocci, rodlike, or filamentous, and many of the bacteria show gram-negative cell types.

8.04.3.2.1 Sulfur oxidizers

Strictly anaerobic sulfur oxidizers are found in the photosynthetic purple (*Chromatiascea*) and green bacteria (*Chjlorobiaceae*) and in the cyanobacteria (Ehrlich, 2002, table 18.3, p. 557); there are also chemosynthetic autotrophs that use hydrogen as an energy source. Some species operate under extreme environments (at high salinities, at high or low pH), or are thermophylic (up to 110 °C); many identified from hot springs or ore deposits. The oxidizing activity is coupled to the reduction of available CO_2, with the carbon fixed into bioorganic molecules. In addition, these anaerobes may use nitrate as the terminal electron acceptor reducing it, via nitrite, to NO, to N_2O to N_2, meaning that these microbes can exist in virtually all surficial environments. Thiobaccilli, the family Beggiaatoaceae, and in the Archea, *Sulfolobus* and *Acidianus*, are among the most widely studied (Ehrlich, 2002, table 18.2, p. 554).

Natural bacterial oxidation processes are exploited industrially. The beneficiation of metal sulfide mineral ores and waste materials via bioleaching has been used to recover very small quantities of gold intimately sequestered within pyrite, FeS_2. Acidic pH and a consortium of bacteria including thiobacillii and *Metallogenium* are utilized. A lixiviate, ferric iron (Fe^{3+}), which is consumed in the reaction, has been shown to be essential (Mustin *et al*., 1992). For specifics on the many sulfur-oxidizing microorganisms used on copper, iron, manganese, and mercury, mineral sulfides, see Ehrlich (2002, pp. 642–657).

The thiobacilli are autotrophs that produce sulfate (or H_2SO_4) directly from the oxidation of H_2S, the gas that may be produced by other bacteria or by volcanic emanations. Other bacteria, and including some Archea, may accumulate elemental S^0 when H_2S is in short supply, and some sulfur oxidizers can start with S^0 rather than H_2S, and produce SO_4 irrespective of the oxygen tension (London and Rittenberg, 1967), whereas species operating in partially reduced environment only oxidize to S^0. When the sources of sulfur are unlimited, most photoautotrophic species oxidize to S^0, which may accumulate intracellularly (purple sulfur bacteria) or extracellularly (green sulfur bacteria). The elemental sulfur accumulated by one species, *Chlorobium*, is readily available to the cell that produced it but not to other individuals in the population nor other species. Scanning electron microscope studies on this bacteria showed that the sulfur was concentrated in hollow proteinaceous protruding tubes on the cell surface, an example of the construction of an organic edifice to contain inorganic, by which we mean "mineral," deposits! (Douglas and Douglas, 2000). Polysulfides ($S_{n-1}SH^-$), but not free sulfur, have also been detected, and are probably an intermediate, in the course of sulfide oxidation to SO_3 and onto SO_4 in some species (Aminuddin and Nicholas, 1974).

8.04.3.2.2 Sulfate reducers

Sulfate reducers, originally identified as the bacterial species—*desulfovibrio, desulfomaculum*, and *desulfomonas*—have restricted nutrition unable to degrade organic materials below acetate. Recent investigations have vastly expanded the number of species that anaerobically use aliphatic, aromatic, and heterocyclic organic molecules, and some use H_2 as an energy source to reduce sulfate.

Archea sulfate reducers can also use simple organic molecules (glucose) as well as more complex substrates, adding to the previously identified methanogenic activity possible in these cells. Some varieties were isolated from the hot (to 110 °C) vent areas in the Pacific (Jannasch and Mottl, 1985; Jorgansen *et al*., 1992), although low-temperature (10 °C) species occur in sediments (Ghiorse and Balkwell, 1983). For more details on the metabolism and range of all these microbes, see Ehrlich (2002, pp. 549–620) and Banfield and Nealson (1997).

8.04.3.2.3 Formation of elemental sulfur

There is widespread deposition of elemental sulfur from H_2S or SO_4 sources by bacteria. Native sulfur in the cyrenaician lakes of Libya, North Africa, is part of a cycle in which sulfate-reducing bacteria utilize the SO_4 available in the waters, and reduce it to H_2S, and associated photosynthetic bacteria oxidize the H_2S to S^0 (Butlin and Postgate, 1952). Sulfur-containing nodules covered with crystalline gypsum, $CaSO_4{\cdot}2H_2O$, are found in the shallow edge

waters of Lake Eyre, South Australia (Ivanov, 1968 pp. 146–150). These nodules and the waters contain active sulfate-reducing bacteria and thiobacilli (Bass-Becking and Kaplan, 1956), and it is assumed that the sulfurogenesis is aided, if not actively the product of bacterial activity. Fumerolic hot springs with H_2S are also localities where oxidization to S^0, or in some cases to H_2SO_4, is bacterially mediated. The result is not only sulfur production but adjacent rock and any other materials, dissolve promptly in such acidic environments (Ehrlich, 1996).

8.04.3.2.4 Sulfate biomineralization

There are a few species that utilize sulfate minerals to form their hard parts. Spangenberg (1976) describes the epidermal intracellular (vesicle) formation of varying size statoliths of gypsum, $CaSO_4{\cdot}2H_2O$, in the jellyfish *Aurelia arita*. This citation is a modern follow-up to the identification by Fischer in 1884. Statoliths of barite, $BaSO_4$, in *Charandother protoctista* (Schroter *et al*., 1975) are recorded by Lowenstam and Weiner (1989, table 4.1, p. 52, table 5.1, p. 76), who suggest that the mineral enabled the animal to respond to gravity by aiding location determination, which makes sense as the specific gravity values for these two sulfate minerals are: 2.3 for gypsum, and 4.5 for barite.

Acantharia. The acantharia use $SrSO_4$, the mineral celestite, or celestine (Butschli, 1906), to biomineralize a remarkably regular geometric test consisting of 20 spines. These creatures were described as early as 1858 by Muller (commented on in Thompson (1942) and illustrated in Schewiakoff (1926). The spines are radially distributed: 10 pairs of spines, each one a single crystal with the elongation of the spine parallel to the mineral crystallographic axes. The spines may grow to 1.5 mm long with a diameter of 38 μm (Schreiber *et al*., 1959). The ranges of geometric patterns in the hard tissue of acantharia were presented by Popofsky (1940) and Reshertnjak (1981) with the most recent compilation listing over 150 species (Bernstein *et al*., 1999).

Acantharians are exclusively marine, planktonic, single-celled protozoa, 0.05–5 mm in diameter, and show a wide range and often multiple types of symbionts (Michaels, 1988, 1991). Common in tropical and subtropical waters worldwide, they do occur in lower numbers in temperate and polar seas (Caron and Swanberg, 1990). Originally detected by dragging nets of fine mesh in shallow (200 m depth) open ocean, the early reports on distribution gave conflicting results: patchy low distribution, less than 1% of the total mineralized biomass, but up to 70% in the Ligurian Sea, and occasionally exceeding the concentrations of radiolarians, foraminifera, and diatoms (Bottazzi *et al*., 1971). The different assessments were at least partially the result of dissolution of the acantharia skeleton that requires special preservation techniques (Michaels, 1988). $SrSO_4$ is highly undersaturated in the oceans (Whitfield and Watson, 1983), and early estimations were affected by the collection system since a particular catch might be analyzed only after month-long storage (Bottazzi *et al*., 1971). Further, as Odum (1951) commented, strontium, as the major constituent of acantharia, is also found in trace amounts in the carbonate minerals of other life forms. The growth of acantharia is a prime example of biologic accumulation against a gradient, since the level of strontium is 8–10 mg (8,000 μg) per liter in seawater. It is now known that barium, and a variety of other elements (calcium, manganese, nickel, zinc, lead, arsenic, and bromine), can be detected in acantharia (Brass, 1980). The average Ba/Sr molar ratio is 3×10^{-3} (Brass and Turekian, 1974), but the value varies for different samples: those from the Pacific were 2% lower than those collected from the Atlantic Ocean. It has been suggested that deceased acantharia undergoing dissolution at depth would release more barium than strontium to the waters, and these creatures may play a larger role in barium than in strontium cycling.

It should be noted that celestine has been found in hydrothermal deposits and in evaporates (Skinner, 1963), although these occurrences may not necessarily be biologically mediated. The mineral also has a rather high specific gravity, 3.98.

8.04.3.3 Iron Biomineralization

The essential element iron is not only utilized by all living systems but bacteria materially assist the nucleation of minerals that contain ferric and ferrous forms. After a brief consideration of the roles of iron in bacterial systems, we discuss the magnetotactics, the bacteria that biomineralize with euhedral nanosized particles of magnetite, Fe_3O_4, and greigite, Fe_3S_4.

8.04.3.3.1 Roles of iron in Archea and Bacteria

Fe^{2+} will auto-oxidize to Fe^{3+} in air above pH 5. However, at lower pH, where Fe^{2+} is the dominant form, many Bacteria and Archea species hasten the reaction with the energy gained in the transformation, Fe^{2+} to Fe^{3+}, to fix carbon. Under anaerobic conditions there would be no oxidation without bacteria in light or in the dark. Some anaerobes can use nitrate as the electron acceptor

coupled with Fe^{2+} oxidation (Benz *et al.*, 1998). These prokaryotes can facilitate mineral deposition, and are considered by some researchers as prime candidates to explain the widespread banded iron formations found in the Precambrian (Widdel *et al.*, 1993).

Fe^{3+}, common in surficial oxic environments, forms hydroxides, complexes formerly called "limonite" (a collection of rust-like precipitates, chemically designated as $Fe(OH)_3$). The aggregate has now been shown to contain several oxyhydroxide species, FeOOH, and the oxides, hematite, Fe_2O_3, and magnetite, Fe_3O_4, and some new minerals have been identified (Schwertmann and Fitzpatrick, 1992, tables 1–3, pp. 10–11).

The ferric forms are stable in neutral or slightly alkaline solutions, soluble in acids, but can only be reduced within a reasonable time frame to Fe^{2+} via bacterial action. Therefore, in oxic environments iron is usually present in Fe^{3+}-containing minerals, i.e., hematite, and unavailable to living forms, thus presenting a limit to growth. To provide for such situations many microbes produce chelators known as siderophores with very high binding constants for Fe^{3+} (10^{22}–10^{50}) relative to those for Fe^{2+} (10^{8}) (Reid *et al.*, 1993). Note that plants have similar needs and utilize similar products and mechanisms. Fungi and microorganisms located on the roots of plants are symbionts that aid in the selection of essential elements from the environment and make them available to the plant. Cells convert the transported ferric compounds into ferrous ions and into heme as well as nonheme complexes. Many microorganisms are involved with iron reduction and oxidation (Lovley, 1987) but only a few biomineralize with magnetite, a mixture of the two oxidation states, in spite of the widespread availability of iron.

8.04.3.3.2 Bacterial iron mineral formation

Bacteria are major mediators in the deposition of many nonmagnetic oxyhydroxide iron minerals such as ferrihydrite and goethite, but some form the iron oxide magnetite, Fe_3O_4 (see Figure 6), or the magnetic iron sulfides, greigite, Fe_3S_4, and pyrrhotite, Fe_7S_8. The location of these minerals may be intracellular (biologically controlled), or extracellular (biologically mediated) (Mann *et al.*, 1992) (Figure 7). The best-studied extracellular producers are *Geobacter metallireducens* and *Shewanella putrefaciens.* Both are rod shaped, gram negative, freshwater chemoheterotrophic forms, meaning that they utilize organic carbon compounds from a range of sources (Lovley, 1987). A halotolerant iron-reducing bacterium has been shown to produce nonstoichiometric magnetitic particles with the mineral composition between magnetite and maghemite (Hanzlik *et al.*, 1996).

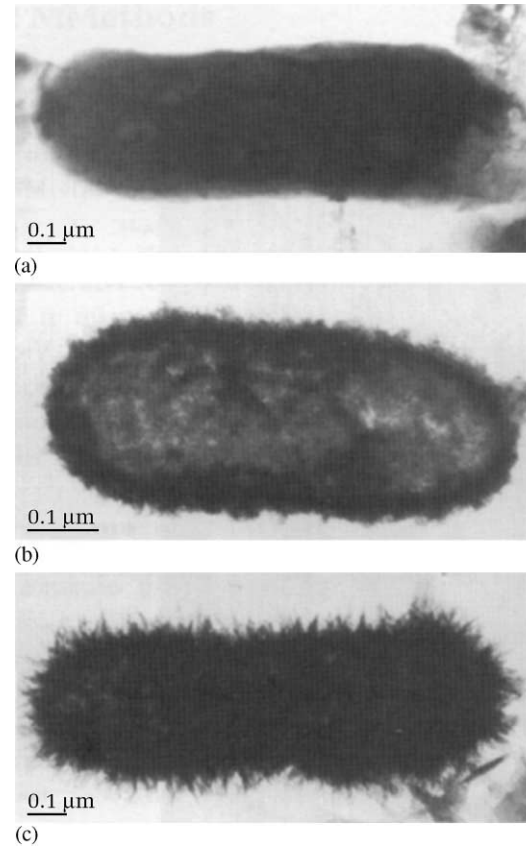


Figure 7 Progressive stages of microbially mediated iron oxide mineralization of a rod-shaped bacteria. Scale bars = 0.1 μm: (a) original bacteria, (b) early deposition of amorphous hydrated iron oxide (ferrihydrite), and (c) intense deposits of acicular magnetite (source Mann *et al.*, 1992, photo 2, p. 118).

Some anaerobic sulfur-reducing bacteria can also use iron as the sole electron acceptor. Since all anaerobic sulfur-reducing bacteria respire H_2S, this product together with a source of iron from an iron-rich environment could aid in the extracellular nucleation and production of greigite, pyrrhotite, or nonmagnetic sulfides, such as the two polymorphs of FeS_2, pyrite, and marcasite. Intracellular magnetite production studies detected temperature-dependent fractionation of oxygen in the formation for Fe_3O_4 and water consistent with that observed with extracellular magnetite produced by thermophyllic Fe^{3+}-reducing bacteria (Mandernach *et al.*, 1999). The minerals formed will depend on the pH, Eh, temperature, and the source and availability of iron, sulfur, and oxygen (Freke and Tate, 1961; Rickard, 1969).

8.04.3.3.3 Magnetotactic bacteria

The processes that create the magnetic particles within bacteria, the magnetotactics, are

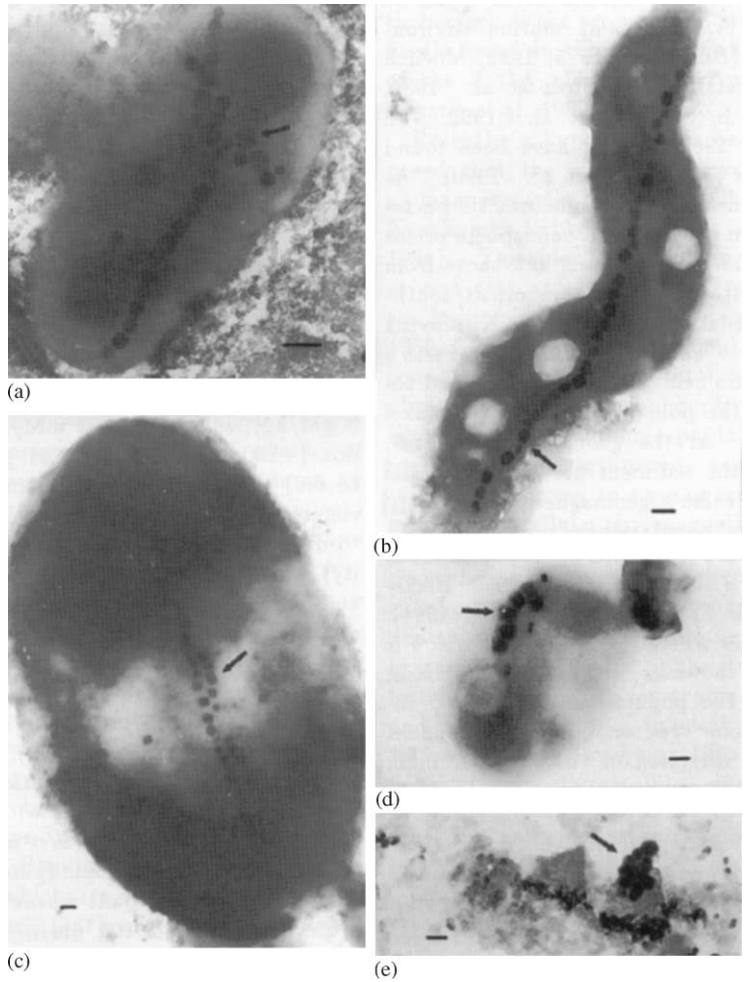


Figure 8 TEM of the magnetotactic bacteria of the Pettaquamescatt estuary: (a) coccoid from 3.6 m, (b) vibrio from 7.2 m, (c) large rods with double row of magnetite crystallites, (d) moribund bacteria on surface of the sediment, (e) particles from 1 m depth. Scale bars = 100 nm (source Stolz, 1992, p. 138).

biologically controlled (R. P. Blakemore and N. A. Blakemore, 1990; Bazylinski, 1996). The particles ranging from 30 nm to 120 nm in diameter are well crystallized, and each is a single crystal with distinctive crystal morphology, and occasionally several crystallites appear within one bacterium and usually become aligned (Mann, 1985). Although all magnetotactics described so far are gram-negative motile bacteria, it is possible that a magnetotactic Archea may exist (Bazylinski and Moskowitz, 1997). Found in highest numbers where an oxic environment changes to anoxic in fresh, brackish, or ocean waters, the magnetotactics show species specificity for geographic as well as depth locations.

The crystallites grow within an organic membrane bound vesicle, or sac, the magnetosome. First a precursor iron oxide forms that matures into a single magnetic domain crystal of a magnetic iron mineral (Balkwill *et al.*, 1980; Mann, 1985). The morphology of the individual crystallites is maintained within a species (Figure 8). Cubes, parallelepipeds, tooth, and arrow-shaped crystallites, some with truncated hexagonal or cubo-octahedral faces as well as the crystallographic growth patterns of the grains have been investigated using high-resolution transmission and scanning electron microscopy. The crystals are usually magnetite or greigite, but there is one magnetotactic species that has both minerals and another that contains nonmagnetic pyrite (Mann *et al.*, 1990). The size and linear arrangement of the crystallites into a chain of magnetic particles, or two chains in some cases, orient so as to enhance the dipole moment of the bacterium. The enhancement probably overcomes any thermal or other forces that might interfere and prevent detection of the Earth's geomagnetic field. Bacteria orient themselves toward the sediments, rather than the oxidized surface waters, with the direction of orientation distinct for northern and southern hemispheres. Alignment is not so simple as this

might suggest. Magnetotactics swim up and down the geomagnetic field using their flagella to find optimum oxygen concentration locations, and probably move when the oxygen concentration changes. The biological control of internal mineral formation and the choice of the mineral species allow the bacteria to use both magnetotaxis and aerotaxis to maximize their habitat (Frankel *et al*., 1997).

8.04.3.4 Carbonate Biomineralization

Of all the minerals that have been associated with biomineralization, carbonates are the most obvious. From the coralline materials found in atolls to the shells of mollusks and gastropods, the average person knows these creatures, and possibly that the "hard materials" contain calcium, if not that the mineral is calcium carbonate, $CaCO_3$. It is the carbonate minerals that provide the distinctive shapes allowing immediate recognition, and the precise compositions are often the means by which one designates a particular invertebrate species.

Lowenstam and Weiner (1989, pp. 8–11, table 2.1) clearly show that carbonates dominate in biomineralization. They even occur in plants and fungi. The volume percent of limestone and marbles is well documented from the Precambrian to the present. Whether these rocks are inorganic precipitates or festooned with fossils, many of the living creatures had biomineralized with calcium carbonate, is usually clear. Indeed, some strata are composed entirely of calcium carbonate shells. We present examples of carbonate mineral deposition in cyanobacteria, corals, coccoliths, foraminifera, mollusks, echinoids, and the arthropods.

Although many calcium carbonate deposits are well known geologically, and the intimate association of bioorganic molecules with the mineral materials fully appreciated for geochemical investigations on stable isotopes, identification of the precise mineral species has often been ignored. Bulk inorganic chemical analysis in early investigations documented the presence of calcium and the term "calcification" was, and is, widely used. This designation does provide important chemical information on the cation but not on the anion. There are many calcium-containing biomineralized tissues that are not carbonates, e.g., calcium sulfates, and oxalates, and a chemical analysis cannot distinguish between the several possible calcium carbonate mineral species, calcite, aragonite, and vaterite (see Section 8.04.2.1.3). Further, because the mineral crystallites in biomineralized systems are usually tiny, and optical microscopy was the only technique generally available until the twentieth century, accurate identification was often impossible. Biominerals, difficult to assess, were labeled "amorphous." It was not until the 1920s that they were adequately evaluated with X-ray diffraction analyses, and the mineral species were accurately determined. The more sensitive spectrographic methods (IR, Raman, ICP-MS) allow for more specific investigations. The stretching and bending modes of the carbonate anions can be evaluated by identifying the species of calcium carbonate mineral from its structural character, and the polymorph determined. The latter may show a novel biomineralization, and perhaps a new invertebrate species.

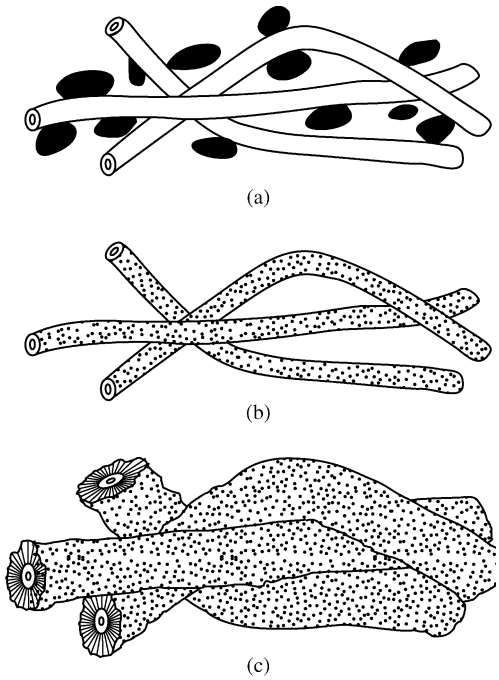
Another important point about calcium carbonate biomineralization is that more than one of the three polymorphs may be present in close proximity within an individual sample and each may be a different calcium carbonate polymorph (mollusks provide such an example), or the mineral may change over time. In many species the larval stage is aragonitic, while the adult biomineral is calcite. The different minerals in biomineralized tissues play specific roles in the proper functioning of the organism.

The uptake and incorporation of other elements, magnesium or strontium, in the calcite have been investigated and shown the bioavailability of these elements. In modern ocean waters, the content and range of magnesium in biotic and abiotic deposited minerals were virtually identical in studies by Carpenter and Lohmann (1992). However, there was a more rapid strontium uptake in the calcium-rich biomineral. The composition of the calcium carbonate mineral deposited is a function of kinetics and related to the metabolism of the organism.

8.04.3.4.1 Cyanobacteria

Cyanobacteria, one of the earliest creatures to biomineralize, are also one of the most pervasive and abundant life forms on the planet. Although they usually occur in the upper oceans where they form mats, and hundreds of species are well known for the deposition of calcium carbonate minerals, they are also found in lakes, in the oxygenated layer in the upper horizons of soils, and associated with fungi and other creatures that bore into limestones or other rock masses (Riding and Voronova, 1982; Ehrlich, 2002). Together with calcifying algae and a number of other mineralizing taxa, cyanobacteria markedly expand at the end of the Precambrian (Lowenstam and Weiner, 1989, p. 233).

The calcifying varieties of cyanobacteria do so extracellularly in and on a mucilaginous and fibrous polysaccharide sheath (Figure 9(A)).



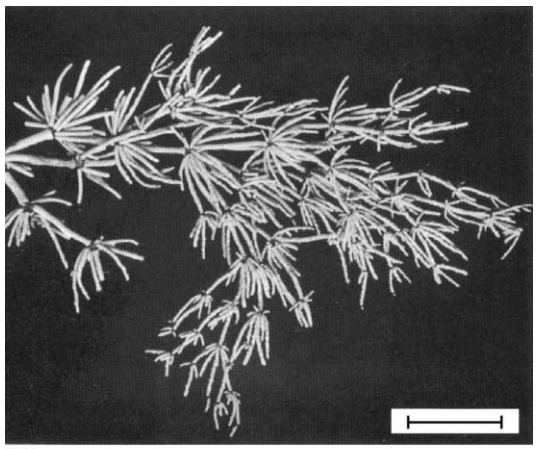


Figure 9 (A) Sketch of the three stages of $CaCO_3$ deposition associated with filamentous bacteria: (a) trapping sediment, (b) calcification of the sheath, and (c) encrustation of the sheath (source Pentecost and Riding, 1986, figure 51, p. 75). (B) Articulated coralline algae. Scale bar 1 cm (source Simkiss and Wilbur, 1989, figure 7.5A, p. 94).

The nucleation of a calcitic or aragonitic form is species specific, and they grow where conditions are also favorable for abiotic carbonate deposition. The filamentous cyanobacterial mats may passively accumulate and bind local sedimentary particles that become entombed through cementation with additional calcium carbonate. Distinguishing between calcium carbonate precipitates adjacent to actively metabolizing cyanobacteria versus cell directed mineralization, i.e., via bioorganic molecular templates, is often moot. It is probable that both processes take place intermittently if not sequentially with shifts in temperature and composition, alkalinity or salinity, of the surrounding fluid media, especially when the waters are effectively saturated with respect to carbonate and calcium ions. Where the waters are in rapid motion, as in springs and waterfalls, $CaCO_3$ deposition may be aided by evaporation while bioprecipitation of carbonates is also taking place (Golubic, 1973, 1983).

Tuffaceous carbonate deposition ascribed to actions of cyanobacteria is known from the arctic to the tropics, and even in caves where light is restricted (Pentecost, 1978, 1985b). The mineral in freshwater mats is usually of lower magnesian content than that in the oceans possibly responding to species specificity but certainly to a difference in the availability of magnesium (Folk, 1974). Studies of the isotopic variability of carbon and oxygen in coralline algae samples indicate that some species are at equilibrium with the oceanic carbonate while others are not (Lee and Carpenter, 2001). The same may well be true of cyanobacteria. The contributions toward fractionation of stable element isotopes by any biomineralizing form during sequestration of carbonate minerals are not obvious nor straightforward.

Oriented calcite crystals of different morphologies enhanced through epitaxy (growth of the mineral continues in crystallographic register on a preformed inorganic template), as well as completely unstructured and disordered mineralized mats have been described. The mineral expression suggests a range from total to no biological direction or control (Pentecost and Riding, 1986). Secondary, and perhaps even tertiary, precipitation of calcium carbonate and/or silicate from the activities of accompanying biomineralizing creatures would lead to the varied microfabrics that have been described for ancient sediments. The textures are undoubtedly related to multiple stages of deposition, even if predominantly due to cyanobacteria (Wray, 1977). Modern investigations of bacterial biomineralization can determine more precisely the species of cyanobacteria by employing the latest advances in genetic techniques and matching methods (Dale, 1998).

The appearance of marine calcified cyanobacteria marking the base of the Cambrian suggests a major change on the Earth, either in the environmental availability of appropriate elements, or a shift in biological capacities that enhanced mineral precipitation. Mineralized reef deposits fortuitously provide a permanent geological

record of the existence and activity of cyanobacteria. The reduction of extensive carbonate deposition post-Cretaceous and the variations recorded in today's marine environments have made cyanobacteria one of the foci for marine geochemical analysis. Wary researchers anticipate diagenetic changes in ancient carbonate mineral deposits when stromatolites were abundant (Walter, 1976; Riding, 1977; Golubic, 1983), but similar care should be exercised in modern reef investigations. Whenever and wherever living creatures expire, their mineralized materials become exposed to a different environment. Addition or subtraction, or modification, of both organic and inorganic constituents, should be anticipated and the evidence sought for in the samples. Using high-resolution techniques (TEM, SEM), the possible morphological or chemical effects attributable to local conditions and diagenesis should be investigated before geochemical analysis to assure accurate and meaningful assays.

Because of the widespread geographic locations and geologic persistence of cyanobacteria, volumes of work have been published on this group of organisms. The reader may find the following references of some advantage in searching for particulars on species and environments of mineral deposition: Brock and Madigan (1988); Carr and Whitten (1982); Rai (1997); and Sebald (1993).

8.04.3.4.2 Cnidaria (coelenterates)

The group Cnidaria includes jellyfish, anemones, and hydras, mobile invertebrates, most of which sparingly mineralize at only a few anatomical sites with specialized functions. For example, the gravity detectors, or statoliths, in the hydrozoa mineralize with magnesian–calcium phosphates or calcium sulfate. However, another group of organisms in this phylum form the reefs, fringing many islands and continents today as they have in the past. These obvious, large geostructures are the mineralized product of sessile, or fixed, cnidarians—the corals. The corals create massive carbonate deposits in the relatively shallow waters of the circum-equatorial regions where salinities are at least 27 ppt and temperatures remain at or above 15 °C year round. Some thousands of miles in extent, the coral reefs are a unique ecological niche that contains many other biomineralizing invertebrates such as sponges, mollusks, and gastropods.

Corals. Among the most important members of the class Anthozoa are the scleratinian corals each of which secrete their own calcified exoskeleton. Most are colonial but some deeper-water (and lower-temperature) species are isolated individuals. Their skeletons mineralize with calcite or aragonite, and sometimes both calcium carbonate polymorphs occur in different anatomic sites in the same animal (Lowenstam, 1964). One member of this class, Octocoralia, produces individual mineralized spicules, or spicular aggregates whose distinctive morphologies are the basis for separating species (see below), while another produces massive or branching but coherent structures (Figure 9(B)). The massive reef building forms contain symbionts within their endodermal tissues. Known as zooanthellae, these unicellular dinoflagellate algae are essential to the metabolism of the coral, including mineralization of their skeletons. Trench and Blank (1987) identified *Symbiodinium*, the gymnodinoid dinoflagellate, and showed that this intimate, indeed essential, relationship was pervasive throughout all reef corals and other benthic marine cnidarians. Using modern genetic techniques on extracted ribosomal DNA, the diversity of symbionts is being confirmed (Gast and Caron, 1996). In sea anemones, the concentrations of different symbionts show a dependence on habitat, illustrating how they could be used to signal specific benthic conditions (Huss *et al.*, 1994; Secord and Augustine, 2000). The possibility for interference with the viability of coral reefs by global climate change is a relatively new arena of investigation (e.g., Lindquist, 2002).

The functional parts in the body of a reef coral have a protective surface of ectoderm which is external but adjacent to the specialized cell layer that gives rise to the mineralized structures (Johnson, 1980). Once the coralline larvae (free-swimming stage) settle onto a surface, a base plate of bioorganic molecules is formed (Wainwright, 1963) and calcite deposits as spherulite interspersed with small rodlike granules (Vandermeulen and Watabe, 1973). The granules, all about the same size, do not coalesce to form larger structures. The two morphological aggregations of calcite suggest that the mineral may nucleate and be retained in spaces defined by an organic membrane. In the second stage of larval mineralization, large aragonite crystals similar to those seen in the adult skeleton are deposited on top of the base plate.

The mineralized portions of an adult scleratinian coral are composed of bunches of bladed aragonite crystallites elongated along their *c*-axes forming spherulites. The spherulites show a dense center, presumably the site of initial mineral nucleation, and on electron microscopic examination the centers were shown to be calcite for the species *Mussa angulosa* (Constantz and Mielke, 1988). The aragonite crystals have distinct morphologies used to define distinct genera. It appears that the mineral deposition is under local control, possibly the result of chemically specific bioorganic

molecules produced by the cell that later disappear. Lipids, phospholipids, and proteins have been detected on analysis of the coral body (~0.02 wt.% of each of these compounds relative to the skeletal weight), and may assist with mineralization. The proteins are characterized by high concentrations (~50 mol.%) of acidic aminoacids, especially aspartic acid, glutamic acid, and γ-carboxyglutamic acid, the latter biomolecular species that is also detected in vertebrate tissues (Hamilton *et al*., 1982).

Other taxa that biomineralize. Spicules formed from magnesian calcitic (calcite that incorporates some magnesium into the structure, Mg-calcite) are found in the Octocoralia and Gorgoniacea. They may occur in the axis, or in the cortex, of the skeletons of these colonial corals as well as in the tentacles with morphologies distinct from the anatomical sites. The orientation of the Mg-calcite crystallites can differ within a particular spicular aggregate or the mineral may form as a single crystal, similar to the calcite mineral deposited in echinoderms. There are examples of functional adaptations of spicular biomineralization. The sea whip (*Leptogorgia virgulata*) has a plant-like appearance and a skeleton composed of the protein collagen reinforced by Mg-calcite spicules. The sea fan (*Melithacea ochracea*) has anatomizing branches of jointed segments that are dense fused aggregates of calcitic spicules alternating with protein segments rich in collagen. It would appear that the design of the mineralized skeleton enables them to withstand the forces of tidal currents with flexible as well as mineralized portions, securing their survival. In the Gorgoniidae family of the Gorgoniacea, calcium phosphate has been identified in the axial spines and holdfasts (Macintyre *et al*., 2000) in addition to the Mg-calcite spicules throughout the coral. In *Pocillipora damicornis*, the skeletal organic constituent is chitin, a polysaccharide (Wainwright, 1963).

The mineralization mechanisms displayed by Cnidaria are extraordinarily varied and, although a relatively primitive invertebrate group, they present highly specialized biological systems, including the incorporation of symbionts that show genetic distinctions related to the ecologic niche occupied by the coral.

8.04.3.4.3 Coccoliths

The most abundant $CaCO_3$ depositing organisms are algae, members of the phylum Haptophyta, which produce a flagellum used during their motile stage, and surface scales that cover their cells. Some species mineralize the outer portion of the scale, especially the coccolithophorids. They account for most of the oceanic carbonate sedimentations and chalk deposits worldwide (Lowenstam and Weiner, 1989, p. 68). The discoid fenestrated double-rimmed scales, less than 10 μm in diameter, of these unicellular photosynthetic planktonic algae are mineralized with calcite. *Emiliania huxleyi* is probably the best-known species. The calcitic mineral deposits on an organic template intracellularly. Similar to inorganic calcite the $[10\bar{1}4]$ faces of the usual calcite cleavage rhomb may be displayed in some coccoliths, while other species do not show any calcite crystal forms, and a few species mineralize with aragonite or silica. This range of minerals with different compositions, and different habits expressed by the minerals, is used to delineate species and suggests that the biomineralization process expresses different degrees of intracellular as well as extracellular control (Green, 1986).

Emiliana huxleyi: intracellular calcification. Using *E. huxleyi* as the example, we outline the sequence of intracellular, intravesicle calcification. The first stage is the formation of a flattened vesicle adjacent to the Golgi apparatus in the cell. The vesicle accumulates polysaccharides and proteins (De Vrind-de Jong *et al*., 1986) forming a base plate, the site of mineral nucleation and deposition that may commence before the scale is extruded. The remarkable mineralized scale structures formed by *E. huxleyi* are composed of a fitted collection of upwards of 20 calcitic segments with anvil-like shapes that are smooth surfaced and behave as a single calcite crystal (Wilbur and Watabe, 1963) (Figures 10(A)–(C)).

To further emphasize the exquisite specificity of mineral deposition, each initial 40 nm rhomb of calcite formed in the plate is oriented crystallographically with the *c*-axis parallel to the base of the segment and aligned radially. The *a*-axes of the calcite are aligned vertically to the plane of the coccolith plate (Watabe, 1967; Mann and Sparks, 1988). Protrusions develop as the mineral is deposited and the calcite segments thicken and fuse. The extracellular coccoliths festooning the surface of the cell display anvil forms (Figure 10(B)) that precess in a clockwise direction with the calcite {104} face always to the right-hand side; this means that there is chirality expressed in the construction. The anvil-shaped units overlap in the completely formed ring (see developmental scheme in Figures 10(A) and (B) and micrograph of the amazing final product; Figure 10(C)).

In other coccoliths species the intracellular vesicles may be mineralized and completed after the scale has been extruded onto the cell surface but still covered by an organic membrane. In the genus *Chyrsotila*, nonmotile cells produce a mucilaginous sheath in which spherulitic aragonite crystallites form, which eventually encapsulate the cell (Green and Course, 1983). There are

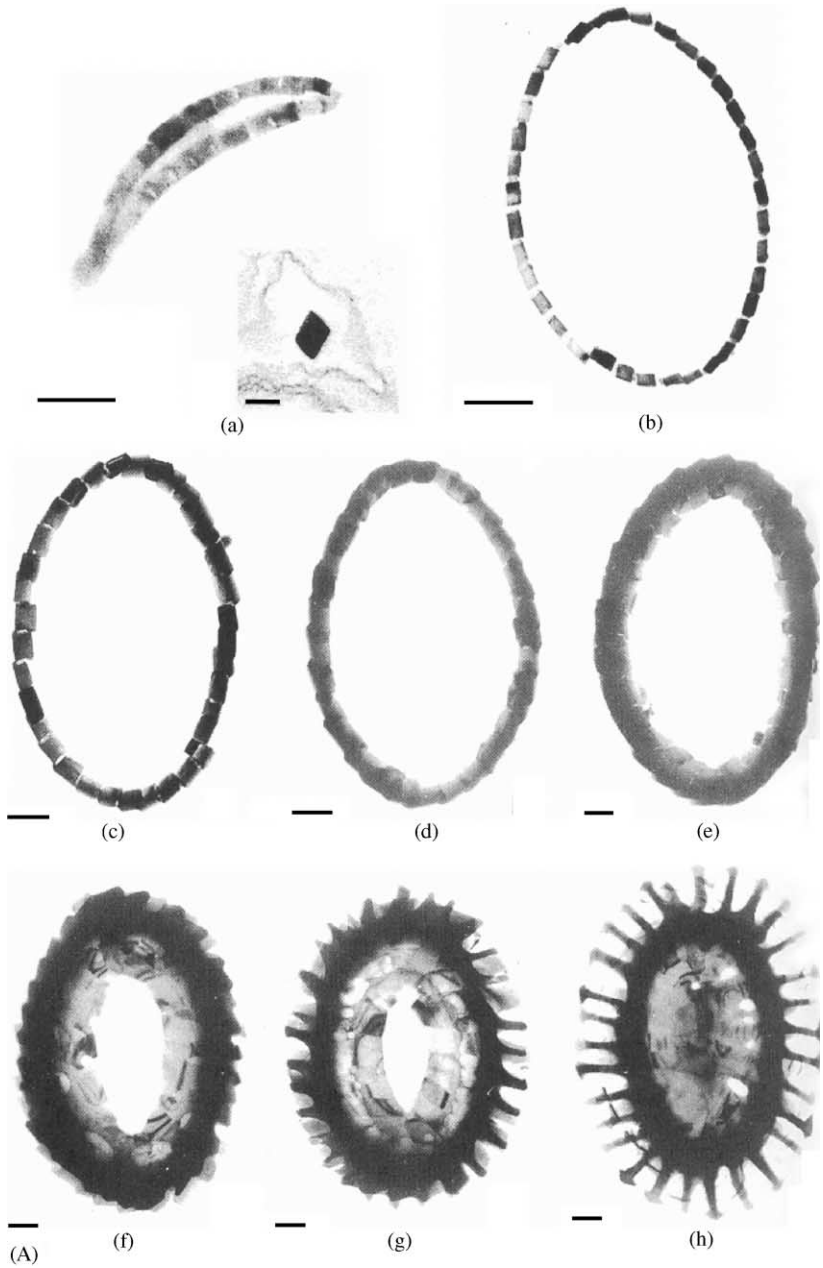


Figure 10 (A) Stages in the formation of *E. huxleyi*: (a) precoccolith ring, (b) mineral growth stages, (c)–(e) continued growth and then overlap of individual segments, (f)–(h) development of extensions from the initial ring. Scale bars for (a)–(d), (g), (h) = 0.2 μm, inset (a) = 0.5 μm. Scale bars for (e) and (f) = 0.1 μm (source De Vrind-de Jong and De Vrind, 1997, figure 17, p. 286). (B) Sketch of a cross-section through the *E. huxleyi* coccolith cell. The dark colored mineralized sections are produced internally and then moved externally (source Simkiss and Wilbur, 1989, figure 6.3, p. 68). (C) An SEM of a complete adult coccolith showing the juxtaposition of the coccolith plates into a sphere (source Mann, 2001, figure 8.9, p. 147).

species that form silica instead of calcitic cysts (Green *et al.*, 1982). Delicate structures with deliberate morphology and crystallographic orientations displayed by the mineral and a precision growth sequence are also characteristic of echinoderm exoskeletons.

The complicated intracellular process means that the *E. Huxleyi* cell must accumulate Ca, CO_2, or HCO_3^-, for the inorganic phase, as well as the carbon and nitrogen to create the organic molecules by a regularized, programmed cycle. The coccolithophorids are remarkable dynamos that illustrate the complexity of biological mineralization we only partially understand (see Mann (2001) for more details on calcification mechanisms).

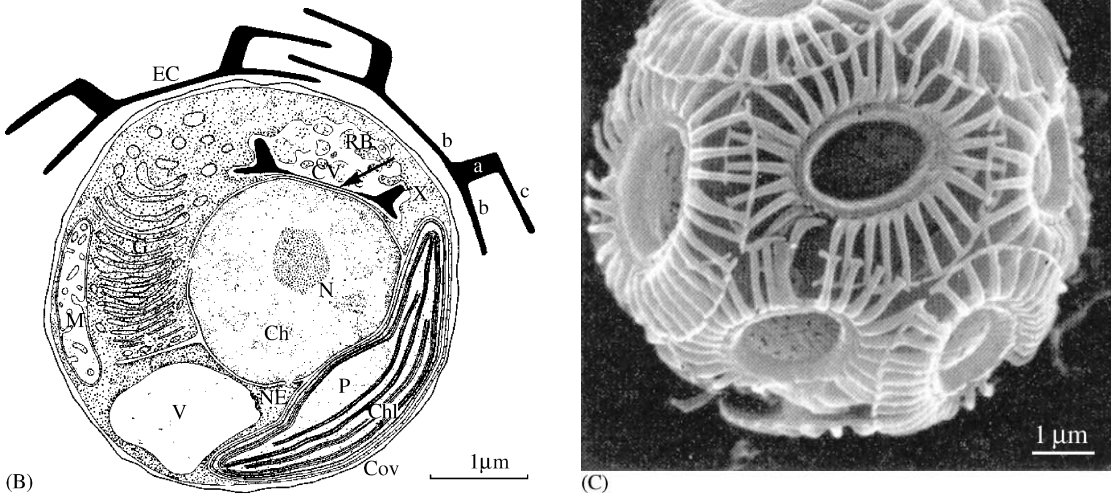


Figure 10 (continued).

8.04.3.4.4 Foraminifera

Planktonic species that occur with the coccolithophorids and nektonic mollusks, foraminifera, are another biogenic carbonate-forming species in open oceans. A significant constituent of the biomaterials that formed the rocks typical of Early Mesozoic to the present, and because of their preservation and morphologic diversity, foraminifera have played a prominent role in biostratigraphy, providing both age and environmental information. The *Catalogue of Foraminifera* (Ellis and Messina, 1940) (now upwards of 70 volumes) describes over 3.5×10^4 species with over 4,000 living today, most of which are benthic, not planktonic. Divisions within the foraminifera are based on the materials used to construct their shells.

One group, the Allogrominia, has membranous shells composed of organic macromolecules and inorganic material picked up from their environment and attached on the exterior surface. Some of these species are so selective that they choose a particular mineral. Another group actually cements whatever it can find with acid mucopolysaccharide and proteins, using its pseudopodia to strategically place the accumulations.

Some foraminifera form calcitic, others aragonitic mineralized tests. The ancient, now extinct, Fusulinia constructed its shells out of microcrystalline calcite with unique morphologies (Green *et al.*, 1980). The Miliolina form magnesian-calcite crystals, 1–2 µm long and 0.1 µm wide within vesicles, which are then haphazardly arranged on their exterior surface, while another species makes a shell from opal (Resig *et al.*, 1980). Most of the Rotaliina group of foraminifera mineralizes with magnesian calcite. The magnesium content may vary but the *c*-axes of the crystals are oriented perpendicular to the inside surface of the shell wall.

Constructed in a modular manner, each chamber of a foraminifera is preformed with an organic layer that subsequently mineralizes. In some cases a differentiating protoplasmic bulge produces two layers and calcite crystals nucleate on both surfaces of the membrane, first in patches that laterally fuse within a few hours and after tens of hours the final dimension of the chamber is achieved. Each new chamber is of larger dimensions and the chamber walls are a composite of organic and inorganic compounds. The growth of the mineral crystallites may be epitaxial (continuation of crystal growth in preordained crystallographic directions) once the signals for nucleation were inculcated through proteinaceous macromolecules generated by the cell (Addadi *et al.*, 1987). One other insight into the range of biomineralization in this phyla comes from the test formed by Spirillinacea which, in polarized light or with X-ray diffraction, appears to be as a single magnesian-calcite crystal (Towe *et al.*, 1977). Other foraminifera taxa utilize aragonite to mineralize their structures.

Of the many different minerals and mechanisms of mineralization expressed in the foraminifera, we must add the fact that they contain photosynthesizing symbionts, dinoflagellates, and chlorophytes, whether they are carnivorous or herbivorous forms. Further, during growth some mineral crystals show dissolution, suggesting that individual crystallites do not grow in isolated

compartments, and there might be some "diagenesis" taking place during their lifetimes. Any resorption or secondary deposition will depend on the bioavailability of specific elements, and the relationships with the symbionts. The organic constituents, although of exceedingly small amount (0.2 wt.%), in the foraminifera are predominantly acidic macromolecules and resemble those found in many mineralized tissues (King and Hare, 1972). They may function at different biochemical levels as: (i) nucleators, attracting cations such as Ca^{2+}, or (ii) merely directing, the organization of mineral crystallites into an appropriate texture. By studying the mineral and patterns of growth relative to the organic frameworks, the distinctions between taxa and species can be delineated (Towe and Cifelli, 1967).

8.04.3.4.5 Echinoids

There are five major subdivisions of the echinodermata with distinct anatomical expressions, but characteristically all show a fivefold symmetry. In each subdivision there are forms that biomineralize their tissues; some forms have rigid tests while others have articulated structures. The mineral, magnesian-calcite, appears as granules, spicules, or spines with the $MgCO_3$ content ranging from 5% to 15% (Chave, 1952, 1954; Raup, 1966). The calcified skeletons are usually smooth externally but spongy and riddled with cavities which may become secondarily filled with mineral and therefore appear solid. There is no obvious internal organic sheath around the individual crystallites, so the mineral behaves as a single crystal (Towe, 1967) even with its high magnesia content (Schroeder *et al*., 1969).

Studies of the mineralization of the larvae of sea urchins (they can be conveniently grown in synchronous culture) have made it possible to utilize molecular biologic techniques to determine gene expression, protein synthesis, and macromolecular organization as well as the sequences of mineral deposition (Benson *et al*., 1987). These are some of the earliest forms to be studied and illustrate the connections between genetics and biomineralization. Stem cells responsible for spicule formation can be isolated and produce normal spicules *in vitro* (Kitajima and Okazaki, 1980); since spines regenerate the secondary mineralization process can also be studied (Ebert, 1967).

Spicule formation in the larval stage of the sea urchin commences when mesenchyme cells migrate into special locations where they fuse forming a syncytium that produces a membrane-bound vacuole. The spicule morphology is directed by the size and orientation of the vacuole, which aggregate and connect through stalks (Beverlander and Nakahara, 1960). Several sites of mineral deposition start but resorb and ultimately only one granule remains per vacuole. As it enlarges, a tri-radiate form is produced that reflects the crystallography of the magnesian-calcite: the long axis of the spicule is aligned with the *c*-axis of the mineral. Cross-sections through the spicule show concentric layers of organic glycoproteins in concentrations only 0.1% by weight of the spicule that effectively compartmentalize the mineral deposition (Benson *et al*., 1983).

The adult mineralized spicules use the larval spicules as a nucleus extending the arms into branches that join into fenestrated plates. At the macrostructural level the spine plate segments show infilling of secondary calcite (Figure 11). The entire skeleton is covered by epithelia and any space within the mineralized portions contains a variety of cells, such as phagocytes and sclerocytes, which are capable of resorbing and reprecipitating mineralized tissue when the skeleton is broken (Loven, 1982).

The crystals forming the plates are aligned with their *c*-axes either perpendicular or parallel to the plane of the plate depending on the species and appear to be single crystals (Raup, 1959). As well, spines behave as single crystals with *c*-axes aligned along the length (Raup, 1966) and, if broken, regenerate to their former length by elongating, connecting, and fusing to restore the characteristic structural pattern (Davies *et al*., 1972).

The regeneration of biomineralized skeletal tissues by echinoids using magnesian-calcite is reminiscent of that observed for the precipitation of calcium phosphate in regenerating fractured vertebrate bones (Section 8.05.3.7.2). These organisms and their cell systems are completely

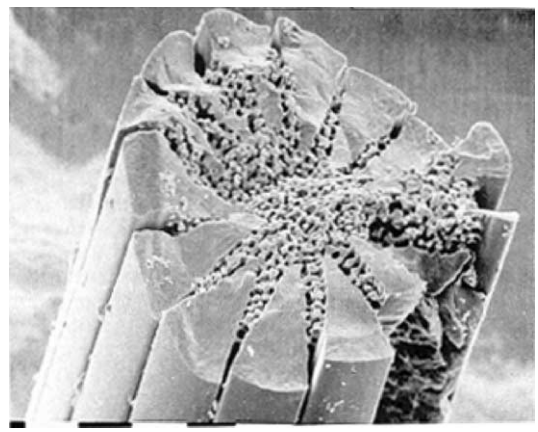


Figure 11 An SEM image of the cross-section through the spine of a sea urchin showing secondary mineral infilling. Diameter of spine is ~1 mm (source Lowenstam and Weiner, 1989, figure 8.2.A, p. 125).

different from vertebrates, but they act in similar fashion and the processes have the same end: a mineralized form that continues to function.

8.04.3.4.6 Mollusks

The biomineralized shells of mollusks, especially the common bivalves, oysters, clams, and mussels, have been well studied possibly because they are easily found in shallow marine waters, where they become a food source for humans as well as animals. One of the largest groups of invertebrates originated in the earliest Cambrian and had a rapid expansion in the Ordivician (Stanley, 1973, 1976). Of the five out of seven taxa in the phylum that mineralize 17 different mineral species have been identified from a range of anatomic sites (Lowenstam and Weiner, 1989, table 6.1, pp. 90–93). Mostly they mineralize with calcite or aragonite, but the third polymorph of $CaCO_3$, vaterite, is found in the freshwater snail egg capsules of gastropods, which also precipitate goethite and opal in their radula. Further, weddellite, $CaC_2O_4{\cdot}2H_2O$, fluorite, CaF_2, and amorphous calcium phosphate are found in the gizzard plates of some species of gastropods. Only a general overview of the aragonite and calcite shell mineralization in bivalves will be presented, along with the interesting anatomical biomineralized structure—the formation of magnetite teeth in chitons, another member of the mollusk family.

Bivalves. The physical expressions of the mineralized portions of modern and fossil bivalves are similar, and faithfully record incremental growth stages. Mollusks, therefore, have been used to study not only the history of the life form, but also the effects of environmental influences, including climatic changes on their growth and development. Any exoskeletal alteration in general shape or size, and interruptions in mineralization due to variations in salinity, temperature, or tidal cycles, can be detected, and correlated with some external event (Seed, 1980). Whether burrowing in soft sediment or attached to a hard surface by the byssus, the macro- and micromorphology and chemical constituents of bivalve shells from far-flung reaches of the oceans have been sampled. The early workers were cognizant of the adaptation strategies of living forms to the environment (Clarke and Wheeler, 1922; Vinogradov, 1953). For example, in the 1920s it was known that the mineralogy of the shells was one of the ways they responded to the physical environment: forms that grew in the colder waters, or at higher salinity, deposited calcite rather than aragonite.

Was it possible that the physical milieu dictated the genetic determinants, and benefited the choice of mineral phase, or was the geographic expansion to different environments the instigator in shifting the mineral precipitated an example of convergent adaptation? Today we know that the mineral phase dictates the uptake of other elements: magnesium is a prominent element in solid solution in calcite, whereas strontium is more readily incorporated in aragonite. However, we note that all mineralogical variations in bivalves are associated with differences in shell architecture.

Bivalve shell architecture. The simple double-capped shell so typical of these species with macroscopically visible incremental growth lines hides a complicated mineralization system (Seed, 1980). There are different patterns of ultrastructure, distinctive to species, and to freshwater forms (Saluddin and Petit, 1983). Whatever the mineralization patterns, they arise through the actions of an organic structure produced by epithelial cells, the mantle. The mantle in the bivalves is composed of a gel-like organic molecular complex with three folds and two grooves. The groove closest to the shell surface is the site where specialized macromolecules, mostly proteins, are secreted, polymerize and form the periostracum (the outer shell covering that contains in addition to water insoluble proteins and chitin) (Waite, 1983). The periostracum varies in thickness, ultrastructure, color, and texture between species; the inner portions are sites of mineralization, i.e., calcitic or aragonitic deposition takes place interior to an organic layer that protects the animal from the external world. The shell is thus a layered structure, with interior cells the site of mineralization, and the calcium and carbonate for the mineral is transported either through or between the cells that produce the periostracum (Figure 12(A)). The cells actively involved in mineralization are elongated with obvious and numerous organelles: mitochondria, golgi, and endoplasmic reticula (Wilbur and Jodrey, 1952; Crenshaw, 1980). The shell thickens vertically and enlarges (horizontally) as the animal grows. Growth hormones and calcium transport proteins have been identified as important in the formation of mollusk shells, whether the process takes place on land or in water (Doterton and Doderer, 1981).

A cross-section through the growing shell edge shows several prismatic layers of $CaCO_3$ separated by organic layers. When the two polymorphs of $CaCO_3$ are present in the same shell, they are separated by ultrathin organic sheets with different compositions; in addition, each mineralite may be enveloped by biomolecules with different compositions (Watabe, 1965). The myostracum, site of muscle attachments in bivalves, is always mineralized with aragonite, adding to the complexities of accurate determination of the mineral in different

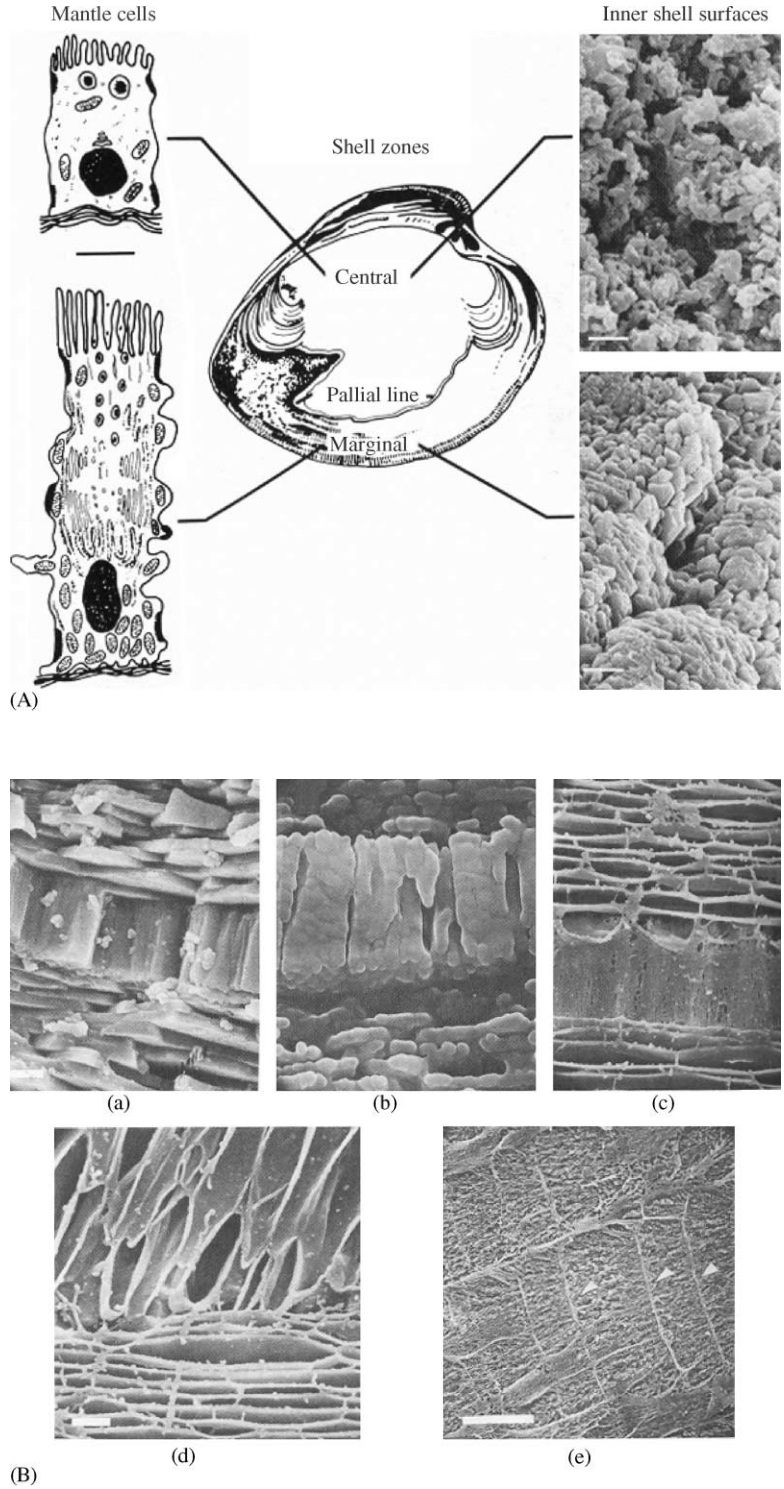


Figure 12 (A) Zones, cells, and appearance of the mineralized portions of the shell of *Mercinaria mercinaria*. Scale bar with sketches of cells 3 μm. SEM images after removal of organic with sodium hypochlorite solution, upper = 0.5 μm, lower = 0.1 μm (source Crenshaw, 1980, figure 2, p. 118). (B) SEM images of the various layers in bivalves, *Mytilus californianus* air dried and fractured sections: (a) pallial myostratum; (b) polished and etched section in which the organic matrix exposed by etching has collapsed over the dried surface; (c) polished and etched section obtained by critical point drying showing organic matrix in high relief above etched surface; (d) transition from prismatic (top) to nacreous layer (scale bar (a)–(d) = 1 μm); and (e) prismatic layer of *Mercinaria mercinaria* showing prominent growth lines (arrows) formed by concentrations of organic matter (scale bar = 10 μm) (source Clark, 1980, plate 1, p. 608).

portions of the shell as it is retained during the shell enlargement.

Over 50 types of shell ultrastructures have been catalogued with different size layers, patterns, and orientations of calcite or aragonite in the layers depicted (Carter, 1980; Carter and Clark, 1985; Boggild, 1930) Figure 12(B). The biomineralization processes appear to be directly responsive to an anatomical site. Such specificity suggests sophisticated genetic control not only on the production of the bioorganic molecules but also for the mineral phase, its site, and orientation. For example, tablet crystals of aragonite in nacre in one bivalve species may have uniform height in all the layers but show variable or different heights and arrangements in another species. The length, height, and shape of the aragonite tablets may vary in a third species from one layer to another layer. The implications of such refinements have been used to gain some general understanding of the mechanisms of biomineralization in invertebrates (Weiner, 1986).

Chitons. Another class of mollusk is the chitons. The shell is not solid as in the bivalves but composed of a series of eight overlapping plates mineralized by aragonite. The plates arise from a mantle-like mass that also produces the girdle and aragonitic spicules. The spicules initially form within vesicles of the epithelial cell system and protrude beyond these enclosures as they enlarge. The mineralized chitons have an additional layer between the periostracum and the inner shell layer, the tegument, in which spherulites and other organs, e.g., light sensing (Haas, 1976), are aligned parallel to the plate face. For details of the anatomy of an adult mineralized shell, see Beedham and Truemann (1967).

The chitons have another and quite different anatomical mineralized site, teeth; these teeth contain magnetite. Since these creatures inhabit the intertidal and subtidal zones on tropical limestone coasts, or other rocky areas, they utilize the teeth for obtaining food, the algae that cling to the rock surfaces. There are other organisms that grow magnetite-containing teeth (Lowenstam and Kirschvink, 1985), and in order to provide a unique insight into a novel biomineralizing system, we describe the chiton teeth that illustrate specialized invertebrate structures.

Teeth of chitons. Chiton teeth are arranged in transverse rows consisting of a central tooth and eight pairs of flanking teeth along the radula, a ribbon-like structure in the mouth portion of the chiton (Figure 13). One end of the radula moves providing an area of 8–10 rows of teeth for scraping. As teeth abrade they are discarded, the rate dependent on species, and within a couple of days new magnetite teeth move into place having been produced at the same rate at the other end of the radula (Nesson, 1968; Kirschvink and Lowenstam, 1979).

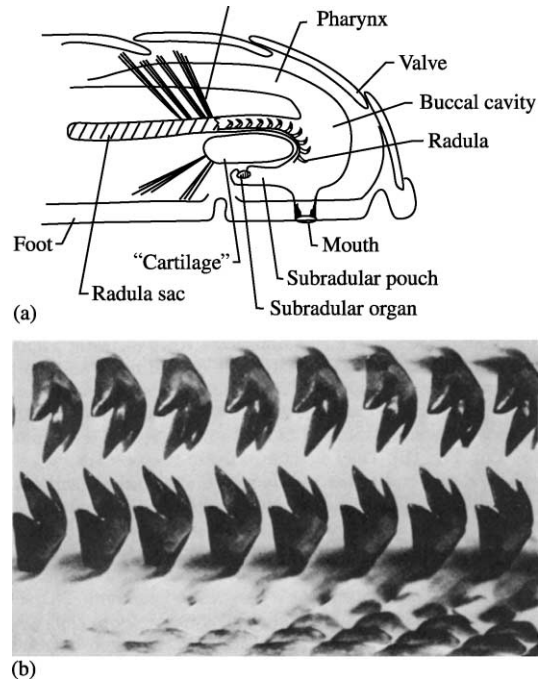


Figure 13 Chiton teeth. A sketch of the radular organ in the chiton mouth and a micrograph showing the shape and rows of magnetite teeth. Each tooth is less than 20 μm in length (source Nesson and Lowenstam, 1985, figure 1, p. 336).

Each tooth with its mineralized cap has a flexible stalk through which it is attached to the radula. Studies in *Chionida* showed that the original organ was defined, and the mineralization sequence orchestrated, by epithelial cells. The cells first create the housing of a mature tooth, then the framework of α-chitin, organic molecules on which iron hydroxides precipitate. The source of iron is ferritin, an iron–protein complex found in the blood. In which the protein encloses the iron oxyhydroxide ferrihydrite ($5Fe_2O_3{\cdot}9H_2O$). Ferritin is brought to the tooth location and stored until iron is extracted and transported in a reduced, soluble form to the mineralizing sites (Nesson, 1968). The first mineral to be precipitated in the tooth is ferrihydrite (Towe and Lowenstam, 1967), a poorly crystalline phase with light brown color, that is a precursor to the nucleation and growth of magnetite. A thin layer of another iron hydroxide phase, lepidocrocite (γ-FeOOH), forms on the inner surface of the enlarging magnetite layer. Note that magnetite occurs on the rasping and pointed edge, as well as on the concave portion of the tooth surface, while the central core and most of the remainder of the tooth is filled with dahlite, carbonate hydroxylapatite or francolite, the fluorine-containing carbonate apatite. A similar mineral sequence

occurs in another chiton, *Cryptochiton*, except that amorphous hydrous ferric phosphate with trace amounts of amorphous silica form the tooth interior. The selection of magnetite, and/or apatites to produce a strong structure is appropriate for the rasping function of this specialized organ compared to the soft calcium carbonate mineralized tests.

For additional information on magnetite, and other iron mineral species see Section 8.04.2.4, and for iron biomineralization and mechanisms see Section 8.04.3.3.2.

8.04.3.4.7 Arthropods

The arthropod domain includes insects, the largest group of living creatures but a group that has not so far been known to be obviously biomineralized. It is certainly true that specialized anatomical sites within this group of organisms contain mineral materials and future investigations could add to our present list. Mobile arthropods may be found anywhere, on land, in freshwaters and ocean waters, and have need of bodily protection, as they are the prey of other invertebrates and vertebrates. Some arthropods, e.g., crayfish or lobsters, like mollusks, and fish, have become important constituents of the human diet. The life cycle and nutritional requirements of these creatures have become well documented so that they can be economically "farmed" (Nowak, 1972; Marx, 1986).

The Crustacea show a wide range of biominerals in specialized organs within their segmented bodies (Lowenstam and Weiner, table 7.1, pp. 112–113). The cuticle, or carapace, the external covering that is shed, or molted, as the creatures age and grow in size, may be mineralized with calcite, aragonite, or calcium phosphate mineral materials. These creatures also produce gastroliths, a much more heavily mineralized organ that functions as an internal storage site for the calcium required in the seasonal regeneration of the carapace. Usually composed of amorphous calcium carbonate (calcite in crabs and lobsters), in insects other calcium minerals, such as calcium oxalates and calcium citrate (in addition to the carbonate or phosphate species), are also found. Copepods show some opal in their mandibles, presumably an advantage for crushing and grinding prey, and several crustacea, including the trilobites, perhaps had calcite lenses. Bees are assumed to harbor magnetite for navigation (Gould *et al*., 1978).

Crustacea exoskeleton, the carapace. The carapace of Crustacea strengthens their exoskeleton through mineral deposition (calcium carbonate or calcium phosphate) in, or on, a fibrous organic template. The first stage is the secretion of protein fibrils, 2.5–5 nm in width, that aggregate in a special manner with chitin, an aliphatic polysaccharide, β(1-4)-N-acetyl-D-glucosamine, which closely resembles cellulose, and is also found in fungi. β-Chitin was recognized as early as 1811 in mushrooms and in insects, and by mid-century the name "Chitine" was applied to arthropod cuticle materials. A relatively recent book summarizes the chemistry and molecular characteristics of chitin including industrial and medical applications (Muzzarelli, 1977), while the molecular biology of chitin has since been investigated by Blackwell and Weih (1980) and Atkins (1985). Chitin, the organic constituent in nacre biomineralization sites in mollusks, is illustrated by Mann (2001, p. 104). In arthropods, the fibrous protein polysaccharide mixture forms layers which stack with the fiber direction 180° out of phase between adjacent lamellar arrays forming a very tough, amazingly resilient "skin".

The total amount of mineral within the matrix layers varies with species (Greenaway, 1985) and from place to place within the cuticle of an individual (Huner *et al*., 1978). Prenant (1927) measured the ratio of calcium phosphate to amorphous or crystalline carbonate in the tissues, and suggested that phosphate may act as an inhibitor to the formation of a crystalline carbonate phase. Hegdahl *et al*. (1977) showed that the cuticle of the crab *Cancer pagurus* contained three distinct layers, each with different ultrastructural organizations and percentages of calcite. It would appear that the mineral selectively associates, and may be nucleated and localized by the specific proteins or the chitinous polysaccharides that characterize each of the species (Roer and Dilliman, 1985; Degens *et al*., 1967).

Gastroliths. The initiation of the crustacean molt cycle takes place below the cuticle (Greenaway, 1985). However, before shedding a new organic molecular composite is formed and some or all of the mineral in the old carapace is resorbed. The calcium released during the molt is retained in a separate storage site and structure. A mineralized button, known as a gastrolith, is created in the cardiac stomach of crabs and lobsters from where the element will be mobilized to mineralize the next and larger shell. Since the amount and availability of calcium depends on habitat, terrestrial and freshwater crustaceans maximize calcium recycling. These forms, when they consume their cuticle, must have at their disposal some "hardening" materials, at least for their mouth parts, as they lose every means of protection, including their ability to feed, when the cuticle disappears. Gastroliths in freshwater crayfish are composed of spherical aggregates of amorphous $CaCO_3$. This ultrafhin, poorly

crystalline, solid form probably proves advantageous as a more crystalline aggregate would not be as easily dissolved with rapid mobilization of the calcium (Travis, 1976).

Other members of the crustacea sequester calcium as calcium phosphate in a gland, the hepatopancreas, where needle-shaped rods up to 300 Å or concentric granules to 4 μm diameter have been detected (Chen *et al*., 1974; Becker *et al*., 1974). In one species the granules have a high concentration of lead, and it has been suggested that these gastroliths may also play a role as a detoxification site (Hopkin and Nott, 1979). Calcium pyrophosphate (amorphous) was identified in the hepatopancreas of gastropods (Howard *et al*., 1981) and yet another biomineralization form and system, extracellular phosphatic concretions, is found in the crustacean *Orchestia cavimana*. The calcium phosphate form may be a problem. Not only is this mineral less soluble but its localization requires that this crustacean create a customized extracellular transport system to move the calcium to the newly forming epithelial cuticle; however, the phosphate is a more durable (hard) mineral than carbonate. Perhaps this species is not a biological oddity, but a trial run for biomineralization in bone where mineral dissolution and reprecipitation is essential to the proper functions of vertebrates.

Calcium is not usually stored in the blood in these creatures, although a freshwater land crab *Holthusiana transversa* shows hemolymph cloudy with microspheres of calcium carbonate after shedding, presumably awaiting new skeletal deposition sites. Specialized storage mechanisms are not unique to crustaceans. A calcium phosphate compound is found in an insect, the larval form of *Musca autumnalis*. Mineral containing granules accumulate in tubules, and resorb, coincidental with the pupation of a fully developed larva (Grondowitz and Broce, 1983; Grondowitz *et al*., 1987).

The arthropods present us with an intriguing range of biomineralization mechanisms that suits their peculiar bodily needs and functions. Biomineralization activities change on demand during their life cycle and clearly have been modulated to maximize a particular form and function. Sequestering and recycling of calcium for "hardening" their carapaces, an essential part of their defense mechanism.

8.04.3.5 Silica Biomineralization

Silica biomineralization is the incorporation of the extremely fine-grained, and virtually amorphous varieties of opal, a hydrated form of the common, but highly insoluble species that has the formula $SiO_2{\cdot}nH_2O$ (see Section 8.05.2.2.1). Opal is related to the very common SiO_2 mineral species, quartz. Oceans are at present undersaturated with respect to opal (Broecker, 1971) possibly because of the biological formation of animals with silicified skeletons such as the diatoms. These delicate structured creatures, which proliferate in the upper photic zone, dissolve at depth. Therefore, only robust siliceous skeletons such as sponge spicules are retained in sediments that accumulate in deep waters, although some diatoms survive on the continental shelf under zones with high productivity. The initial deposition of the amorphous hydrated silica, opal, converts first to opal-CT and eventually to crystalline quartz (Kastner, 1981).

The oldest sponge spicule accumulation has been found at the Precambrian–Cambrian boundary (Sdzuy, 1969) (Figure 14). The beginning of extensive siliceous deposits of radiolarian skeletons marks the mid-Ordivician. Cherts derived from shallow-water deposits of radiolarian skeletons, and sponge spicules are found throughout the Paleozoic (Lowenstam and Weiner, 1989, p. 245) and suggest that seawater may have been silica saturated at this time. Diatoms become quantitatively important constituents of the marine planktonic community in mid-Cretaceous along with the first appearance of silicoflagellates ~100 Myr ago, while dinoflagellates evolved in the Paleocene just after the massive Cretaceous extinction. The huge explosion of diatoms extracting silica from ocean waters, especially in the Cenozoic, has been cited as the reason for the decrease in the level of siliceous mineralization in radiolarians and siliceous sponges (Harper and Knoll, 1975), but reduction of silica biomineralization has not been detected in the silicoflagellates, and some other families (Tappan, 1980). The expansion of these geologically modern biomineralizers may affect the global ocean silica saturation levels, which may account for a decrease in: (i) the accumulation of siliceous marine deposits, (ii) the number of species of radiolarians, and (iii) the concentration of mineral per individual radiolarian skeleton (Moore, 1969).

Freshwater diatom accumulations, diatomites, are part of the radiation of this family of siliceous mineralizing species in the Cenozoic (Round, 1981). Along with members of the plant kingdom, such as the angiosperms with silica in their seeds, and the grasses (see Section 8.05.3.6), silica biomineralization expanded to the land more or less at the same time as diatoms proliferated in the oceans.

8.04.3.5.1 Radiolarians

The extensive sampling during the Deep Sea Drilling Project (1969–1979) has expanded

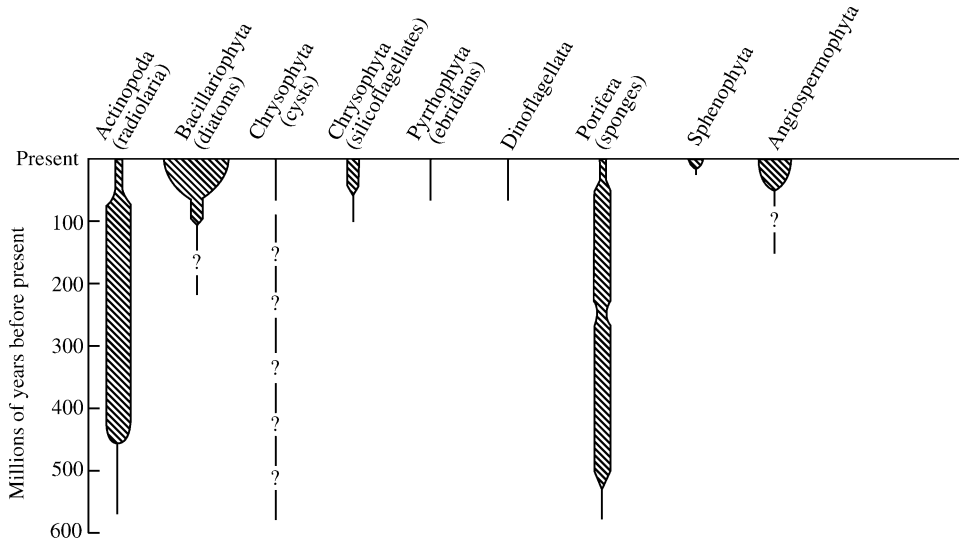


Figure 14 The stratigraphic ranges of invertebrates with silica mineralized hard parts (source Lowenstam and Weiner, 1989, figure 12.4, p. 245).

the number of varieties of the beautiful and delicate structures formed by the radiolarians. Originally depicted by Haeckel (1899–1904b) from the Challenger expedition, the range of structures has delighted lay and scientific observers ever since (Figure 15(a)). Similar to other invertebrates radiolarian mineralized remains, especially the intricate structural detail has been, and is, used to distinguish between families and species. Planktonic photoautotrophs, most radiolarian species, live in the top few meters in normal composition seawater. Their well-preserved skeletons have been part of cherts since mid-Ordivician and throughout the Paleozoic. These deposits are the sites not only of the silica skeletons but they were also often cemented by silica, suggesting that the oceans may have been saturated with silicon during that period (Lowenstam, 1972; Broecker, 1974; Whitfield and Watson, 1983).

The frothy appearance of a live radiolarian (Anderson, 1986) (Figure 15(b)) belies the mineralizing material, opal. Produced by the uptake of monomeric silicic acid, followed by dehydration and polymerization, the bioprocessing that creates these creatures was difficult to study because of the differential hardness of the silica mineral and the organic components. However, employing $Ge(OH)_4$, as a substitute for $Si(OH)_4$, has now elucidated the rates of uptake of silicon as well as the mechanisms of biomineralization. With its longer-lived isotope ^{68}Ge (half-life 282 d) relative to ^{31}Si (156 min), the transport, sequence, skeletal production, and insight into other biologically controlled processes have been investigated (Azam and Volcani, 1981). An outline of these mechanisms is presented under diatoms (Section 8.04.3.5.2); here we merely reiterate that growth and proliferation of all the organisms that mineralize with silica have customized systems that present some interesting chemical conundrums.

The modern radiolarian skeletal frameworks appear less robust. Typically their tests show dissolution and dissociation. This is diagenesis taking place within a living form, and though often encountered it may not be recorded; it is difficult to document that which is missing. However, examination with scanning or high-resolution microscopy of the sample, and attention to the individual test morphology, or alteration, should be a regular part of sample preparation before any geochemical analysis.

Through examination of the recently obtained Cenozoic deposits, the record of radiolarians is sufficiently complete to provide us with a remarkable picture of morphologic diversity. The amount of information now available affords insight into the evolution as well as extinction of a few taxa (Figure 15(c)) and the ability to study their distinctive morphologic variance, growth, and development, as well as biomineralization processes, all useful to paleontologists, biostratigraphers and to those interested in biomineralization.

8.04.3.5.2 Diatoms

Of all the biomineralized species that are used as geobiological indicators, the best studied are the diatoms. As the base of the food chain for many eukaryotic organisms, the group also presents opportunities to geochemists investigating environmental issues. Diatoms are abundant today in hypersaline marine to freshwater

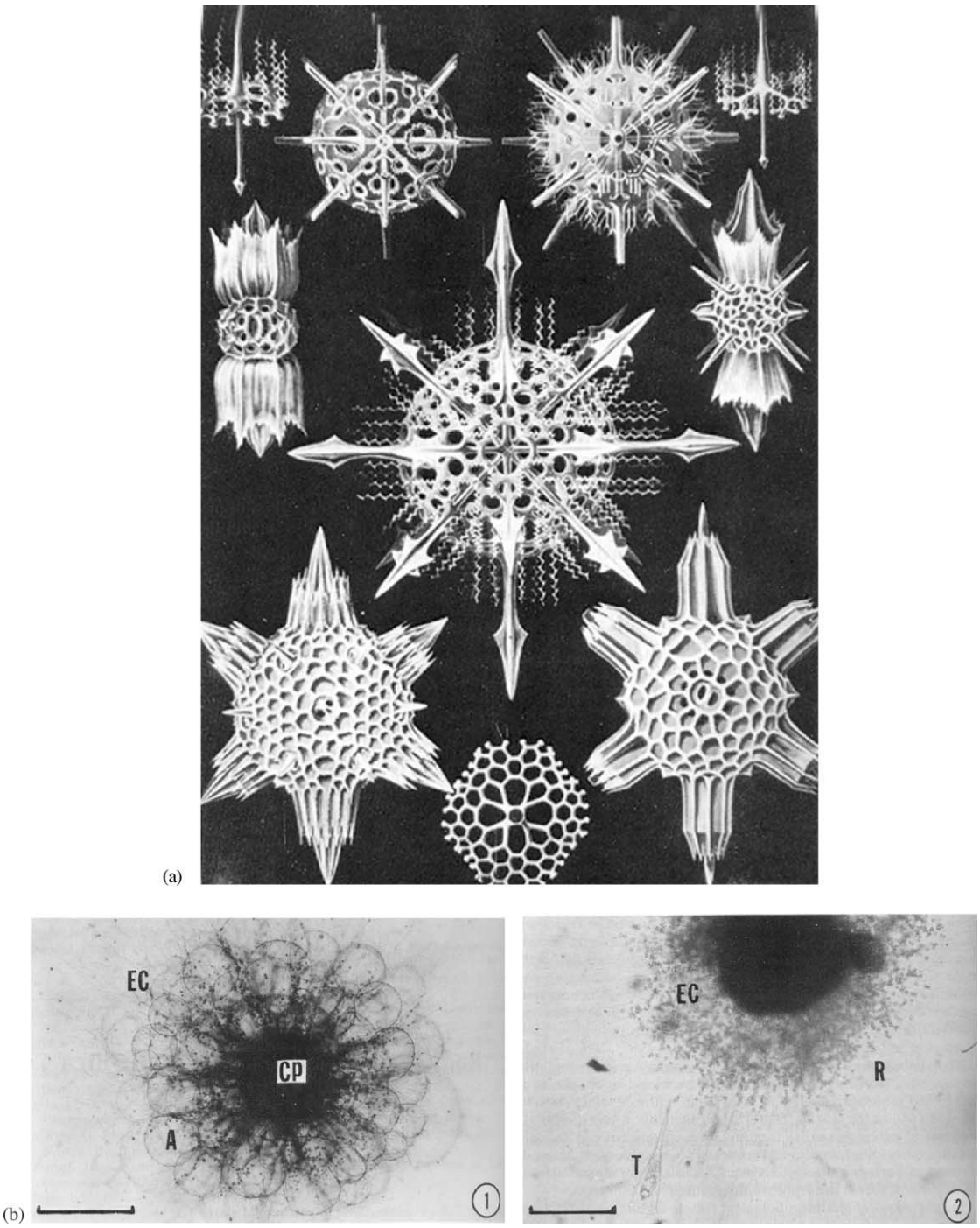


Figure 15 (a) Radiolarian morphologies as depicted by Haeckel (1899–1904b). (b) Light micrographs of fresh radiolarians showing frothy appearance: (1) *Thalassicolla nucleata* A = cytoplasmic bubbles which are penetrated by algal symbionts and enclose captured algal prey, EC = extracellular capsular cytoplasm, and CP = central capsular region; (2) a member of the Astrospaeorida family of radiolarians showing the spherical organization of the cytoplasm (scale bar = 0.5 mm) (source Anderson, 1981, figure 13.1, p. 348). (c) Evolution of radiolarian forms over time: *Lithocycla* (1–4) and *Didymocryrtis* (5–11) (source Reidel and Sanfilippo, 1981, figure 12.6, p. 332).

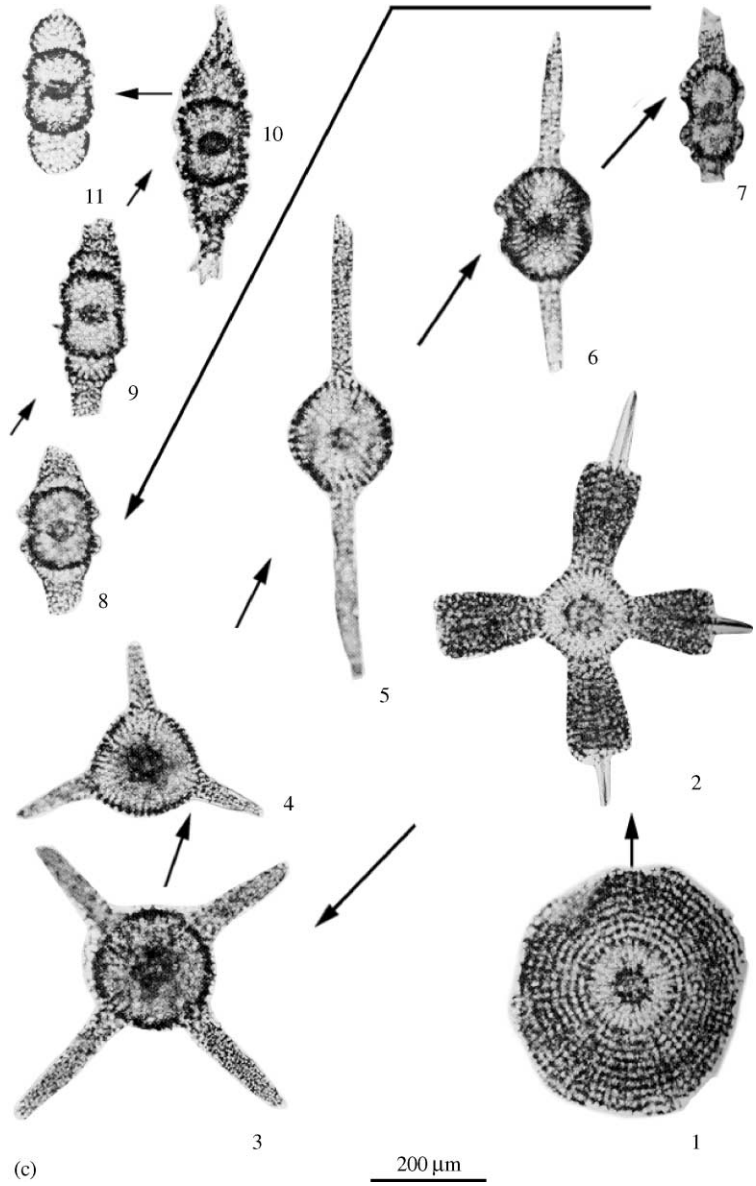
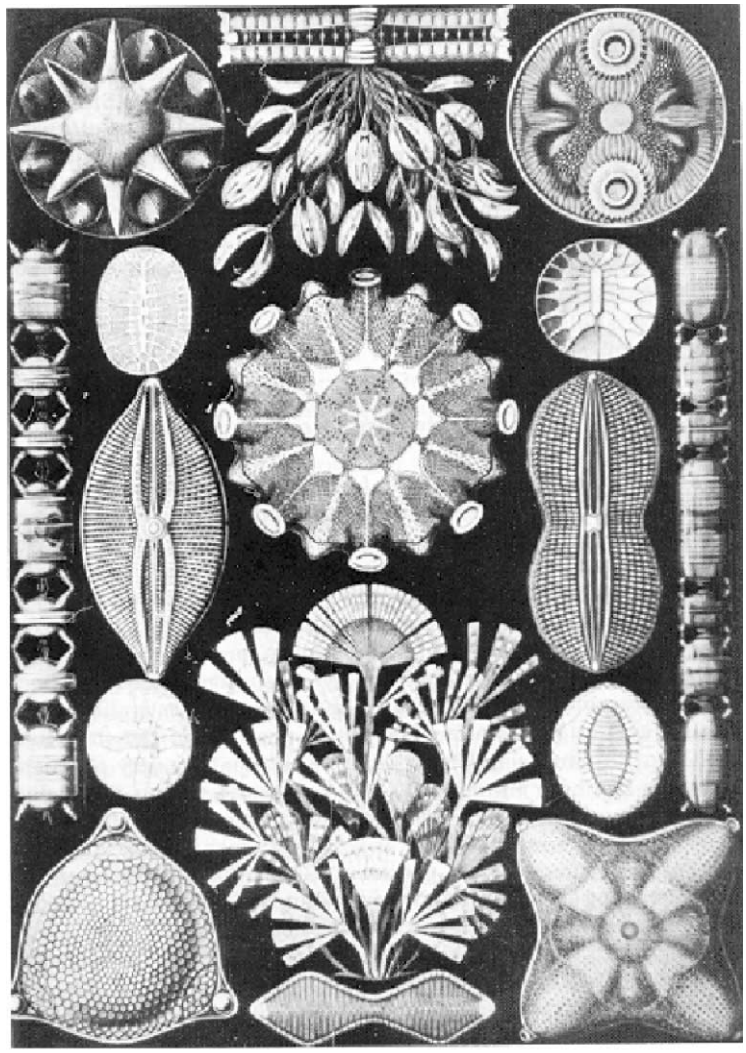


Figure 15 (continued).

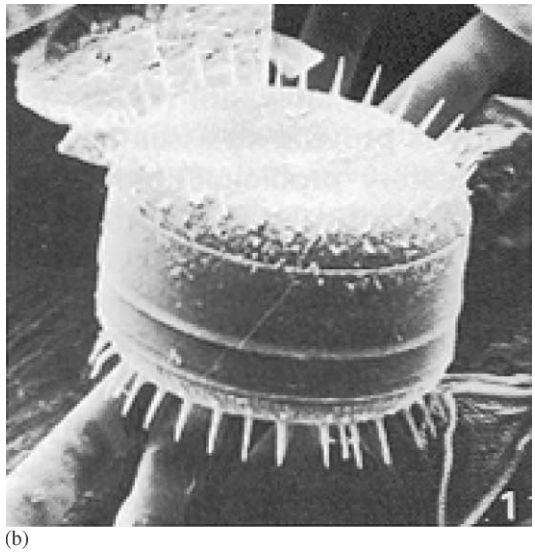
environments. Phototrophic diatoms mineralize with opal similar to radiolarians. Accumulations of diatom-rich sediments may be a source of silica-rich solutions which, through diagenesis and metamorphism, formed siliceous veins, layers, or pods in sediments, a process analogous to the accumulation of plant materials that give rise to oil and gas deposits.

The diatom's delicate structures, similar to radiolarians, were originally presented by Haeckel (1899–1904a) (Figure 16(a)) and over 10^4 species, modern and fossil, have been described (Locker, 1974). The variety of the delicate mineralized structures not only identify species but have also been used to determine the ecology of their depositional environment, freshwater or salt water (Crawford and Schmid, 1986).

The mineralized structures of tiny silica diatoms (<50 μm in diameter) appear smooth even at the highest magnifications reflecting the amorphous state of the mineral. Diatom tests are multipartite, a combination of specialized forms described by Li and Volcani (1984, p. 519) as "box-and-cover-like structures" with the parts known as valves and girdles (Figure 16(b)). The valves are often elaborately patterned with ribs, processes (tubes), and pores, while the girdles form bands that may vary in number. There are two major groups of diatoms based on the pattern of the perforations on the valves: pinnate diatoms



(a)



(b)

Figure 16 (a) Diatom morphologies as depicted by Haeckel (1899–1904a). (b) Whole cell of the centric diatom *Stephanodiscus* showing the mineralized "box and cover" structure with a protruding ring of spines, 1,000× (source Round, 1981, figure 5.1, p. 100).

that show bilateral symmetry, and centric diatoms with tri- or circular form. Silicification of the valves varies with species from almost none, i.e., the structure is purely organic, to heavily silicified, while the girdle portion characteristically contains mineral.

There is a complicated morphogenesis of these siliceous structures. The initial uptake of silicate, probably as silicic acid, moves into the cell and becomes associated with the endoplasmic reticulum and most prominently with a specialized membrane-bound vesicle known as the silicalemma. This vesicle occurs in all organisms that form siliceous structures (Sullivan and Volcani, 1981; Round, 1981). Mann (2001) constructed a simplified schematic for the diatom *Conscinodiscus wailesii* to illustrate the interplay of specialized and sequential cell activities: elongated vesicles are secreted but stay attached to the cell wall. Close-packed and distributed with regular polygonal symmetry, silica deposition takes place not within but between the vesicles on the vesicle membranes. The initially isolated silica deposits fuse into a silica web with pores representing the opening, or internal void of the vesicles. The three-dimensional silica scaffold is directed through precise placement of the vesicles until the full diameter of the diatom is a series of concentric fenestrated shells with specific nanoscale geometric patterns (Mann, 2001, pp. 134–135).

Diatoms not only have a siliceous mineralized skeleton but also require silicon for synthesizing their DNA, and transcriptional proteins, making their entire metabolism dependent on silicon. It would appear that in the process of producing the silica mineralized structures, sufficient silicon becomes sequestered for all requirements. The original silica pool created within the organism (up to 500 mM silicon has been measured in the solution within cells, considerably above the 3.5 mM L^{-1} in inorganic solutions which autopolymerize) is distributed as granules along the preformed vesicle membrane. The membranes, composed of polycarbonates and acid polyprotein molecules, play several roles: they act as the templates aiding the placement of appropriate amounts of silica in precise arrays and thereby prevent premature silica precipitation (Lowenstam and Weiner, 1989). The biomineralization process in diatoms is an exercise in control on the uptake, localization, and deposition of opaline silica. Studies comparing the water chemistry surrounding diatoms show that biological activity keeps the mineralizing system out of equilibrium with the surrounding environment. Freshwater and oceanic diatoms may also incorporate locally available aluminum and iron as they produce their delicate, distinctive tests.

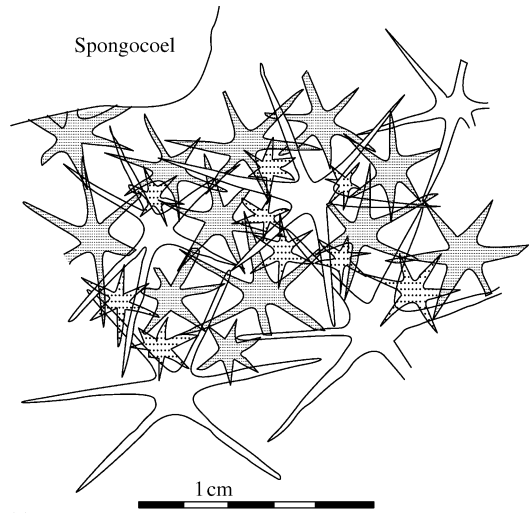
8.04.3.5.3 Sponges

Found in all aquatic environments these filter-feeding organisms may occur on soft or on hard substrates at a variety of depths. Some members are entirely organic, others form mineralized skeletons that are calcareous, found in association with other $CaCO_3$ biomineralizers, i.e., corals; and there are siliceous forms, the demosponges (Finks, 1970). The mineral portions form as spicules, which are distinctive to a species, and some sponges produce secondary mineral deposits, which leads to dense and rigid structures, generally typical of deeper water forms. Modern sponge lineages, mostly soft sponges, derive from Early Carboniferous as few of the Paleozoic families survived the Frasne/Famenene (Devonian) extinction (Reitner and Keupp, 1991, p. v). Before sponges with rigid calcareous skeletons were rediscovered in the 1960s (Hartman and Goreau, 1970), there had been differences of opinion on an appropriate inclusive classification to cover the entire range of such a diverse group of organisms (see discussions by van Soest (1991); see also Reitner and Keupp (1991) and Hartman (1981)).

The oldest heteractinid calcareous genus *Eiffelia* was described from the well-preserved spicules in the Burgess shale of British Columbia (Cambrian age) by Walcott (1920) (Figure 17(a)). These skeletons were composed of three different sizes of hexaform spicules, regularly and spherically distributed but other tri-stellate, Y-shaped, tetra- and octaform spicule species were described. By the Pennsylvanian marked knobby calcareous overgrowths that occasionally became fused and obscured, the initial calcareous spicules in a grossly tubular form with a variety of substructures were described (Rigby and Webby, 1988) (Figure 17(b)).

The carbonate spicules, initiated in the outer or dermal part of the organism, become interior in later forms of these organisms. The process of secondary mineralization leads to a diversity of macrostructures, many centimeters in height (Rigby, 1991). Localities in the Silurian of North America and the Devonian of Australia show an explosion of such structures, but the morphological expressions are different suggesting ecological specificity. Similar structures are seen in modern localities (Rigby and Webby, 1988).

In siliceous sponges the spicule-forming site is known as the desma. An organic biomolecule defined structure located in the ectosomal regions near the surface of the growing sponge; it had been defined in 1888 (Sollas, 1888). Successive layers of silica (opal) are deposited as rods that become decorated with symmetric or asymmetric distributed sidepieces. These spicules may fuse joining several mineralized desmas to produce



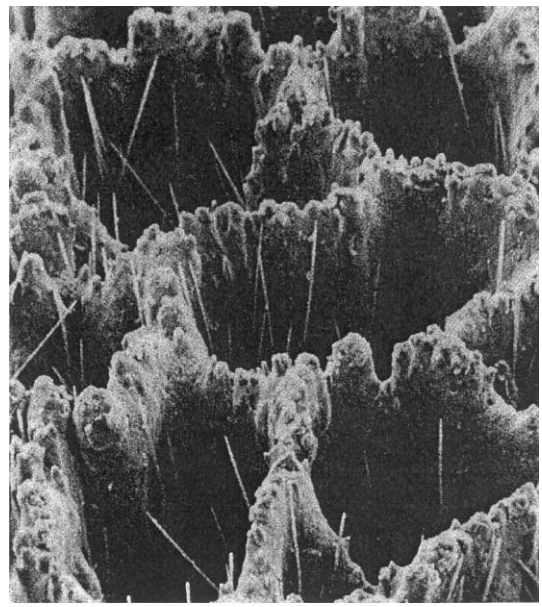


Figure 17 (a) Drawing of the skeleton of a sponge, *Eiffelia globosa* from the Middle Cambrian Burgess shale of Canada illustrated in Walcott (1920). Three ranks of spicules are shown: the first order is unpatterned, the second is loosely stippled, while the third is dark stippled (source Rigby, 1991, figure 2, p. 84). (b) Photomicrographs of *Wewokella solida* Girty 1911 from the Pennsylvanian Deese formation, Oklahoma, 4× (source Rigby, 1991, figure 7B, p. 88). (c) SEM of a cleaned surface of a sclerosponge skeleton showing silicious needles embedded in, and protruding from, the aragonitic skeleton, 100× (source Hartman, 1981, figure 16–28, p. 488).

a complex rigid skeletal mass (Hartman and Goreau, 1970; Hartman, 1981). A similar process occurs in carbonate-mineralizing sponges. In the sclerosponges, a modern group, there is a species that has very thin silica needles in a massive calcareous (aragonitic) structure (Hartman, 1981) (Figure 17(c)).

Studies of sponge lineages show evolutionary patterns with convergent adaptation and mimicry across groups in both the calcareous and siliceous varieties. The amazing reappearance of the same basic structural elements, after a lengthy hiatus, even by the geologic timescale, and of diverse spicule morphology that enlarges and may fuse,

producing rigid forms, suggests that sponges, like many other invertebrate mineralized species, adapt to exploit ecologic niches today as they did in remote times.

8.04.3.6 Plant Biomineralization

8.04.3.6.1 Introduction

Biomineralization, or the precipitation of mineral as a result of the metabolic functioning of a living organism, is a process present in all five kingdoms of life, and is widely practiced by plants (Figure 18). Both unicellular and multicellular plants biomineralize producing structures known as "phytoliths," utilizing a range of chemical compounds with various mineralogies. The morphology of these structures varies as well: some plant biomineral structures are intricate and ornate, while others are simply massive. Investigations of the mineral fractions that sequester in plants are sparse, and to date they are mostly related to anthropological interests for identifying specific agricultural varieties.

8.04.3.6.2 Plant biominerals

Plant-tissue biominerals are overwhelmingly of three chemical types: calcium oxalate, silica, and calcium carbonate. Of these types, calcium oxalate is thought to be the most prevalent and widespread (Webb, 1999). Opaline silica (Geis, 1978) and calcium oxalate (Finley, 1999) have been reported within plant tissues from hundreds of families in the plant kingdom, while calcium carbonate is widely described in many plant species. In addition to these compounds, which form common minerals, several oxides have been reported in isolated plant species, although the number of plant species is small, i.e., <4% of total mass (Duke, 1992). They include magnesium oxide throughout the tissues of *Stellaria media* (chickweed), calcium oxide in the leaf of *Corylus avellana* (hazel), iron oxide in the seeds of *Cannabis sativa* (marijuana), and aluminum oxide as well as manganese oxide in the seeds of *Gossypium* spp. (cotton) (Duke, 1992). A few additional compounds have been noted, but <0.5% of total mass: potassium nitrate is found in the leaves of *Piper betel* (betel pepper); calcium sulfate is present in the roots of *Echinacea* spp. (coneflower); and potassium chloride can be found in the calyx of *Physalis peruviana* (ground cherry) (Duke, 1992). In addition, some metal–organic associations have been noted. Phytoferritin, a central iron-containing core surrounded by a polypeptide shell, has been found in the plastids of some higher plants and in the seeds of legumes (Webb and Walker, 1987). Aluminum and silicon were found to be co-deposited in the needles of *Pinus strobus L.* (Eastern white pine) growing on acid soils in Muskoka, Canada, with aluminum accumulation increasing toward the needle tip (Hodson and Sangster, 2002), the areas of heaviest silica biomineralization (Figure 19).

Calcium oxalates in plants. Calcium oxalate sequestration in plants is found in two chemical states related to different levels of hydration, and the stable formation of two distinct minerals: whewellite, the monohydrated calcium oxalate, $CaC_2O_4{\cdot}H_2O$, and weddellite, calcium oxalate dihydrate, $CaC_2O_4{\cdot}2H_2O$ (Gaines *et al.*, 1997, p.1011). These minerals were found as well-formed isolated crystals in *Opuntia microdasys* (Pricklypear) and *Chamaecereus silvestrii* (Unnamed cactus), respectively (Monje and Baran, 1996, 1997) and both minerals are also commonly found in human urinary calculi (Gaines *et al.*, 1997, p. 1011). Calcium carbonate occasionally occurs as isolated deposits, such as within the seeds of *Magnifera indica* (Mango) (Duke, 1992), and both calcite and aragonite have been known as co-precipitates with silica in plants. For example, Cystoliths in the Acanthacea plant *Beloperone californica* contain both calcium carbonate and silica (Hiltz and Pobeguin, 1949), and the prickly emergences of *Urtica dioica* (Stinging nettle) are silica structures upon which calcite has been bioprecipitated (Thurston, 1974). Similarly, the endocarp of the *Celtis occidentalis* (Hackberry) fruit is composed of a reticulate opaline network, within which aragonite has been precipitated (Jahren, 1996) (Figures 20(A) and (B)), and developmental studies showed that vilification is complete by the time calcification begins (Jahren *et al.*, 1998), indicating that the plant exerts strategic control over the timing and the composition of biomineral precipitation (Table 1).

Silica in plants. Mineral precipitation is a fundamental cellular activity for many photosynthesizers and the vast majority of the dry mass of a biomineralizing organism is in the mineral, which is dense, having a higher specific gravity than the organic constituents, or the fluids that make up the great volume of the tissues. Diatoms are a striking example of this, with concentrations of 95% (dry weight) silica in their walls (Kaufman *et al.* (1981); see Section 8.04.2.2); another extreme example is *Bambusa* spp. (Bamboo) (Figures 21(A) and (B)), which may consist of 70% silica (dry weight) (Jones *et al.*, 1966). The dry mass of rice husks at harvest contains up to 20% silica (Garrity *et al.*, 1984), and the amount of calcium oxalate is in excess of 10% (dry weight) within the bark of *Quillaja sapona* M. (Soapbark) (Duke, 1992).

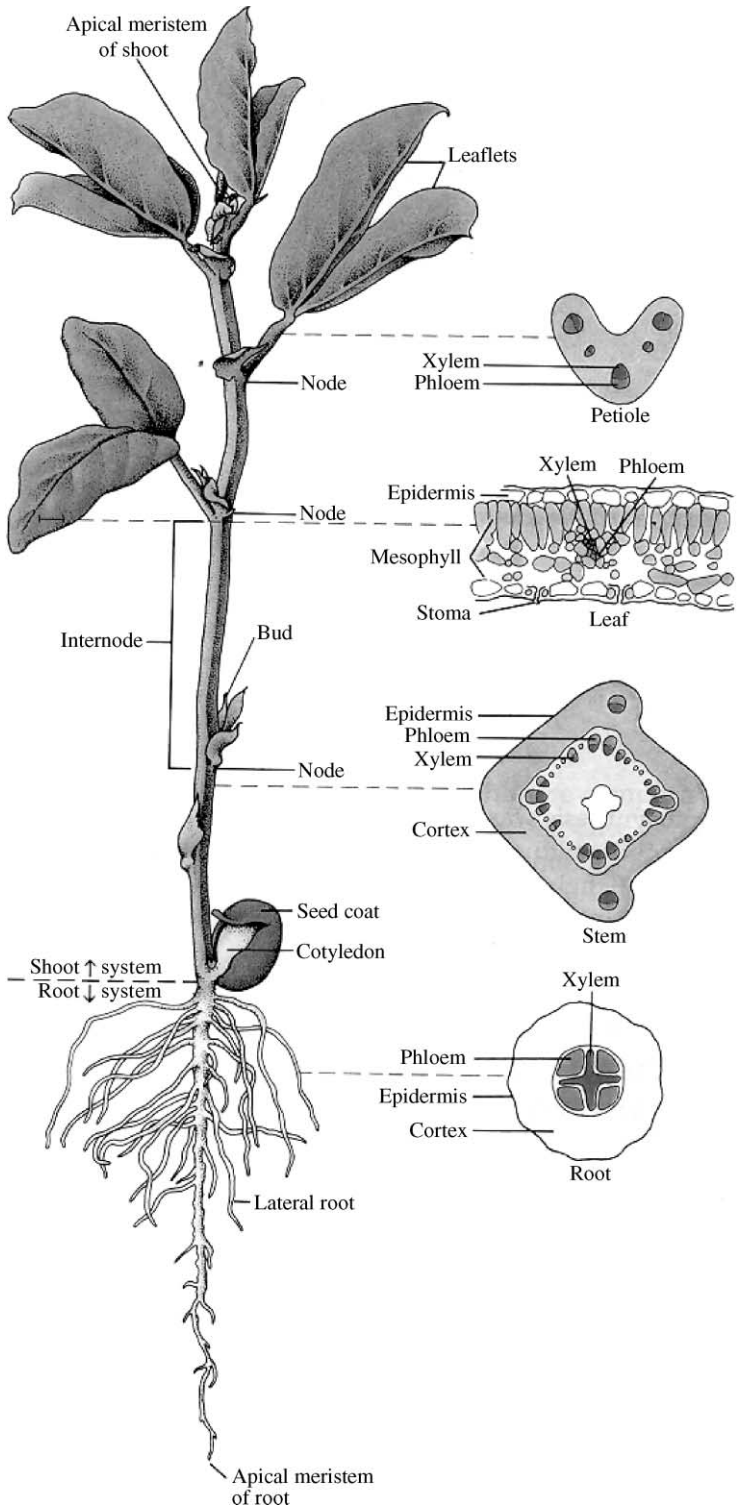


Figure 18 Diagram of a young broad bean illustrating the anatomy (source Raven *et al*., 1999, p. 8).

The amount of mineral offers structural advantages. Among the *Bambusa* whose hollow stems are reinforced by silica are the tallest members of the Poaceae. Commonly viewed as grasses, some bamboo achieve tree-like stature, while the vast majority of grasses are less heavily silicified, and commonly are less than 1 m in height. Throughout much of Asia, heavily silicified bamboo is strong enough to use as a building material, possibly a long-standing practice. One interesting illustration

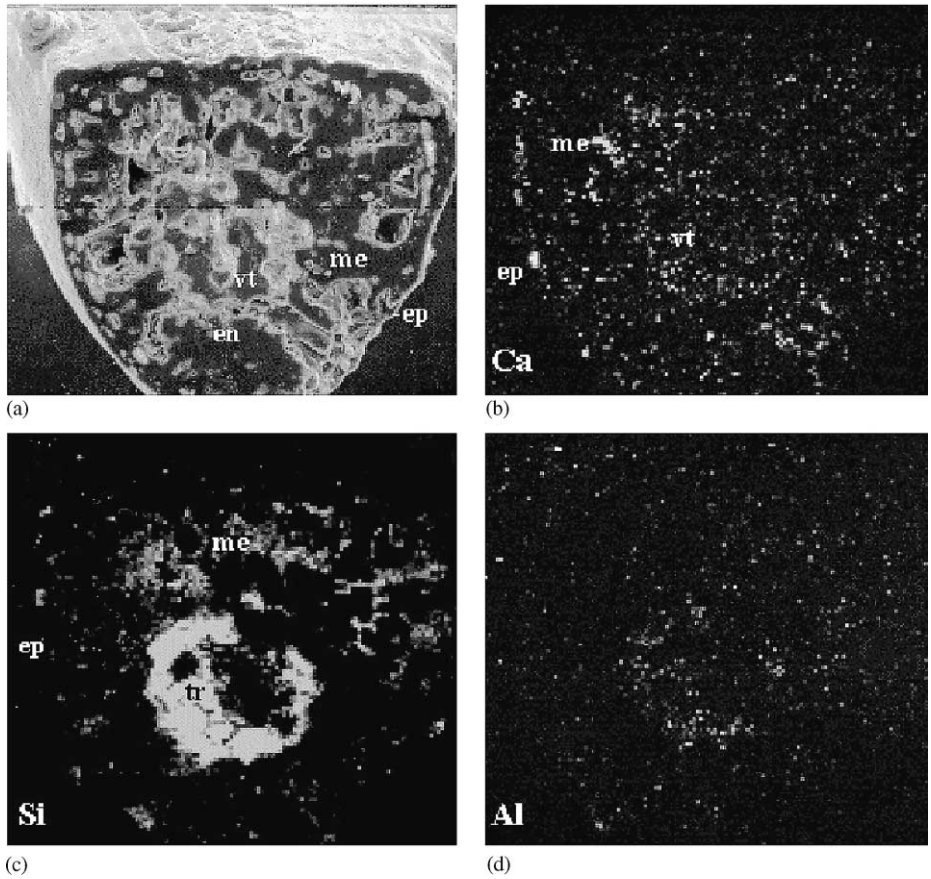


Figure 19 SEM and energy dispersive analysis micrographs (175×) showing mineral distribution, Ca, Si, and Al, localization in a frozen, planar transverse section 1 mm behind the tip in a second year needle of *Pinus strobus* (Eastern white pine): (a) secondary electron image, (b) calcium distribution, (c) Si distribution, and (d) Al distribution. Abbreviations: endodermis (en), epidermis (ep), hypodermis (hy), mesophyll (me), transfusion tissue (tr), vascular tissue (vt), and xylem wall (xw) (courtesy of M. J. Hodson and A. G. Sangster, unpublished collection).

of this concerns the "Movius Line," or the imaginary geographical line drawn for the Lower Paleolithic which divides Africa, the Near East and Western Europe with their developing hand axe technologies from Eastern Europe and Southeastern Asia, where "chopping-tool" industries and use of casually retouched flint and chert flakes were dominant. Several suggestions have been offered for this discontinuity. Eastern regions were subject to chronological, geographical, and other barriers, and there were different requirements between the separated peoples. One provocative explanation includes the favored use of bamboo as a raw material (Schick and Toth, 1994). Bamboo was, and is, plentiful in these regions and its high silica content gives crude tools fashioned from splitting bamboo glass-like sharpness. Under this hypothesis, stone tools take on a secondary role, their main purpose being the initial processing of bamboo. Because the biomineralized silica bundles found in bamboo are recognizable by their morphology and elemental composition, it may be possible to place the early phases of bamboo processing in the archaeological record by examining crude stone tools for bamboo phytolith residue (Jahren *et al*., 1997).

8.04.3.6.3 Phytoliths: indicators of the environment and paleoenvironment

The eventual decay of organic tissues releases plant biominerals into the soil, where they can be recognized as evidence of plants by paleobotanists, paleontologists, archaeologists, and other environmental historians (Pearsall and Piperno, 1993; Piperno, 1988). Extensive genetic influence is exerted over all aspects of plant morphology, rendering different species of plants distinct in their shape, size, and collection of accruements. As an extension of this, most plant phytoliths are distinctive of plant family, and many can be used to determine plant genus or even species.

Phytoliths can be grouped broadly by their morphology (Figures 22(A) and (B)) with great accuracy: monocotyledon (herbaceous annual

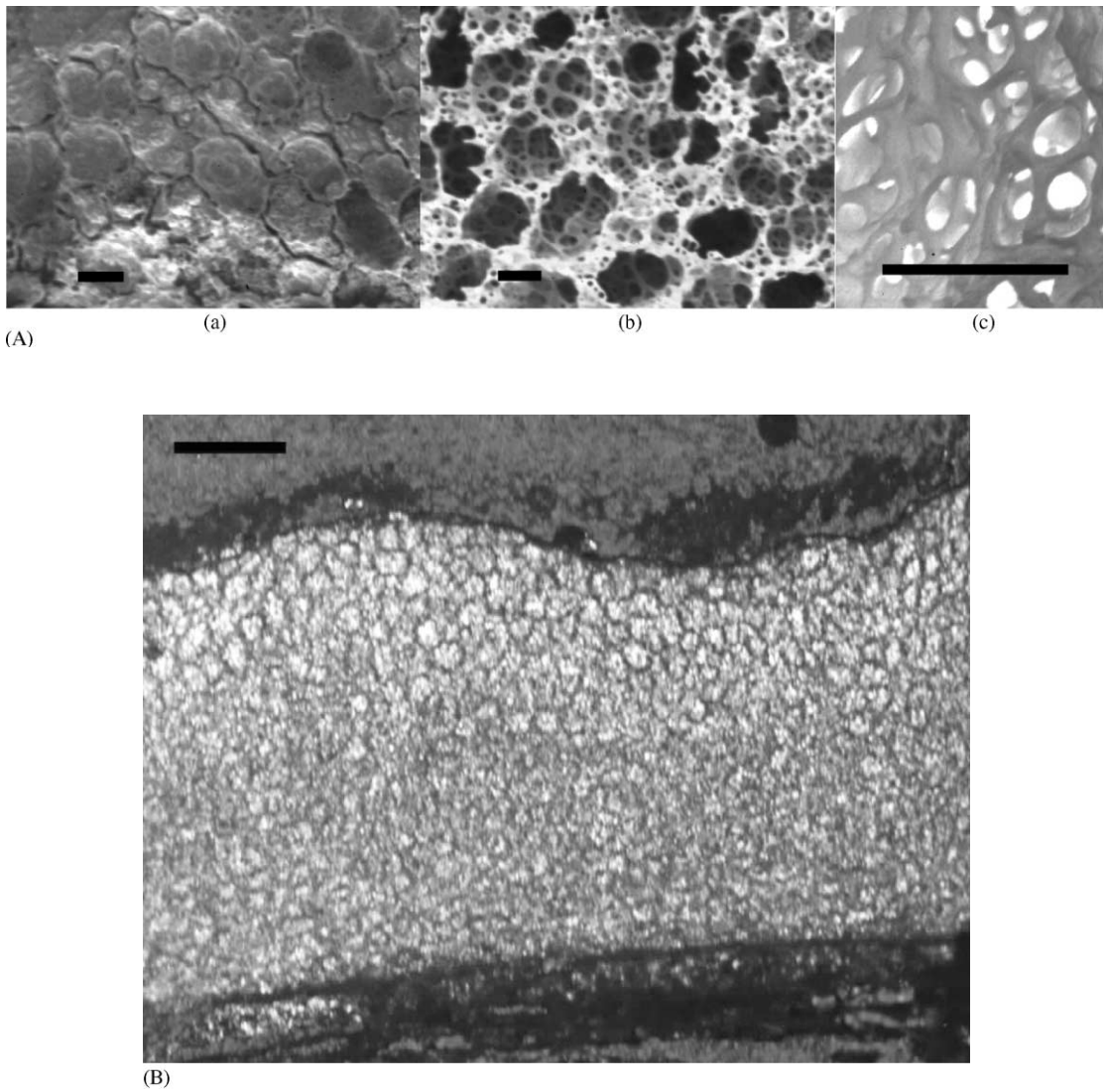


Figure 20 (A) SEM images of *Celtis Occidentalis* (Hackberry) endocarp. Aragonite deposited in a: (a) honeycomb pattern, (b) the inner silica scaffolding, and (c) organic matter occluded within the silica (scale bar = 10 μm) (source A. H. Jahren, unpublished collection). (B) Petrographic thin section of *Celtis Occidentalis* (Hackberry) endocarp, note honeycomb pattern (scale bar = 100 μm). Photograph taken with crossed polars and quartz plate inserted (source A. H. Jahren, unpublished collection).

plants) are recognizable and distinct from dicotyledon (angiosperm tree) phytoliths. Furthermore, both groups are distinct from gymnosperm phytoliths, which include conifer trees. Recognition of changes in the relative abundance of each group in archaeological and geological records has illuminated continental-scale changeovers from grassland to forestland through geological time.

The diversity and abundance of phytolith assemblages can be used to determine the composition of paleoplant communities. A study across the Great Plains of the US showed that modern phytolith assemblages displayed a geographic pattern consistent with modern grassland composition (Fredlund and Tieszen, 1997a). Because the species composition of grasslands is observed to change systematically with environmental parameters such as temperature, the study also suggested that phytolith assemblages from prehistoric grasslands could be used to reconstruct paleoclimate conditions.

For example, phytoliths from the Black Hills, South Dakota (complemented with stable isotope data) show that regional vegetation changed from C3 grasslands to mixed C3/C4 grasslands between 11 kya and 9 kya, and that the presence of C4 vegetation was stable into the Late Holocene (Fredlund and Tieszen, 1997b). Use of phytolith assemblages reveals that grassland and steppe ecosystems were widespread in Washington State

Table 1 Biomineralization in plants.

Mineral	*General distribution*	*Specific occurrences*[a]
Calcium oxalate ($CaC_2O_4{\cdot}n\,H_2O$)	215 Families, including both gymnosperms and angiosperms (Mcnair, 1932)	*Chamaecereus silvestrii* (Unnamed cactus) (Monje and Baran, 1996) *Opuntia microdasys* (Pricklypear) (Monje and Baran, 1997) *Phaseolus vulgaris* (Common bean) (Zindler *et al*., 2001) *Quillaja saponaria* M. (Soapbark) (Duke, 1992) *Sida rhombifolia* (Teaweed) (Molano, 2001) *Vitis* spp. (Grape) (Webb *et al*., 1995)
Silica ($SiO_2{\cdot}n\,H_2O$)	>100 Families of tropical plants, including both gymnosperms, angiosperms and pteridophytes (Piperno, 1991)	*Arundo donax* (Giant reed) (Mulholland, 1990) *Bambusa* spp. (Bamboo) (Jones *et al*., 1966) *Bouteloua curtipendula* and *Panicum virgatum* (Sideoats and Switchgrass) (Ball and Brotherson, 1992) *Phalaris canariensis* (Annual canarygrass) (Perry *et al*., 1984) *Thalassia testudinum* (Turtlegrass) (Brack-Hanes and Greco, 1988) *Zea* spp. (Corn) (Doebley and Iltis, 1980)

[a] Not a complete list.

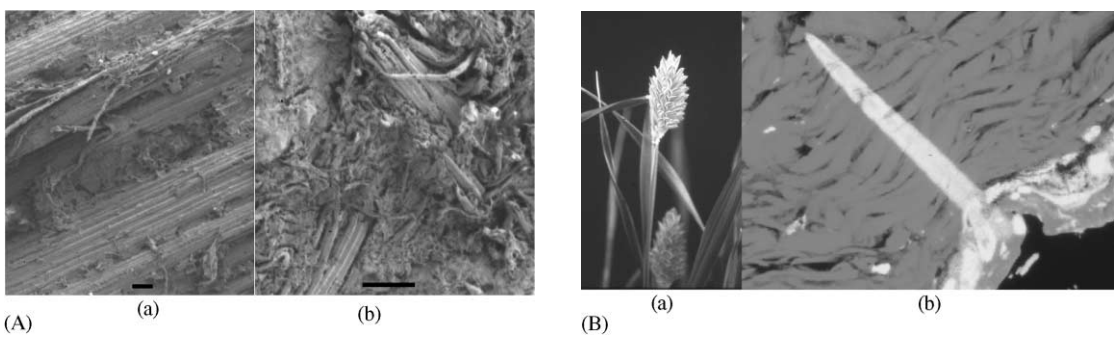


Figure 21 (A) SEM images of silica phytoliths from *Bambusa indigena* (Bamboo) (a), and of the surface of a chert tool that had been used as a wedge during the chopping of Bamboo stems (b). Note the silica phytoliths fused to the tool edge (scale bar = 30 μm) (source A. H. Jahren, unpublished collection). (B) Inflorescence of the grass *Phalaris canariensis* (Canarygrass) (a), and a siliceous hair from the inflorescence of this grass species penetrating the skin of a mouse (b). The hair is stained with fluorescein and is 50 μm long with a midpoint diameter of 3 μm; there is a fracture near the tip of the hair; the tissue is stained in acid fuschin (courtesy of M. J. Hodson, R. J. Smith, A. Van Blaaderen, T. Crafton and C. H. O'Neill, unpublished collection).

during the Pleistocene, compared with the forested ecosystems of the region today (Blinnikov *et al*., 2002).

Phytolith assemblages show a clear turnover from savanna to forest ecosystems in south central Brazil ~4 kya (Alexandre *et al*., 1999) and indicate that the closed forest in the Amazon Basin has existed since 4.6 kya, but prior to this time the forest composition and species abundance fluctuated widely (Piperno and Becker, 1996), leading researchers to hypothesize that climate change stabilized the vegetation during the middle Holocene.

Occasionally, phytolith analyses suggest a reinterpretation of the evolutionary effects of vegetation change. The changes in tooth morphology of herbivores during the Miocene are often attributed to expanding grasslands and the development of phytolith-resistant dentition. However, phytolith assemblages from northwestern Nebraska showed that open C3 grasslands dominated the landscape between 25 Ma and 17 Ma, millions of years before the observed changes in tooth morphology of regional herbivores (Stromberg, 2002).

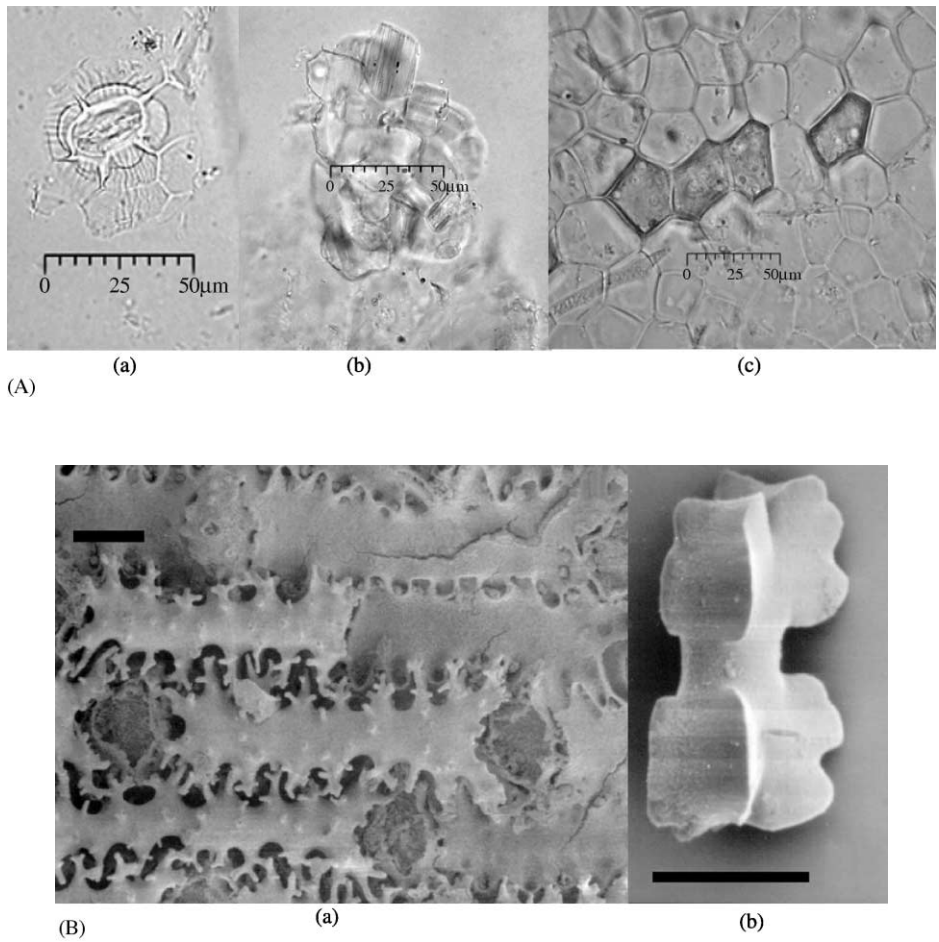


Figure 22 (A) Light microscope images of plant phytoliths (scale bar = 50 µm). Silicified stomate from *Cordia lutea* (Flor de overo) (a), irregularly sized blocky crystals of calcium carbonate from *Brownea grandiceps* (Rose of Venezuela) (b), and silicified epidermal cells (dark cells near center) from *Licania longistyla* (Licania) (c) (courtesy of D. M. Pearsall, unpublished collection). (B) SEM images of silica phytoliths from common crop plants. *In situ* dendriforms from *Triticum monococcum* (Wheat) inflorescence (a), and a bilobate cross-body phytolith from *Zea mays* (Corn) (b) (scale bar = 10 µm long) (courtesy of T. B. Ball, unpublished collection).

Variability in critical environmental parameters has been shown to affect the total height, biomass, and leaf area (among other aspects) of a growing plant. However, extreme variations in soil texture, light levels, and water availability did not alter the morphometry of phytoliths in two grass species: *Bouteloua curtipendula* (Sideoats grama) and *Panicum virgatum* (Switch grass) (Ball and Brotherson, 1992). Further, crop plant phytoliths (Figure 22(B)) can be differentiated from wild plant phytoliths by recognition of genus- and species-specific morphologies (Cummings, 1992). One example is the suite of recognizable traits in *Zea Mays* (Corn) phytoliths compared to those of wild grasses (Piperno, 1984). The morphology and taxonomy of New World domesticated plants has been extensively documented (Piperno, 1985) and used to explore the history and significance of various crop species to the peoples of this region (Piperno, 1991). Phytolith material extracted from residues on prehistoric pottery, and from human dental calculus, presented an opportunity to evaluate cooking practices and nourishment levels, allowing archaeologists to characterize and understand past civilizations in terms of agrarian lifestyles (Pearsall and Trimble, 1984). Much of what is known about the importance of *Zea Mays* (corn) in the New World has been interpreted from thousands of years of phytolith-bearing sites (Bush *et al.*, 1989). The mixed record of phytoliths from both wild and cultivated plants was used to interpret environmental change as the result of agricultural activities. On another continent Kealhofer and Penny (1998) found that the Late Holocene transition to rice agriculture in Thailand was accompanied by a reduction in dry-land forest and the subsequent

establishment of secondary-growth forests. Plant biominerals are incorporated into the geologic record when plant communities are subjected to natural fires, or by slash-and-burn agriculture practices.

Some authors have warned against overinterpretation of the phytolith record: Carter (2002) compared phytolith assemblages with pollen extracted from sediment cores and showed that trends in vegetation through time were not only disparate, but completely opposite. Additional contextual information led Carter (2002) to conclude that the phytoliths reflected a very local vegetation signal, whereas widely distributed pollen was more representative of a larger geographical signal. Also, phytoliths, like most minerals, are subject to diagenesis. This may be a particular problem in highly weathered and actively weathering soils. Boyd *et al.* (1998) have developed a specialized methodology for preparing and interpreting phytolith assemblages from wet tropical sites.

Radiocarbon dating using plant biominerals. Carbon associated with plant biominerals has been explored as a substrate for radiocarbon dating in Quaternary phytolith samples. The ^{14}C content of calcium carbonate biomineralized in Hackberry endocarps over the past 120 yr parallels the observed ^{14}C variations of atmospheric CO_2 during that time span, indicating that these common phytoliths faithfully record the year in which they formed (Wang *et al.*, 1997). When compared to other ^{14}C dating substrates from Quaternary archaeological and geological sites, Hackberry endocarp carbonate yielded ages that compared favorably with those obtained by more established means (Jahren *et al.*, 2001; Wang *et al.*, 1997). Additional studies have focused upon carbon in the organic matrix occluded within the plant biomineral as a potential substrate for radiocarbon dating. Pigment from rock art near Catamarca, Argentina has been sampled and analyzed for its mineral content. These analyses revealed that plant material was used as a pigment binder, resulting in calcium oxalate and calcium carbonate from local cacti incorporated into the paint. By extracting calcium oxalate phytoliths from the pigments and using occluded organic carbon for ^{14}C analyses, Hedges *et al.* (1998) were able to hypothesize a Quaternary age for the rock paintings.

Stable isotope investigations on plant biominerals. In contrast, only a limited number of studies have investigated the stable isotope composition of plant biominerals as a source of paleoenvironmental information. Silica extracted from the Eocene paleosols of Axel Heiberg Island in arctic Canada were found to have notably high stable oxygen isotope value, leading to the suggestion that the mineral represented an accumulation of plant phytoliths, which presumably formed from isotopically enriched leaf water (Kodama *et al.*, 1992). Jahren *et al.* (2001) found that the oxygen stable-isotope composition of aragonite in Hackberry endocarps from 101 North American sites was well correlated with the oxygen isotope composition of environmental water at the sites where the trees grew. They used this correlation to interpret the oxygen isotope composition of fossilized endocarps from Pintwater Cave, Nevada as resulting from summer influx of isotopically heavy monsoon precipitation during the Early Holocene. Organic matter occluded with silica phytoliths has also been used as a substrate for carbon isotope analysis. Stable carbon isotope composition of phytolith-occluded organics was found to be in agreement with values from paleosol organic matter, and isotopic trends through time reflected expanded C4 plant communities and regionally warmer conditions in the Great Plains during the middle Holocene, relative to the present (Kelly *et al.*, 1998).

8.04.3.6.4 Funghi and lichen biomineralization

Plant biomineralization influences the fungal communities that coexist with plants, and often confers advantages to the decomposers. There are several reports of fungal biomineralization that mimic plant biomineralization, occurring only when fungi are grown directly on plants. In compost fungi, much of the hyphae actually consist of calcium oxalate crystals that originate within cell walls (Arnott and Webb, 1983). Calcium oxalate crystals encrust the hyphae of the fungi, *Cyathus striatus* and *Cyathus olla*, common decomposers of plant debris (Tewari *et al.*, 1997). Calcium oxalate crystals have been found on the aerial hyphae of the fungus *Agaricus bisporus* when it was grown on natural plant substrates (Whitney and Arnott, 1987). Lichens (a symbiotic association between a fungus and an alga) may have the ability to biomineralize as well: within lichen communities, the lichens with the greatest capacity to weather volcanogenic sediment showed weddellite (dihydrate calcium oxalate) and calcite, as well as many bacteria (Sancho and Rodriguez, 1990; Easton, 1997).

8.04.3.7 Vertebrate Biomineralization

8.04.3.7.1 Introduction

In all vertebrates, including humans, the calcium phosphate mineralized endoskeleton is not only the basis of species identification, but plays two distinct major roles in this phylum. The skeleton is the scaffold with sites for muscle

attachment permitting vertebrates their peculiar stance and mobility, and bones act as storehouses for the elements required for all facets of metabolism (Skinner, 2000). In forensics, or in paleontological studies, distinctive features of each bone or tooth tells its own story of growth, development, aging, trauma, and diagenesis.

There are 32 teeth and over 200 bones in an adult human, each a separate organ with its own essential role. These organs are composed of mineralized tissues, composites of bioorganic, and mineral that record the chemistry of what has been ingested, i.e., the C4 or C3 characteristics of the diet, as well as the overabundance of certain, perhaps hazardous cations, e.g., copper (Pyatt and Grattan, 2001), or shortfall of essential elements, i.e., calcium, that may lead to rickets, usually expressed in the abnormal appearance and function of the long bones.

There is at present worldwide concern with personal and public health. Coupling genetic and nutrition information it is possible to discriminate the predisposition, or susceptibility, of some disorders of the vertebrate hard tissues. For example, using animal models the aberrant production of tooth enamel, amelogenesis imperfecta (Dong *et al*., 2000), is now traceable, and osteoporosis (Avioli, 2000), which may have previously been attributed solely to nutrition or inactivity, is being re-examined in light of molecular genetic predisposition. Mindful that genetic abnormalities are not confined to current populations of humans or animals, a major caveat in evaluating samples for geochemical investigation is to discriminate between "normal" and diseased or "pathologic" tissues. The distribution and composition of the familiar, and common, calcium bioapatite mineral in teeth and bones, relatively easily obtained, is usually extracted from the organic constituents before being examined. Teeth, because of their distinctive forms, functions, and high degree of mineralization, are an important source of samples, while bones present some drawbacks. The background on human mineralized tissues, briefly outlined below, should allow us to make more informed choices for geochemical investigations.

8.04.3.7.2 Bones and bone tissues

The formation of a mature skeleton composed of a series of individual bone organs of various shapes ultimately depends on cellular control and the deposition of mineral within an organic matrix, and the creation of specialized tissues. The mineral, a calcium phosphate, closely approximates hydroxylapatite, ideal formula $Ca_5(PO_4)_3(OH)$, described in Section 8.04.2.3.

The mineral in skeletal tissues (see Section 8.04.2.3) is found as part of several different textures, e.g., woven, lamellar, haversian, trabecular and cortical bone that can be discriminated optically, or histologically, and at higher resolutions using TEM or SEM (Figure 23). The most dense, most highly mineralized tissues are found in the external portions of bones, and especially the shaft of the long bones in tetrapods, whether warm or cold blooded. The textural term for these tissues is cortex or cortical bone (Figures 24(a) and (b)). Interior to the cortex is an area of less dense tissue which becomes more porous grading into the marrow cavity where only mineralized spicules, or trabeculae, are located. This mineralized tissue is known as trabecular bone. The marrow, largely fat, is where the blood tissues are produced.

The fact that bones have hollow centers is an expression of the clever mechanical design of these organs. Bones and bone tissues fulfill their structural function while protecting the marrow where essential cellular material is generated (Wainwright *et al*., 1976). Sufficient bone stiffness is achieved by increasing the amount of calcium phosphate mineral relative to the organic components in the tissue. Each bone is an independent structure in which the amount and distribution of mineralized tissue suits its size and functional needs while minimizing the organ weight. Programmed construction of each mineralized organ conserves mass and provides a mechanical advantage, the strength required, for example, to prevent buckling in the long bones of land-based vertebrates (Albright, 1987). As animals increase in size, the skeleton becomes a greater percentage of body weight. The skeleton of a mouse (20 g) is 5% of its body weight, that of a 70 kg man is 14% of its body weight, while a 1,000 kg elephant has a skeleton 27% of its body weight. The largest mammals that exist today are aquatic, and dwell in buoyant environments. One of the largest captured, a blue whale, weighed 203 t, with a skeleton that was 15% of its body weight (Kayser and Heusner, 1964).

Bones are successful living structures, because they are composed of tissues, biomolecules, and mineral, maintained by a highly integrated system containing several different cell types with specialized functions.

Biomineralization of bone: the bone morphogenic unit. Bone tissues have an auto-inductive cell cycle that keeps these essential organs viable throughout the life of the animal. Tissues are resorbed and regenerated within restricted sites in the cortex or trabeculae, known as bone morphogenic units (BMUs), a few micrometers in diameter (Urist and Strates, 1971). Three specialized cells are part of the unit; they interact responding to constantly changing conditions so that small portions of the tissue resorb and re-form without jeopardizing the function of the organ.

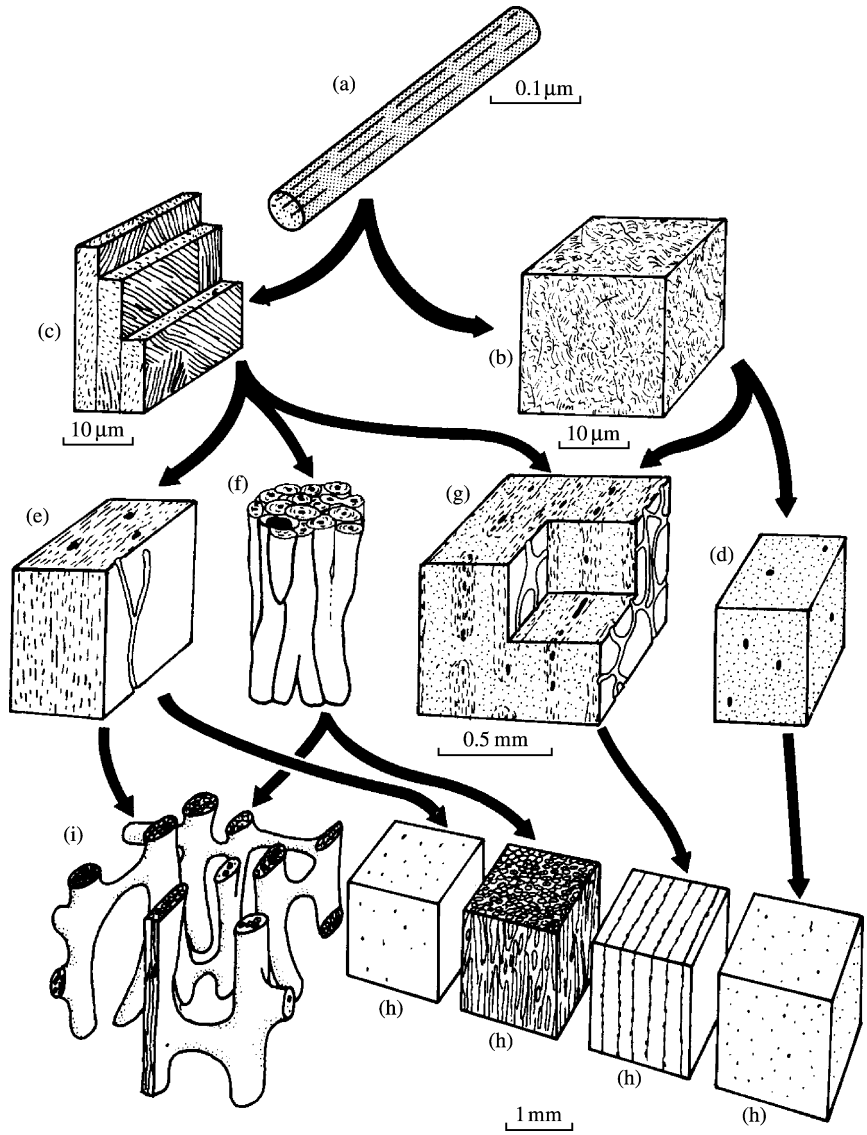


Figure 23 Mammalian bone at different levels of resolution: (a) Collagen fibril with associated mineral. (b) Woven bone (random collagen distribution). (c) Lamellar bone showing separate lamellae with collagen organized in domains with preferred orientation alternating in adjacent lamellae. (d) Woven bone with blood channels shown as dark spots, woven bone stippled. (e) Primary lamellar bone orientation indicated by dashes. (f) Haversian bone, a collection of haversian systems are shown as a longitudinal structure. Each system has concentric lamellae around a central blood channel. Darkened area represents an empty (eroded) portion of the section which will be reconstituted with new bone. (g) Alternation of woven and lamellar bone. (h) Various orientations of heavily mineralized (cortical, or compact) bone. (i) Trabecular, or cancellous, bone (Wainwright *et al*., 1976) (reproduced by permission of Hodder Arnold from *Mechanical Design in Organisms*, **1976**).

The cells are connected via the cardiovascular system that permeates each organ and transfers nutrients and wastes as required.

A BMU is generated, and a cycle commences, when circulating hematopoetic stem cells from the marrow are recruited, fuse, and differentiate into multinucleate osteoclasts (Figure 25(a)). Enhanced by circulating hormones, osteoclasts resorb a portion of mineralized tissue a few micrometers in size, creating lacunae, or depressions, which subsequently fill in with organic matrix produced by cells called osteoblasts (Figure 25(b)). These cells originate from stromal mesenchymal cells and migrate to the sites vacated by the relocation or death of the osteoclasts. Mineralization of the refilled area takes time depending on the animal species and, in some disease states, may take months (Mundy, 1999). There is another, and perhaps the most important, cell in the bone morphometric

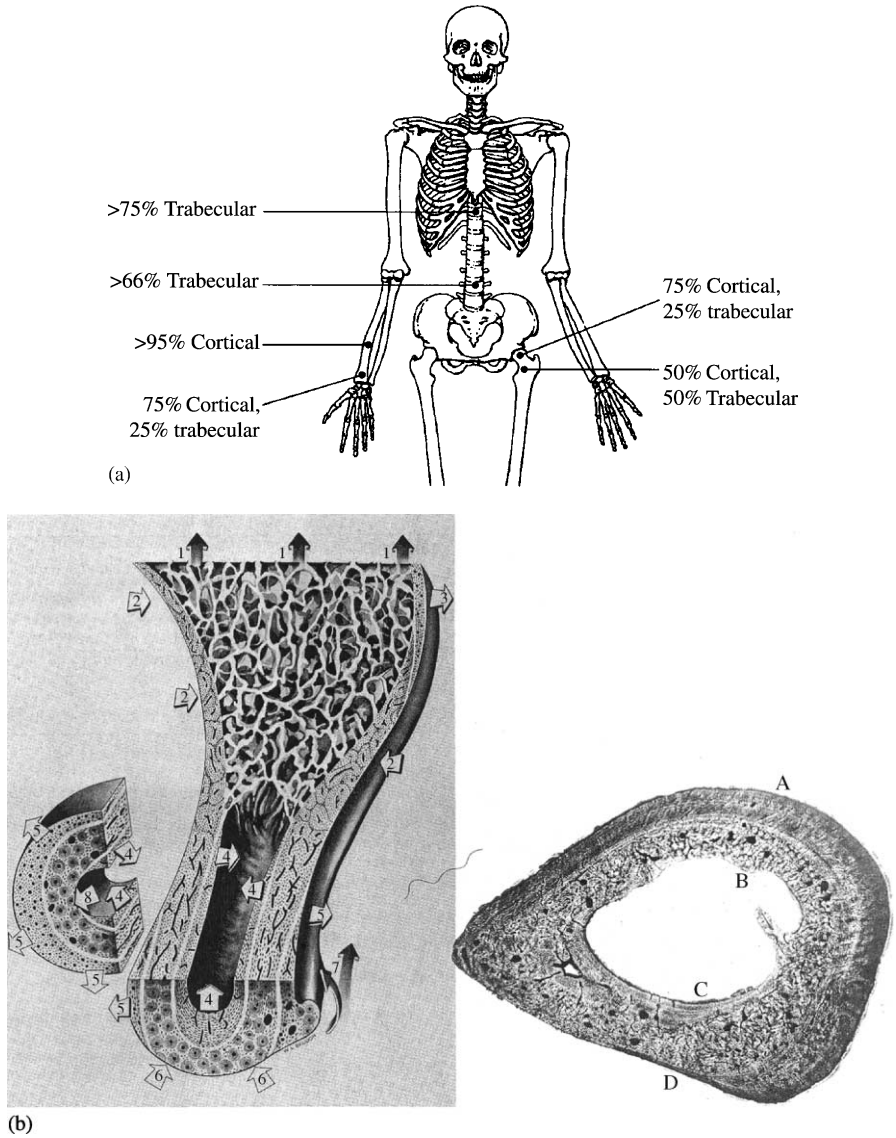


Figure 24 (a) Diagram of the human skeleton showing the percentages of cortical and trabecular bone in different bone organs (source Mundy, 1999, figure 2, p. 31). (b) Composite diagram through a long bone illustrating the tissue distribution and the changes that will take place as a bone grows and remodels. The longitudinal cross-section shows diagramatically areas of cortical and trabecular bone with directions of growth and/or resorbtion (numbers with arrows show the directions). A cross-section of an actual long bone is on the right-hand side of the diagram. It presents the appearances one observes of bone tissue changes: A = at the periosteum where there is active bone deposition, B = in the marrow cavity showing the response to bone resorption, C = at the opposite side of the marrow cavity showing lamellar bone deposition, and D = external planing of a portion of the bone in response to organ shape changes (source Enlow, 1963, figure 53, p. 111).

unit. Osteocytes (Figure 25(c)) are osteoblasts that become embedded in mineralized matrix. They remain viable, connected through pseudopoda and small blood vessels to each other, and the cardiovascular system to obtain nutrients, circulating small molecules, including hormones, and output any wastes. The detection of external signals, and the production of specific chemical species in the life span, days to weeks, of the osteocyte, the ultimate gateway for maintaining living and metabolizing bone tissue, is critical to the processes involved in bone biomineralization (Skinner, 1987).

A similar cycle acts when bone organs restructure during growth and development from the fetus to the adult except the tissue resorption and deposition are displaced to accommodate revisions in the shape of the organ (Enlow, 1963; Figure 24(b)). To achieve the adult size of the long bones, a separate mineralizing system, known as endochondral ossification, is generated. The system has specialized cells and organic

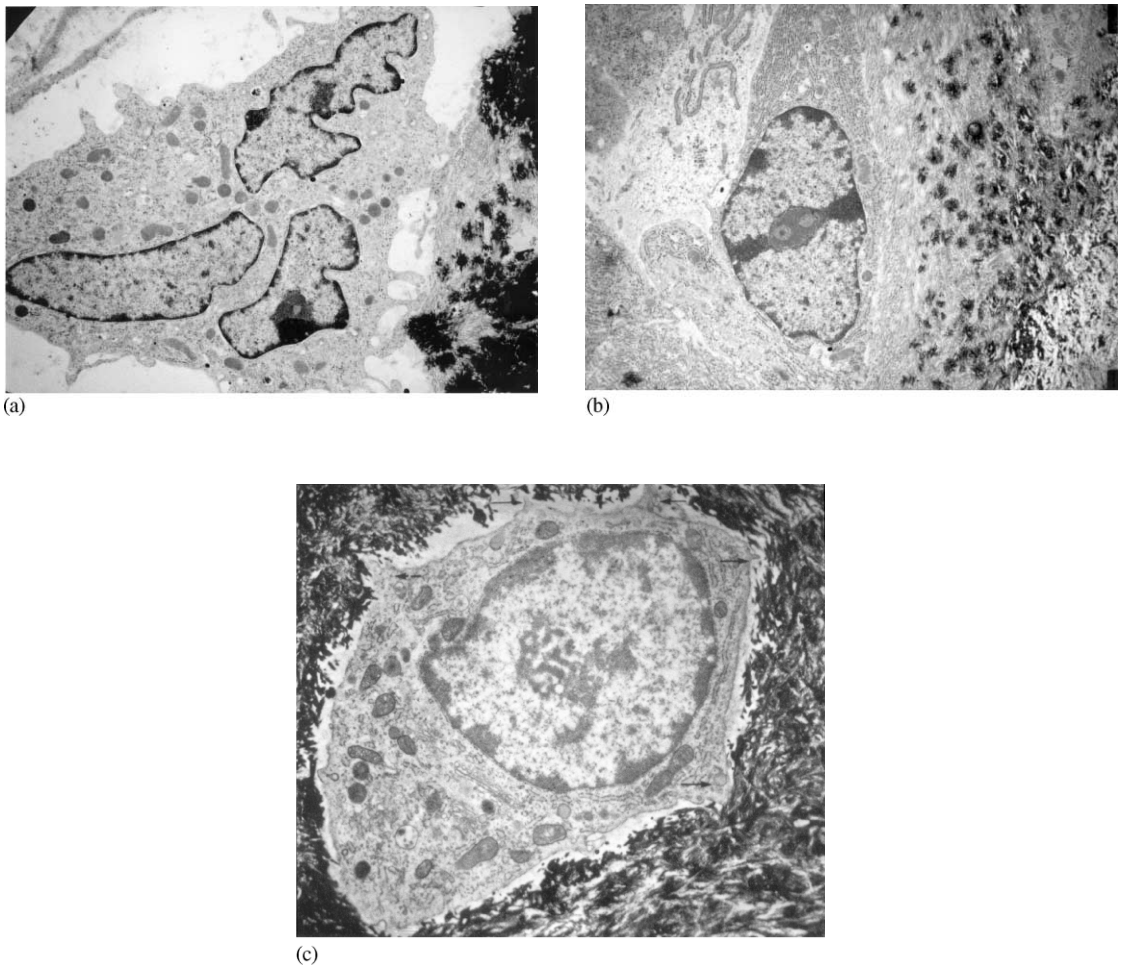


Figure 25 TEM microradiographs of individual bone cells: (a) osteoclast, a multinucleate cell; (b) osteoblast, mononucleate with surrounding matrix only partially mineralized (dark spots in fibrous matrix); (c) osteocyte, mononucleate, completely embedded in darkly colored mineralized tissue. Scale: the nuclei of these cells are ~5 μm in diameter (courtesy of Lynn Ann Neff, Department of Orthopaedics, Yale University).

components, and a growth plate with distinctive morphology to accommodate the extension and calcium phosphate deposition necessary to achieve the adult size of the organ (see Section 8.04.3.7.3).

When mineralized tissues are dissolved, both the organic and inorganic fractions become available for redeposition or redistribution. Some of the breakdown products (elements) will immediately reincorporate into new bone tissue, but others may move elsewhere to other body systems and cycles. Tissue reworking, the bone remodeling required during growth of the organ or the consistent tissue turnover throughout life via the BMU, provides many different elements stored in the apatitic mineral to the general circulation. The crystal structure of apatite allows the incorporation of a wide variety of cations and ions beyond the essential calcium and phosphate needed for apatitic bone and tooth mineral formation. Indeed, most of the elements in the periodic table can be found in solid solution within the precipitated bioapatite or adsorbed on the exceptionally large surface area that characterizes bone crystallites (Table 2; Section 8.04.2.3). The chemical composition of bone mineral can be used to evaluate some of the element concentrations in the environment in which it formed (Leeman, 1970; Larsen *et al*., 1992; Leethorp *et al*., 1994; Skinner *et al*., 2003).

Bone cells and products: collagen. With the advent of high-resolution transmission and scanning electron microscopy, the intimate relationships between the cells (Figures 25(a)–(c)) that form (osteoblasts), maintain (osteocytes), and remodel (osteoclasts) mineralized bone tissues have been depicted and their products and reactions studied. The first extracellular products of the osteoblast are bioorganic molecules dominated by the fibrous asymmetric protein

Table 2 Major element chemistry of geoapatite and bioapatite.

	1	*2*	*3*	*4*	*5*
SiO_2	0.55	0.34	11.9	bd/br	bd/br
Al_2O_3	0.04	0.07	1.7	0.02–0.14	0.00–1.63
Fe_2O_3	0.14	0.06	1.1	(bd, incl.)	(bd, incl.)
FeO	0.24	0.00	bd/br	0.22–1.90	0.00–0.01
MnO	0.08	0.01	bd/br	bd/br	bd/br
MgO	bd/br	0.01	0.03	0.12–1.07	0.00–0.39
CaO	53.91	54.02	44.0	49.80–56.70	60–62.1
SrO	bd/br	0.07	Bd/br	2.20–2.50	0.00–0.17
Na_2O	0.10	0.23	0.06	0.86–7.56	0.00–0.46
K_2O	0.01	0.01	0.05	0.06–1.04	0.00–0.01
REE_2O_3	1.69	1.43	3.75	0.00–0.23	0.00–0.37
P_2O_5	41.13	40.78	30.5	29.20–33.30	36.3–37.6
SO_3	bd/br	0.37	1.8	bd/br	bd/br
CO_2	bd/br	0.05	2.2	5.84–5.98	bd/br
F	2.93	3.53	3.1	bd/br	bd/br
Cl	0.71	0.41	0.04	bd/br	bd/br
Net	101.66	101.40	103.23	NA	NA
-O = F,Cl	1.23	1.58		NA	NA
Total	100.43	99.82		NA	NA

Source: Skinner *et al*. (2003). 1-Granitoid rock, Cougar Canyon, Elko County, Nevada (Chang *et al*., 1996, table 3), 2-Fe-apatite ore, Cerro de Mercado, Durango, Mexico (Chang *et al*., 1996, table 4), 3-Phosphorite average, Phosphoria Fm., CO (Gulbrandson, 1966), 4-"Typical" ranges for bone bioapatite composition (Skinner *et al*., 1972 and Rink and Schwarcz, 1995), 5-"Typical" ranges for enamel bioapatite composition (Cavalho *et al*., 2001 and Brown *et al*., 2002). "bd/br" = below detection limit or below rounding (two significant figures). "bd,incl") = Fe_2O_3 may not be reported separated, included within FeO value.

known as type I collagen (Miller, 1984). One of 11 different composition fibrous proteins in the collagen family (Kuhn, 1987), the molecular biology of type I collagen has been thoroughly investigated (Skinner (1987, pp. 207–208) presents a sketch of these details). It is composed one-third of the amino acid glycine (GLY), significant amounts of the imino acids proline and hydroxyproline, with many molecular repeats of GLY–X–Y where X and Y represent other amino acids on the initial strand. Some globular end regions assist, as does the subsequent hydroxylation of proline and lysine, in the cross-linking of individual protein chains into a triple stranded helical array of uniform size, roughly 1.5 μm wide × 280 μm long, known as a fibril (Miller, 1984). Note that the collagen fibril has a morphology reminiscent of a DNA molecule but is composed of amino acids not nucleic acids, is triple rather than double stranded, and contains no phosphate groups. The triple stranded collagen fibrils are extruded and aggregate extracellularly with a very special 1/4 stagger into fibers. The stagger leads to "holes," which may become sites for the deposition of apatitic mineral. Fibrillar collagen can be detected by tissue staining as the fibril aggregation appears to maximize the association of regions along the chains of positive and negative amino acids (Kuhn, 1987). Figure 26 illustrates, at the ultramicroscopic level, the typical banded pattern of osmium-stained collagen-containing tissue samples dotted with fine mineral deposits. The figure contains an electron diffraction pattern that confirms the presence and identity of the bone mineral as apatitic (Skinner, 1968). The grain size of the mineral deposits range from less than 300 Å in young animals up to 20 nm in maximum dimension for mature cortical bone (Elliott, 2002). The mineral permeates throughout, within as well as on, the collagen and protein–polysaccharide matrix produced by the osteoblasts. So thoroughly and intimate is the association that even after extraction of greater than 95% of the organic fraction, the gross morphology of the bone and texture are retained (Skinner *et al*., 1972).

Matrix mediated biomineralization and other possibilities. The collagen family of proteins is the dominant constituent of most connective tissues in the human, other vertebrates (Mayne and Burgess, 1987), and some invertebrates (Bairati and Garrone, 1985). Collagens other than the types I and II (cartilage), but always with high glycine content, are typically found in articular cartilage, and the many other nonmineralized connective tissues throughout the vertebrate body. Most of the other collagen species are smaller fibrous molecules that form networks with glycoproteins. Highly resistant to proteolysis, and only slowly degraded after death even under nonbiological conditions, several techniques have been developed to evaluate, and to date, the organic and

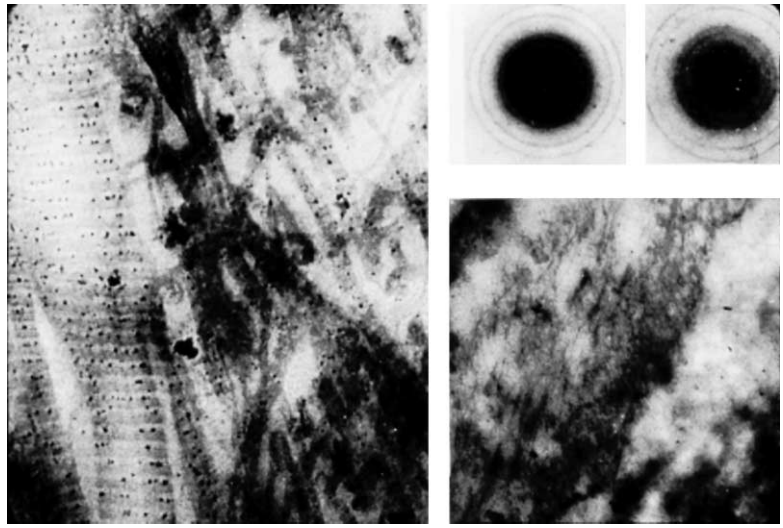


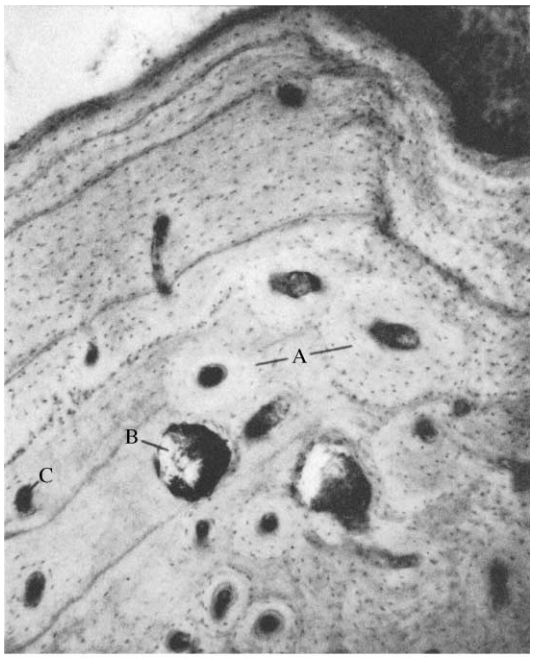
Figure 26 Electron micrograph of partially mineralized collagen. Magnification is $\sim 6 \times 10^4$. An electron diffraction pattern is shown in the inset that identifies the mineral as apatite (after Glimcher, 1959; reproduced in Glimcher, 1984, figure 13, plate 2).

mineral materials in fossils (Hare and Abelson, 1965; Kohn and Cerling, 2002; Trueman and Tuross, 2002).

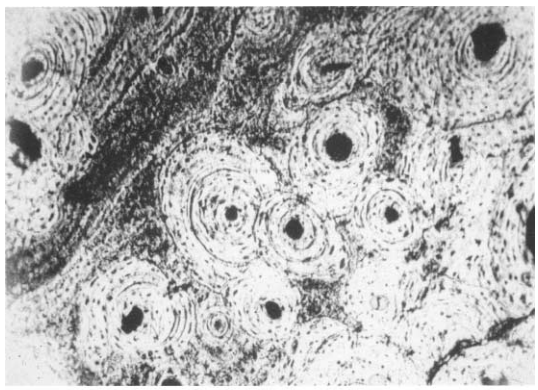
Biomolecules, such as collagen, with spatial as well as charged surficial sites could overcome the nucleation barrier (heterogeneous nucleation) required for mineral deposition, whether the mineral is hydroxylapatite or one of the many polymorphs of calcium carbonate. The strategy, matrix-mediated biomineralization, has been espoused for a long time, although the specificity for consistent and regularized mineral deposition in bone tissues remains elusive (Mann, 2001). A hypothesis that offered such an advantageous scenario relied on the bone cells to control the chemistry of the biomolecular species: the amino acids serine and threonine in collagen, especially if located within the hole regions could became phosphorylated. As phosphate monoesters, they could attract calcium ions providing the bridge between the organic and inorganic (Glimcher, 1959, 1960, 1984). Another proposal for inducing extracellular biomineralization, the type found in bone tissues, invoked small noncollagenous protein molecules produced and secreted by the bone cells. The roles of exogenous molecules to stimulate the resorption, formation, and mineralization of bone tissues are beyond the scope of this review, but reports on their potential are part of the search for treatment of osteoporosis (Nordin, 1971; Wasnich, 1999). Using cell culture techniques and animal model systems, the effects of osteonectin, osteocalcin, calcitonin, and many growth factors, for example bone morphogenic protein #7, or osteogenic protein #1 (OP-1), on the production and regeneration of bone, especially the healing of fractures, are under investigation (Deftos *et al.*, 1999; Friedlaender *et al.*, 1999). It should be pointed out that many small molecules originally described from bone studies have been detected in other tissues and organs where they may play roles in the general metabolism of the body (Hollinger, 1997). Another suggestion of how calcium phosphate mineral is provided to growing bone tissues was by Anderson (1973). He suggested that apatite could become localized within lipid bound sacs or vesicles. Vesicles with lipid membranes will self-assemble in aqueous environments, and are not confined to the eukaryotes. They are prominent at sites of intracellular nucleation of iron oxides and sulfides in magnetotactic bacteria (Section 8.04.3.3.2; Mann, 2001, pp. 70–71). The lipid bilayer of cells, and of vesicles, often incorporates proteins that enhance the transfer of elements across these membranes, i.e., calcium, with the possibility that apatite crystallites could be prefabricated within vesicles. If assembled in the extracellular environment, the mineral could be released at the mineralizing tissue site, and deposit on the collagen. Mitochondria are also regarded as possible sources of mineral for biomineralization (Lehninger, 1983). The possibilities that any or all of these mechanisms act in the mineralization of bone tissues are still niether fully evaluated nor understood (Mann, 2001, pp. 143–144).

In addition to the molecular localization of apatite in vertebrate tissues, we hasten to point out that the quest to understand and control, or at least modulate, the mechanisms of bone biomineralization with its many facets and levels of mineral deposition continue. The intricate

processes that lead to calcium phosphate deposition and specialized tissue formation have been refined over most biogeologic time not only since the first mammals (Halstead, 1969; Tuross and Fischer, 1989). Enlow, one of the early researchers into the histological characteristics of bone tissues, found counterparts of the human textures in the long extinct dinosaurs (Enlow and Brown, 1956–1958) (Figures 27(a) and (b)).



(a)



(b)

Figure 27 (a) Thin section observed with optical microscopy of lamellar bone from a femur of a Rhesus monkey through an area of muscle attachment showing resorption spaces (B), and haversian systems (A) and vascular canals (C), 150× (source Enlow, 1963, figure 32, p. 68). (b) Thin section of a dinosaur (Ceretops) bone showing lamellar and haversian bone similar to the tissue characteristics seen in (a) (source Enlow and Brown, 1956, p. 410).

Analyses of bone. The regeneration of mineralized tissues within all bone organs continues throughout the life of the organism. Therefore, though bones are durable and appear unchanged, the tissues are dynamic during the lifetime of the individual, and the integration of several textures during sampling may contain locally distinct, as well as with variable composition, organic and inorganic materials. A particular BMU will reflect the elements and molecules circulating during the time spanning the deposition phase. By employing markers specific for mineral, such as tetracycline, or fluorine, the sites, and the rates, of mineral deposition have been investigated in a variety of animals, and in human disease (Skinner and Nalbandian, 1975; Figure 28).

Because the external appearance of a bone gives no indication of this continual, internal reworking sampling may not be straightforward. Cortical bone, the more heavily mineralized and homogeneous tissue with slower turnover (species related), is often chosen, but any sample will contain several bone morphogenic units with heterogeneous organic and mineral deposition expressing responses to short-term, i.e., dietary, changes. Analyses of the mineral using multiple samples even from one individual may, therefore, show considerable variability. The results are an aggregate, a summary of the formation, resorption, and redeposition from several bone morphometric units. With such dynamic activity effectively hidden within bone tissue samples, it is not surprising that bone is considered less suitable than enamel, one of the tissues in teeth (see Section 8.04.3.7.5), for evaluating the elemental composition of bioapatites. If the object of the research effort is to establish the range of ingestion and uptake of certain natural, or anthropogenically provided, elements or compounds in a population, bone from known age individuals could indicate bioavailability, and some pollution or contamination (Kohn and Cerling, 2002; Trueman and Tuross, 2002). In the past skeletal investigations focused on the physical expression of bones comparing "normal" bones with those that might have experienced trauma, metabolic disturbances, or disease showing obvious differences (Ortner, 1992). With the advent of the more sensitive analytical techniques, such as scanning electron microcopy with energy dispersive analysis, there are many more investigations of the chemical variations of bone tissues (Skinner *et al.*, 2003; Ceruti *et al.*, 2003).

8.04.3.7.3 Cartilage

Cartilage is a distinctive skeletal tissue that also mineralizes with calcium phosphate.There are multiple locations in the vertebrate skeleton

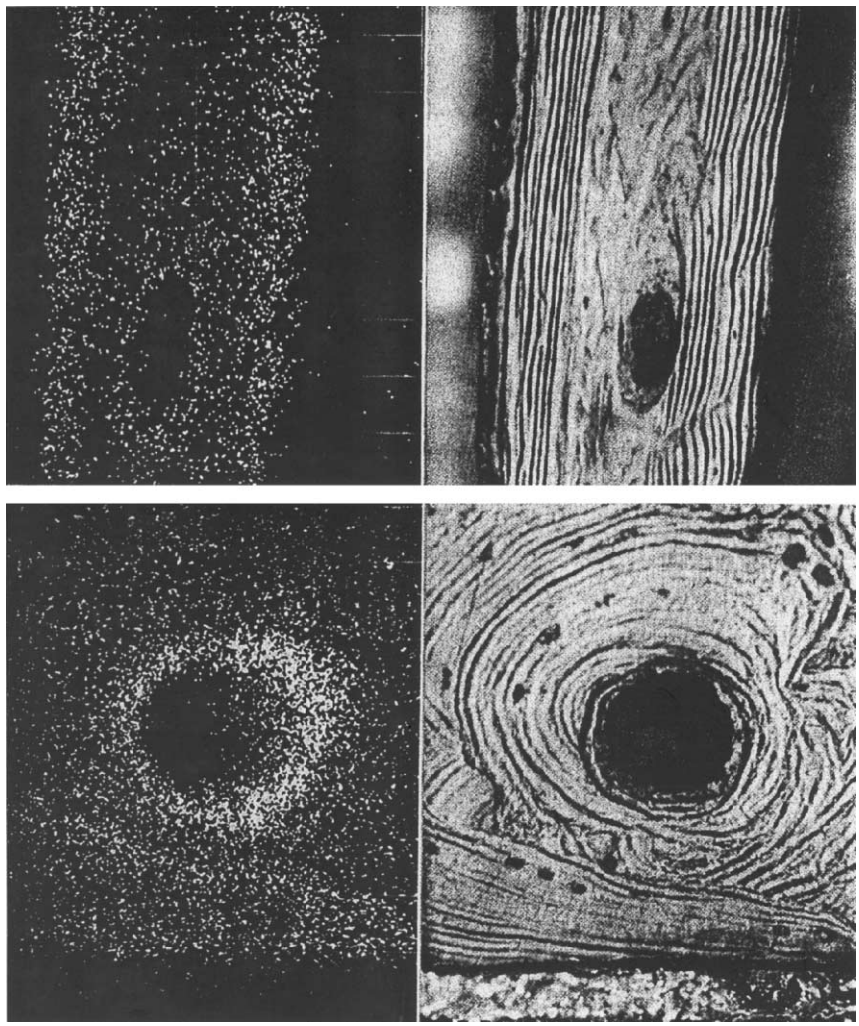


Figure 28 Fluorine uptake in bones. Upper diagrams—mouse; lower diagrams—human (osteoporotic) bones. White dots in left two plates indicate the location of F using electron microprobe emission analysis selecting the Kα wavelength of F. Companion plates on the right are photomicrographs of the same area using general illumination, and show the mineralized tissue structures, lamellar bone mineral deposition in the mouse, and mineral deposition at the center of a haversian system in the human coinciding with the concentration of white dots indicating F in the associated diagram (source Vischer, 1970, figures 1 and 2, p. 28).

that are predominantly cartilage but only some sites biomineralize, e.g., *in utero*, where an aggregation of cells, chondrocytes, proliferate and create a preformed model of a bone. The cartilage will eventually be replaced as part of the normal gestation and maturity sequence of the organ (Ogden and Grogan, 1987; Ogden *et al.*, 1987). This precursor bone formation system, known as endochondral ossification, is typical of vertebrate long bones where elongation of the organ takes place at the growth plate and the cells as they are produced assemble in columns (Figure 29). The chondroblasts produce a water-rich (up to 80%) gel-like aggregate (anlage) of two organic molecular species: proteoglycans, or protein–polysaccharides, and type II collagen, which becomes mineralized with apatite (Lowenstam and Weiner, 1989, pp. 167–175). Sharks and some fish keep such cartilaginous "hard" tissues to maturity (McLean and Urist, 1968; Moss and Moss-Salentijn, 1983), whereas in humans this "cancellous bone" is replaced by "membranous bone."

The other sites where cartilage occurs are not meant to mineralize. Articular cartilage is the shiny slippery textured material found at the ends of many bones, a tissue that facilitates the motion between two bones at the joints. The deposition of mineral in articular cartilage and joints is pathological, and briefly discussed in Skinner (2000). Cartilagenous tissues that occur in the ear, epiglottis, and intervertebral disks are normally not mineralized, and the organic components at these sites are distinct chemically and

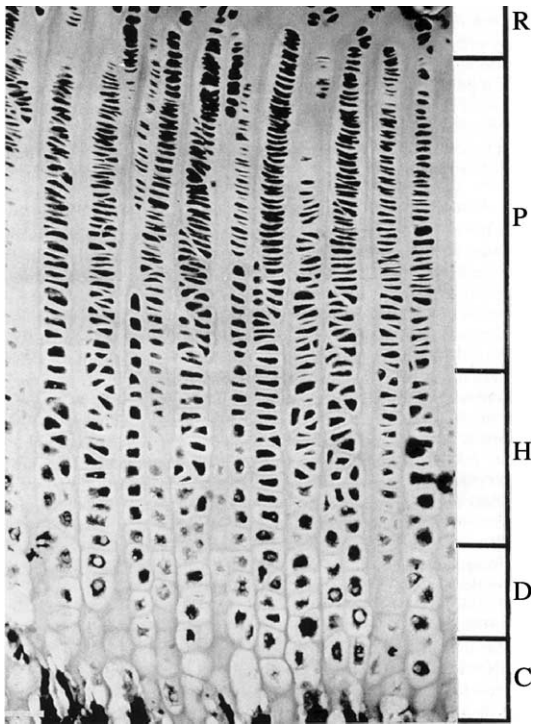


Figure 29 Optical micrograph of epiphyseal cartilage in the femur (large leg bone) of a rabbit showing columns of chondrocytes in the growth plate. The growth plate is divided into zones (C–R) that reflect changes in the chondrocytes during the elongation and mineralization of the endochondral bone (Source Lowenstam and Weiner, 1989, figure 9.12, p.172).

histologically from the mineralized cartilage sites. Miller (1985) compares the collagen compositions and the associated proteoglycan molecules that have molecular weights upwards of 200 kDa. These molecules have a core of hyaluronic acid (a polymer of glucuronic acid and *N*-acetylglucosamine), ~1,500 nm in length. This aliphatic protein base has upwards of 100 protein-linked monomers with noncovalently linked "side chains." Each of the monomers, formed intracellularly by chondroblasts, also has a protein backbone ~300 nm long with tens of negatively charged glycosaminoglycan chains covalently attached through serine and threonine residues. The assembly of such very large molecules, some of which contain sulfated species, e.g., chondroitin sulfate, results in extracellular, negatively charged species which, like bone, provides sites where mineralization commences and continues.

Biomineralization of cartilage. Prior to mineralization, mitochondria in the chondrocytes, cells equivalent to osteocytes, buried in the protein-glycosamino-gel, load up with calcium and phosphorus. Using SEM/EDAX analyses and microdissection, a timed efflux of calcium and phosphorus from mitochondria was shown to be coordinated with mineral deposition (Shapiro and Boyde, 1984). The mineralized tissue produced contained platy hydroxylapatite crystallites similar in size and composition to that in bone, but not uniformly associated with the cartilage collagen (type II). The extracellular cartilaginous matrix with its highly charged anionic polysaccharide chains may attract additional calcium and aid mineral nucleation (Hunter, 1987). However, the matrix also contains abundant lipid bound vesicles (Bonucci, 1967). At the earliest stages of cartilage matrix formation, the vesicles are without mineral but over time accumulate apatite crystallites, which coalesce, and the vesicles disappear leaving a haphazardly mineralized tissue (Ali *et al.*, 1970, 1977). There are slight differences in cartilage mineralization from that of bone: the mineral is not uniquely associated with the type II collagen, and the large amount of proteoglycans provides multiple opportunities for nucleation in the extracellular environment. The vesicles, acidic proteoglycans, and the procollagen type II molecules appear before mineralization and each could be, or could become, dominant as the mechanism of biomineralization, or they could behave cooperatively (Lowenstam and Weiner, 1989, p. 175). Vesicles have been identified as transport packets of mineral during biomineralization in invertebrates (Addadi *et al.*, 1987). In cartilage, acidic phospholipids on the vesicle membrane may act as a site of calcium accumulation (Wuthier, 1984), or nucleate apatite crystallites (Vogel and Boyan-Salyers, 1976). Vesicles could transport calcium and/or mineral to the mineralization front. Studies of biomineralization mechanisms in model animal systems use isotopically labeled elements and molecules, such as ^{45}Ca, ^{31}P, or the sulfur, in the sulfated organic complexes to elucidate the process.

8.04.3.7.4 Antlers

There is one other "bone" which could be useful to geochemists interested in assessing environmental exposures. Antlers, but not horns, are shed annually. Both these organs have a bony core but horn is covered with dead keratinous tissues while antlers are covered initially by velvet, an epidermal tissue with separate blood and neural supply. Once the velvet has been rubbed off, the bony superstructure that has formed and mineralized very rapidly provides the animal with a remarkable headdress. After the breeding season osteoclasts resorb at the base where the antler is attached to the frontal bone of the skull and antlers may be relatively easily obtained for analyses. Male deer, reindeer, and caribou regrow their membranous bone excrescences each year with an increase in the complexity and size of these unique structures

(Halstead, 1974, pp. 98–100). Frank (2003) investigated an odd disease of moose in southern Sweden showing that a molybdenum deficiency in their forage leads to diabetes in these animals.

8.04.3.7.5 Teeth

Introduction. The evolution of dentitions as independent mineralized organs shows that from the earliest vertebrates to modern mammals, there has been a diversity of biomineralized structures and processes (Halstead, 1974). The following discussion focuses on human dentition as an example of the many teeth and oral cavity arrangements found in warm or cold-blooded vertebrates.

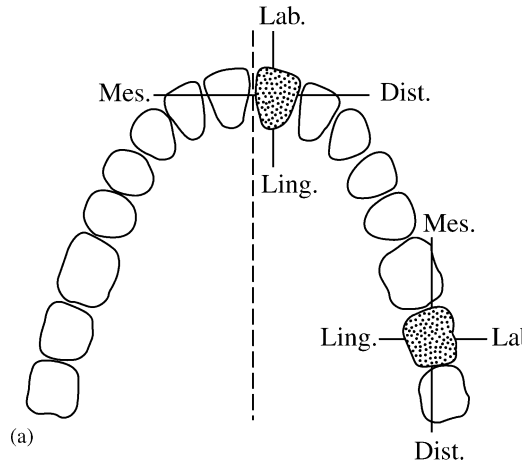
The adult human normally has 32 teeth (Figure 30(a)) with distinctive shapes related to function, a system that parallels the morphological expression of bones in the skeleton. Each tooth should be considered an independent organ. The significance of the macrostructural variety and the biomechanical contributions, especially tooth wear, has become important to those interested in the evolution of vertebrates, particularly the hominids. The relationships of dentition to the skull bones, the mandible and the maxilla, and the size and shape reflect the habitat, and diet, of the species. Oral anatomy, tooth development, mineralization, and demineralization (caries) remain active research areas by a host of dental professionals, as well as biologists, anthropologists, and geologists. In fact, studies of calcification, elucidation of the composition and structure of bioapatites, and biomolecular species, especially collagen, are and have been supported in the US by the National Institute of Dental Health and Craniofacial Anomalies. Obvious malformations such as cleft palate provided some of the first information on genetic abnormalities and hazardous drug ingestion (Goose and Appleton, 1982). Early studies into tooth biomineralization came from Europe (Schmidt, 1921) and markedly expanded in the US, around fluoridation of domestic waters to minimize the formation of cavities (caries), especially in young children (Vischer, 1970). This review of the biomineralization of teeth affords insight into different sites of calcium phosphate deposition and some novel geochemical applications.

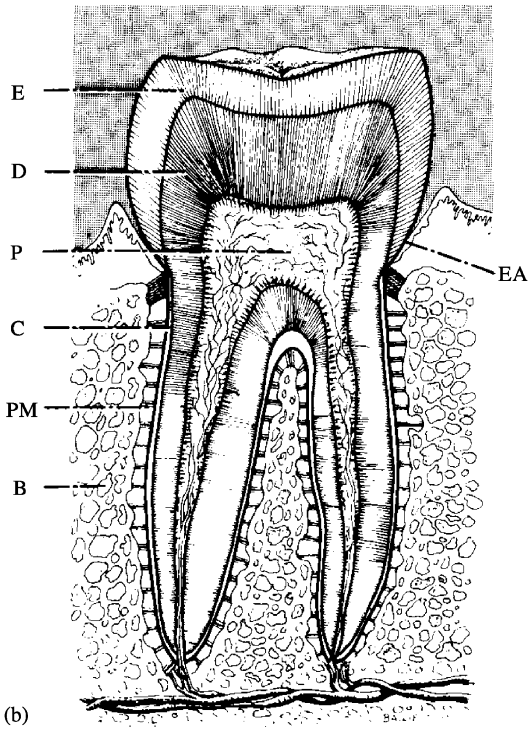
Tooth biology and mineralogy. There are three mineralized tissues in a tooth each with distinct cell systems and different histological expressions. Table 3 compares enamel, dentine, and bone, the relative amount of calcium phosphate to organics and the composition range of the mineral material. Most analyses of the mineral are on samples that have been chemically extracted, or ashed, to remove organic components and water (Skinner *et al.*, 1972, 2003) and reported on the dry-fat-free basis. Many papers report only calcium and phosphorus, calculate the Ca/P ratio, and compare the results with the ideal for hydroxylapatite (2.15 wt.%, 1.67 mol.%). Some analyses include magnesium (always less than 2.5 wt.%), sodium (usually less than 1 wt.%), and chlorine, always less than 0.1 wt.%. Occasionally CO_2 is reported (between 3 wt.% and 6 wt.%) with other elements mentioned when related to specific research activities. Comprehensive elemental analyses that add to 100% are not usual (Skinner *et al.*, 1972).

The tooth structure is created when ectodermal cells, ameloblasts, situated in apposition to the mesodermal dentinoblasts secrete organic matrices, and direct calcium phosphate deposition. The third tissue, cementum, covers the dentine below the gum line in the tooth root (Figure 30(b)). Teeth are anchored into the jaw bones via fibers that start in the cementum (below the enamel cap) and end in the bone; known as periodontal ligaments, these essential supporting guy wires must be discarded when deciduous teeth are replaced by the permanent dentition. Each tooth in its socket represents a multiple mineralization site, forming and forcing resorption of adjacent bone (remodeling) over time. In humans "baby teeth" are evulsed after resorption of the root portion as a new larger tooth is created with new enamel, dentine, and cementum, and erupts into the oral cavity through a jawbone enlarged to the adult size.

Mature enamel is a cell-free tissue while cementum though mineralized is avascular, similar to some bone tissues, and may have developed to serve the local needs, not a primitive, but a derived and modulated tissue system (Poole, 1967). The bulk of the mineralized tooth is dentine, also a vital tissue that contains passageways known as tubules that are intermineralized with apatite. The tubules reach from the pulp cavity with its supply of nerves and blood vessels that enter at the base of the root(s) and extend to the enamel. Dentine has the possibility to repair itself.

The incremental growth of a human tooth is demonstrated in Figure 30(c). The bright nested lines are generated from sequential doses of tetracycline. An antibiotic, tetracycline, interacts with the mineral, recording the sites and rates of deposition at the mineralizing front during tooth gestation of this molar. Tetracycline incorporation can be detected because of its characteristic fluorescence in UV (Skinner and Nalbandian, 1975). Mineralization starts at the dentine–enamel junction, fills in toward the central pulp canal and tapers towards the root(s), while the enamel grows in the opposite direction forming a cap over the entire upper surface. The central canal reduces in size as the organ completes its maturation and erupts into the oral cavity.





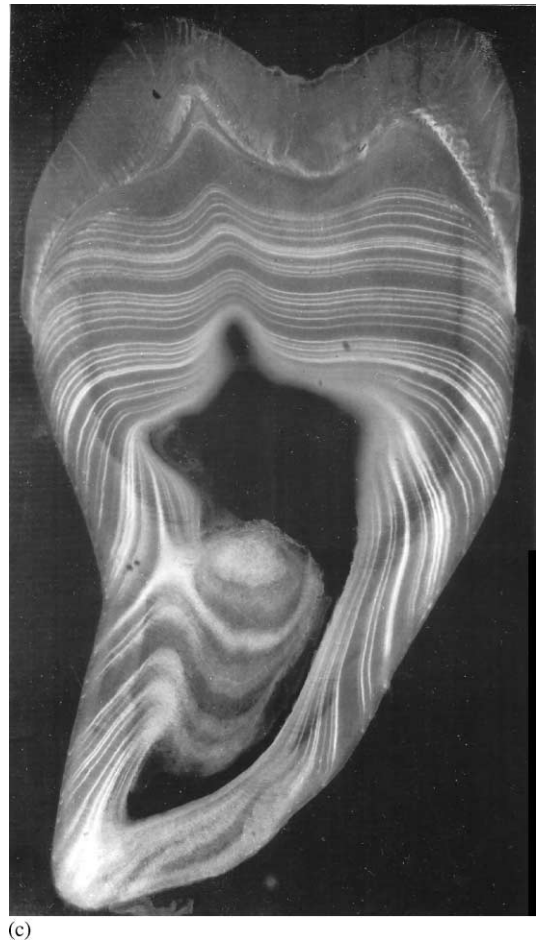


Figure 30 (a) Schematic of the maxillar (upper jaw) dentition of the adult human (source Peyer, 1968, figure 10, p. 16). (b) Diagram through a molar: E = enamel, D = dentin, P = pulp chamber, C = cementum, PM = pericementum muscle attachments, B = bone, and EA = enamel cap finishes with the cementum the mineralized tissue below the gumline (source Goose and Appleton, 1982, figure 8.1, p. 126). (c) Cross-section of molar, comparable to Figure 30(b), showing tetracycline uptake as a series of nested bright lines indicating where and when the antibiotic was ingested, separated by darker areas when no antibiotic was circulating in the system, and therefore was not deposited with the mineralized tissue of the growing tooth (Skinner and Nalbandian, 1975, fig. 4A, p. 386).

Table 3 Bulk composition, density and crystallite size of mineral in bone, dentine, enamel, and the composition of the extracted bioapatites.

	Bone[a]		*Dentine*[a]		*Enamel*[a]	
	wt.%	vol.%	wt.%	vol.%	wt.%	vol.%
Inorganic	70	49	70	50	96	90
Water	6	13	10	20	3	8
Organic	24	38	20	30	1	2
Density (avg) (g cm^{-3})	2.35		2.51		2.92	
Crystal size (maximum)[b] (angstroms, Å)						
Length	300 Å	1,600 Å				
Width	60 Å	410 Å				
Hydroxylapatite composition (wt.% on a dry, fat-free basis)[c]						
Ash	57.1		70.0		95.7	
Ca	22.5		25.9		35.9	
P	10.3		12.6		17.0	
Ca/P	2.18		2.06		2.11	
Mg	0.26		0.62		0.42	
Na	0.52		0.25		0.55	
K	0.089		0.09		0.17	
CO_2	3.5		3.19		2.35	
Cl	0.11		0.0		0.27	
F	0.054		0.02		0.01	

[a] Driessens and Verbeeck (1990: table 8.2, p. 107; table 9.4, p. 165; table 10.5, p. 183). [b] Elliott (2002, table 4, p. 441).
[c] Zipkin (1970, table 17, p. 72).

Once erupted, the cells that produced enamel disappear; hence, there is no possibility of inducing new enamel formation, should cavities develop. Enamel is the only tissue observed in the mouth. Enamel is a hard, composite, tissue, and the most highly mineralized tissue in humans, consisting of over 95% mineral. There is virtually no organic component in enamel (Table 3), although it is essential to creating the patterns distinctive to this mineralized tissue. The ameloblasts secret small (2.5×10^4 MW) hydrophobic proteins known as amelogenins (Fincham and Simmer, 1997) which have high concentrations of proline, glutamine, leucine, and histadine, but no regular repeating [Gly–X–Y] aminoacid sequence typical of the collagen fiber series. There is a self-assembly of 20 nm nanospheres of ~100 amino acid (AA) residues, that appear to assist in the orientation of the crystals parallel to the *c*-axis or long axis of the apatite. Enamelins, acidic glycoproteins (β-pleated sheath structure; Deutsch *et al.* (1991)), enclose each growing crystallite binding to specific crystallite surfaces.

Enamel contains well-oriented apatite crystallites, of sizes up to 1 nm in maximum dimension (Elliott, 2002). Ultra-high-resolution photos of thin sections of developing enamel show the unmistakable hexagonal outlines of apatite (Figure 31(A)). Powder X-ray diffraction analysis which can be used to accurately identify the crystal structural characteristics of any solid shows that the material is clearly apatite and that there is preferred orientation of the crystallites. These analyses reinforce the fact that the mineral of enamel mineral is much better crystallized (more regular in structural character) than the mineral portion of bone or dentine. The size of the crystals can be measured using a variety of techniques (Elliott, 2002).

In all vertebrates the ameloblasts create packets, called prisms, of organic materials with aligned clusters of crystals, arranged in arcuate arrays that are species specific (Halstead, 1974). Differences in the orientation of the prisms and in the degree of mineralization determine the textures observed optically, or with TEM, in enamel (G. Gustafson and A. G. Gustafson, 1967). Depending on the orientation of the mineral-containing thin section being examined, optical microscopy reveals (i) narrow stripes (called the Lines of Retzius) generated by the relationship between adjacent prisms during successive periods of growth in human enamel, or (ii) Hunter–Schrager bands that extend from the dentine–enamel junction ~2/3 of the way toward the surface of the enamel and result from the changing directions of the prisms. Disruptions or variations in amounts of mineral within or in adjacent prisms can also be observed (G. Gustafson and A. G. Gustafson, 1967).

Dentine is less mineralized than enamel with a lower Ca/P, and shows variable composition individual to individual (Rowles, 1967), similar to the other mineralized tissues. Some of the chemical variations reported on teeth may be the result of the pretreatment, the level of analytical

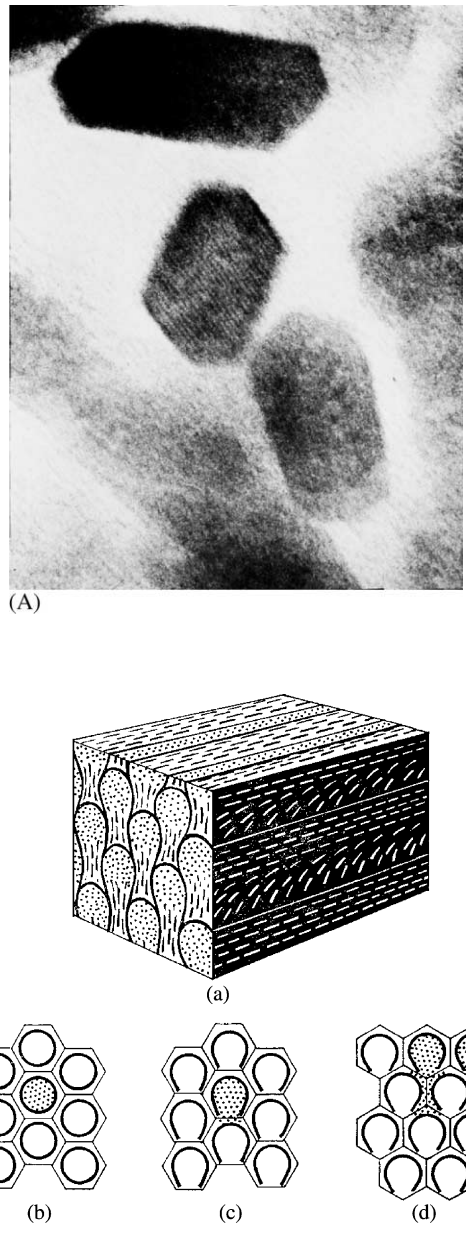


Figure 31 (A) Ultra-high-resolution (1,109,000×) TEM photo of apatite crystallites in enamel (early developing rat enamel) (source Helmcke, 1968, figure 8, p. 143). (B) Diagram showing the distribution of enamel crystallites within prisms and prism arrangements formed by ameloblasts in enamel of vertebrates illustrating the variety of arrays in the tissue depending on the angle of light microscopic observation. Note: different vertebrate species have distinct enamel patterns depending on the sites where crystallites are deposited and the number of ameloblasts producing the prisms. The textures observed not only vary with the orientation of the thin section examined but are distinctive for the ameloblasts contribution to crystallite orientation. For example in "d" the deposition of crystallites in a prism is contributed by four ameloblasts (source Halstead, 1974, figure 12.3, p. 89).

sensitivity, as well as relate to the particular tooth, or portion of the tooth, examined. For example, ingestion of fluoridated water and the age of the individual or of the sample may increase the mineral content postextraction due to dehydration. Using transmission microradiography increased mineralization around dentine tubules, and the formation of secondary dentine after tooth trauma, are detectable. These expressions of mineralization post the original organ mineralization may be large and could contribute to analytical variations. The apatitic mineral in dentine, and cementum, is similar in size and composition range and variations depending on the stage and diet during gestation, plus whatever uptake (usually minor) after maturity of the organs. Fluorine content in human dentine, for example, may increase over time with constant ingestion of fluoridated water reaching a maximum at ~55 yr of age. Enamel, alternatively, is completely formed, in a short time period for an individual tooth, with little, if any, change postcompletion and emplacement of the whole tooth into the oral cavity, including uptake of elements (except as might be briefly adsorbed when flushed by saliva). The post-mortem uptake (passive) of fluorine in both teeth and bone from groundwater is well known. It is an expression of the stability of fluorapatite over hydroxylapatite in Earth environments (see fluorine uptake in bone, Figure 28). Besides, the fluorine content of fossil samples helps determine whether there has been obvious diagenesis. During formation of bioapatites in humans, however, the mineral is a hydroxylapatite with trace quantities of fluorine even in areas where high fluorine waters are ingested.

Over geological time, vertebrates have generated a variety of mineralized structures. Their multiple forms of dental organs and tissues, and replacement modes reflect genetic diversity, probably modulated through adaptations or conversions relative to the habitat and diet of the species. Some of the tissues which can be examined today in lampreys, bony fish, and amphibians are histologically similar to tissues known as aspidin, and enameloid that may have functioned as armor in the earliest Devonian vertebrates (Orvig, 1967; Halstead, 1974). The morphological expressions of these tissues distinctive to vertebrates may be unique, but the mineral matter is invariably the calcium phosphate, apatite.

8.04.3.7.6 Otoliths

There are other mineralized structures that have been used for dating and investigations of the ecology of vertebrates—the otoconia or statoliths—that occur in the ears of mammals, birds, and fish. The predominant mineral in these

structures is $CaCO_3$ in the form of aragonite, but as shown in Figure 32(a) there are some families which possess calcium phosphate otoliths, and many otoliths are a combination of two mineral species.

Otoliths are tiny, often barely visible, mineralized structures that are unattached and do not form part of the skeleton *per se*. They are highly mineralized single, or multiple and fused crystals, of one of the three polymorphs of $CaCO_3$, aragonite, calcite, or vaterite. The crystallites (Figure 32(b)) grow in the canals or labyrinths of the ear ducts and may have several functions: sound detection, as sensors of gravity, or as part of the system used to determine orientation equilibrium of the animal body, making it essential that they be mineralized structures. Otoliths show distinctive morphology that is used to make taxonomic decisions (Nolf, 1985).

Large otolith accumulations in sedimentary horizons led Cuvier in 1836 to suggest that the taxonomy of fish species, e.g., teleosts, could be used to determine the ecological parameters for the marine environment. The otoliths became markers for deciphering ocean sediments comparable to the use of mammal teeth in terrestrial environments. In the ensuing years otoliths or "buttons" from the Jurassic to the present in localities as diverse as the Viennese Basin, Sumatra, Nigeria, New Zealand, the Mediterranean, and England were investigated. Past studies and applications are summarized by Nolf (1985). Nolf, along with Milton and Cheney (1998), have all been concerned with diagenetic alteration of the specimens. The chemical analyses of recent fish otoliths from marine and freshwater sites for trace elements that include both stable and radioactive species provide an independent source of data on migration, maturation, as well as being indicators of pollution (Adami *et al.*, 2001; Gillanders *et al.*, 2001; Hanson and Zdanowicz, 1999; Spencer *et al.*, 2000; Volk *et al.*, 2000). Analyses for specific elements, e.g., lead and strontium, can go beyond bulk determinations as otoliths may record a time series. Their growth rings (Figure 32(b)) are similar to tree rings documenting seasonal changes. Laser-ablation inductively coupled plasma mass spectroscopy (ICPMS) analyses on cross-sections through the collection of rings in the larger, up to centimeter-sized, oval to circular otoliths typical of some species, is a most effective analytic technique.

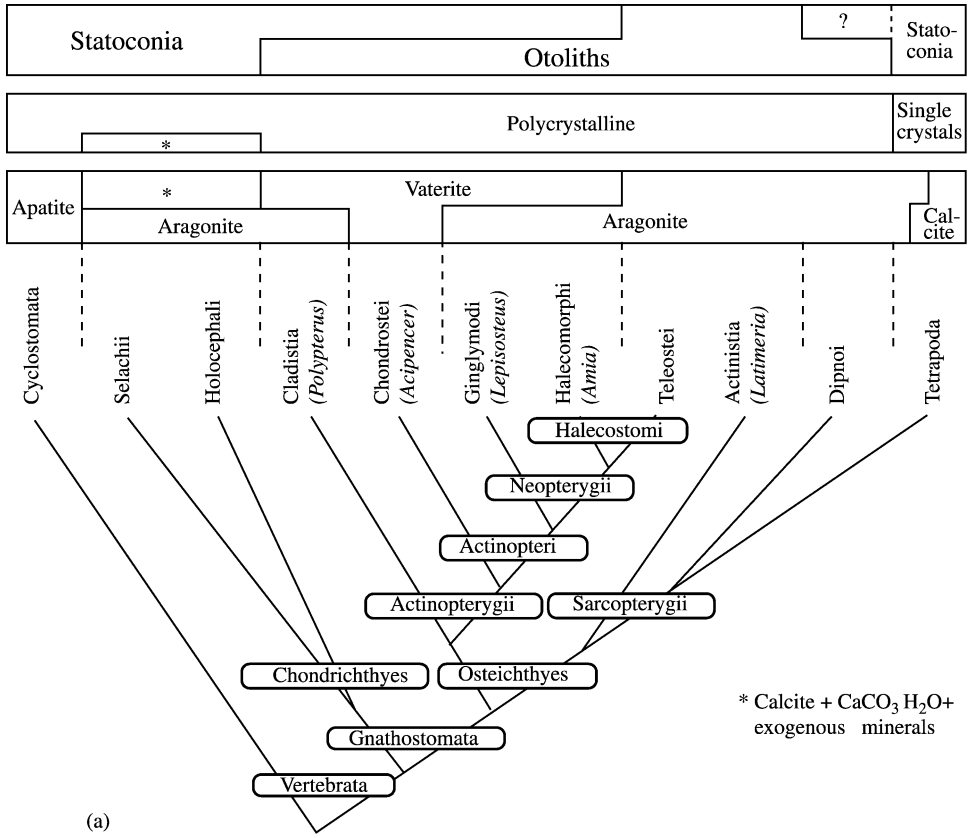
The deposition of mineral matter in fish otoliths may take place in three paired canals designated as saccular, utricular, and lagenar, for the actinopterygians by Norman and Greenwood (1975), or saggita, lapillus, and asteriscus for other species (Nolf, 1985, p. 3). Although there are multiple crystallites, the otoliths may be no more than a 0.01 mm in diameter depending on the species, age, and size of the fish. However, since there will be two of each sort for intact fish, sufficient sample of these biogenic carbonates may not be a problem (Arslan and Paulson, 2002). The rings, a light colored and usually thicker deposit, consisting predominantly of inorganic materials formed during summer and fall alternate with dark, and thin, winter rings that may contain up to 10% organic materials. The organic portion in both light and dark areas is up to 85% otolin, a collagen-like protein.

Interpretations of the otolith data to document climate change or pollution have been careful to consider the bioavailability of specific elements, variations in habitat, or ingestion idiosyncrasies of the specific fish species (Campana, 1999).

8.04.4 SUMMARY: WHY BIOMINERALIZE?

It appears that the capacity for biomineralization has been an evolutionarily widespread and an enduring trait, because it conferred strong and obvious selective advantage to organisms that possessed it. Biomineralization, in this analysis, is a fundamental life process by which animals and plants gain structure and mass, virtually without tissue maintenance cost, drawing on the chemistry of the environment to find strategies for maintenance and defense. Mineralization increases the opportunities for organisms: they can become mobile, producing endoskeletons on which to attach appropriate muscles (vertebrates), and therefore move out of hostile environments, or, if sessile, adapt by strengthening their internal skeletons (gorgonid corals), or produce external protection such as shells (mollusks). Biomineralization also contributes to the classic adaptation to land environments by plants.

Beneficial attributes conferred on biological forms by biomineralization and biominerals are actually twofold: (i) physical, or macrocontributions, the production of skeletal structures that provide integrity and specificity on the organism, and (ii) chemical, or micro- or sub-micro-contributions. In the latter, biomineralization provides a personal storage system from which ions are mobilized to other portions of the living creature if they are necessary: the nutrients that assist or regulate growth or, at the other extreme, ions may be permanently sequestered to avoid toxicity, if they are hazardous. Availability of stored nutrient ions is crucial to the buffering of body fluids and to skeletal and tissue repair activities central to the evolutionary survival and competitive status of an organism. The generation and systemic circulation of hormones or other special molecules could facilitate these activities and probably represent evolutionary advances or adaptations.



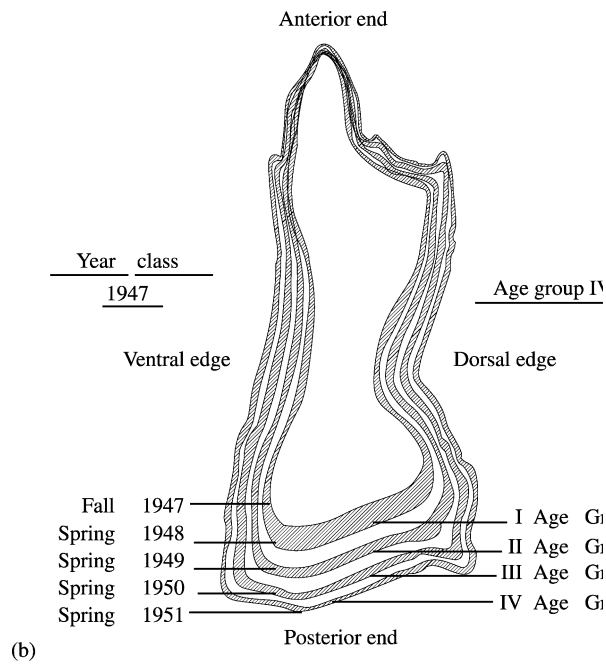


Figure 32 (a) Mineral materials found in the statoconia and otoliths of vertebrates (source Nolf, 1985, figure 1, p. 3). (b) An otolith showing incremental growth stages (source Nolf, 1985, figure 5, p. 5).

8.04.4.1 Physical or Macrobiomineralization Contributions

As animals and plants came to colonize land surfaces, gravity (cf. buoyancy) became a primary force; organisms required a structure, or framework, in order to stand up under the earthward pull of gravity. Minerals became the ideal choice. Incorporating them into the biological structures provided the stiffening agents. Throughout evolution the design and engineering of the composite (inorganic and organic), tissues became integrated into macroscopic, but relatively low-density structures. Produced internally or externally by the animals or plants, biomineralization did not reduce, but rather aided flexibility, if not mobility, for some forms. Once mobile, for example, animals could migrate to more benign habitats, seek different food sources, and avoid becoming prey. Sparsely mineralized carapaces of arthropods protect soft tissues but allow for mobility through an articulated exoskeleton. These creatures can live under water, in the air, as well as on land; mollusks burrow into mud flats to depths commensurate with the mineralization level of their shells. Mineralized internal structures characteristic of coccoliths and diatoms establish their domains, while silica reinforcement of plant stem tissues gives them structural integrity that could never be achieved using soft organic tissues alone. To remain rigid is critical for plants and it provides competitive advantages. Structural support during growth periods increases the plant's ability to compete for light, and provides resilience against heavy rain, strong winds, or trampling by animals.

8.04.4.1.1 Defense and protection through biomineralization

Many defense structures and mechanisms exist in plants, living forms that must survive in fixed locations. Sharp hairs or spikes known as "trichomes" are extensively biomineralized. The stiffness and sharpness of the biomineral allows the structure to effectively stick, lodge within and irritate an animal, even though the animal may possess several times the mass and infinitely more mobility than the plant (Hodson *et al*., 1994). Stinging nettles (*Urtica* spp.) possess complex trichomes on the surfaces of their leaves and stems. The proximal silicious portion of the trichome attaches to a distal calcified portion with a terminal bulb. Upon contact with an herbivore, the bulb is dislodged leaving a beveled silicious hollow that functions like a hypodermic needle. This sharp needle, filled with various irritant chemicals including histamine, acetylcholine, and 5-hydroxytryptamine, may be released into the animal's skin sometimes causing extreme irritation or allergic reaction (Kulze and Greaves, 1988). Silicious spines or sharp needle-like crystals could damage soft mouthparts with instantaneous effect, preventing herbivores from specializing in, and thus obliterating, the biomineralized plant species and communities.

Many plant fruits contain a hard covering for their inner endosperm contents, providing structural protection for the reproductive tissues that will grow into a new plant, while allowing dispersers to enjoy enticing fruit. The hard covering is usually hard because of the incorporation of biomineral: peach-pits contain aragonite, and walnut shells are heavily silicified. Some biominerals may be designed to protect the endosperm as it travels through the acidic gastro-intestinal tract of a dispersing herbivore. Hackberry carbonate endocarps dissolve in low pH solutions, leaving a reticulate silica network, porous enough to let the endosperm germinate after it passes through the herbivore (Cowan *et al*., 1997). Immature stamens of *Philodendron megalophyllum* (Philodendron) are reinforced with calcium oxalate (Barabe and Lacroix, 2001), which may serve to keep these delicate structures intact until pollen is ready for dispersal. In developing fruits, biomineralization shows specific timing (Jahren *et al*., 1998), with the bulk of the mass added at the same time that the endosperm forms. After the mineralized endocarp is essentially complete, the edible fleshy portion of the fruit begins to develop. Studies of dual-mineral plant phytoliths have shown that vilification is complete by the time calcification begins (Jahren *et al*., 1998; Thurston, 1974).

Horses who graze sandy-quartz soils suffer from silica enteroliths, either as a result of phytolith intake, or direct soil intake. Geis (1978) studied three species of opal-bearing Gramineae and found that the amount of opal deposited to the soil annually by root systems and aboveground parts of plants was approximately equal in magnitude, indicating that biomineralization in grasses is not specific to leaves, but results in considerable contribution of phytolith material directly to the rhizosphere. The evolution of horse dental morphology through geologic time is thought to be partially controlled by the impact of phytolith-bearing grasses on horse enamel (MacFadden *et al*., 1999).

8.04.4.2 Chemical or Microbiomineralization Contributions

Biominerals because of their crystal chemistry naturally incorporate certain elements via solid solution in the crystal lattice, and their habits may present morphological (size or twinning)

advantages for the adsorption of specific elements, or molecular species. The result is that the mineral may act as a storehouse for useful or hazardous ions.

Silica may deter animals from eating or contacting (and possibly crushing or damaging) plant tissues, but silica biomineralization may help to alleviate the toxicity of aluminum in plants growing on contaminated soils (Hodson and Sangster, 2000). Plants relieve potential Ca^{2+} toxicity via biomineralization of calcium oxalate (Webb, 1999), but calcium oxalates are also well known for preventing herbivory through chemical restraints.

Calcium oxalate results from the reaction of oxalic acid and calcium ions (McNair, 1932), and oxalic acid itself is an effective deterrent to herbivores in its own right (Arnott and Webb., 1983). At least four different biochemical pathways have been shown to result in the synthesis of oxalate in plants (Raven *et al*., 1982). Utilizing radiocarbon-labeled ascorbic acid administered to roots of *Yucca torreyi* L. (Torrey's yucca); the incorporation into vacuole crystal bundles demonstrated that ascorbate is an important precursor to oxalate in this plant (Horner *et al*., 2000).

The number of calcium oxalate crystals was found to be highest in young leaves and lowest in mature leaves of five tropical plants, indicating that plant organisms use biominerals to preferentially defend their most vulnerable tissues (Finley, 1999). Plants are also able to respond to ongoing herbivory by increasing the amount of biomineralized calcium oxalate crystals: leaves from seedlings of *Sida rhombifolia* (Teaweed) subjected to herbivory had a greater crystal density than those grown under protection (Molano, 2001). However, many herbivores have developed strategies to deal with plant biominerals. Microscopic spherulites of calcium carbonate found in the dung of herbivores may represent transformed plant-derived calcium oxalate (Canti, 1997), suggesting that the original biomineralized material can be transformed and excreted.

To discuss the range of possibilities of "why biomineralize?" we utilized plants, but there are parallels when assessing the possible reasons for biomineralization in other life forms, some of which have been mentioned or alluded to in the body of those sections. Our purpose has been to accentuate the cross-overs between the Earth and the biological sources for nutrients (and hazards) for all forms of life. We underscored the case of biomineralization in plants, because we and all land-based and most marine life must feed upon them. There are, of course, many agricultural examples which have not been included herein. The fact is that biomineralized tissues do reflect the environment and have become one of the important areas for future geochemical research. Each biological form mentioned in this chapter has mineral components, storage sites of elements, and molecules that deserve further investigation.

Biomineralization strategies and the biominerals are, for many good reasons, important to all who inhabit the Earth. The crossovers between the biomineralizers, the chemistry and the morphology of the minerals, the tissue textures, the cells, and the mechanisms that characterize some life forms offer intriguing insights to the physical–chemical laws of nature.

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