Chapter 28

Mineralogy of Bone

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I. Introduction

Medical geology encompasses many scientific endeavors with global activities and impact, but it also includes aspects that are very personal and individual. Local environment is sampled through what is ingested and inhaled whether or not it is salubrious, marginal, or downright unhealthy. Human bodies react to the remarkable range of natural and man-made chemicals that they are exposed to every day. It is common knowledge that a range of nutrients is required, but some of the chemicals can be hazardous to our health. This chapter focuses on bones, and specifically the mineral portion in these tissues, the component essential to the functions of these organs. These discussions illustrate several attributes of the emerging field of medical geology. The scientific information outlined herein is drawn from a diversity of disciplines and expertise, from biophysical and biochemical sciences, from physicians and dentists, and from geologists, mineralogists, and engineers. This knowledge enables us to address the many individual and collective roles that minerals play in the body. A disorder that affects people on every continent, osteoporosis, is presented as an example of how information on minerals can be applied. It is only one of the possible targets of opportunity where mineralogical/geological expertise has, and continues to have, the potential to ameliorate suffering and promote better global and personal health. Selected classic and recent references are included to whet the appetite of those who will make contributions to our knowledge in the future.

II. The Skeleton and Mineralized Tissues

Humans can be distinguished from all other mammalian species by their skeletons which are composed of over 200 bones and 32 teeth. Each of these skeletal components is a separate organ composed of mineral-
ized tissues. Created by the actions of distinct cell systems, the tissues are true composites, an intimate association of extracellular macro- and microbioorganic molecules, and inorganic mineral materials. The tissues are in dynamic equilibrium with a highly controlled fluid whose chemical composition resembles that of the ocean: highly oxidized, with a pH around 7 with sodium, Na⁺, and chlorine, Cl⁻, as the dominant ionic species.

The mineral of bones and teeth is a calcium phosphate that closely resembles the naturally occurring mineral, hydroxyapatite (Gaines et al., 1997), and because of its intimate association with biological activity and molecules it is known as bioapatite. The mineral formed and maintained in the soft tissue or matrix of bones has peculiar attributes. Each mineralized tissue is a remarkable and unique chemical repository whose maintenance is only beginning to be comprehended as part of the dynamic skeletal system.

A. Normal Mineralized Tissues:
Bones and Teeth

The relative amounts of mineral to bioorganic components and the distinct spatial aggregations or structures of the mineral-matrix combination have been objects of investigation for over 250 years. Four different types of normal human mineralized tissues have been described (Glimcher, 1976; Mann, 2000). With increased sensitivity and availability of analytical techniques, the four types have been shown to have discrete cell systems, chemical components, and spatial expressions that change during growth, with age, or with disease. Studies, especially those from different life stages, have allowed us to identify essential participants and gain some understanding of the expected or "normal" state and the reactions required to maintain function appropriate for the skeleton.

Three of the four tissues are found in teeth: enamel, dentine, and cementum (Figure 1) (Miles, 1967), and the fourth is in bone. Subsets of all four types have been described and amplified with each new and more sophisticated analytical technique. Optical examination and specialized methodology on thin sections of mineralized tissues at high resolution has defined the anatomy of their components, the cells, extracellular products, or the typical textures (Figure 1A). Tissues typical of bones (Figure 1B) have woven, lamellar, and haversian textures with mineral distribution and content that varies with tissue age, with nutrition, or with disease (Albright & Skinner, 1987). All three textures may occur in cortical bone, the heavily mineralized portions of bone, or in trabecular bone, the porous and spongy segments of the organ, and are expressions of their dynamic nature. But all bioapatite deposits in humans are not normal and expected.

B. Pathological Apatitic Deposition

In addition to the normal bones and teeth, bioapatite may deposit pathologically, that is, in the tissues of other organs that would normally not mineralize. Nodular apatitic sphaerules may be detected throughout the body. One site where bioapatites may occur is in tumors, both benign and cancerous, where rapid cell production may cause accumulations of dead cells. When a cell dies it releases any phosphate bound to bioorganic molecules into the surrounding fluid. The elevated calcium concentration in the circulating serum is much higher than the calcium concentration within the cell and bioapatite may nucleate. Another likely site for bioapatite mineralization is at scar tissues, the sequelae to tissue trauma, where excessive amounts of the fibrous protein collagen accumulate as a result of the normal cellular repair systems that occur throughout the body. Collagen is the most common protein in the body; a normal biomolecular component of all connective tissues and the dominant protein (approximately 90 wt%) in the organic fraction of bone tissues. Cells that produce collagen are known as fibroblasts; in bone they are called osteoblasts. Many biochemical varieties of the collagen molecule with slightly different amino acid compositions and intramolecular cross links have been described (Miller, 1973; Skinner, 1987), but type 1 collagen is typical of bone, dentine, and cementum. Collagen is not found in enamel.

Pathological bioapatite may be found in the arteries, an expression of cardiovascular disease, and in kidney "stones" (Skinner, 2000a). It may occur in association with other calcium phosphate species (Table 1). For example, calcium pyrophosphate, \( \text{Ca}_3\text{P}_2\text{O}_7\cdot \text{H}_2\text{O} \), occurs in joints (Skinner, 2000a), and octacalcium phosphate, \( \text{Ca}_8\text{H}_2(\text{PO}_4)_6\cdot 5 \text{ H}_2\text{O} \), and/or whitlockite, \( \text{CaMg(PO}_4\text{OH})_2\cdot (\text{PO}_4)_{6\text{\_}} \) in dental calculus, which is a mineral deposit that forms just below the gum line in the soft tissue around teeth (see Driessens & Verbeek, 1990, for a complete discussion of dental plaque mineral constituents).
(A) Sketch of a tooth and a long bone indicating the typical tissues found in these organs. (From Skinner, 2000a, Figure 1, p. 356.) (B) Sketch of a longitudinal section and a cross section through a long bone illustrating the tissue types and textures that occur and the changes that take place during the development of a bone. Arrows indicate the directions of growth and remodeling. (From Albright and Skinner, 1987, Figure 5–16, part A, p. 175.) Numbers at specific sites illustrate the following changes: 1, length increase, typical growth direction of a long bone; 2, thickness or diameter increase that also takes place as the organ changes shape and size. Some initial trabecular tissues become cortical bone tissues. The textures depicted in 2 and 5 are typical of heavily mineralized lamellar bone that results from the remodeling of trabecular tissues into cortical tissues. 3, all bones require local remodeling to achieve final organ size and shape in order to function as part of the skeleton; 4, remodeling is also needed to maintain the internal (marrow) cavity size and shape; 5, extensive remodeling in the mid-shaft of long bones where previous trabeculae have been remodeled into heavily mineralized cortical tissue; 6, the circular patterns of haversian bone express the sites of resorption and re-deposition of mineralized tissues which continue throughout life; 7, an outer protuberance on the bone, probably the site of muscle attachment, which will change as the bone responds to growth and development; and 8, a cross section showing distinct layers of haversian and lamellar bone tissues surrounding the marrow cavity.
To cover all calcium phosphate mineral species that may be found in the human body is beyond the scope of this presentation. An introduction to the usual and predominant mineralizing system (Section III), the submicron characteristics of the mineral (Section IV), and the methodology and techniques used to determine the composition, concentration, and distribution of the mineral in mineralized tissues are presented (Section V). The final section (VI) on the disease osteoporosis illustrates how knowledge of the mineral, distinguishing normal from the abnormal, and mineral dynamics have become foci for medical research.

Although the techniques for studying bones and mineral are similar to those employed for all solid materials, mineralized tissues present special problems. Foremost are the difficulties of obtaining sufficient sample when the object of study is a living human. Initial diagnosis of osteoporosis usually employs noninvasive techniques such as transmission X-ray analyses (radiology) of a bone or bones. Once a clinical diagnosis is made it may be followed, especially today, by examination of mineralized tissue samples in spite of the difficulties of procurement and preparation (Sections V.A, B, C, D). The tiny amounts of tissues can provide the information that is essential in defining and adequately treating the disease in many patients. Whenever mineralized tissue samples are studied, either normal or pathological, the physical and chemical characteristics of the tissues and the mineral fraction benefit the health of future patients and populations.

III. CRYSTAL CHEMISTRY OF THE MINERAL IN MINERALIZED TISSUES

The normal and much of the abnormal or pathological mineral deposits in humans is a calcium phosphate, a member of the apatite group of minerals (Gaines et al., 1997). The bio-deposits conform crystal chemically most closely to the mineral species hydroxyapatite in the ideal formula $\text{Ca}_5(\text{PO}_4)_{3}(\text{OH})$, (hereafter abbreviated as HA), which is described in most introductory mineralogical texts (Klein & Hurlbut, 1985). However, the precise composition and crystal structure of the bioapatite mineral has proved difficult to pinpoint so the term “apatitic” is often used. These difficulties relate to the chemical variability reported from many analyses of the mineral from different mineralized tissues. Further, X-ray diffraction analyses, the criteria for accurate identification of any mineral species, applied to biomineral samples is only partially successful primarily because of the very small grain size of the mineral materials. What has been shown from extensive investigations is that the biomineral, although apatitic, is not the ideal or stoichiometric chemical compound whose formula is presented above. The following formula is a more appropriate presentation:

$\left(\text{Ca},\text{Na},\text{Mg}\left[\right]\right)_x\left(\text{PO}_4,\text{HPO}_4,\text{CO}_3\right)_{y}\left(\text{OH},\text{F},\text{Cl},\text{CO}_3,\text{O}\left[\right]\right)_z$
The brackets indicate vacancies in some lattice sites of the solid to achieve a charge-balanced solid phase.

This complicated chemical solid can be described as follows: bioapatite is predominantly a calcium phosphate mineral most closely resembling the species hydroxyapatite but usually contains many elements and molecular species other than calcium and phosphate that probably contribute to its physical attributes and reactivity, and should be part of any identification. The crystal structure of all apatite minerals is dominated by tetrahedral anions. In the case of hydroxyapatite and bioapatite it is phosphate, the phosphorous-oxygen tetrahedral anionic groups (PO₄)³⁻, that forms the backbone of the structure and bonds predominantly to calcium, but other cation and anion species, vacancies, and complex molecular groups are usually detected on analysis.

A. The Mineralizing System: CaO—P₂O₅—H₂O

The chemical system that defines bioapatite can be simplified to CaO—P₂O₅—H₂O. Figure 2 is an experimentally determined phase diagram. It is a portion of the chemical system that describes the mineralization of bones and teeth, which is a summary of the solid and
TABLE II. The Composition of Normal Mineralized Tissues
A. Bulk Composition of Bone, Dentine, and Enamel

<table>
<thead>
<tr>
<th></th>
<th>Bonea</th>
<th>Dentinea</th>
<th>Enamelb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt%</td>
<td>vol%</td>
<td>wt%</td>
</tr>
<tr>
<td>Inorganic</td>
<td>70</td>
<td>49</td>
<td>70</td>
</tr>
<tr>
<td>Water</td>
<td>6</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Organic</td>
<td>24</td>
<td>38</td>
<td>20</td>
</tr>
<tr>
<td>Denisty (avg.) (g/cm²)</td>
<td>2.35</td>
<td>2.52</td>
<td>2.92</td>
</tr>
</tbody>
</table>

B. Composition of Major Elements and the Ca/P Ratio of the Bioapatites in Three Tissues (Wt% on a dry, fat-free basis)c

<table>
<thead>
<tr>
<th></th>
<th>ASH</th>
<th>Ca</th>
<th>P</th>
<th>Ca/P</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>CO₂</th>
<th>Cl</th>
<th>F</th>
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<tr>
<td></td>
<td>57.1</td>
<td>22.5</td>
<td>10.3</td>
<td>2.18</td>
<td>0.26</td>
<td>0.52</td>
<td>0.089</td>
<td>3.5</td>
<td>0.11</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>70.0</td>
<td>25.9</td>
<td>12.6</td>
<td>2.06</td>
<td>0.62</td>
<td>0.25</td>
<td>0.09</td>
<td>3.19</td>
<td>0.0</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>95.7</td>
<td>35.9</td>
<td>17.0</td>
<td>2.11</td>
<td>0.42</td>
<td>0.55</td>
<td>0.17</td>
<td>2.35</td>
<td>0.27</td>
<td>0.01</td>
</tr>
</tbody>
</table>

aFrom Driessens and Verbeeck, 1990, Table 8.2, p. 107; Table 9.4, p. 165; and Table 10.5, p. 183.
bFrom Zipkin, 1970, Table 17, p. 72.

cThe bulk chemical composition of the tissues and of the included bioapatites found in them, the dominant human mineralized tissues, are listed in Table II. Table IIA lists the bulk composition of the major components in these tissues, and Table IIB presents the range of major elements and the different Ca/P ratios of the bioapatites from three different sources. The measured Ca/P wt% range, from 1.3 to 2.2, differs from the ideal or stoichiometric value for hydroxylapatite of 2.15. Because the values are both above and below the stoichiometric value, several hypotheses for such variations have been proposed.

1. Variations Due to Multiple Minerals

One group of investigators suggested that the biomineral matter was not a single mineral phase. Table I shows that there are a number of calcium phosphate mineral species with different, and in many cases lower, Ca/P ratios than HA. Most of these minerals may nucleate and are stable in the body fluid-tissue environment. The presence, and a variable amount, of a second mineral with lower Ca/P that might occur with HA in bio-deposits has been cited as a possible reason for the compositional variability measured in tissues. The higher Ca/P ratio may reflect the substitution of another anion, e.g., CO₂, for a portion of the PO₄³⁻ (discussed in Section IV.D).

2. Variations Due to Nucleation and Maturation

Another possible reason why the analyses of bioapatite may not show uniform and stoichiometric composition is that the mineral precipitate changes over time. It is well known that phosphate ions (H₂PO₄⁻, HPO₄⁻) are...
usually detected in the fluid phase at initial mineral nucleation. This led to the suggestion that nucleation was a discrete process from growth and maturation of the mineral phase and the composition of the fluid at least locally varied over time as mineralization proceeded (Roberts et al., 1992). Glimcher (1976) suggested that nucleation could be induced by charges from phosphate groups associated with matrix collagen molecules. Alternatively, because the initial solid was so poorly crystalline, Posner (1977) suggested a separate phase called “amorphous” calcium phosphate mineral, octacalcium phosphate was suggested by Brown et al. (1987), and a third suggestion of brushite was made by Neuman and Neuman (1958). All three mineral materials were considered intermediates in the mineralization process before a mature biominal phase that more closely resembled hydroxylapatite was produced. Even during the investigations of the basic mineralizing system, the synthetic calcium-phosphate-water investigations that delineated the formation of HA showed this stable and ubiquitous phase which was most often associated with other solid phases and had incongruent solubility (Van Wazer, 1958; Skinner, 1973a).

A variety of chemical techniques for determining the Ca/P ratio in the synthetic chemical system and early mineral deposition confirmed that initial precipitates produced by adding calcium to phosphate-rich solutions, or vice versa, was a calcium phosphate mineral with higher phosphorous (P) content than that of stoichiometric hydroxylapatite (and therefore a lower Ca/P). Although the presence of amorphous and brushite as initial phases has not been confirmed, this question remains under study (Brown, et al., 1987; Roberts et al., 1992; Kim et al., 1995; Aoba et al., 1998).

3. Variations Due to Substitutions Within the Mineral

Putting aside the nucleation and maturation processes and identification of the initial precipitates, biomineral has been mostly thought of as a single calcium phosphate phase whose variations in Ca/P may be accommodated via substitutions and/or vacancies within the solid. The opportunity for elements and molecular species other than calcium (Ca), phosphorus (P), oxygen (O), and hydroxyl (OH) to occur in mineralized tissues is a most important consideration from the perspective of medical geology.

A multiplicity of chemicals exist in the natural environment, and it is probable that at least some of them will become part of the dynamic mineral systems found in bones and teeth. Detailed investigations of the apatite crystal structure, presented in Section IV, enable us to discuss the ability of this species to incorporate a variety of chemical species. Each of these incorporations, known as solid solution, could alter the Ca/P ratio of the solid. For example, in geological environments, sodium (Na), lead (Pb), or strontium (Sr) may substitute for some of the calcium which means an accurate analysis for Ca/P that would be slightly lower than stoichiometric HA. Alternatively, if an anionic group, such as sulfate (SO₄), became incorporated in place of some of the phosphate (PO₄), the Ca/P would be above the stoichiometric HA value. Calculation of a Ca/P ratio, or a cation/anion ratio, from a chemical analysis will depend not only on which chemical elements and species were available when the mineral formed but also on the completeness of the data used in the calculation. If only calcium and phosphate are measured, for example, no matter how accurately, calculation of a Ca/P for bioapatite may be misleading. The following section on crystal structural details of mineral apatites will allow investigators to more fully appreciate the possible uptake and incorporation of specific elements, or molecular species, into bioapatites.

IV. The Crystal Structure of Calcium Apatites

The structure of apatites, a common group of naturally occurring minerals in many rock types throughout the world, is distinguished by hexagonal symmetry (Gaines et al., 1997). Figure 3A depicts one projection of the three-dimensional arrangement of the atoms that make up the apatite structure. This view is down the unique c-axis and depicts a plane perpendicular to c. The rhombohedral outlines are the disposition of two of the three a axes (directions at 120 degrees apart) and the atoms are precisely placed conforming to the crystallographic characteristics of the repeating unit, or unit cell, of the apatite structure.

To further illustrate the distribution and importance of the tetrahedral orthophosphate groups (PO₄³⁻) that form the backbone of the apatite structure, Figure 3B presents another view of the structure. It is a projection 90° to the c-axis along one of the a axes. The yellow phosphorus atom is in tetrahedral coordination surrounded by two white oxygen atoms in the same plane as the phosphorus and two light purple oxygen atoms, one above the plane, the other below it. Phosphate
FIGURE 3 The crystal structure of hydroxyapatite, ideal formula $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$. (A) Projection down the c-axis, showing the distribution of all the atoms in the unit cell. (B) Projection down the a-axis of the unit cell, note the tetrahedral orthophosphate groups. (C) Projection down the a diagonal, a different view of the three-dimensional arrangement of atoms in the unit cell. (D) Crystals of hydroxyapatite, note the hexagonal prismatic morphology.
anionic groups coordinate with calcium cations to produce a charge neutral solid when a singly charged species, OH\(^{-}\) or F\(^{-}\), the blue atom site, is added. Figure 3C is the view down the diagonal between two \(a\) axes. Note the distribution of the calcium atoms and the channelways where the OH\(^{-}\) or F\(^{-}\) occur parallel to the \(c\)-axis direction. The prismatic hexagon morphology typical of many apatite mineral samples shows up in Figure 3D. It is a selection of single crystals of mineral apatite produced during investigations of the simplified chemical system. These crystals are synthetic hydroxyapatites and are up to several millimeters in length.

There are two distinct sites for cations and they are discretely colored orange and raspberry red. One is connected to the tetrahedral phosphate (PO\(_4\)) backbone oxygens and the atoms in channelways (OH, F, or Cl) that parallel the \(c\)-axis, and the other is related to the trigonal \(a\) axes in the center of the cell. The two calcium sites are designated Ca1 and Ca2 in Figure 4. When the channels are occupied by OH\(^{-}\) the mineral is known as hydroxyapatite, and when occupied by F\(^{-}\) the name is fluorapatite, which is another member of the calcium apatite mineral group.

An amazing number of different composition minerals, each a separately named mineral species, may form with the apatite structure type. These minerals show mixed chemistries with very minor variations in structural detail that are related to the amount and exact placement of particular elements. Because of the importance of the chemical variations to our medical purview and the fact that many of these naturally occurring minerals have been studied in great detail, the sections below present examples to show what elements find their way into bioapatites and where they may be located. Bioapatite composition mirrors elemental bioavailability.

A. Substitutions in the Phosphate Backbone

Though the most common minerals are probably the phosphate apatites, there are arsenate or vanadate minerals that form with virtually identical apatite-like crystalline structures. The AsO\(_4\)^{3-}, and VO\(_4\)^{3-} anions have a charge and size very similar to the PO\(_4\)^{3-} group. Naturally occurring arsenate or vanadate apatites usually contain lead (Pb) rather than calcium. Lead phosphate apatite, the mineral pyromorphite with ideal formula Pb\(_5\)F\(_3\)(PO\(_4\))Cl, has all cation sites occupied by lead, but chlorine, Cl\(^{-}\), takes the place of the OH in the channelways (see Section IV.C).

Other tetrahedral anionic groups, sulfate SO\(_4\)^{2-}, and silicate SiO\(_4\)^{4-}, although of different charge, may also form minerals with apatite structure type, and small amounts of these groups as well as arsenate and vanadate may be found in the phosphate apatite minerals. Britholite-Y, a naturally occurring mineral, is one of a series of phosphate apatites that contain silicon substituting for phosphorus, but there is a second cation in addition to calcium, yttrium (Y). Both OH and fluorine (F) are present in the channelways.

B. Substitutions in the Cation Sites

There are two different cation sites, or distinct lattice locations, known as Ca1 and Ca2 in hydroxyapatite (Figure 4). The site designated Ca1 bonds to nine oxygen atoms of the tetrahedral phosphate groups while Ca2 bonds to six phosphate oxygens and one OH ion. Many other cations, especially those with double positive charge similar to Ca\(^{2+}\) such as Sr\(^{2+}\), can be accommodated in these sites. Table III lists the elements by charge and by size that could substitute in apatites.

Lead apatite, the mineral pyromorphite, has an apatite structure but lead is a large ion relative to calcium. However, small amounts of lead occur in the predominantly calcium bioapatites. Lead isotope \(^{207}\)Pb has been used to discriminate between the possible sources. Lead from leaded gasoline, or uranium, for example, might be taken up in human tissues. Lead may also occur in high concentrations indoors. It might be found as a component of dust distributed about the living areas with the possible source local soils tracked.
inside a residence or be from lead-based paint. Some from either source could be ingested and become sequestered in bones or teeth. These exposures make lead a "silent" hazard, a potential danger especially for young children who constantly put their fingers in their mouths. The term silent is used because Pb is not visible, and detection and amount determined in the environment or in the body is impossible without specialized analytical methods and tools. Therefore biological uptake and sequestration of lead in bones, which may be cumulative, is not monitored. Lead exposure goes unrecognized unless some distinctive disease or signal, as described in a study on schoolchildren (Mielke, 2003), is recognized, evaluated, and related to lead levels.

Strontium apatite, ideal formula (Sr, Ca)₅(PO₄)₃(OH), has been designated a separate mineral species and a member of one of the subsets within the apatite group, because the amount of Sr is greater than 50% of the total cations present. Studies on naturally occurring rare-earth-element-substituted apatites show coordinated substitution: when rare earth elements (REE) with a charge of 3⁺ are incorporated in the structure, there may be a substitution of Sr⁺⁺ for part of the phosphorus. With both substitutions, a charge balance comparable to the original association of Ca⁺⁺ and P⁺⁺ in the calcium apatite structure can be maintained. Another coordinated substitution is when an REE⁺⁺ is incorporated along with Na⁺⁺. Charge balance may be achieved because the cations are distributed at both sites in calcium apatites (Hughes et al., 1991b).

Some elements prefer one cation site over the other. A study of REE-containing apatite minerals by Hughes et al. (1991b) demonstrated that some of the REE preferred the Ca⁺⁺ site while others preferred the Ca⁺⁺ site, provided charge balance was maintained by substitutions at the anionic sites or with additional and differently charged cations. Site designation for particular elements can be determined using high-resolution X-ray diffraction analyses, paramagnetic resonance, thermoluminescence, or infrared spectroscopy (Suitch et al., 1985).

Apatite samples can have different compositions in spite of coming from similar sources or sites. Although it is possible with modern techniques to show elements at specific sites in the crystal structure of geological samples, the tiny crystallites of bioapatite are too small for such detailed investigations. It is worthwhile to reiterate that bioapatite composition in one bone may not be identical to another bioapatite forming elsewhere at the same moment or at different times. The fluid-cell-matrix-mineral system composition is unlikely to be constant from one moment to another, much less from year to year as the human ages, resides in different geographic localities, and ingests water or food from different sources.

There is another set of concerns that relate directly to cation substitution in bioapatites: bone seeking α-emitting radionuclides and ionizing radiation exposures of humans. During atomic bomb tests a half century ago in the southwest United States there was a scare related to fallout of radioactive nuclides, especially ⁹⁰Sr. The
anxiety was based on the similarities in behavior of calcium and strontium, the half-life of the nuclide (28 years), the prevailing wind direction toward the more heavily populated east, and the fact that American dietary calcium came mostly from milk products with the largest consumers being children who were actively putting down new bone. The worldwide average of $^{89}$Sr was shown to be about 0.12 microcuries per gram of calcium in man or 1/10,000 of the acceptable permissible level at that time. This suggested that the atomic bomb circulating $^{90}$Sr was not a global hazard. A remarkable study on baby teeth in mid-western communities of the United States compared the strontium concentrations with adult bones the late 1950s. These investigations showed that bioapatites discriminated against strontium during formation and concluded that only those individuals who obtained their total food supply from restricted areas (with low calcium in the rocks, soils, or waters) were at risk (Eckelman et al., 1957). The furor over the nuclide hazard eventually collapsed when it was realized that strontium bioavailability was overshadowed by calcium and no one, especially children, was likely to be at risk in the United States or abroad (Eckelman et al., 1954; Fowler, 1960).

Massive doses of radioactive elements from nuclear explosions such as the Chernobyl disaster are locally extremely hazardous as they spread the radionuclides in the soils and the plants that animals and humans ingest. However, the potential for incorporation in mineralized tissues at a hazardous exposure level of several potentially harmful radioactive materials has yet to be documented in spite of extensive surveys (Fabrikant, 1988).

**C. Substitutions in the Hydroxyl Site**

The hydroxyl (OH) site in the calcium apatite crystal structure can be fully occupied by fluorine or chlorine. As end members in that chemical system, they form the independent minerals fluorapatite and chlorapatite. These halogen species are the predominant forms of apatite found in sedimentary, metamorphic, and igneous rocks (Hughes et al., 1989). Bromine (Br) or iodine (I) can be incorporated in the apatite structure type, but mineral species in which the halogen site is filled entirely by bromine or iodine have not been found naturally.

Bioapatites originally precipitate and remain mostly as hydroxylapatite in human bones and teeth because of the predominance of aqueous fluid and OH concentration relative to the halogens in the human body. When higher amounts of fluorine become available the element can become incorporated substituting for part, at least, of the OH. For example, 1 mg L$^{-1}$ fluorine added to drinking water, an amount that has become standard for many of the reservoirs that supply water to populations across the United States, appears to reduce caries and may lead to a reduction in the incidence of osteoporosis (Watts, 1999). On the other hand, the regular ingestion of greater than 100 mg L$^{-1}$ of fluorine over a long period of time by humans leads to disease. Such high amounts of fluorine in local waters and agricultural products grown in soils irrigated with high-fluorine-containing water, or through industrial exposure, may result in fluorosis (Vischer, 1970; Finkelman et al., 1999). Whether the mineral matter in the bone and tooth tissues of such exposed human populations is partially fluorapatite, i.e., a mixture of the two separate apatite species, or whether each apatite crystallite has both fluorine and hydroxy in its channel sites is unknown. Fluoride has been used to treat osteoporosis (see Section VLB.1 and Chapter 12, this volume).

Chlorapatite, the calcium phosphate apatite mineral in which all the channelways are filled with chlorine, has not been identified in mineralized tissues in spite of the high concentration of chlorine (Cl) in body fluids, but small amounts of chlorine can be detected on analyses of bioapatites.

**D. Carbonate (CO$_3^{2-}$) in Apatites**

One additional chemical constituent that is often detected in apatite analyses becomes important when discussing bioapatites, and that is carbonate. Two carbonate-containing calcium apatite minerals, dahlrite and francolite (Table I), have been described from phosphorites, fine-grained sedimentary deposits mined for fertilizer on many continents. These phosphate minerals are associated with the common calcium carbonate minerals calcite and aragonite (Gaines et al., 1997). Neither calcite nor aragonite has been identified in bone tissues, but because many bioapatites show higher than stoichiometric Ca/P ratio and CO$_3^{2-}$ on analysis, the suggestion is that CO$_3^{2-}$ substitutes either for PO$_4^{3-}$ or for OH$^{\text{-}}$ in the apatite. The carbonate ion CO$_3^{2-}$ has a different charge and size than the dominant phosphate groups (PO$_4^{3-}$). It is a planar trigonal ion with a diameter of 0.24 nm and does not easily fit in the crystal structure. The amount and disposition of CO$_3^{2-}$ within the apatite crystal structure is, and has been, a topic of great interest for some time (McConnell, 1973; Skinner, 1989; Elliott, 1984, 1994).
If carbonate, \( \text{CO}_3^{2-} \), is present in the crystal lattice other ions, such as triply positively charged cations, it might take the place of calcium so that local charge disruption could be balanced by coupled substitution or by vacancies in the lattice. The inclusion of \( \text{CO}_3 \) could compromise the ideal architecture of the apatite backbone and the channelways. Such destabilization may account for the very fine-grained nature of bioapatites.

The association of \( \text{CO}_3 \) with bioapatite is not particularly surprising. The molecular species \( \text{CO}_3 \) or bicarbonate, \( \text{HCO}_3^- \), are produced along with many others during cell metabolism and could adsorb on the high surface area of the tiny crystallites. Early deposition of mineral takes place under acidic conditions where orthophosphate species may aid the nucleation of bioapatite whereas other ions in the fluid, such as bicarbonate, may be inhibitory (Glimcher, 1998).

The carbonate ion and its distribution is not of major concern for this medical geology purview except that its presence makes us aware of the necessity to consider both the physical and chemical aspects of the mineralizing system. Carbonate probably aids incorporation of other elements into the lattice. Bioapatite precipitates are aggregates of crystallites, which means that the mineral mosaic of many crystallites can each present a slightly different composition and size. The lower crystallinity, and variable composition, of carbonate-containing-bioapatites may be an irritation preventing precise designation of the mineral phase, but it is a positive advantage for the biological system. The high surface area of the crystallites facilitates their dissolution as required for the dynamic bone mineral formation-resorption system. Nature has utilized a solid phase that fulfills several functional roles required for bone (Skinner, 2000b). Bioapatites record exposures of living creatures to the environment and particularly the bioavailability of elemental species in our diets.

This brief summary does not do justice to investigations with a variety of techniques which include electron and X-ray diffraction analysis, infrared, polarized infrared and Raman spectroscopy, solid state carbon-13 nuclear magnetic resonance spectroscopy, and most recently atomic force microscopy. These have been and are used to detect and quantitate the amount of carbonate within the crystal lattice of a single-phase mineral or bioapatite or as an adsorbed species.

From the above selected examples and Table III, the very wide range of elements and molecular species that can be accommodated within the apatite crystal structure is summarized. Table IV lists the levels of elements essential to proper body function. The match is quite remarkable. Many elements and chemicals entering the human body may become associated with, or become part of, the apatitic mineral matter.

### V. Analysis of Apatitic Biominerals

To ascertain the ranges of included elements and species in bioapatites, the mineral matter must be extracted and concentrated. The techniques devised for separating mineral from the associated organic and cellular materials can, in many cases, further complicate the assays. The other option is to analyze the tissues keeping both the mineral and organic fractions associated. Either way there are special techniques required to prepare the
sample for analysis. Preparation of the mineral phase and the main methods of analysis, diffraction, will be presented followed by the sample preparations necessary for examining whole tissues. Histology is the general name for the host of techniques using optical and/or scanning electron microscopic (SEM) analyses to investigate thin sections of tissues.

A. Sample Preparation: Mineral

The opportunity to examine the mineral separately from surrounding organic materials, whether examining pathological aggregates from arteries or from normal bone tissues, is a non-trivial undertaking (Kim et al., 1995). Bioapatites have individual mineral grains of the order of 1 x 2 x 25 nm and are loosely associated one with another as porous aggregates with random crystallite distribution or alternatively on and in a fibrous protein matrix. The latter is characteristic of bone tissues where the crystallites often align parallel to the length of the collagen molecules (Skinner, 1987). Aware that there is a range of composition of bioapatites and mindful of their very small grain size, care must be exercised. In addition it is necessary to record the specific tissue and site in the organ or exactly where a pathological deposit is located. The age of the individual and the date of sampling are also critical because different cell systems and tissue textures are encountered in every bone (Weiner & Wagner, 1998).

In all normal bone the intimate association of mineral with matrix proteins and other proteins shows variations dependent on source (Miller, 1973) Table II gives average amounts, but the mineral concentration as well as distribution also varies at submicron levels (Rey et al., 1996). The chemical variations detailed above reinforce the possibility that the mineral itself may also vary with growth and maturation, especially in bone where the entire structures, e.g., haversian bone, are constantly resorbed and re-deposited over time (Skinner, 1987). The chemical analysis of mineral that may attract and absorb transient ions from the surrounding fluids is only an indication of the compositional range of the tissue at a specific moment in time.

Analyses of mineralized tissues are not only compromised by the poor crystallinity of the mineral but by the presence of non-mineral components. A sample of enamel with over 96 wt% mineral, less than one percent protein (enamelin), and the most highly mineralized normal tissue in the human body, is the preferred choice for mineral analyses. Enamel is also examined because it has a very restricted period of formation, and because no cells remain at maturity the final tissue is not reworked. Dentine, the mineralized tissue adjacent to enamel and the major tissue in the tooth, is maximally 75% mineral when fully mature, with collagen about 20% of the total, plus some small molecular protein species, and fluid. Bone tissues, especially in the first stages of formation, and spicular, or trabecular bone, found adjacent to the marrow cavity (Figure 1B), may contain less than 50% mineral per unit area. To accurately ascertain the mineral composition and structure, it is important to extract the mineral portion from these organic moieties.

The usual way to separate the mineral fraction has been to immerse the entire sample in sodium hypochlorite or bleach. Most of the organic matter will eventually dissolve, and the time it takes depends on the size and porosity of a particular sample relative to the amount of bleach. Smaller sample size allows for a more rapid dissociation of the mineral from the intimately associated organic molecules. To concentrate the mineral fraction from the heavily mineralized cortex of a long bone will require a long soak and much decanting and re-suspending of the tissue sample in the bleach. This procedure could alter the amount of mineral, especially as smaller crystallites are likely to be more soluble.

More exotic systems of extracting the organic moieties from the mineral phase using chemical methods have been suggested. Refluxing with ethylene diamine, (Skinner et al., 1972), but it is a lengthy procedure and possibly dangerous because the solution pH is 12 or greater. An interesting observation on careful chemical extractions is that the morphology of the organ or sample, whether bone or tooth, will likely remain after virtually all of the organics (>95%) have been removed. This is an illustration of the permeation of the biological macromolecules and how difficult it is to completely extract all of the intimately associated organic phases. Pathologic spherulitic apatitic samples also have ultrasmall size, not only of the aggregates, but of the crystallites within them.

Several other methods of extraction have been employed. One technique, called "ashing" subjects tissues to elevated temperatures, above 500°C and often to 1000°C, for at least an hour. Researchers who wish to assure complete disappearance of the bioorganic portion advocate up to 10 or more hours at elevated temperatures. The extended high temperature treatment certainly eliminates any organic components, but it also re-crystallizes the mineral phase. An X-ray diffraction analysis of the ashed sample (see Section V.B) provides a sharp diffraction pattern of hydroxyapatite that is comparable to well-crystallized geologically obtained mineral. Occasionally, two mineral phases are detected with the identity of the second phase dependent on the
bulk composition of the sample, the temperature, and duration of ashing. For example, above about 200°C adsorbed water and any carbonate will be lost and other calcium phosphates, CaHPO₄ or Ca₃P₂O₇, appear (Table 1). This indicates that the bulk Ca/P composition was lower than stoichiometric HA. At greater than 1000°C, tricalcium phosphate, Ca₃(PO₄)₂, may take the place of the pyrophosphate. The relative amounts of the two species at a known temperature can be used to calculate a Ca/P for the sample. If the Ca/P is greater than 2.15, tetracalcium phosphate Ca₄(PO₄)₂O may form. The appearance of different mineral phases reflects the experimental conditions that the mineralized tissue sample was subjected to, not the presence of the second phase in the low-temperature-produced tissues. An alternative, low-temperature ashing using activated oxygen, has also been employed to remove the organic constituents from mineralized tissues. X-ray or electron diffraction techniques on the extracted materials will be used to determine the crystal chemical characteristics of the mineral phase (Kim et al., 1995).

B. X-ray and Electron Diffraction

Unambiguous identification of most mineral materials utilizes diffraction techniques that can easily determine the species based on the unique crystal structural characteristics of the compound (Klug & Alexander, 1954). The powder diffraction method may be used for the identification of any crystalline materials. Either X-ray or electron diffraction may be employed, and the choice is usually dependent on instrument availability and the specifics of the sample. Vast databases have accumulated over the past hundred years since the techniques were elucidated. The International Union Committee of Diffraction has a compendium of X-ray diffraction data on crystalline compounds, both organic and inorganic, which contains a subset for natural and synthetic mineral materials including the apatites.

The tiny crystallite size and variable composition of bioapatites are fully expressed in the X-ray diffraction analysis of the extracted materials. Well-crystallized (without vacancies and >0.5 μm in average size) hydroxyapatite geological samples give many discrete diffraction maxima from which one easily calculates unit cell parameters. Comparing a mineral apatite X-ray powder diffraction pattern (Figure 5) with bioapatite, e.g., cortical bone mineral, the latter has few and broad maxima. A poorly crystalline compound makes it difficult to determine any compositional details other than to say the sample gives a pattern consistent with an apatite (Skinner, 1968) and focuses attention on the detection of any additional solids. Enamel is perhaps the only biomaterial that provides sufficient diffraction detail to calculate lattice parameters of a calcium apatite. Table V presents the results of calculations from powder diffraction data of several different apatites. Electron diffraction does not necessarily afford more precise results over X-ray diffraction as the level of crystallinity is the important criterion for producing diffraction. The advantage of electron diffraction is that the beam may be more finely focused, and a small mineral crystal aggregate within a thin section might be separately examined rather than extracting the mineral to obtain a pure mineral sample for powder diffraction.

C. Sample Preparation: Mineralized Tissues

To examine the mineral phase in its biological surroundings requires different sample preparation. The
### Table V. Calculated Unit Cell (Lattice) Parameters for Synthetic Apatites, and Bioapatites

<table>
<thead>
<tr>
<th></th>
<th>a-Axis</th>
<th>c-Axis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyapatite (Synthetic) 315°C, 2 Kbars H₂O pressure</td>
<td>9.421</td>
<td>6.882</td>
<td>Skinner (1968)</td>
</tr>
<tr>
<td>CaO/P₂O₅ 1.61</td>
<td>9.416</td>
<td>6.883</td>
<td>Skinner (1968)</td>
</tr>
<tr>
<td>CaO/P₂O₅ 1.12</td>
<td>9.415</td>
<td>6.880</td>
<td>Skinner (1968)</td>
</tr>
<tr>
<td>600°C, 2 Kbars H₂O pressure</td>
<td>9.422</td>
<td>6.8819</td>
<td>Skinner (1968)</td>
</tr>
<tr>
<td>CaO/P₂O₅ 1.61</td>
<td>9.422</td>
<td>6.883</td>
<td>Bell &amp; Mila (1979)</td>
</tr>
<tr>
<td>CaO/P₂O₅ 1.12</td>
<td>9.4174</td>
<td>6.8855</td>
<td>Young &amp; Holcomb (1982)</td>
</tr>
<tr>
<td>100°C Precipitation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Reitveld calc.)</td>
<td>9.418</td>
<td>6.875</td>
<td>Gaines et al. (1997), p. 856</td>
</tr>
</tbody>
</table>

|                      |         |        |            |
| Carbonate Apatites (Synthetic)² |         |        |            |
| Low temperature (<100°C precipitates, air-dried) |         |        |            |
| Direct (adding calcium acetate into a solution of ammonium carbonate+phosphate) | 9.373  | 6.897  | Labarthe et al. (1973) |
| 13.8% CO₃            |         |        |            |
| Inverse (adding ammonium solution into calcium acetate) 10.4% CO₃ | 9.354  | 6.897  | Labarthe et al. (1973) |
| Direct using sodium carbonate | 9.440  | 6.880  | LeGeros et al. (1968) |
| Direct using sodium carbonate and fluoride 22.1% CO₃ | 9.268  | 6.924  | LeGeros et al. (1986) |
| High temperatures 1.455 CO₃, the maximum determined | 9.367  | 6.934  | Rey et al. (1991) |

|                      |         |        |            |
| Bone                 |         |        |            |
| Ashed cortical       | 9.419   | 6.886  | Skinner et al. (1972) |

|                      |         |        |            |
| Enamel               |         |        |            |
| Human                | 9.421   | 6.881  | Carlstrom (1955) |

²The wide variations in the values may be partially attributed to differences in composition of the starting materials and to the methods of preparation, e.g., direct versus inverse, under low temperatures for carbonate apatite synthesis.

See Elliott, 1994, p. 234–248 for details on precipitation mechanisms and discussion of IR, X-ray diffraction analyses, and possible locations of the carbonate ion in the apatite lattice.

Techniques must minimize any alteration of the tiny crystallites while maintaining the organic and cellular framework in which the mineral matter is distributed. Thin sections are prepared for examination with optical and electron microscopic techniques so that morphological relationships typical of the different tissue types and chemical observations on the mineral phase can be assayed (Section V.D).

The techniques to prepare biological tissues as thin sections parallel the methods employed in petrology (the study of rocks) (Blatt & Tracy, 1996). The mineralized tissue sample is embedded to obtain a plano-parallel thin section, from 50 to less than 10μm thick. The thickness will depend on the information desired and the method of analysis. Embedding preferentially employs plastics, rather than paraffin, the typical media for soft tissue sections examined in pathology laboratories (Malluche et al., 1982). Plastic is required because the juxtaposition of tiny crystals of hard mineral with the soft organic matter and cells minimizes differential hardness of the medium put to sectioning.

After obtaining a fresh tissue sample the first act is to soak the pieces in alcohol solution about 10 times the sample volume for a few hours or overnight refreshing the solution several times. Occasionally formaldehyde is used but formalin has to be buffered or the solution will be acidic and at least some mineral crystallites may be lost or dissolve during immersion. The alcohol soak effectively lowers the water and fat content in the tissue, stops further biological degradation, and stabilizes the organic components. The second stage is to embed the whole sample with not too viscous epoxy materials such as...
as methylmethacrylate, or other commercially available plastics such as Spurr™ or Epon™. The viscosity must be appropriate to facilitate penetration throughout the sample, and probably will require a vacuum to maximize efficiency. Most mineralized tissue laboratories will have an automated embedding system that takes the tissue through the extraction and embedding procedures and applies vacuum and heat and a “hardener” to ensure a fully homogeneous block of embedded sample. Any holes, effectively pockets of air that might incorporate dust or foreign materials, must be avoided especially if the analysis will use SEM coupled with energy dispersive analysis (SEM/EDXA) to measure the elemental composition of the mineral. Once embedded the whole tissue piece is ready for sectioning and polishing.

Sectioning requires a sharp knife, usually diamond or carborundum blades, to cut the plastic embedded tissues without tearing the crystallites from their organic matrix. Once cut the thin section is mounted on a glass or plastic slide for microscopic study, usually without the addition of a cover slip. Grinding to assure planoparallel surface for high-resolution electron microprobe elemental analysis may be essential, but it almost always results in smearing and disrupting the crystallites, so this level of the preparation procedure again requires care. It is wise to constantly view the section under a microscope with at least 400x magnification. Any surface
roughness will cause interference in elemental analyses, although background corrections can be applied to the raw data (Reed, 1993).

Every laboratory that performs mineralized tissue section analyses, usually the pathology departments of hospitals, has their own sterile procedures. To prepare uniformly parallel, non-artifact-containing hard tissue thin sections is an art, not a science. Once made, sections may be stained to identify osteoid, cell walls, nuclei, etc., or the calcified portions for specialized investigations, but such treatments would compromise analysis of the mineral phase. Careful, sensitive attention to the preparation of any section is necessary for accurate documentation of the tissue components, their structures, and elemental components.

D. Histomorphometry

To study the variations in the textures found in all mineralized tissues, bone, teeth, or pathological materials, optical investigations at different magnifications are usually required. Optical light microscopy using transmitted light at approximately 40x magnification is sufficient to determine areas of mineralization in a tissue section. The mineral matter will be non-translucent and easily distinguished from organic components. By employing a polarizing light microscope, with the crossed polarizers in place, the mineral portions will show variations in birefringence on rotation of the stage, which is the characteristic used to identify any crystalline mineral (Nesse, 2004). The mineral calcite, for example, can be easily differentiated from hydroxylapatite because calcite has a much higher birefringence. However, to thoroughly document the tissue components and study the remodeling of bone tissue, combinations of SEM and optical and electron microscopy are usually employed.

Some of the distinct morphologies observed in normal and pathological mineralized tissues are next. Figure 6A is tissue section cut longitudinally through the upper end of a long bone. Dense mineral matter and cortical bone surrounds the shaft and the open marrow cavity. It also outlines the trabecular tissue in what appears to be the porous head of the femur. The arcuate patterning of trabeculae throughout the head is an expression of mineral deposition conforming to stress in this organ; the bone tissue distribution responds to mechanical strain (Albright & Skinner, 1987). In Figure 6B two tissue sections through vertebrae of different ages illustrate changes in trabecular thickness and patterning. The vertical struts thicken with age as the tissues respond to the effects of gravity from our vertical posture.

Figure 7A is an x-ray transmission microradiograph of cortical bone tissue from a dog tibia. It illustrates variations in mineral density in the several osteons (the circular patterns), typical of haversian bone. Variation in the levels of gray in this section reflect different amounts of mineral per unit area and detected at higher magnification because of the mineral impeding the transmission of x-ray energy. Figure 7B is another view of the same area but illuminated with UV light. Three of the osteons show two circular dark lines around the central vascular opening, the pattern resembling an archery target. The lines are due to the incorporation of the antibiotic tetracycline as the mineral deposits and the molecule glows under UV light. The two lines mark two separate doses of tetracycline allowing the rate of mineral deposition, and the growth of osteons in the cortex, to be determined (Skinner and Nalbandian, 1975).

Figure 8A is an image that shows spherulitic (pathologic) calcium phosphate deposits in breast tissue. The higher resolution (x1200) and use of back scattered electron imaging (one of the modes available with SEM) shows these tiny deposits. Figure 8B is an electron micrograph at still higher magnification (x25,000) of the dentine-enamel junction illustrating the different size, shape, and aggregation of hydroxylapatite crystals in these two tissues. At magnifications greater than x200,000, transmission electron micrographs have shown hexagonal outlines of the early-formed enamel crystals.

Weiner and Wagner (1998) recently reviewed the multiple levels of structural organization seen with histological examination and some of the physical contributions of the mineral crystallites. Bone organs require strong and flexible tissues, so they do not buckle and break when subjected to torsion, tension, compression, and instant and consistent responses to applied stresses for proper organ function. Although histology demonstrates the physical textures and mineral distribution during growth, development, and aging, the incorporation of chemicals may well alter the reactions of the mineral, and hence the mineralized tissues (Bronner, 1996; Skinner et al., 2004). A brief discussion of osteoporosis and outlining some treatments for this disease will illustrate how our present understanding of bone physical properties and bone mineralogy are benefiting medical care.

VI. OSTEOPOROSIS

Osteoporosis is a metabolic bone disorder characterized by reduction in the volume of bony tissue per unit
FIGURE 7  Ground section of the cortical portion of a rib from a dog given two doses of the antibiotic tetracycline. The tetracycline is incorporated at the time of deposition of bone tissue (Skinner and Nalbandian, 1975, Figure 3). Magnification 175x

A: In transmitted light, the darker the color the more mineral present, an expression of decreased transmission of light. Note variations in bone mineral density between the several haversian systems in the section. Some contain more mineral than others.

B: Same area in ultraviolet light (tetracycline glows in UV light) which shows two fluorescent rings (concentric dark circles) in the haversian systems marking the times when two doses of tetracycline were added to the diet as bone tissues were developing.

FIGURE 8  Microradiographs taken with the scanning electron microprobe, backscattered images of breast tissue samples. (A) Calcium phosphate spherules deposited in breast tissue. Note the size of the spherules at this high resolution (magnification x1200) relative to the heavily mineralized calcium phosphate deposit (white areas) on the right. Section thickness is 6μm. (From Poggi, et al., 1998, Figure 3.) (B) The dentin-enamel junction in a tooth showing the typical small crystallites in dentine (left) and the larger crystallites in enamel (right). (From Goose and Appleton, 1982, Figure 2.6.) Enamel crystallites are about 100μm in length.
A

**FIGURE 9** Osteoporosis examined by transmission X-ray analysis. The first lumbar vertebra from five autopsy subjects illustrates the possibility of quantifying the level of mineral in a bone (from Barzel, 1970, Figure 2B). (A) Top row vertical sections through the lumbar vertebrae showing changes in the distribution of mineral (white) from homogeneous fully mineralized tissues on left part of the figure to more porous on the right where the trabeculae thicken and appear more vertically oriented in the samples from older individuals. Middle row transverse section through the vertebrae shows increased porosity and disorder of the vertical sections. Bottom row transverse sections have been extracted and are now fat-free and dry. The porosity increase and the density of the mineral per unit area can be measured. Formula used to calculate the apparent density of columnar sections is weight/volume, g/cc. (B) Transmission X-ray of the entire vertebrae illustrating the apparent density differences that might be visible on radiologic examination (from Barzel, 1970, Figure 4, left half). The several vertebrae depicted in this figure coincide with the mineral density calculated for the sections in Figure 9A.

Volume of bone (Figure 9). The disease has been a topic of investigation for over a hundred years (Arnold, 1966; Barzel, 1970; Riggs & Melton, 1995). The tissue reduction leads to fragility of the bone organs and may cause an osteoporotic individual to sustain a fracture, perhaps without obvious trauma, pain, or other warnings. The main concern is that the usually elderly patient will be at high risk for additional fractures. In osteoporosis there is a normal mineral/collagen ratio, just less min-
eralized tissue per unit area which distinguishes the disorder from osteomalacia where mineral is reduced in amount relative to the bioorganic matrix. There is, as might be expected, no known cure for osteoporosis. It is part of the normal aging processes and pervasive in postmenopausal women and older men. The disease is considered a health issue in the United States and in other developed countries. It has become an important area for basic and clinical research with the incidence of fracture considered a signal of disease (Figure 9). Onset of different types of osteoporosis, the relationship to nutrition, exercise, or other potential contributing factors, and the results of a variety of treatments are the factors considered in the many epidemiological studies in several countries.

Bone mass is genetically programmed for an individual but may be modulated qualitatively and quantitatively by environmental factors. Reduction of bone tissue density in the vertebrae, a hallmark of osteoporosis, and subsequent vertebral fracture is estimated to be as high as 1 out of 4 women by age 65–70 (Wasnich, 1996; Eastell, 1999). This expression of compromised bone strength results in debilitation and pain with normal body movements and often premature death. Medical attention now must go beyond bone quantity (mass) into bone quality: architecture and rates of turnover (Chestnut et al., 2001) that involve molecular biochemistry and the relationships of the inorganic with organic constituents.

Osteoporosis, a focus of public attention when bone loss was recorded for the astronauts subjected to weightlessness, is also a consideration from long periods of inactivity such as for disabled people in wheelchairs or bedridden. The dynamic bone tissues respond to normal wear and tear. A cadre of metabolic disorders that result in bone loss in the young as well as in the old have been identified (Avioli, 2000). The accumulated knowledge from abnormal situations has illuminated the complicated array of physical and chemical interactions necessary to maintain a viable skeleton. However, what constitutes effective treatment or, best of all, prevention of osteoporosis, remains elusive. A multiplicity of approaches that include not only pharmacologic intervention, but genetics (Econs, 2000), diet (Marcus et al., 1996), and exercise (Riggs & Melton, 1995) are addressing the disease.

A. Detection of the Disease

Throughout the skeleton and its interrelated body systems there are dynamic changes but probably none are so obvious a sign of aging as the bend of the spine, also known as dowagers hump, because it is often typical of older women. Figure 6B and Figures 9A and B depict the remarkable differences at the tissue level in the distribution of trabeculae in affected vertebrae. A diagnosis of osteoporosis is inferred from a clinical examination and confirmed by radiologic examination using X-ray radiographic transmission analyses. A radiograph of the forearm, or leg bones, which is available as a result of an accidental fracture, may present poorly mineralized, inhomogeneous, or “porous” bone and tissues that are reminiscent of these spine photos.

The density level, or mineral content, of bones can be measured using these radiographs, and such examinations are noninvasive (Johnston et al., 1996). Digital radiographic techniques can quantify the mass of any bone or portion thereof. The results are compared with results from persons of like race, stature, and age to estimate the degree of osteoporosis and the potential risk for future fractures for a specific patient. What is actually measured is the mineral concentration per mass of bone (Figure 10).

High-resolution radiographic analysis methodology, computerized axial tomography (CAT) scans, has also been used to show local differences in mineral density and distribution in the cortical bone, or the number, size, and organization of the trabeculae in a bone. These measurements are a reminder that each bone has its own biominalization system, which is independent during formation and must be maintained at a certain level to be a contributing and effective part of the skeleton.

Radiological and densitometry analyses are useful in aiding diagnosis, but treatment for an individual depends on the patient’s distinctive metabolic status. These data may inform on whether the osteoporosis is due to lowered amount or to overproduction of certain hormones that might influence the level of circulating calcium or phosphorus, for example. There are many other factors that can impact the several cells unique to the bone tissue system that must be considered in mineral formation and maintenance.

An initial designation of osteoporosis via radiographic survey and density of mineral amount per unit area may be followed with a bone biopsy. A small portion of tissue usually from the iliac crest (hip) is extracted by syringe and prepared for histological examination. Histomorphometry techniques examine texture and quantify tissue components, such as the number of the essential cells, specific hormones, and proteins, and estimate the level of bone formation and resorption. Does the tissue show normal amounts and distribution of mineral? Are there sufficient osteoblasts, the bone forming cells
FIGURE 10  Graphs comparing the average (over 1000 person years) of fracture risk versus bone mass and fracture risk versus age. (From Johnston et al., 1996, Figure 1.)

present, or alternatively, bone tissue resorption accelerated with a high concentration of osteoclasts (Frost, 1973). These two options are the yin and yang of bone remodeling, the dynamic system that underlies the viability of all skeletal organs (Urist, 1965; Mundy, 1999).

The onset of osteoporosis is asymptomatic and prevention is at least partially associated with an adequate diet and the intake, transport, and uptake of calcium, protein, and calories as well as vitamin D. The evaluation of personal choices, such as consumption of calcium-containing foods or specific trace elements via supplements may not be easily assessed for a particular patient. A variety of elements, hormones, enzymes, and proteins are essential in the complicated integration of other organs and cycles of the body system for maintaining a functional set of dynamic skeletal tissues (Figure 11). From the production of Vitamin D, or parathyroid hormone, to the absorption of calcium at the intestines, and recycling of phosphorus by the kidney, research has shown that all are important and must be properly integrated in order to arrive at the best treatment for a particular patient with osteoporosis (Avioli, 2000).

FIGURE 11  Sketch illustrating the proposed contributions to age-related bone loss in women. The interactions of vitamin D, hormones, and activity level affect the uptake of calcium, bone turnover, and cell function, which if not in balance, may cause bone loss. (From Eastell, 1999, Figure 1.)
B. Treatments for Osteoporosis

Over the past 50 years investigations from the cell level to animal model systems have been utilized to assess treatment regimens. Although some therapies may appear promising in animals, they may not translate to effective treatments for humans (Jee, 1995). Much of the research has involved tests using pharmacologic agents that act on several body systems and effect the balance of circulating calcium and phosphorus. This seems a sensible approach applicable to all the bones in the body rather than attempting to construct treatment for specific bones.

The goal of osteoporosis treatments is to assist the body toward normal bone growth and repair and specifically to provide for normal mineral formation and retention. Because the mineral is our focus, but only one component in a complicated and multifaceted system, the following three sections on pharmacologic treatments focus on the composition and relative amounts of mineral to bring out some of the contributions provided by this basic science presentation.

1. Fluoride

The first thrust for making fluorine a therapeutic agent was put forward by dental scientists. Their studies led them to suggest that if the mineral in dental tissues was fluorapatite it would be less susceptible to caries as fluorapatite was the more stable apatite (Vischer, 1970). To increase the formation of fluorapatite would require incrementing the amount of fluorine ingested that would then become part of the bone mineral as the inorganic portion of the tissues should respond to the nutrients supplied. However, it was known that some geographic areas with high fluorine in the waters led to a disease known as fluorosis. The simple hypothesis of more fluorine belies other aspects of the complicated bone tissue system. Fluoride may not only form fluorapatite, it may affect bone cells and the formation of the organic matrix. To obtain the optimum amount of fluorine to benefit human bones requires not only bioapatite production and composition, but also consideration of tissue recycling.

In the presence of small amounts of fluorine there was an increase in mineral matter and therefore the density of bone tissues. One study showed that fluorine was mitogenic for osteoblasts and a clinical investigation produced dramatic sustained (years) increases in bone mineral density when administered in doses of 20–30 mg fluoride daily (Riggs & Melton, 1995). However, other clinical studies showed no effect and the cortical portion of the long bones decreased in density relative to an increase in trabecular bone density. The disturbance of cortical tissues could be modulated by increasing Ca and Vitamin D intake or by arranging F-free periods during treatment (Watts, 1999). These interesting insights into the use of fluoride supplements as a possible means to increase fluorapatite in bone tissues also showed that only some of the fluorine found its way into bioapatite. When new mineral was formed little if any fluorine was partitioned into already existing bone mineral. The predominant bioapatite phase on bulk analyses remained hydroxylapatite.

Fluoride may be rapidly absorbed from the stomach but 50% will be eliminated via the kidneys within a few hours. Serum fluoride levels between 0.1–0.25 mg L−1 and doses greater than 70 mg daily (35 mg fluorine) produce grossly abnormal bone (Riggs & Melton, 1995). In areas of China with naturally high fluorine in the environment, telltale signs of brown spots (fluorosis) on the teeth and bent legs and bodies attest to its interference in the strength and architectural character of normal mineralized tissues (Finkelman et al., 1999). There is a narrow therapeutic window for fluorine. Domestic water levels at 1 μg–L−1 achieve dental benefits and may also provide some osteoporotic relief, but what is the level and length of treatment, or which cooperative treatments might be paired with fluorine ingestion to ensure no abnormal demineralization, and strong bone?

In shark teeth whose enameloid tissue shows the highest fluorine mineral content for any vertebrate, the uptake unfortunately appears to be related to genetics and phylogeny and not to the fluoride concentration in the aqueous environment (Aoba et al., 1997). The appropriate dose and best schedule for human fluorine ingestion have not been fully determined. The possible impact of fluoride on bone and on other body systems will require much longer and more comprehensive investigations before fluoride can be part of routine treatment of postmenopausal or other types of osteoporosis.

2. Bisphosphonates

Bisphosphonates are a group of compounds, analogues of the pyrophosphates, where the phosphorus atom of the phosphate group connects directly to a carbon atom. The chemical arrangement of several pharmacological bisphosphonates that are used to treat osteoporosis approximates the following general structure:
OH \quad R_1 \quad OH \\
O = P - C - P = 0 \\
OH \quad R_2 \quad OH

where R1 and R2 represent other chemical substituents (Fleisch, 2000). These phosphate-containing chemicals adsorb onto mineral surfaces and are not readily degraded chemically or enzymatically, an attribute that makes them useful markers of mineral materials. Specific formulations with easily detectable elements in the “R” groups (such as radioactive species in these adducts) have been employed in nuclear medicine experimentation. However, other, non-nuclear-containing bisphosphonates are useful in treatment of osteoporosis because they act locally by preventing the resorption of calcium phosphates by osteoclasts. Some bisphosphonates may inhibit osteoclast activity or cause apoptosis (cell death), but the major effect is to slow down the dissolution of bioapatite and therefore reduce bone remodeling, although the action is usually transient.

Only 5% of an oral dose of a bisphosphonate is absorbed from the gut, and the amount will be lower in the presence of calcium or other divalent ions. The drugs, usually taken in the morning before any food is consumed, will mostly be excreted by the kidney. Because bisphosphonates bind to the mineral they may become buried in the tissue becoming inactive. It is estimated that roughly 25% of the amount bound will be lost from the skeleton after 10 years, an indication of the slow rate of bone tissue turnover.

There are side effects of bisphosphonate ingestion: some tissues may not mineralize properly, and an increased amount of non-mineralized matrix may be the locus for fracture of the bone. Oral administration may also cause some soft tissue side effects but intravenous bisphosphonates have been effective in patients with Paget’s disease (Watts, 1999). Current research using animal model systems and high-resolution transmission electron microscopy show that bisphosphonates may increase the width of individual apatitic crystallites in the tissues but not markedly increase the mineralization level. Such results beg additional questions. One might be phrased as follows: In the composite tissues typical of bone where the physical and chemical properties of all the components must be maintained to ensure proper and continued interactions and organ function, is the major effect thus far noted with bisphosphonates, increasing crystallite size, an appropriate and useful contribution for long-term treatment of osteoporosis?

3. **Hormones**

The most familiar pharmacologic treatment for postmenopausal osteoporosis is to augment the lowered production of estrogens in older women. Although the maximum level of skeletal bone density is affected by nutrition and usually achieved by the age of maturity (25–30 years), estrogen was shown to be important in producing and keeping the calcium levels in the circulation adequate for proper skeletal mineralization. For aging individuals the addition of estrogens, originally prescribed to relieve hot flashes and vaginal dryness, was shown to increase bone mineral density in the spine by 5–10% after age 65. Progesterone, or one of the progestins, was added to the estrogens in some formulations. Long-range clinical studies on the combined pharmaceuticals have shown no bone tissue benefits. Most recently these clinical studies have noted an increase in cardiovascular disease and breast cancer rates. This is most unfortunate and a sad commentary on hormonal replacement therapy that has been followed for many years by hundreds of thousands of women.

Although linked with skeletal health, the mechanisms for a direct action of the hormones on bone tissue remains under study. Early clinical investigations indicated reduced bone loss perhaps through suppression of osteoclastic bone resorption. Estrogens bind at receptors on the nuclei of target cells in both men and women and activate genes that affect the several different pathways required to maintain adequately mineralized bone tissues. For example, the normal production of growth factors and cytokines involved in calcium homeostasis may be altered without appropriate amounts of estrogens and other hormones. Estrogen acts at the kidneys and in the bowel, and via feedback mechanisms may sensitize the remodeling system bone cells to be more receptive to mechanically induced electrical signals (Watts, 1999).

The “normal dose” of 0.625 mg daily of conjugated estrogens, usually equine derived, was lowered to 0.3 mg to prevent bone loss (Watts, 1999). With a half-life of 10–18 hours, estrogen compounds are easily metabolized by several different tissues once absorbed. From a series of investigations it became known that the stable equilibrium for the hormone between estrone, the dominant form, estradiol, the active form, and other conjugated and esterified forms, is rapid. Estrogens circulate by binding to sex hormone-globins or albumin, the dominant circulating protein in blood and in tissues, but only the unbound form is biologically active. Estrogen compounds are excreted in
the small intestine, and become less active in the process.

The side effects of tenderness, fluid retention, weight gain, occasional generation of deep vein thrombosis, and pulmonary emboli are some of the reasons that discouraged many women from starting or continuing the use of the estrogens post menopause.Raloxifene (Evista) acts to modulate the effect of estrogens on the surface of cells. It is an estrogen-acceptor modulator, and thus far it is the only drug approved that has been shown to prevent bone loss by causing differential expression of estrogen-regulating genes. The actions of estrogen and future use are cloudy at present and their potential contribution to minimizing bone tissue loss needs further study (Chlebowski et al., 2003).

4. Summary

Bisphosphonates, possibly useful for reducing bone mineral resorption; hormones, particularly estrogen, certainly important in calcium-mineral dynamics; and fluoride, thought to increase the stability of the mineral phase; all have shown modest success for retaining or restoring mineral in bones by a variety of clinical investigations. Together with exercise and a diet that includes vitamins and certain trace elements, the battle to understand and relieve if not prevent osteoporosis continues. The lack of consistent and sustained benefits from the proscribed regimens on osteoporosis-affected populations makes it clear that additional experimental protocols are needed and will require lengthy clinical evaluation. Any new techniques or pharmacologic agents in these ongoing research efforts will provide useful data on the mineral, its roles and reactions.

SEE ALSO THE FOLLOWING CHAPTERS

Chapter 5 (Uptake of Elements from a Biological Point of View) · Chapter 29 (Inorganic and Organic Geochemistry Techniques)

FURTHER READING


Bell, L. C., and Mika, H. (1979). The pH Dependence of the Surface Concentration of Calcium and Phosphorus on


