2

Skeletal Biology

> It is true, if we come to torture a bone with the Fire, it seems to confess that it consists of all the five Chymical Principles...
>
> Clopton Havers (1691)

2.1 Introduction

Chapter 1 has shown us that the skeleton must withstand very high forces; because our muscles can only contract a small percentage of their length, we must amplify movements using levers that "spend rather than save forces," as Borelli put it. In this chapter we begin to develop an understanding of how the biology of bone and cartilage provides tissues that can support these large forces day in and day out for a lifetime.

As more is learned about the mechanics of the skeleton, it becomes increasingly clear that mechanical demands heavily influence what happens biologically. Most of what we know about the basic cell- and tissue-level mechanisms that enable bone to adjust to mechanical forces has been learned since the 1960s. Before then, skeletal research was focused on bone chemistry, for two reasons. First, mineral metabolism is of great significance in physiology and medicine, and there was intense interest in this subject in the middle of the twentieth century. Second, bone is a difficult tissue to study histologically because it is calcified. Although significant histologic studies of bone have been conducted sporadically throughout this century, most histology laboratories still only work with decalcified bone. Quantitative analyses of histologic sections of mineralized bone became common only in the 1970s. Biochemists, on the other hand, could study bone mineral with existing methods. They soon realized that bone served as a mineral reservoir for the rest of the body, particularly with regard to calcium and phosphorus. For quite a long time, the research of physiologists and biochemists on this aspect of the skeleton strongly influenced medical interest in bone. Bone biology was seen largely in terms of its role in transferring mineral to and from the skeleton. In this view, calcium was removed from the reservoir through bone resorption by cells called *osteoclasts.* Calcium was put into the reservoir by cells called *osteoblasts*, which laid down

new bone. These two kinds of cells were thought to work independently of one another and to be separately controlled by hormones. To greatly simplify the picture, if the serum calcium concentration fell, *parathyroid hormone* was released and stimulated osteoclasts to resorb some bone and put its calcium into the bloodstream. If serum calcium became too high, another hormone called *calcitonin* was released to produce the opposite effect. If the balance between resorption and formation of bone was not sufficiently controlled by the hormonal system, bone loss and *osteoporosis* (literally, "porous bones") resulted.

Coincidentally with the advent of more quantitative studies of bone's remodeling dynamics, in the second half of the century much more became known about the mechanical properties of bone. The "old" bone biology, dominated by interest in mineral metabolism, began to be replaced by a "new" bone biology based on broader perspectives. The lamp of this new bone biology has been fueled by contributions from many individuals, but the flame was kindled by an orthopaedic surgeon named Harold M. Frost. Using simple laboratory techniques, he began in the 1960s to make histologic sections of bone and look at them with an independent mind. He deduced that osteoclasts and osteoblasts usually do not work independently, but are coupled together in teams, with osteoblasts automatically following osteoclasts and replacing the bone that they removed. Frost called these teams of bone cells *basic multicellular units*, or *BMUs*. He went on to make many other observations and deductions about bone biology. Initially, his ideas were poorly understood and often rejected, but through the years many of his concepts have been accepted in one form or another. Central to these ideas is the concept that the skeleton is primarily a mechanical organ, rather than a calcium reservoir. Although bone plays an important role in calcium homeostasis, the main job of osteoclasts and osteoblasts is now seen as maintenance of the mechanical integrity of the skeleton. How this is done by teams of cells communicating by chemical messengers and other means, while still fulfilling the metabolic roles of the skeleton, is the principal focus of current orthopaedic research. It is also an important cornerstone of this book.

Our purpose here is to briefly summarize bone and cartilage biology as a foundation for our study of skeletal tissue mechanics. What does the skeleton look like inside? What is it made of? How are bones initially formed? How do they grow? How does a fractured bone heal? These are the questions that are addressed in this chapter.

2.2 The Shapes of Bones

While the shapes of our bones are quite variable, comparisons with those of other animals show that the architecture of a vertebrate skele-

ton is fairly conservative and stereotypical. We usually can recognize a femur or a tibia, and we recognize that they have a characteristic relationship, regardless of what animal they came from. Yet within these broad architectural constraints, the skeletal morphology is quite variable, and we can imagine a rubber archetypical skeleton being stretched and molded to create such diverse skeletons as those of a flamingo, a bat, an elephant, a whale, or a human.

Not only is skeletal architecture conservative, but so too is the topology of individual bones within a species. Thus, a physical anthropologist can recognize an isolated bone, or even a fragment of a bone, as belonging to a human rather than one of the other primates. These constraints on architecture and topology give the impression that bones are determinate, static structures. On a scale of centimeters, this is approximately true. However, if we increase the resolution of our investigation, and focus on scales of microns to millimeters, we see that bone is a highly dynamic tissue, continually adjusting to its physiologic and mechanical environment by changes in its composition and microscopic architecture. An important principle of skeletal physiology is that bones are able to sense the mechanical loads which they bear and modify their structures to suit changes in these loads. This principle is called *Wolff's law*, and we study it in detail in Chapter 6.

2.3 Types of Bone Tissue

Figure 2.1 is a sketch designed to show the general features found in most long bones, such as the humerus or metatarsal. This figure illustrates the relationships between many of the structural features of bones described in this chapter.

The nonmineralized spaces within a bone contain *marrow*, a tissue composed of blood vessels, nerves, and various types of cells. The chief function of the marrow is to generate the principal cells present in blood. The internal presence of marrow is a nearly universal feature of bones (the ossicles of the inner ear are an exception). The relationship of marrow and bone is both biologic and physical: they share common stem cells, and marrow never exists outside of bone. Bone can be made to form outside the normal skeleton (e.g., in a muscle) by implanting osteogenic materials, but when this happens, a space containing marrow automatically forms in the nodule of extraskeletal bone. Moreover, marrow itself is a highly osteogenic material and can stimulate bone formation if placed in an extraskeletal location (Tavassoli and Yoffey, 1983).

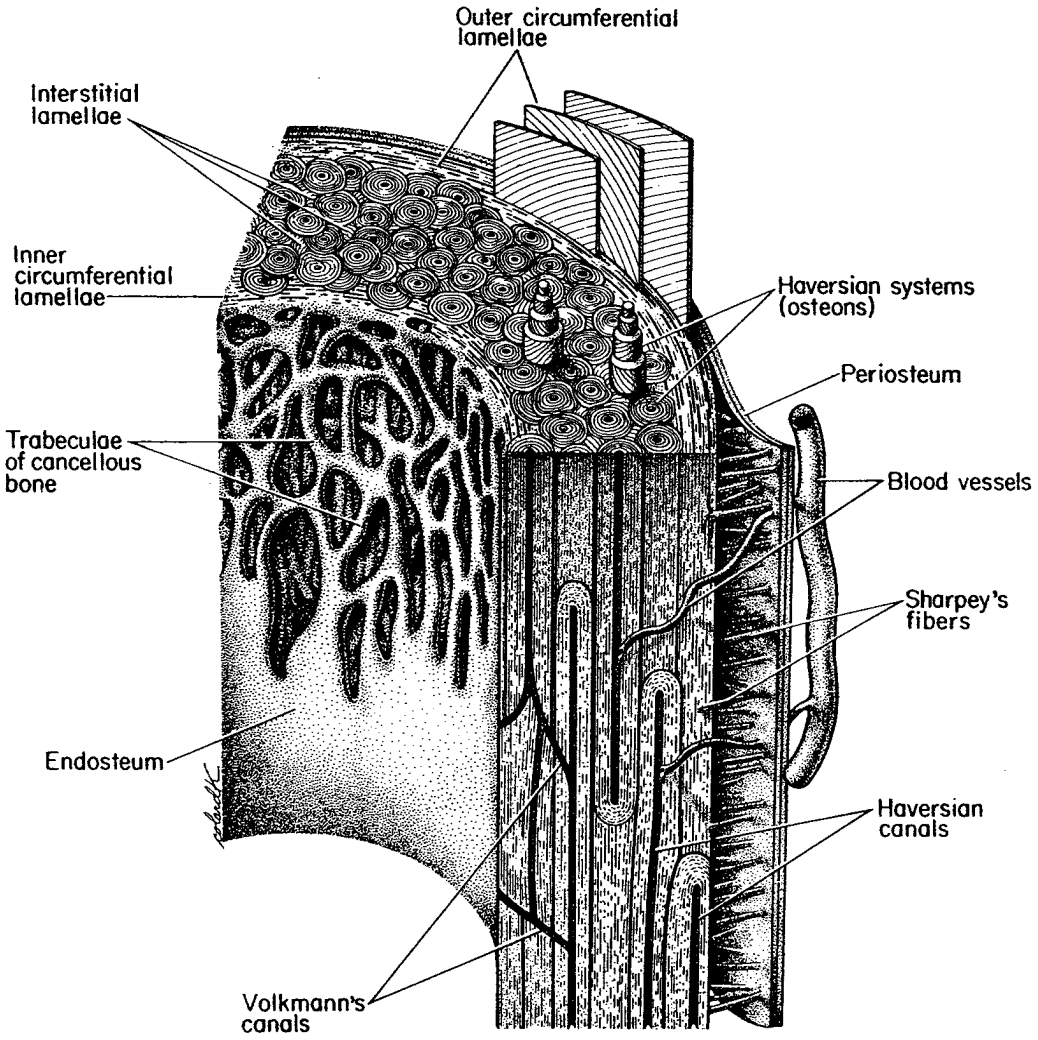

FIGURE 2.1. Sketch of some important features of a typical long bone. (After Benninghoff, 1949.)

Trabecular Vs. Compact Bone

If you were to slice open a dozen bones of various sorts and wash away the marrow with a high-pressure stream of water, it would be clear that the remaining bone is of two distinct kinds, as determined by porosity (the volume fraction of soft tissues). While in principle the porosity of bone can vary continuously from zero to 100%, in fact most bone tissues are of either very low or very high porosity, with little bone of intermediate porosity. These two types of bone tissue are referred to as *compact bone* and *trabecular bone*, respectively.

Trabecular bone (also called *cancellous* or *spongy* bone) is porous bone found in the cuboidal bones (e.g., vertebrae), the flat bones, and the ends of long bones (Fig. 2.2); its porosity is 75%–95%. The pores are interconnected and filled with marrow. The bone matrix is in the form of plates or struts called *trabeculae*, each about 200 micrometers (μm) thick. The

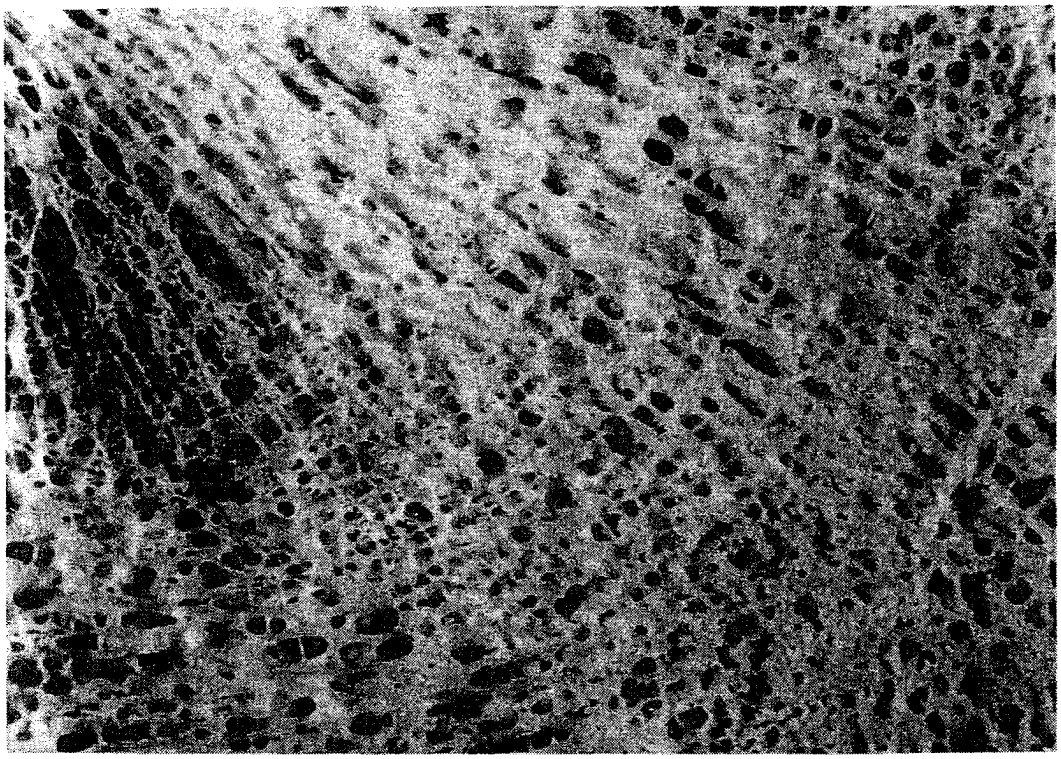

FIGURE 2.2. Example of trabecular bone structure in the distal end of a human femur. Field width, ~1 cm.

arrangement of the trabeculae is variable. Sometimes they appear to be organized into orthogonal arrays; often, they are more randomly arranged.

Compact bone is the dense bone found in shafts of long bones and forming a *cortex* or shell around vertebral bodies and other spongy bones (Fig. 2.3). Hence, it is also called *cortical bone*. Its porosity is 5%–10%, and its pores consist of spaces categorized as follows

Haversian canals are approximately aligned to the long axis of the bone, contain capillaries and nerves, and are about 50 μm in diameter (about the diameter of a human hair). Haversian canals are named after an English physician, Clopton Havers (1691).

Volkmann's canals are short, transverse canals connecting Haversian canals to each other and to the outside surfaces of the bone. These canals also contain blood vessels and probably nerves. They are named after Richard von Volkmann (1830–1889), a surgeon and early advocate of Lister's antiseptic surgical methods.

Resorption cavities are the temporary spaces created by osteoclasts in the initial stage of remodeling (described later). Resorption cavities are about 200 μm in diameter.

It is important to remember that bone is a **dynamic** porous structure; its porosity may change as the result of a pathologic condition or in a

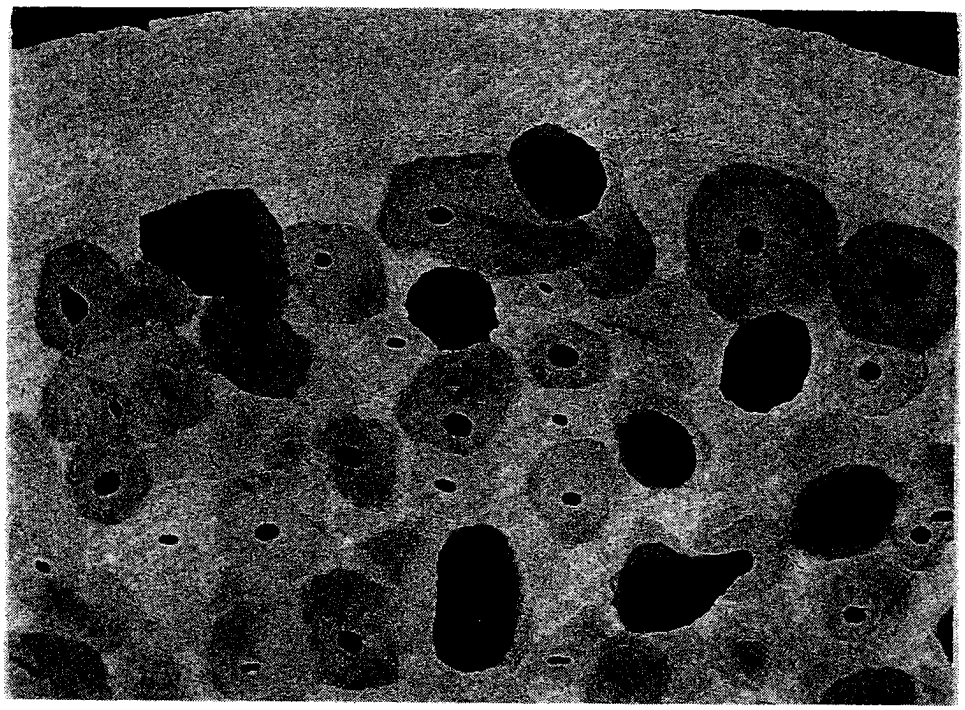

FIGURE 2.3. Microradiograph (X-ray image of a thin cross section) showing compact bone. Haversian canals and resorption spaces are *black;* note variable mineralization of osteons. Outermost region (at *top*) contains well-mineralized primary bone. Field width, ~2 mm. (Courtesy of Dr. Jenifer Jowsey.)

normal adaptive response to a mechanical or physiologic stimulus. Trabecular bone may become more compact, or compact bone may become more porous. Such changes strongly affect bone's mechanical properties.

Lamellar Vs. Woven Bone

Examining compact and trabecular bone at a still finer scale of resolution, it is evident that each may contain two major types of bone tissue.

Lamellar bone is slowly formed, highly organized bone consisting of parallel layers or lamellae comprising of an anisotropic matrix of mineral crystals and collagen fibers. Two fundamentally different kinds of "plywood" architecture coexist within the general complexity of bone's lamellar structure (Giraud-Guille, 1988). The first of these schemes corresponds to the classical view of lamellar structure: the collagen fibers are parallel in each lamella and change direction by 90° at the lamellar interface. That is, as a lamella is built up, layers of collagen are put down in one orientation. Then, when the next lamella is started, the orientation of collagen fibers suddenly changes so that they are laid down at right angles to the previous direction. In other regions of the bone, this orthogonal plywood-like structure is replaced by helicoidal plies

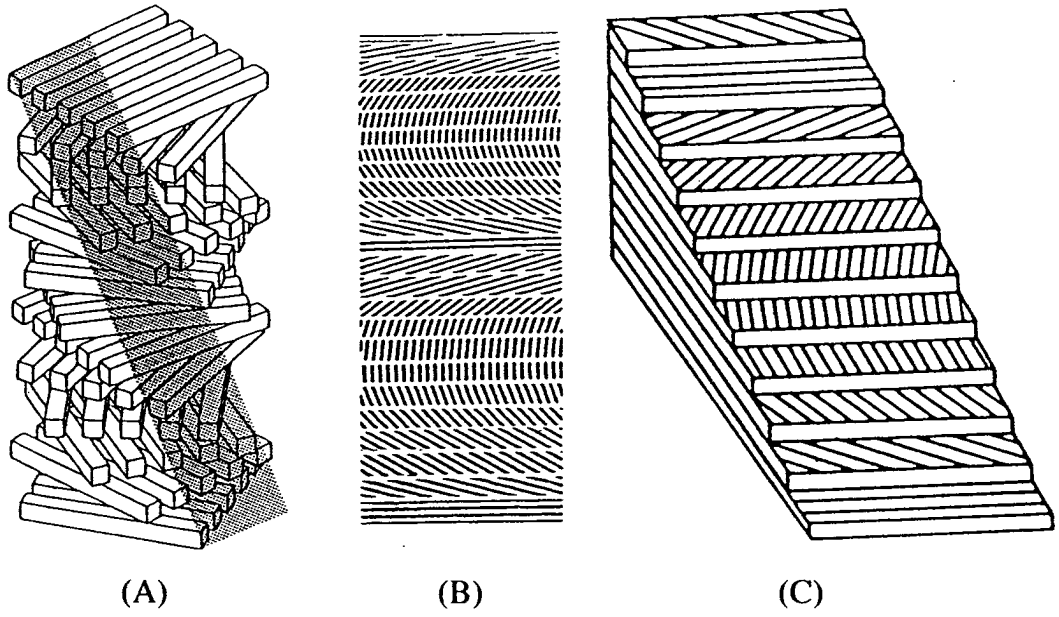

FIGURE 2.4A–C. Diagram of helicoidal plywood structure. **A** Three-dimensional structure. **B** Visual effect of arches seen when an oblique section (as indicated by the *shaded plane* in **A**) is cut. **C** The arches seen in an oblique section are formed by the helicoidal lamellae. (Reproduced with permission from Neville, 1984.)

(Fig. 2.4). In this scheme the collagen fibers **continuously** change their direction, so that in a sense there are no individual lamellae. However, the bone still shows a lamellar structure because as the orientation of the collagen fibers rotates through 180° cycles, the fiber orientation repeats itself, and layers appear when a histologic section is examined microscopically. In her landmark paper, Giraud-Guille (1988) showed that both these architectures are present in human compact bone, but their relative distribution and interspecies variation have not been determined.

Both these forms of lamellar structure give rise to *birefringence*, the capacity of some fibrous structures to interact with polarized light. When a histologic section of bone is transilluminated with polarized light and viewed through a polarizing filter oriented perpendicular to the vibration plane of this incident light, the section appears dark (i.e., the observer sees a "dark field") except where collagen fibers are parallel to the plane of the section. These collagen fibers rotate the light's plane of polarization so it is no longer perpendicular to the viewing polarizing filter. Therefore, the light is not blocked and reaches the viewer's eyes. Thus, in a bone section observed in a polarizing microscope, transversely oriented fibers are bright and longitudinally oriented fibers are dark. In the 1960s, Ascenzi and his co-workers used this phenomenon to categorize osteons as having bright, alternating, or dark lamellae (corresponding to their collagen fibers exhibiting mostly circumferential, alternating, or longitudinal orientation). These

classifications are shown as parts a, b, and c, respectively, in Fig. 2.5. A dark "iron cross" pattern superimposed on the brighter osteons shows where the lamellae lie parallel to the polarization directions of the upper or lower polarizing filters.

Woven bone is a quickly formed, poorly organized tissue in which collagen fibers and mineral crystals are more or less randomly arranged. Woven bone may become more highly mineralized than lamellar bone, which, mechanically speaking, may help compensate for its lack of organization. Rats and other small animals also have *fine-fibered bone* in the cor-

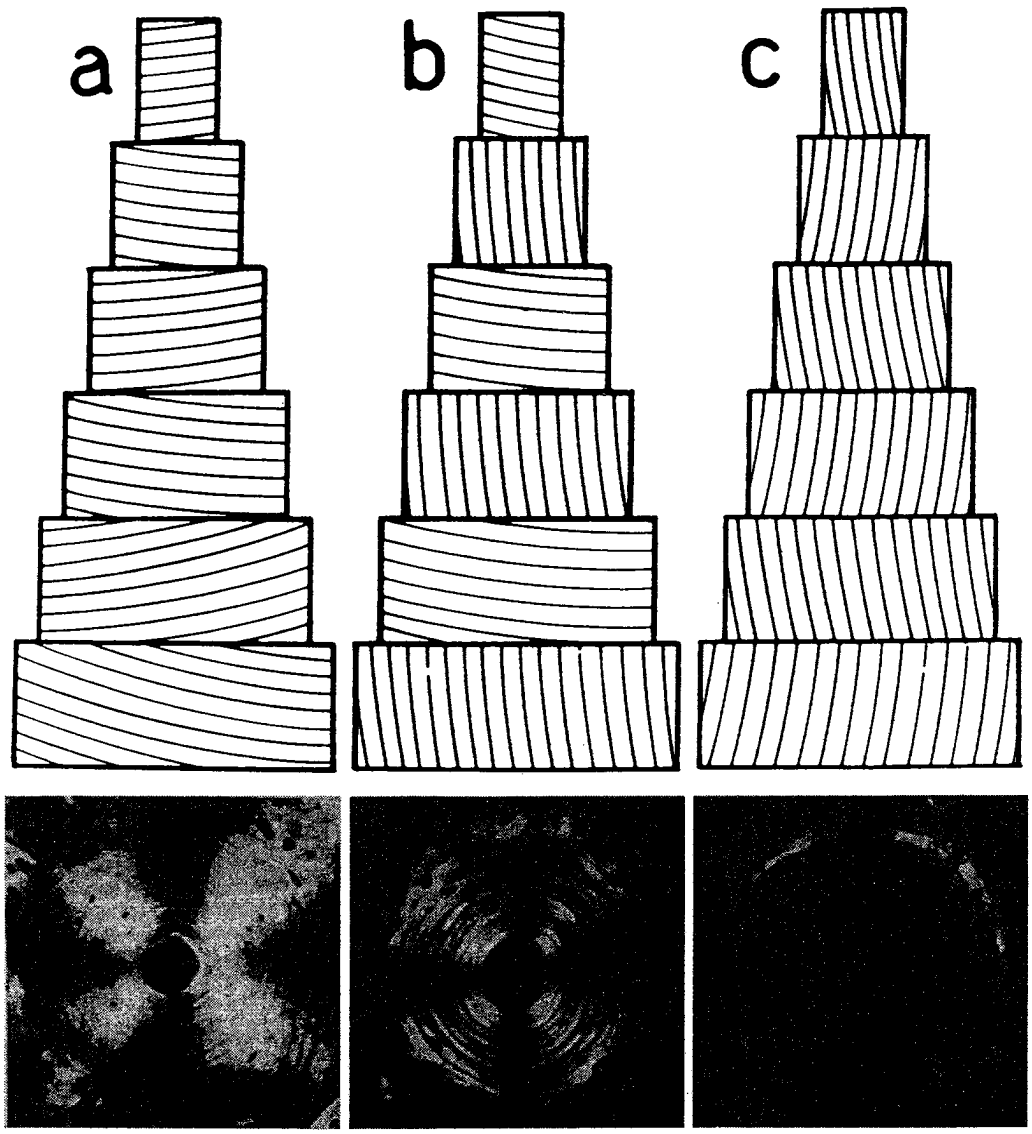

FIGURE 2.5. Three osteon types as defined by Ascenzi and Bonucci. Photomicrographs at *bottom* show appearance in plane-polarized light; diagrams *above* show hypothesized fiber arrangements in successive lamellae. *a*, Type T or transverse (i.e., circumferentially wrapped) fiber orientatio; *b*, type A or alternating fiber orientations; *c*, type L or longitudinal fiber orientation. (Reproduced with permission from Ascenzi and Bonucci, 1967.)

tices of their bones. In this bone the rather randomly arranged collagen fibers are smaller and closer together than in woven bone, so the tissue appears to be more organized. It may be that the lamellar organization of bone can vary continuously, depending on factors such as how fast it is made. The important generalization is that woven bone can be made more quickly than lamellar bone, but is weaker.

Primary Vs. Secondary Bone

Compact bone may be further characterized as **primary** or **secondary** bone.

Primary bone is tissue laid down de novo on an existing bone surface, such as the periosteal surface, during growth. It may be of two general types:

Circumferential lamellar bone, in which the lamellae are parallel to the bone surface. Figure 2.6 is a schematic diagram of circumferential lamellar bone beneath the periosteal surface. Blood vessels are incorporated into the lamellar structure such that each is surrounded by several circular lamellae, forming a *primary osteon* with a *primary Haversian canal* at its center.

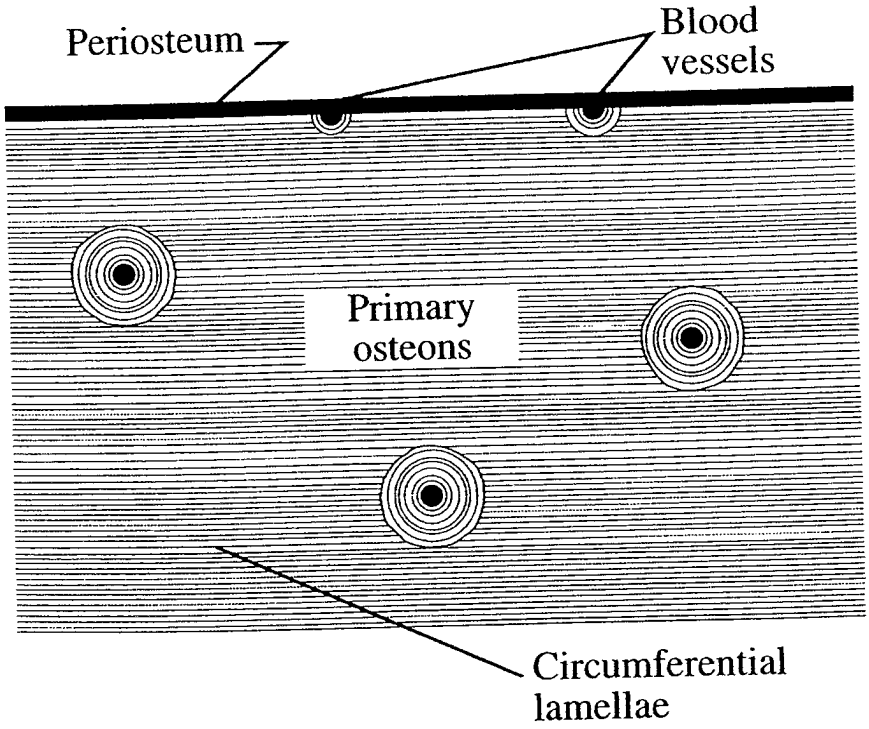

FIGURE 2.6. Sketch of primary circumferential lamellar bone structure. Primary osteons form when blood vessels on the bone surface become incorporated into the new periosteal bone. They usually have several concentric lamellae, but their cement line is not scalloped.

Plexiform bone, in which the rate of formation is greatly increased by continually constructing a trabecular network on the surface and filling in the gaps. The result is a mixture of woven bone (the trabeculae) and lamellar bone (the filled-in spaces). Plexiform bone contains rectilinear residual vascular spaces, which often produce a "brick wall" appearance. Figure 2.7 contains a low-magnification view of this structure. It typically occurs in large, fast-growing animals like cows. Racehorses, which put enormous stress on some of their bones, have a similar kind of bone that may have exceptional fatigue resistance (Stover et al., 1992).

Secondary bone results from the resorption of existing bone and its more or less immediate replacement by new, lamellar bone. This process is known as remodeling; it is discussed in detail later. In compact bone, secondary tissue consists of cylindrical structures known as *secondary osteons* or *Haversian systems* (Fig. 2.8). These are about 200 μm in diameter, and consist of about 16 cylindrical lamellae surrounding a central *Haversian canal*. The boundary between the osteon and the surrounding bone is known as the *cement line*. In adult humans, most compact bone is entirely composed of secondary bone, which may include whole osteons and the remnants of older osteons that have been partially resorbed (*interstitial bone*). Trabecular bone in adults is also mostly secondary bone, and this is true from an even earlier age, because it is remodeled, or "turned over," more rapidly than compact bone. However, the remodeling of trabecular bone rarely produces osteons because they usually do not fit inside individual trabeculae.

FIGURE 2.7. Photomicrograph of plexiform bone. Field width, ~500 μm.

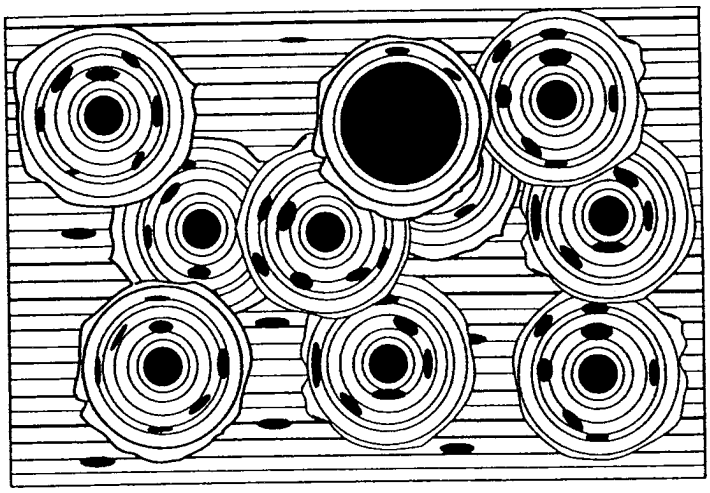

FIGURE 2.8. Schematic diagram of secondary osteons on a field of primary bone. One osteon is still forming; it has overlapped the Haversian canal of an existing osteon.

2.4 Composition of Bone

Bone is composed of collagen, water, hydroxyapatite mineral, and small amounts of proteoglycans and noncollagenous proteins.

Collagen is a structural protein, widely distributed throughout the animal kingdom, that can spontaneously organize itself into strong fibers. More than a dozen types of collagen have been identified. The predominant collagen in bone is type I, which is also found in tendons, ligaments, and skin. Collagen gives bone flexibility and tensile strength. It also provides loci for the nucleation of bone mineral crystals, which give bone rigidity and compressive strength.

Mineral in bone consists almost entirely of *hydroxyapatite* crystals, $Ca_{10}(PO_4)_6(OH)_2$. The individual crystals are rods or plates with hexagonal symmetry, measuring about $50 \times 50 \times 400$ angstroms (1 micrometer [μm] = 10,000 angstroms [Å]) . Bone mineral is impure, containing many structural substitutions (e.g., carbonate, fluoride, citrate). These impurities are governed by the composition of body fluids and in turn affect the solubility of the bone mineral.

Ground substance of bone consists of *proteoglycans* (formerly called *mucopolysaccharides*). In particular, *decorin* and *biglycan* are small species of proteoglycans found in bone. Although the specific role of the proteoglycans in bone is unclear, decorin is known to modulate collagen fibril assembly. Proteoglycans may also function to control the location or rate of mineralization in bone through their calcium-binding properties.

Noncollagenous proteins include a number of molecules whose functions are also unclear. The most abundant noncollagenous protein is *osteocalcin*, which is secreted by osteoblasts and appears to be important in the mineralization of new bone. It also is a chemoattractant for bone cells, and

TABLE 2.1. Volumetric composition of whole bone in dogs

Component	Site or specific molecule	Volume, %
Water, 25%	Bonded to collagen	60
	Other	40
Organic matrix, 32%	Collagen	89
	Proteoglycan	1
	Other organic molecules: e.g., osteocalcin, <1%; osteonectin, <1%	10
Apatite mineral, 43%	In gaps between collagen ends	28
	Intrafibrillar	58
	interfibrillar	14

From Robinson and Elliott, 1957.

assays of its serum concentration are an excellent method of noninvasively determining rates of bone turnover. Other noncollagenous proteins in bone include *osteopontin* and *osteonectin.*

Some of the *water* in the calcified bone matrix is free, and some is bound to other molecules. The mineralization of *osteoid* (the organic portion of extracellular bone) displaces part of its water. Therefore, the water content of new bone tissue changes as it mineralizes.

Table 2.1 gives the approximate composition of bone tissue by volume in dogs, measured in primary or secondary lamellar bone several months after formation.

Quantitative Representation of Bone Composition

We have seen that bone is composed of three primary substances: water, mineral, and an organic matrix that is largely collagen. Clearly, these three substances have distinctive physical properties. Therefore, factors such as the strength of bone depend on the relative amounts of each substance. (Obviously, mechanical properties also depend on the architectural organization of each substance, but we discuss these in later chapters.) It is useful to consider here some variables that quantify the composition of bone.

Let V_T refer to the total volume of some region of bone. This volume can be divided into two parts: the hard, bony matrix (V_m) and voids filled with soft tissue (V_v). Thus,

$$V_T = V_m + V_v \tag{2.1}$$

The volume fractions of these two portions are known, respectively, as the bone volume fraction

$$B_V = V_m/V_T \tag{2.2}$$

(often abbreviated BVF in the bone literature), and the porosity,

$$p_v = V_v/V_T \tag{2.3}$$

Clearly, $B_V + p_v = 1$. In cancellous bone, $p_v > 0.5$ and is largely marrow; in cortical bone, $p_v < 0.5$ and is largely composed of Haversian and Volkmann's canals. The smallest voids in bone, consisting of octeocyte lacunae and canaliculi, are usually included in V_m rather than V_v. The material inside V_m is often called the bone **tissue** to distinguish it from the term "bone," which usually refers to a region large enough to contain Haversian canals or other voids.

Another commonly used variable is bone's **apparent density**, ρ, which is defined as the mass of a volume of bone divided by its volume. Here, we are speaking of the mass of both the hard and soft tissues inside V_T. Typically, ρ would be measured simply by machining a cube of bone, weighing it, and dividing the mass by the cube's calculated volume. If ρ_m is the density of the bone tissue and ρ_v is the density of the soft tissues in the void spaces, then

$$\rho = (\rho_m V_m + \rho_v V_v)/V_T \tag{2.4}$$

$$\rho = \rho_m B_V + \rho_v p_v \tag{2.5}$$

As $B_V = 1 - p_v$

$$\rho = \rho_m - (\rho_m - \rho_v)p_v \tag{2.6}$$

Note that apparent density depends on both the porosity of the bone (p_v) and the density of the bone tissue (ρ_m). Based on measurements of cortical bone, the value $\rho_m = 2.0$ g/ml is suggested. Because the soft tissues in the voids are almost entirely water, $\rho_v \cong 1$ g/ml. Later, we rearrange Eq. 2.6 to estimate porosity from apparent density measurements.

If we now turn our attention to the composition of the bone tissue (i.e., the material in V_m), we may write

$$V_m = V_o + V_a + V_w \tag{2.7}$$

where the subscripts o, a, and w refer to the organic matrix (largely collagen), the mineral, and water, respectively. Representing the densities of the organic, ash, and water fractions of the bone tissue with the appropriate subscripts, we can also write

$$\rho_m = (\rho_o V_o + \rho_a V_a + \rho_w V_w)/V_m \tag{2.8}$$

Currey (1990) has suggested the values $\rho_o = 1.1$ g/ml and $\rho_a = 3.2$ g/ml. Of course, $\rho_w = 1.0$ g/ml.

If we dry a specimen of bone as it comes from the body in an oven at 100°C and then weigh it, its **dry mass** is $m_d \cong \rho_o V_o + \rho_a V_a$ because we evaporate not only the free water in the bone tissue but also that in the soft tissues. If we then ash the specimen by placing it in a furnace at about 800°C for 24 h, evaporating the organic matrix, the **ash mass** will be $m_a = \rho_a V_a$. The ratio of these two masses is called the **ash fraction**:

$$\text{Ash fraction} = m_a/m_d = \rho_a V_a/(\rho_o V_o + \rho_a V_a) \tag{2.9}$$

The ash fraction is a measure of the degree of mineralization of the bone tissue. Unlike apparent density, it is independent of the porosity of the bone. Typically, ash fractions are about 0.65 ± 0.03.

Basic Stereology

Stereology has been defined as "...methods for the exploration of three-dimensional space, when only two-dimensional sections through solid bodies or their projections on a surface are available." (H. Elias, quoted in Underwood, 1970). Stereology is extremely useful in measuring variables related to the structure and composition of bone. In the foregoing paragraphs, we referred to weighing specimens to determine apparent density and calculating porosity from this variable. A more direct way to measure porosity is to cut the specimen into histologic sections and measure the void volume fraction from the two-dimensional (2-D) image (Fig. 2.9). There are three ways to do this.

The first method is to measure the areas of all the voids in the image, or a set of images, and divide the total void area (A_v) by the entire area of the imaged bone (A_T). It can be shown that the 2-D or areal porosity (A_v/A_T) approaches the 3-D or volumetric porosity ($p_v = V_v/V_T$) as more and more measurements are accumulated (Fig. 2.9A),

$$V_v/V_T = A_v/A_T \tag{2.10}$$

provided A_v/A_T is averaged over a large enough sample. The second method is to randomly position "test lines" (Fig. 2.9B) on the image and measure the fractional length of the test lines that falls on void spaces. This length fraction, L_v/L_T, is a one-dimensional measure of void volume fraction, the mean value of which approaches the 2-D and 3-D values as the accumulated length of test lines increases.

Finally, one can use a "zero-dimensional" measurement technique in which a grid of points is randomly placed over the image and the fraction of the points that falls on voids, P_v/P_T, is recorded (Fig. 2.9C). In the limit of many measurements, one expects that

$$\text{Porosity} = p_v = V_v/V_T = A_v/A_T = L_v/L_T = P_v/P_T \tag{2.11}$$

In practice, P_v/P_T is commonly used if the measurements are being made "by eye" or if a computer is being used for image analysis. In the latter case, each test point is a pixel (picture element) in the computer image. The problem is programming the computer to recognize void space pixels, which is usually done by making the bone tissue light or dark in relation to the void space and using a threshold gray scale value to partition the pixels into tissue and void groups. This is not a trivial problem, however, and point counting by a human observer is often the most reliable method, especially if the void spaces need to be categorized (e.g., as Haversian or Volkmann's canals).

The equivalence of volume fraction measurements in zero, one, two, and three dimensions does not apply to another important variable in bone histomorphometry: internal surface area. Bone resorption and formation can only occur on bone surfaces, which can be external surfaces (i.e., periosteal or endosteal surfaces) or internal surfaces (i.e., Haversian or Volkmann's canal surfaces, or trabecular surfaces in cancellous bone). Measurements of internal surfaces are usually expressed per unit volume of bone; this variable, called *spe-*

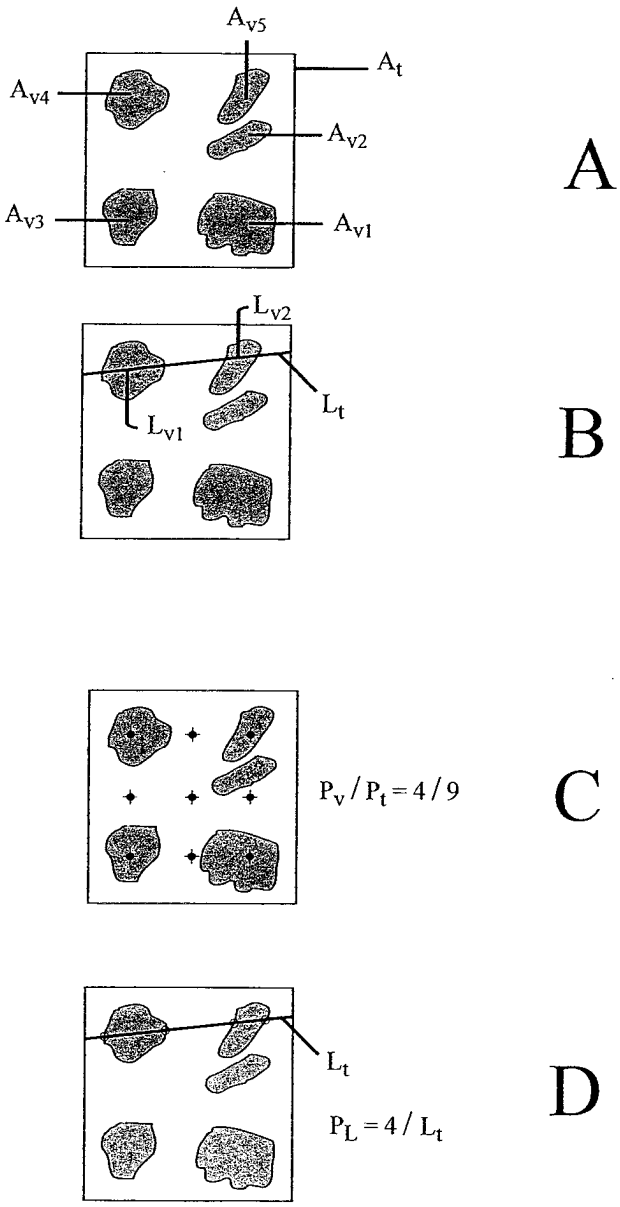

FIGURE 2.9A–D. Basic stereologic methods. *Shaded regions* represent soft tissue spaces in two-dimensional sections of bone. If i = 1, 2, 3, etc., A_{vi} = area of the *i*th void, L_{vi} = length of test line within *i*th void, P_v = number of test points falling in voids; P_t = total number of test points, and L_t = total length of test line. Images **A**, **B**, **C**, and **D** are further described in the text.

cific surface (S_V), has units of mm^2/mm^3 or mm^{-1}. The two-dimensional equivalent of S_V is the perimeter of voids in the image divided by the total area of the image, L_A. The one-dimensional version is the number of test line intercepts with void surfaces divided by the total length of test lines (P_L, Fig. 2.9D). These three measurements of specific surface area are related as follows:

$$S_V = (4/\pi)\, L_A = 2\, P_L \qquad (2.12)$$

where it is again assumed that sufficient measurements have been made of L_A or P_L for the mean to provide a good estimate of S_V. It is also important to

realize that porosity measurements are independent of the shapes and orientations of the voids, but specific surface measurements are not. Consequently, test lines that uniformly represent all orientations are used to measure *average* specific surface, while test lines oriented in particular directions can be used to quantify the anisotropy of bone's internal structure.

2.5 Bone Cells

So far we have discussed the extracellular matrix, that part of bone which is outside of living cells and can be mineralized. Formation and maintenance of the matrix is carried out by the cells that make up a small but critical percentage of the bone volume. There are four types of bone cells. These cells fall into two categories: those that resorb bone, and those which form or have formed bone. The "resorbers" are closely related to *macrophages*, cells that migrate throughout all tissues of the body to remove debris and pathologic material. The "formers" are closely related to cells like *fibroblasts*, which produce structural molecules in other tissues.

Osteoclasts are the cells that resorb bone (Fig. 2.10). They are multinuclear because they are formed by fusion of cells called *monocytes* originating in

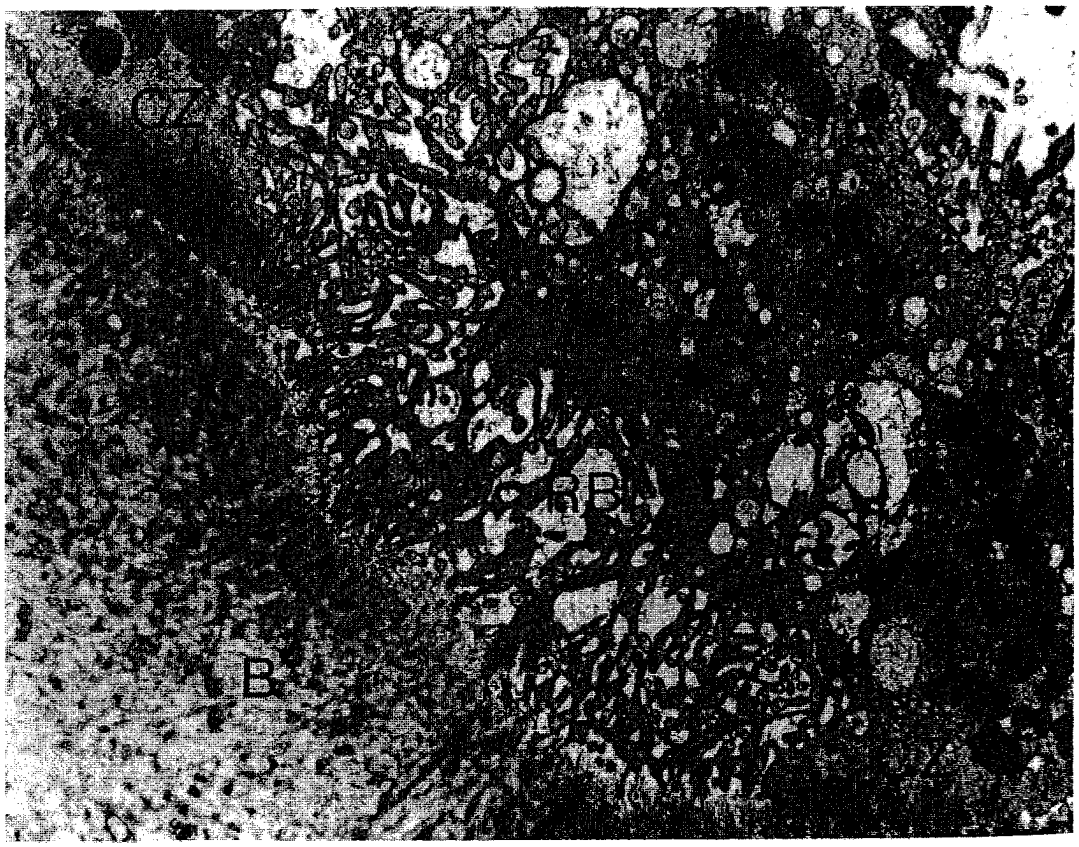

FIGURE 2.10. Electron photomicrograph of a portion of an osteoclast *(upper right)* resorbing bone *(lower left)*. *CZ* labels clear zones where the cell is sealed to the bone surface; *RB* labels the ruffled border where the cell releases chemicals that break down the calcified bone matrix *(B)*. (Reproduced with permission from Borysenko and Beringer, 1984.)

the hemopoietic portion of the bone marrow. Resorption occurs along a highly invaginated *brush* or *ruffled border* of the cell, which is sealed to the bone surface by a peripheral *clear zone.* Osteoclasts erode their way through bone at a rate of tens of micrometers per day by first demineralizing the adjacent bone with acids and then dissolving its collagen with enzymes. These destructive chemicals are manufactured by the cell and transported to the ruffled border in portable intracellular chambers called *secretory vesicles.*

Osteoblasts are mononuclear, cuboidal cells that produce osteoid, the organic portion of the bone matrix. Figure 2.11 is an electron photomicrograph showing the interface between an osteoblast (at top) and the bone surface

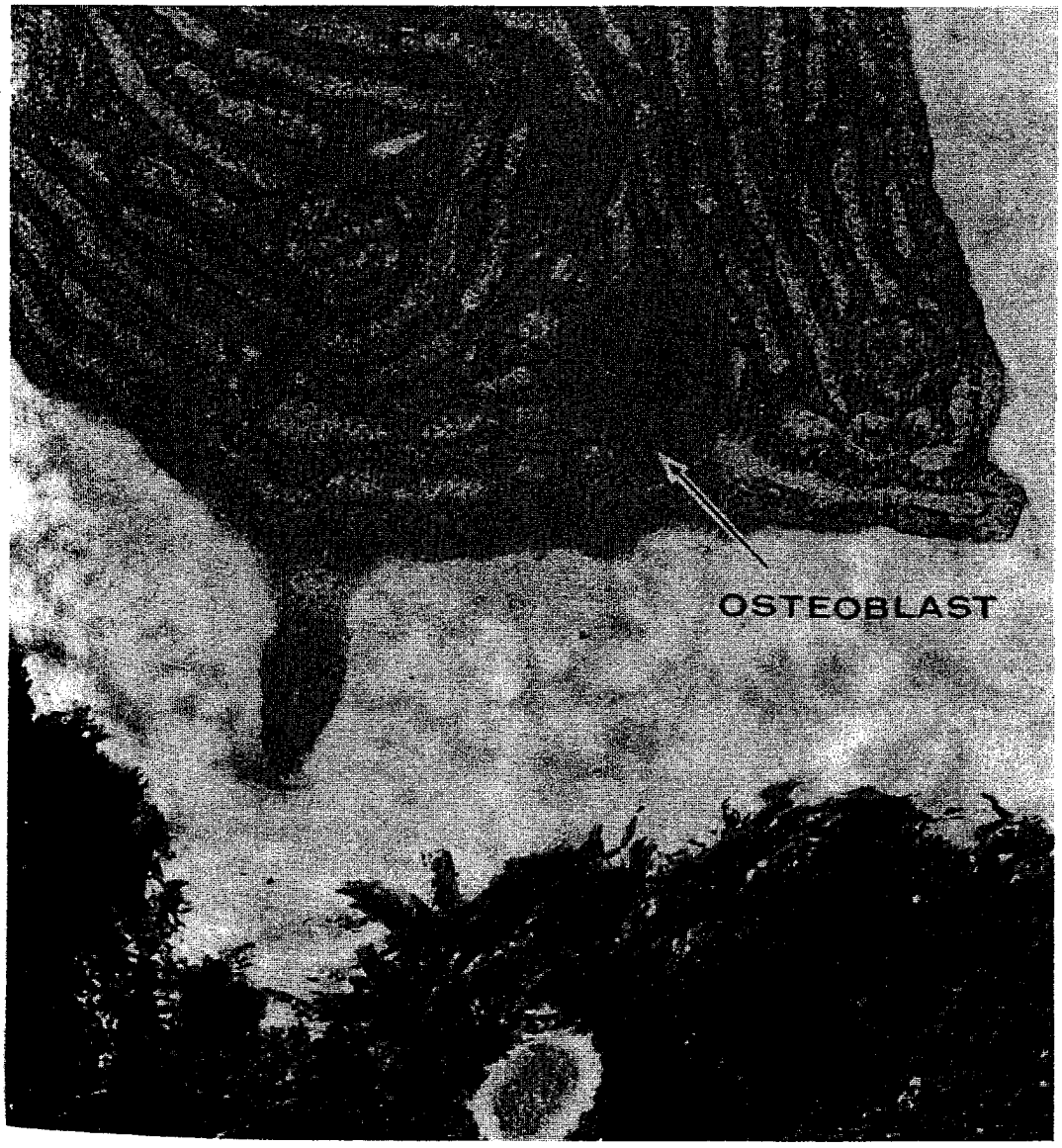

FIGURE 2.11. Electron photomicrograph of osteoblast forming bone. Dark material at bottom is mineralized bone. The lighter, interposed material is osteoid, production of which necessitates the extensive rough endoplasmic reticulum in the cell. A portion of a process protrudes from the cell; another is seen in cross section in the bone matrix. (From Reddi and Anderson, 1976, by copyright permission of The Rockefeller University Press.)

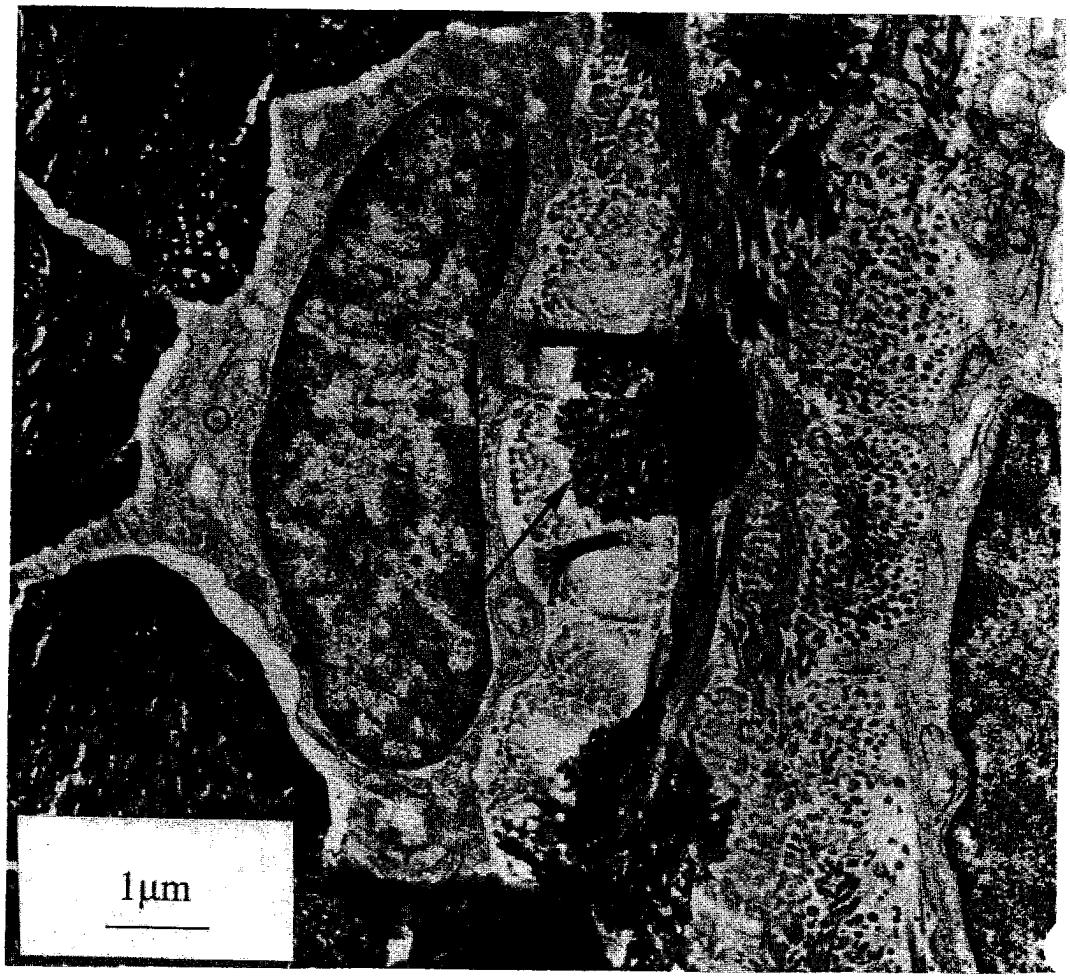

FIGURE 2.12. Electron photomicrograph of osteoblast forming bone (*far right*). It is burying another osteoblast (*O, left center*), which will become an osteocyte. Note cell processes at *far left* and collagen fibers both parallel and normal to the page. *Black material* is mineral; *arrow* indicates patch of new mineral in osteoid between the cells. (Reproduced with permission from Cooper et al., 1966.)

(below). Osteoid contains collagen and noncollagenous proteins, proteoglycans, and water. Much of the water is replaced by mineral as the osteoid calcifies. The boundary between osteoid and calcified bone is called the *mineralization* or *calcification front*. Collagen precursors and the other organic matrix molecules are produced in the osteoblast's rough endoplasmic reticulum. Also shown is a *cell process* extending from the osteoblast into the bone that it has helped produce. This process connects to an *osteocyte* within the calcified matrix, as described next.

Osteoblasts are differentiated from cells known as *mesenchymal cells*. Depending on where the bone formation occurs, these mesenchymal cells come from the deep or *cambium* layer of the *periosteal membrane* or from the stromal tissue of bone marrow. Differentiation of mesenchymal cells into osteoblasts is a multistage process requiring 2–3 days an

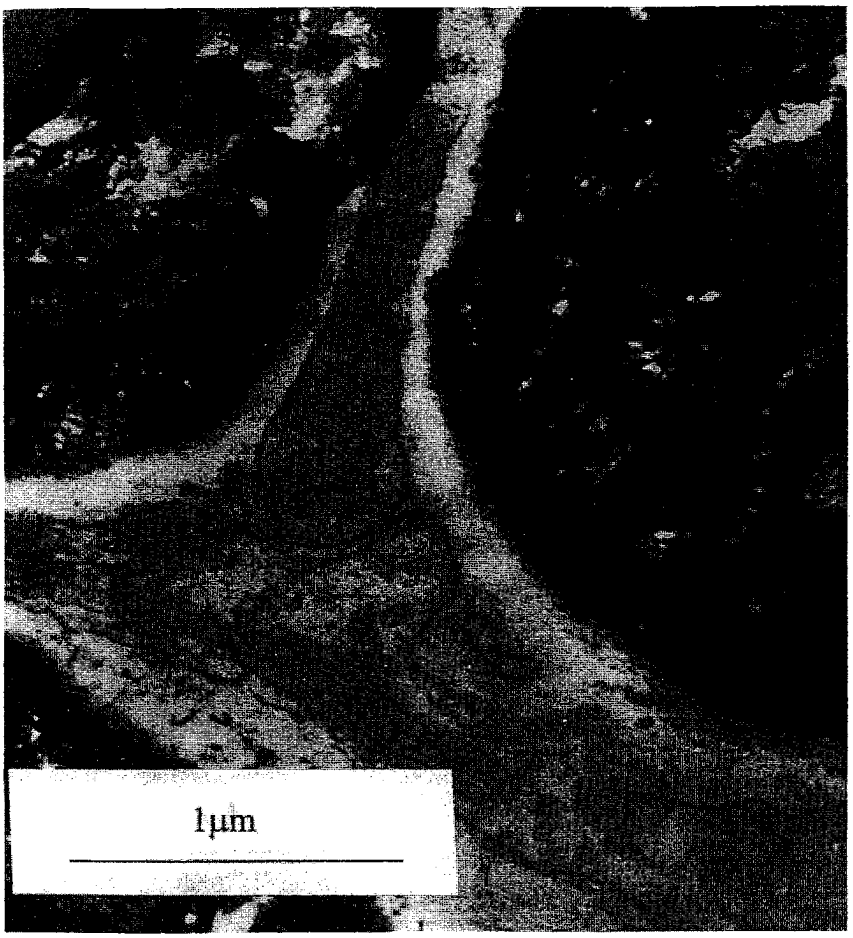

FIGURE 2.13. Electron photomicrograph shows junction between two osteocyte processes. (Reproduced with permission from Cooper et al., 1966.)

appears to be triggered by mechanical stress to the tissue. Osteoblasts lay down osteoid at a rate of about 1 μm/day. This is called the bone *apposition rate*.

Osteocytes are former osteoblasts that have become buried in the bone which they and their neighbors have made. Osteocytes sit in cavities called *lacunae* and communicate with each other and with osteoblasts via processes passing through tunnels called *canaliculi* ("little canals"). Figure 2.12 shows a recently buried osteocyte and one of the osteoblasts that helped bury it. The black regions are calcified bone. The "paths" through these regions indicate the location of canaliculi containing intercellular processes. As shown in Fig. 2.13, processes from adjoining cells are connected by *gap junctions*, implying that these cells communicate and exchange substances to a significant degree. Figure 2.14 shows numerous osteocytes and their network of processes at a much lower magnification. There are about 15,000 lacunae per cubic millimeter (mm^3) of bone (Mullender et al., 1996), but because of their small size, the lacunae and canaliculi occupy only about 1% of the bone volume. Their surface area, on the other hand, is huge. Johnson (1966) estimat-

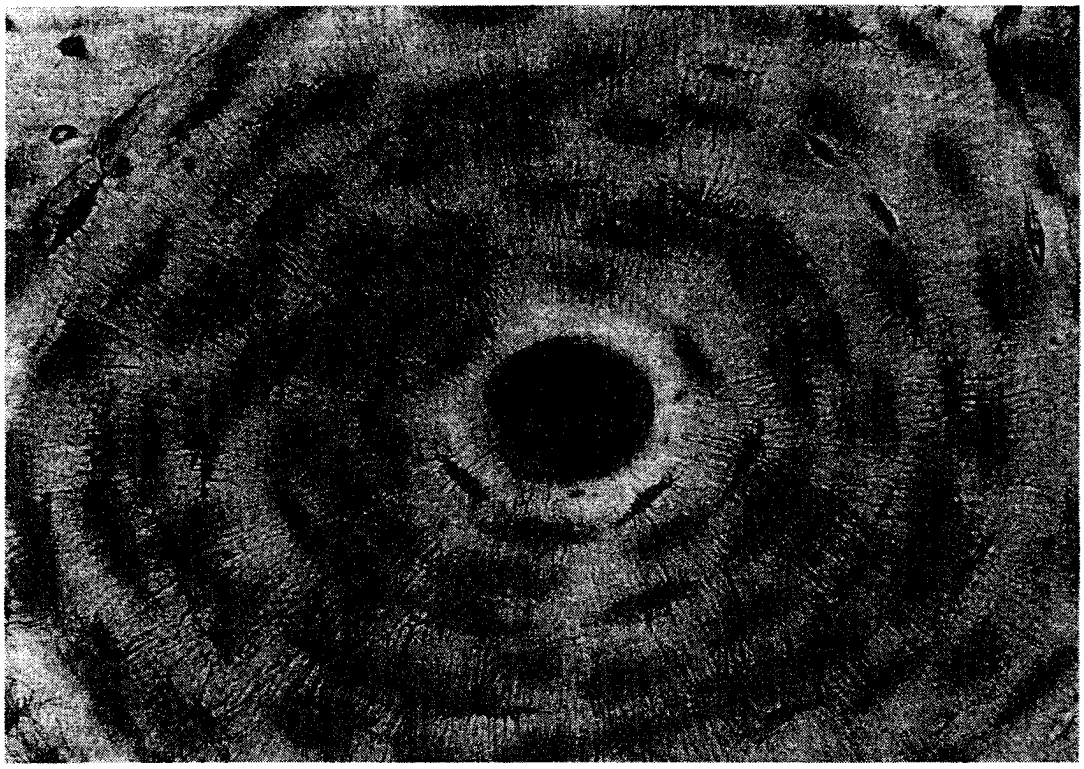

FIGURE 2.14. Photomicrograph shows the network of osteocyte lacunae (*dark ellipses*) and canaliculi (*fine dark lines* radiating from lacunae) within an osteon in basic fuchsin-stained, undemineralized cross section of equine third metacarpus. Field width, ~200 μm.

ed the total skeletal surface area of canaliculi in an adult male skeleton to be 1200 m^2, compared to 3.2 m^2 for Haversian and Volkmann's canals and 9 m^2 for cancellous bone surfaces. This intimate contact with virtually every nook and cranny of the skeleton is one reason that osteocytes are thought to be important in transporting mineral into and out of bone, and perhaps in sensing mechanical stress. However, their physiologic functions and significance are as yet poorly understood.

Bone lining cells are, like osteocytes, "retired" (quiescent) osteoblasts. These are the osteoblasts that escaped being buried in newly formed bone and remained on the surface when bone formation ceased. As production of bone matrix stops, bone lining cells become quiescent and flattened against the bone surface, but they do not form a continuous, gapfree barrier over the bone. They maintain communication with osteocytes and each other via gap-junctioned processes, and also appear to maintain their receptors for parathyroid hormone, estrogen, and other chemical messengers. Like osteocytes, they are thought to be responsible for transfers of mineral into and out of bone (Parfitt, 1987) and for sensing mechanical strain. They are also believed to initiate bone remodeling in response to various chemical and mechanical stimuli (Miller and Jee, 1992).

Box 2.1 Technical Note
Bone Histology

Microscopic examination of biologic tissues requires that they be infiltrated and embedded in a solid medium to retain proper shape and cellular anatomy during subsequent sectioning. Paraffin, a waxy mixture of hydrocarbons commonly used to manufacture candles, has been the traditional medium of choice because of its low melting point and its miscibility with other organic compounds used to dehydrate and fix the tissue. Manufacturers now add plastic polymers and dimethyl sulfoxide to the paraffin to improve its infiltration and cutting characteristics. In the histology lab, small pieces of tissue, usually about a cubic centimeter in size, are immersed in a mold containing melted paraffin that is allowed to solidify. The embedded "tissue block" is then mounted in a microtome and thin sections (approximately 5 μm thick) are cut, mounted on glass slides, and examined under light microscopy. Skilled histologists can cut and mount several ribbons of serial sections an hour; what they cut is essentially a block of soft wax with cellular and extracellular material of similar softness suspended within.

Because of their hardness, bones and teeth present special problems. Obviously, processing these tissues in the standard fashion would result in broken microtome blades, shabby sections, and haggard histologists. To circumvent the problem, diagnostic histology laboratories first demineralize the sample to make it soft enough for processing using standard embedding and sectioning techniques. Methods of decalcification (the traditional name despite the fact that phosphorous is being liberated along with the calcium) have been in use since the turn of the century, and usually employ solutions of acid or chelating agents to solubilize and extract the mineral. Acetic, chromic, formic, hydrochloric, and nitric acid have all been used successfully; ethylenediaminetetraacetic acid (EDTA) is the current chelating agent of choice. Although such methods make paraffin embedding possible and are adequate for routine examinations of the cellular elements in tumors and marrow, they do not render the complete microscopic picture, particularly in terms of bone as a dynamically changing structural tissue. To learn about bone as a tissue, researchers in the field usually use intact mineralized sections that require specialized equipment and procedures to process.

Before the advent of plastics in the 1930s, the only way to prepare acceptable mineralized sections of bone was by grinding down nonembedded rough-cut sections, most often by hand. Wet sandpaper was often used to prepare sections, especially thick sections of 100 μm or more; Frost's seminal discoveries were made using this method. For thinner sections, the specimen was sometimes sandwiched and ground with abrasive powder between two glass plates. Surprisingly beautiful histologic specimens only 5 μm thick can be produced in this way, however the technique is time consuming, tedious, and runs the risk of destroying trabecular architecture. Things changed for the better when ethyl and methyl methacrylates were introduced as embedding media around 1940. Much like paraffin embedding, samples of bone could be immersed and infiltrated with solutions of liquid media that were then polymerized. These hard plastics were a much closer match to the material properties of bone and thin sections (<10 μm) could be cut directly from the tissue blocks using specialized heavy-duty microtomes, or thick sections (100 μm) could be prepared without grinding or damage using a diamond wafering saw. Several new polymers and resins are available today for embedding and processing hard mineralized tissue.

In the later half of the twentieth century, examinations of undecalcified (and frequently unembedded) histologic sections of bone have elucidated the cellular machinery responsible for normal bone growth and repair. Histologic manifestations of metabolic bone diseases have been well defined. Dr. Frost and his colleagues developed staining and analysis techniques for mineralized sections of bone that enabled clear identification of pertinent cells, endosteum and periosteum, osteoid, cement lines, and canaliculi, as well as patterns of mineralization. These methods, combined with fluorochrome labeling (see Box 3.2), have given us insight into the complex cellular activity responsible for maintaining the mechanical integrity of our skeletons throughout life.

2.6 Cartilage

Like bone, cartilage comes in different forms, each suited to a particular application. For the sake of simplicity, we define three types of cartilage.

Hyaline cartilage is the most prevalent type of cartilage in the adult. It is found in the ventral ends of ribs, in the tracheal rings, and covering the joint surfaces of bones, where it is known as *articular cartilage*. In addition, the growth plates are composed of this cartilage (the growth plate is described in Section 2.7).

Elastic cartilage is the variety of cartilage found in the external ear, eustachian tubes, and epiglottis. It has greater opacity, flexibility, and elasticity than hyaline cartilage, and is yellowish rather than white. Its extracellular matrix is permeated with dense, branching elastic fibers unique to this type of cartilage.

Fibrocartilage is the type of cartilage occurring in intervertebral disks, the pubic symphysis, and in the bony attachments of certain tendons. It also may form when hyaline cartilage is damaged.

Composition of Cartilage

Articular cartilage is most often hyaline, although some joints contain fibrocartilaginous disks (e.g., the menisci of the human knee). The extracellular matrix of articular cartilage is composed mostly of type II collagen and proteoglycans manufactured by cartilage cells (called *chondrocytes*), and then assembled outside the cell into a mesh of collagen interwoven with aggregated proteoglycan molecules called *aggrecan*. Like bone cells, chondrocytes live in chambers called lacunae. Sometimes single or multiple chondrocytes are found in "bags" within the matrix called *chondrons*.

Unlike bone cells, chondrocytes are encapsulated not in mineralized bone but in a viscoelastic extracellular matrix of collagen fibers and proteoglycan molecules. Although they are active metabolically, producing collagen, proteoglycans, and proteolytic enzymes, there is no direct cell–cell contact, nor do these cells have processes to help them communicate with

each other. The mechanism for intercellular signal transduction, and how chondrocytes coordinate their activities, if they do, are unknown.

Cartilage matrix is ordinarily not mineralized and is about 70% (by mass) water. Approximately 40%–70% of the dry weight of the matrix is collagen and 15%–40% is proteoglycans. Chondrocytes make up less than 5% of the volume of articular cartilage.

Type II collagen is the predominant species (about 80%) of collagen found in articular cartilage, but several other types are also present (Table 2.2). Type XI collagen forms a core for the type II collagen fibril, and probably controls fibril growth. Type IX collagen, which is really a form of proteoglycan molecule, is found periodically along the type II collagen fibril and is covalently cross-linked to it. It is probably important to the structural integrity of the collagen fibril assembly, and it has been suggested that disruption of the type IX–type II bond may be one factor leading to the degeneration of articular cartilage and a condition called *osteoarthrosis.*[1]

Type VI collagen is found in small amounts in the pericellular region of the chondrocytes. Although its function is not precisely understood, it is thought to be an adhesion molecule that brings stability to the cell–extracellular matrix system. Type X collagen is found in small amounts within type II collagen fibers. However, it is primarily found forming a network around hypertrophic chondrocytes in the growth plate, where it is probably involved in controlling mineralization of the cartilage matrix. Types XII and XIV have also been recently discovered in cartilage, and may provide cohesion between the extrafibrillar matrix and collagen fibers.

Proteoglycans in aggregate form are the other primary polymer in the matrix of cartilage. These are giant molecules composed of a central hyaluronic acid chain to which are attached (by *link proteins*) side branches called *core proteins*. These core proteins are in turn the attachment points for *glycosaminoglycans* (GAGs) (Fig. 2.15). The GAG chains consist

TABLE 2.2. Types of collagen in articular cartilage and their functions

Collagen type	Function
II	Structural and mechanical; primary constituent of articular cartilage
VI	Pericellular adhesion molecule
IX	Fibril association; stabilizes type II
X	Hypertrophic zone of growth plate; role in calcification postulated
XI	Core of type II; controls fibril growth

[1]Osteoarthrosis is a term applied to joint degeneration that has a mechanical rather than an inflammatory cause. This condition is more commonly referred to as osteoarthritis, even though the "itis" in that term implies that inflammation is the initiating cause.

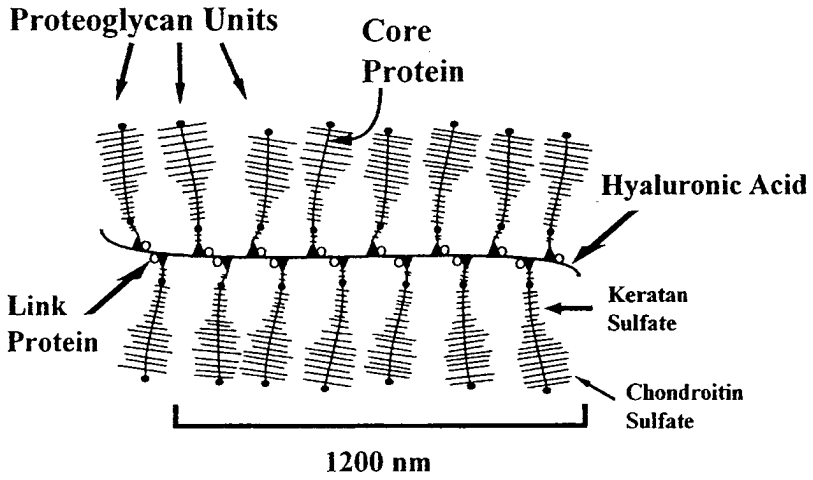

FIGURE 2.15. Diagram of proteoglycan aggregate structure.

of repeating disaccharide units in which a sugar is linked to a hexosamine. Chondroitin 4-sulfate, chondroitin 6-sulfate, and keratin sulfate, all of which carry a negative charge, are the three predominant GAGs found in cartilage. These molecules are covalently bound to the core protein to form the proteoglycan monomer which, when linked to the hyaluronic acid molecule, become the proteoglycan aggregate known as *aggrecan*.

Several smaller species of proteoglycan are also found in articular cartilage. *Decorin* "decorates" the collagen fibrils and apparently controls fibril assembly. It probably functions to reduce fibril diameter, particularly close to the articular surface, where it is found in greater abundance. *Fibromodulin* is found in increasing amounts in the cartilage matrix away from the chondrocyte, and seems to regulate fibril assembly. Other proteoglycans (lumican, epiphycan, versican, neurocan, and perlecan) are found in varying amounts in different kinds of cartilage.

Mechanical Significance of Cartilage

Articular cartilage has unique chemical properties allowing it to serve as a bearing surface. It is able to transfer loads from one bone to another while simultaneously allowing the load-bearing surfaces to articulate (i.e., roll and slide over one another) with very low friction. This behavior is studied in detail in Chapter 7. Here, however, we may ask, "How can a material that is 70% water and not mineralized support loads that are several times body weight?" The answer to this question lies in the proteoglycan portion of the cartilage matrix. Specifically, the proteoglycan aggregate *aggrecan* serves this function. The proteoglycan monomers attached to the hyaluronic acid backbone are all highly negatively charged because of the chemical structure of the sulfated GAGs that compose them. This charge causes the aggrecan molecules to repel one another and branch out into something with the appearance of a bottlebrush. It also causes the aggrecan to be very hydrophilic. This attraction for water molecules

resists the tendency for water to flow out of the cartilage when it is compressed. As the "bristles" of the brush restrain the water, they are placed in tension, and when the compressive load on the cartilage is removed the aggrecan molecules reattract water to replace whatever has managed to escape. The large size and negative charge of the aggrecan molecule give cartilage its hydrophilic properties. Thus, compressive loads are borne by a volume of water that cannot escape, rather like a rubber hot water bottle filled with water. In this case, however, the water is constrained not by an outside membrane but by an internal structure that electrically confines the water molecules.

Organization of Articular Cartilage

Collagen and proteoglycans are dispersed differently through the thickness of the articular cartilage, with more collagen toward the joint surface and more proteoglycan in the deeper layers of cartilage matrix. This arrangement works well mechanically. Large tensile stresses within the articular surfaces and at the edges of joint contact areas are resisted primarily by tangentially oriented collagen fibers. The compressive and hydrostatic stresses found in the deeper layers of cartilage are resisted by the incompressibility of water, which is held in place by the hydrophilic aggrecan molecules.

The matrix of collagen fibers in cartilage is not organized randomly. Instead, the structure varies from place to place in response to functional demands, some of which are mechanical (Fig. 2.16). The surface layer, the *lamina splendens*, consists of closely packed, small diameter (~30-nm) fibrils, tangentially arranged. The lamina splendens acts as a barrier membrane to enzymes and large molecules such as hyaluronic acid. Thus, small molecules like glucose and hemoglobin in the synovial fluid can penetrate the lamina to nourish the chondrocytes deeper in the cartilage, but hyaluronic acid, the large, lubricating molecule, remains on the surface of the cartilage. Mechanically, the horizontal fiber arrangement allows the lamina splendens to resist the high tensile and

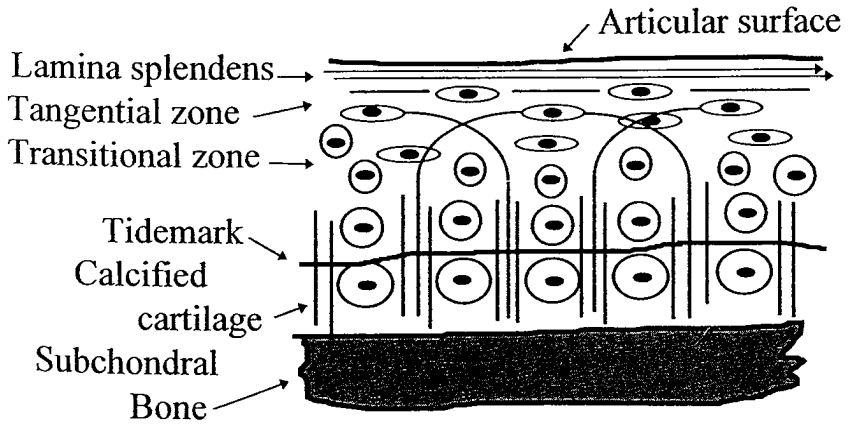

FIGURE 2.16. Sketch of articular cartilage structure.

shear forces in and around the joint contact area. However, with age, gaps appear in the lamina, allowing synovial fluid to carry in enzymes (e.g., collagenase or hyaluronidase) that can break down the cartilage matrix.

The lamina splendens is only a few micrometers thick and merges gradually into the *tangential zone* in which the collagen fibrils are slightly larger, although still arranged parallel to the articular surface. This layer is more cellular than the lamina, containing small, flattened chondrocytes. The tangential zone also contributes to the tensile stiffness of the cartilage matrix.

The *transitional zone* (or radial zone) contains collagen fibers that are larger than those in the tangential zone and also are more widely spaced and obliquely or pseudorandomly arranged. The chondrocytes tend to line up in radial columns (i.e., perpendicular to the surface), particularly in the deeper layers of this zone. These chondrocyte columns are parallel to the surrounding collagen fibers, and the chondrocytes within them actively synthesize structural molecules that replenish the cartilage matrix.

This zone ends at the *tidemark*, beyond which the cartilage has calcified with hydroxyapatite—the same mineral as found in bone. The calcified cartilage layer may serve to make the change in elastic modulus between articular cartilage and bone more gradual. In addition, collagen fibers from the transitional zone seem to cross the tidemark and reinforce against shear between stiff (calcified) and compliant (uncalcified, articular) cartilage layers.

For many years, investigators have used a simple method to visualize the orientation of collagen fibers in articular cartilage: simply sticking a pin into the cartilage surface at multiple points over the surface of a joint reveals the pattern because the material splits parallel to the fiber direction. Figure 2.17 illustrates the articular surface at the distal end of the human knee after this has been done.

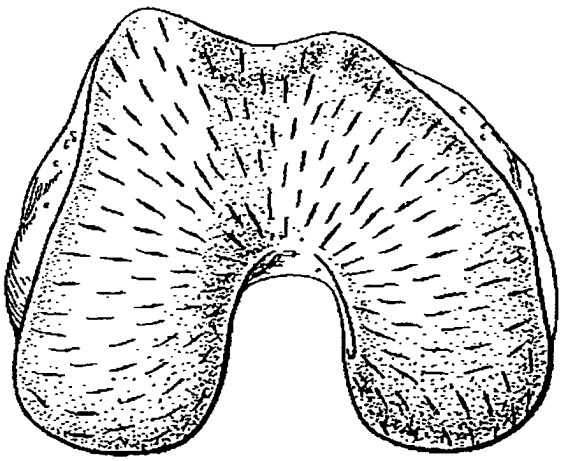

FIGURE 2.17. "Split line map" of the articular surface on the distal end of the human femur.

Noncalcified articular cartilage has no nerves and no blood vessels (calcified cartilage has both). The chondrocytes are nourished by diffusion through the extracellular matrix, which is largely water. This passive transport of nutrients may be aided by flows induced by mechanical loading, but the dependence on passive transport rather than a blood supply limits the capacity of cartilage to repair large defects. Thus, although chondrocytes are constantly resupplying the extracellular matrix with collagen and proteoglycans as it degrades, the capacity to repair large amounts of damage is apparently absent.

The Role of Cartilage in Growth

Suppose that you were given the job of designing the mechanism by which a child's bones are to grow longer. The bones must do this while the child is active and loading them. The simplest solution might be to put osteoblasts on the ends of each bone, and let them form a layer of new bone there each day. The obvious problem with that solution is that the forces and motions between the bones would crush and grind the osteoblasts to bits. An alternative would be to put the osteoblasts inside the bone and let them "push out" new bone matrix, expanding the volume from within. Some early bone scientists thought this was how longitudinal growth worked, but eventually it became clear that the mineralized bone matrix is too rigid for this to happen. Bone can only be formed by cells on a bone surface because cells can only extrude new matrix into an unmineralized, "soft tissue" space. But how can such a soft tissue support loads during growth?

The solution to this dilemma is cartilage. Because it is largely water, cells can "push out" new matrix, expanding its volume, yet it is a tissue that can support very high loads **if they are primarily compressive**. As is described in Chapters 6 and 7, there is evidence that nature has arranged a sort of pact between cartilage and bone such that the former handles stresses that are mainly compressive (hydrostatic) and the latter shear (deviatoric) stresses.

This remarkable capacity for growth of a load-bearing material makes cartilage especially suitable as a skeletal substance during development. Indeed, most of the skeleton is first formed in cartilage models, which later are replaced by bone. The occurrence of cartilage is more restricted in postfetal life, but it continues to play an indispensable role in the longitudinal growth of children's bones and in the maintenance of articular surfaces in adults.

2.7 Longitudinal Growth of Bones

Longitudinal growth of bones occurs in a nonmineralized region of growth near, but not at, each end of the bone. This region is called the *growth plate* or *physis*. The physis separates the bony *epiphysis* from the bony metaph-

ysis. New cartilage is constantly formed by chondrocytes within the growth plate. It turns out that cartilage is viscous enough that cells within it can extrude more cartilage matrix, increasing its volume. This would increase its thickness except that the cartilage on the metaphyseal side of the growth plate mineralizes and becomes part of the metaphyseal bone, so that the length of the metaphyseal and diaphyseal parts of the bone is increased, while the thickness of the growth plate remains constant.

The growth plate may be divided into zones, each representing a stage in the life cycle of its chondrocytes (Fig. 2.18). (The number of these zones can vary, depending on the reference consulted.) In the following description, you can imagine that you are traveling down through these zones and into the metaphysis at a given point in time, leaving the upper surface of the growth plate behind. Alternatively, you can imagine that you are sitting at a point which is fixed in relation to the bone as a whole, and watching your surroundings change as time passes, so that the top of the growth plate leaves you behind.[2] Forming a clear mental image of this relativity between time and position will help you understand the process by which bones grow longitudinally. The growth plate's zones are, from the top (i.e., the epiphyseal side), as follow.

1. **Reserve or resting zone:** Cells of moderate size are scattered irregularly throughout this zone, which is anchored to the bone of the epiphysis and receives nourishment from epiphyseal blood vessels. These cells are not chondrocytes and are not really "resting," but are dividing slowly to provide chondrocytes for the remainder of the growth plate. Such cells are often termed *stem cells*. When they divide, one of the resulting pair remains a stem cell, and the other differentiates into a cell with a different function, in this case a chondrocyte.
2. **Proliferative zone:** Here, the chondrocytes divide repeatedly and arrange themselves in columns. They also become disklike, so that they look somewhat like a stack of coins. They produce proteoglycan and type II collagen and other molecules needed for the surrounding extracellular matrix. The collagen fibers are aligned parallel to the cell columns.
3. **Hypertrophic zone:** As they stop proliferating, the chondrocytes are said to "mature." They accumulate glycogen in their cytoplasm and secrete copious amounts of matrix, increasing the volume of the surrounding substance. They also *hypertrophy*; they increase their intracellular volume. Because the growth plate is radially constrained by bone around its perimeter, its volume changes are expressed primarily in the longitudinal direction. It is thought that the chondrocytes in this zone are in the early stages of *apoptosis* (programmed cell death). They stop producing cartilage matrix and begin producing molecules that prepare the adjacent cartilage for calcification. They also increase in size.

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[2]Recall Einstein's famous question, "Does Zurich stop at this train?"

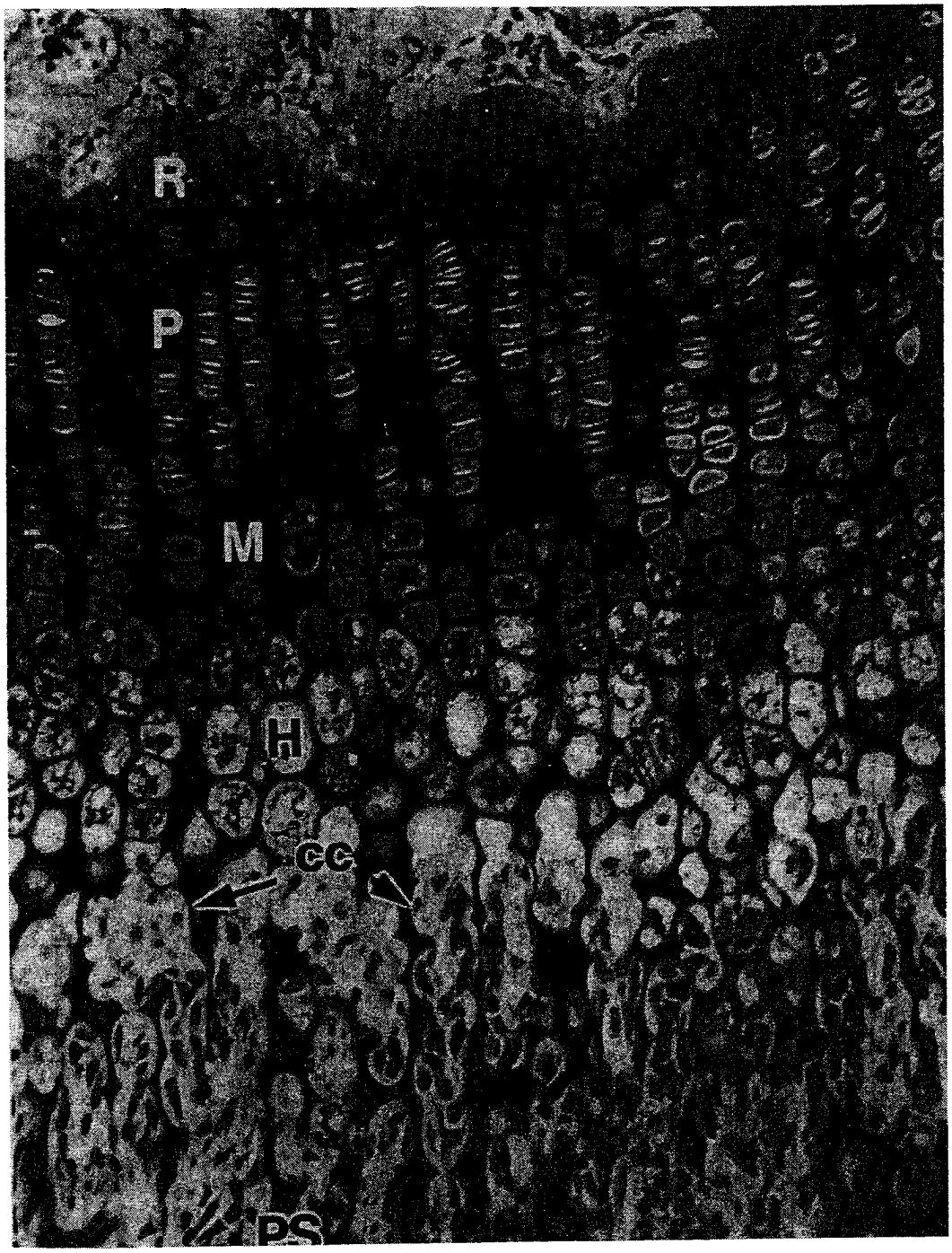

FIGURE 2.18. Zones of the growth plate: *R*, resting; *P*, proliferating; *M*, maturing; *H*, hypertrophying chondrocytes; *PS*, primary spongiosa of the metaphysis. Calcified cartilage cores in the newly formed trabeculae are labeled *cc*. (from Jee, 1988.)

4. **Zone of provisional calcification:** In this zone the degenerating chondrocytes continue to hypertrophy. As they reach the limit of this zone, where the growth plate ends and the metaphysis begins, the chondrocytes die. Simultaneously, the surrounding cartilage matrix is calcified.

The calcification mechanism is imperfectly understood, but a working hypothesis for this process is as follows. The hypertrophying chrondrocytes synthesize type X collagen and a protein called *chondrocalcin*, which may help initiate calcification. Proteoglycans may also be disassembled by enzymes released by the degenerating cells. Mineral crystal formation commences within *matrix vesicles*, which are intact spherical membranes extruded from the hypertrophic chondrocytes. These vesicles contain the enzyme ATPase, which provides energy to transport calcium ions into the vesicle against a concentration gradient. In addition, other enzymes in the vesicles cleave phosphate and calcium from compounds to which they are bound, allowing them to form crystals. The initiation of apatite crystal growth is the most important function of the matrix vesicle. As the levels of calcium and phosphate increase, amorphous calcium–phosphate aggregates form, eventually spreading beyond the vesicle wall and accumulating more mineral by *epitaxy* (crystal growth that imitates the form of the substrate). At this point, apatite crystals begin to grow in such a fashion that they are integrated with the collagen molecules in the matrix, and water is displaced as the cartilage matrix mineralizes.

Development of Metaphyseal Trabeculae

The columns of enlarged lacunae left by the dead chondrocytes form the basis for tunnels, allowing blood vessels and cells from the metaphysis to gain access to the calcified cartilage at the bottom of the growth plate. As each chondrocyte hypertrophies, it dissolves some of the cartilage around it, so that only narrow walls, or *septa*, are left between the enlarged lacunae. Cells called *septoclasts*, another monocyte descendant, but perhaps not identical to osteoclasts, resorb the *septa* at the bottom of each chondrocyte column (Lee et al., 1995). Blood vessels from the metaphysis then invade these tunnel-like spaces, which are separated by columns or "trabeculae" of calcified cartilage. Other osteoclast-like cells (called *chondroclasts* by some authors) resorb some of the calcified cartilage on the walls of the tunnels. Osteoblasts then lay down small amounts of bone on these calcified cartilage trabeculae. This remodeling activity begins to convert calcified cartilage to bone and marks the transition from growth plate to metaphyseal "bone." At this early stage in the formation of spongy bone the tissue is called *primary spongiosa*; it still contains considerable calcified cartilage within the trabecular cores. As time goes by, and remodeling continues to replace the cartilaginous portions of the trabeculae with the metaphyseal bone, the trabecular bone becomes the *secondary spongiosa*. Eventually, the trabeculae become entirely bone. Those trabeculae in the center of the metaphysis are eventually entirely resorbed to form the medullary canal, with an arch of trabecular struts remaining to transmit loads from the central portion of the growth plate out to the cortices of the diaphysis.

Growth of the Physis

In the newborn, the physis is typically a flat, more or less circular plate. As the child grows and the bones become larger, the physis must increase in diameter. This increase is achieved by cell division at its circumference in a region called the *zone of Ranvier.* In addition, the physis loses its flat shape and becomes curved, often with a complex system of ridges, valleys, and lappets. The physis, being composed of cartilage, is weak in shear and tension, and physeal injuries in children are relatively common; the epiphysis of the proximal femur sliding off the shaft at the physis (*slipped capital femoral epiphysis*) is a well-known example. One consequence of the development of an irregularly shaped contour to the physis in the growing child is that the complex interlocking geometry of the epiphysis and metaphysis confers some protection against failure of the physis in response to shearing forces.

Most long bones have two physes, one at each end (although some bones, such as the metacarpals and phalanges, have only one functional physis). Usually one of these contributes more than the other to longitudinal bone growth. For example, in the femur the distal physis contributes more to growth, whereas in the tibia the proximal physis is more active. Historically, the relative growth rates of the two physes of a long bone have been measured by comparing bones of different age with respect to some naturally occurring marker in the diaphysis, usually the entrance point of the nutrient artery.

Closure of the Physes

As a child matures, bones reach their adult length and the physis is no longer required. At this point, the physes "close" by ossification, connecting the epiphysis to the metaphysis with bone. The blood circulatory systems of the epiphysis and metaphysis, formerly independent, also unite. This region of bone remodels over many years, but the physeal "scar" or "ghost" (a plate of bone at the site of the former physis) may persist into old age.

Although we generally consider the role of the physis to be longitudinal growth of bones, its ability to *stop* longitudinal growth by closure is also important. In animals that lack an ossified epiphysis (many reptiles, e.g., crocodiles), there is no physeal closure and the bones continue to increase in length indefinitely, so that great longevity becomes associated with great size.

Closure of the various physes occurs in a specific sequence, and by examining a radiograph (usually of the left hand and wrist) one can determine the *skeletal age* of an individual by observing which physes have closed. Skeletal age is not the same as chronological age; the growth plates close earlier in some individuals than others. In girls, physes close several years earlier than in boys; this contributes to the shorter average stature of women compared to men.

2.8 Modeling and Remodeling of Bone

We have just described the way in which most bones grow in length. This kind of bone formation, in which cartilage is formed first, calcified, and then replaced by bone, is known as *endochondral ossification*. Another mode of bone formation, in which osteoblasts make bone directly, is called *intramembranous ossification*. So-called "flat bones" (skull, scapulae, pelvis) are formed in this way. The word "intramembraneous" refers to the fact that in the embryo, this kind of ossification occurs adjacent to membrane-like layers of mesenchymal cells that differentiate into osteoblasts. The same sort of direct formation of bone by osteoblasts occurs in periosteal modeling, where it is still called "intramembraneous bone formation" because the osteoblasts and their precursor cells are found in the deep layer of the periosteal membrane. If the osteoblasts are not part of a membrane (i.e., if they are on an endosteal, trabecular, or Haversian canal surface), the bone formation is said to be *appositional* rather than intramembraneous.

Modeling Vs. Remodeling

As the long bones develop, their shafts must grow in diameter as well as length. We have seen how growth in length throughout childhood is accomplished by continuation of endochondral ossification in the growth plate. Growth in diameter is accomplished by periosteal intramembranous ossification. However, as growth occurs, it is not enough just to add material to make the bone organs longer and larger. The bones must also be shaped in various ways. That means that bone must be **removed in some places while it is added in others**. This sculpting, involving osteoclastic activity (bone resorption) in some places and osteoblastic activity (bone formation) in others, has become known as *modeling*. In addition, fatigue damage must be repaired inside bones and their internal architecture must be adjusted to varying load conditions, both while the skeleton is developing and throughout the remainder of one's life. This repair involves removal and replacement of bone in particular places by the coupled actions of osteoclasts and osteoblasts working at the same site. This replacement process has become known as *remodeling*.[3]

Thus, modeling and remodeling refer to the actions of osteoblasts and osteoclasts in reshaping and replacing portions of the skeleton. They are distinguished from one another in several ways:

1. Modeling involves independent actions of osteoclasts and osteoblasts. Remodeling involves sequential, "coupled" actions by the two types of cells.

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[3]These terms were coined by Frost. Be aware, however, that many orthopaedists and bone scientists refer to both modeling and remodeling activities, as defined here, as "remodeling."

2. Modeling results in change of the boneís size, shape, or both; remodeling does not usually affect size and shape.
3. The rate of modeling is greatly reduced after skeletal maturity. Remodeling occurs throughout life, although it too is substantially reduced after growth stops.
4. Modeling at a particular site is continuous and prolonged, whereas remodeling is episodic, with each episode having a definite beginning and ending.

Modeling

Modeling is necessary because the longitudinal growth process does not produce a bone of correct geometry, and because each growing child loads his or her skeleton in a somewhat different way, requiring each skeleton to be "customized." Modeling may be resorptive or formative. Examples of modeling include the following.

Metaphyseal modeling to reduce bone diameter: As some long bones grow longer, the diameter of the cylinder of bone created beneath the growth plate must be reduced to create the diaphysis. This change is accomplished by osteoclasts working continuously on the periosteal surface of the metaphysis to cut the shaft down to size (Fig. 2.19, top). This action is most important in bones with a widely flaring metaphysis such as the proximal tibia; in other bones, diametric growth of the physis is coordinated with longitudinal growth in such a way that removal of bone in the lower metaphysis is unnecessary (e.g., metatarsals).

Diaphyseal modeling to increase bone diameter or alter curvature: As a child continues to grow, long bone shafts become larger in diameter, accomplished by slow, continuous addition of bone to the periosteal surface by osteoblasts. Simultaneously, bone is removed from the endosteal surface (Fig. 2.19, middle). In addition, the curvature of long bones is usually adjusted during growth. This is accomplished by increased formation and resorption on the sides of the bone such that the cross section "drifts" sideways relative to the ends of the bone (see Fig. 2.19, bottom). This phenomenon may increase or decrease bone curvature as required in specific bones. It also serves to correct the curvature resulting from a poorly reduced[4] fracture.

Modeling of a flat bone: As a child grows, the bones of the skull must increase in size to accommodate the increasing size of the brain. This enlargement cannot be accomplished simply by addition of bone at the suture lines between the cranial bones because the plate's radius of cur-

[4]Physicians say that a fractured bone is "reduced" when the fracture surfaces are placed against one another such that the bone fragments are returned to their original positions and orientations.

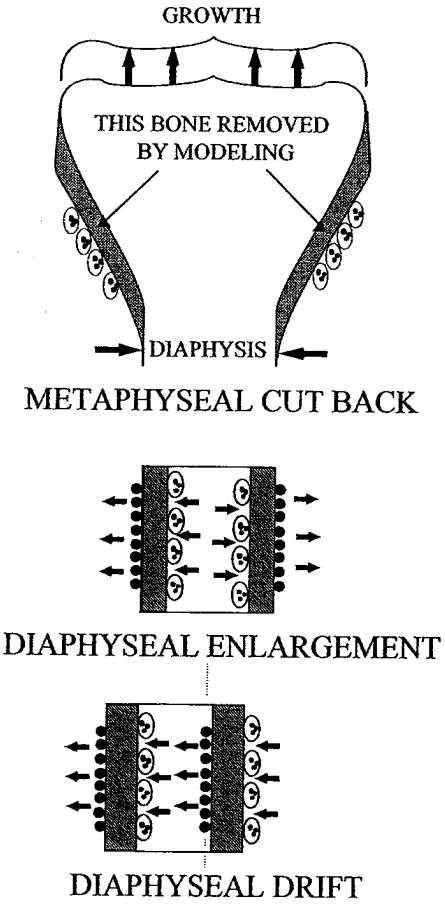


FIGURE 2.19. *Top:* Resorptive modeling beneath the growth plate to form the diaphysis from the metaphysis. *Middle:* Formative (periosteal surface) and resorptive (endosteal surface) modeling to enlarge the diaphysis. *Bottom:* Modeling to "drift" the diaphysis to the left (thereby altering diaphyseal curvature).

vature must also be adjusted. Modeling of these bones (resorption on the inner surface, formation on the outer surface) is required for proportionally correct increases in cranial size. Simultaneously, modeling transforms the facial features of a child into those of an adult.

Remodeling

Remodeling removes a portion of older bone and replaces it with newly formed bone. This process repairs microscopic damage and prevents accumulation of fatigue damage that could lead to fatigue fracture. It has been hypothesized that remodeling also mechanically "fine tunes" the skeleton to increase its mechanical efficiency.

ARF and BMUs

Remodeling is accomplished by teams of osteoclasts and osteoblasts that work together in basic multicellular units, or BMUs. A BMU consists of about 10 osteoclasts and several hundred osteoblasts. There are three principal stages in

a BMU's lifetime: *activation, resorption,* and *formation* (ARF). Activation occurs when a chemical or mechanical signal causes osteoclasts to form (by fusion of monocytes) and begin to remove bone somewhere on or in the skeleton. The osteoclasts resorb a volume of bone in the form of a ditch (on bone surfaces) or tunnel (in compact bone) about 200 μm in diameter that progresses along the surface or through the cortex, moving at about 40 μm/day. After the osteoclasts have passed a particular point, osteoblasts are differentiated from mesenchymal cells over a period of several days and begin to replace the resorbed tissue. Formation is much slower than resorption. The resorption period is about 3 weeks in humans; the formation or refilling period is about 3 months. The total remodeling period is about 4 months.

Osteonal Remodeling

When a BMU tunnels through compact bone, it creates a secondary osteon. Figure 2.20 shows the leading portion of an osteonal BMU in longitudinal section, tunneling to the right. A few osteoclasts may be seen on the resorbing surface. At left, osteoblasts and osteoid line the refilling surfaces. The "head" or front end of a BMU contains a capillary "bud" to supply nutrients, and probably to supply the progenitor cells for osteoclasts and osteoblasts. Because osteonal BMUs become isolated deep within the cortex, this vascular supply must be maintained. Therefore, the tunnel cannot be entirely refilled, and each BMU leaves a new Haversian canal in the bone. Within each Haversian canal are two capillaries: a "supply" and a

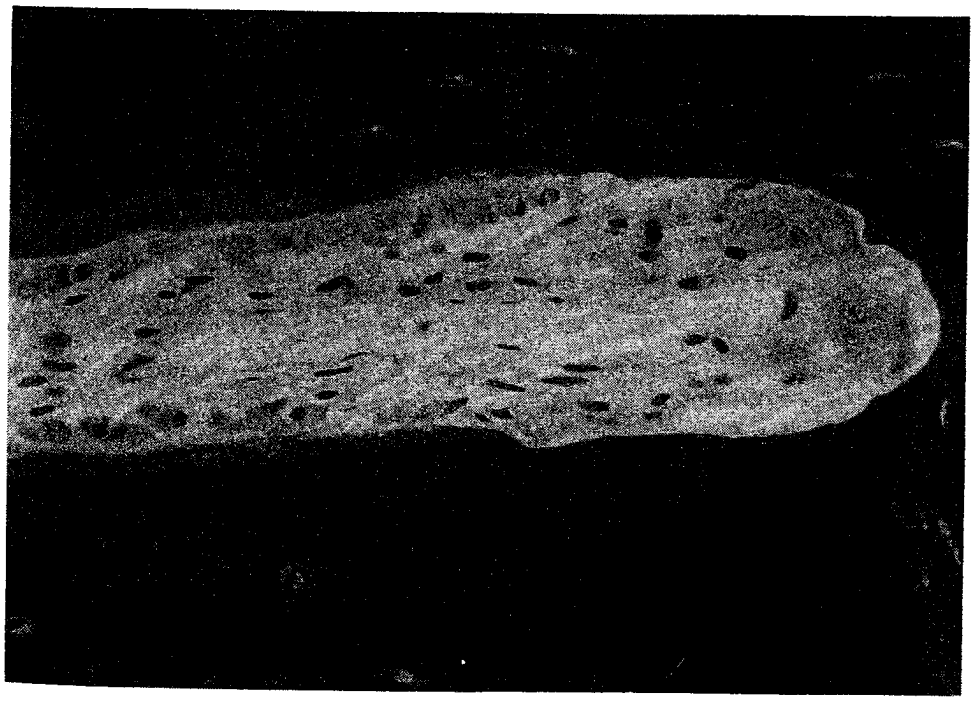


FIGURE 2.20. Photomicrograph of an osteonal basic multicellular unit (BMU). Two multinuclear osteoclasts are visible at *right,* tunneling through the bone; osteoblasts are on bone surfaces to left. Field width, ~1 mm. (Courtesy of Dr. Jenifer Jowsey.)

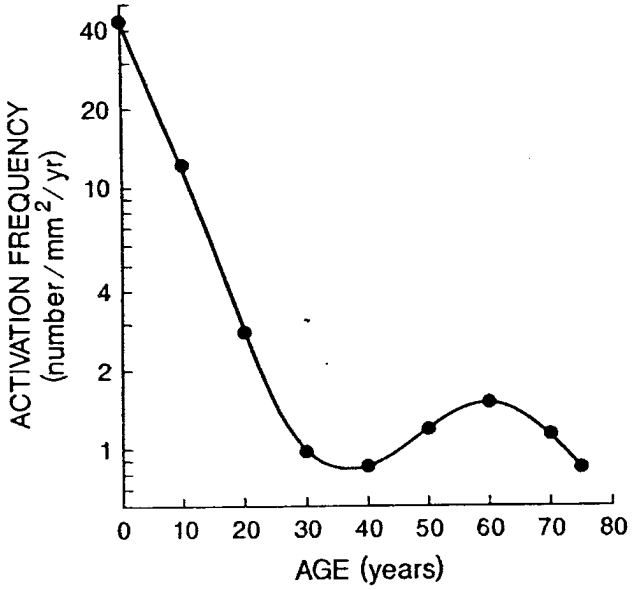


FIGURE 2.21. BMU activation rate vs. age for human ribs. (From data by Frost, 1964b.)

"return" vessel. These vessels connect with the vasculature in the medullary canal or on the periosteal surface.

In human adults, osteonal BMUs replace about 5% of compact bone each year. Figure 2.21 shows how the rate of remodeling changes with age in the human rib. It is very high in children, is reduced in young adults, then rises and falls again in older individuals. (The gender of the individuals in the graph was not reported, but the rise after age 50 probably results from the effects of menopause in women; estrogen inhibits bone remodeling.)

Trabecular Remodeling

Trabeculae are remodeled similarly, except that the BMUs work on their surfaces, digging and refilling trenches. Imagine the lower half of the BMU in Fig. 2.20 moving over the surfaces of the trabeculae seen in Fig. 2.2. (Because a BMU is about the same diameter as a trabecula, it cannot tunnel longitudinally within a trabecular strut without constantly breaking out along its sides.) BMUs replace human adult trabecular bone at a relatively high rate, about 25% each year. In fact, the rate of bone turnover varies widely throughout the skeleton. Table 2.3 demonstrates this using data from various skeletal sites in young adult dogs. It can also be seen that the remodeling rates in dogs are much higher than in humans.

Endosteal and Periosteal Remodeling

In principle, remodeling can also occur on endosteal and periosteal surfaces. Endosteal and periosteal remodeling could be responsible for the observation that long bones expand radially with age in adults as well as children. This expansion would be accomplished by arranging for forma-

TABLE 2.3. Cancellous bone turnover rates in young adult dogs

Site	Turnover rate (%/yr)
Lumbar vertebrae	205
Thoracic vertebrae	167
Cervical vertebrae	121
Mandible	105
Skull	60
Calcaneus	120
Proximal humerus	174
Proximal femur	138
Proximal radius	127
Proximal tibia	112
Carpals	31

After Jee et al., 1991.

tion to exceed resorption on the periosteal surface, and vice versa on the medullary canal surface. However, these effects could be produced more efficiently by modeling, and the issue is unresolved for lack of data.

Bone Structural Units Are Produced by Remodeling

The packet of new bone produced by a BMU is called a *bone structural unit*, whether the remodeling occurs in cortical or trabecular bone or on an endosteal or periosteal surface. Thus, a secondary osteon in cortical bone is a bone structural unit. There is no common name for trabecular bone structural units, although they are sometimes called trabecular osteons or bone packets.

Remodeling and Cartilage

Surfaces covered by cartilage that has not calcified cannot be resorbed by osteoclasts, and cannot serve as a working surface for osteoblasts, so they cannot be modeled or remodeled. The same is considered to be true of surfaces covered by osteoid.

Cellular Events in Modeling and Remodeling

Modeling and remodeling involve some combination of four basic cellular events: migration, mitosis, differentiation, and expression of the mature phenotype (e.g., matrix synthesis by osteoblasts or matrix resorption by osteoclasts). Some of these events are governed by hormones or other chemicals originating outside the skeleton (e.g., parathyroid hormone [PTH] or estrogen). At a local level, most of these complex changes are controlled by proteins called *cytokines* and others

called *growth factors*, which are produced by cells present in and around bone surfaces. For example, cell migration may be directed by *chemotaxis*, in which a cell moves itself up a chemical concentration gradient. Bone matrix itself is known to contain more than a dozen cytokines. Other cytokines affecting bone remodeling are synthesized by bone marrow cells.

Skeletal Envelopes and Senile Bone Loss

There are four *skeletal envelopes*, surfaces on which modeling and remodeling occur: *trabecular*, *endosteal*, *periosteal*, and *Haversian*. The first two are bathed in marrow, and remodeling that occurs on them is usually characterized by an excess of resorption relative to formation. This endotrabecular deficit of bone replacement during remodeling is the reason for normal, age-related bone loss that occurs in men and women after age 30–35. The periosteal envelope experiences formative modeling or remodeling in which resorption generally removes less bone than formation produces. Such processes partially compensate for involutional (i.e., senile) bone loss. The osteonal, or Haversian, envelope also contributes to involutional bone loss. A Haversian canal must be created for each new osteon, increasing the porosity of compact bone until a level of porosity is attained at which each new BMU removes an existing Haversian canal before creating a new one.

The reason why a deficit in formation occurs during remodeling on surfaces adjacent to marrow is unknown. There is, however, an association between a remodeling deficit and the amount of fat in the marrow (Minaire et al., 1984; Martin et al., 1990).

Regional Acceleratory Phenomenon

A sudden transient increase in the rate of remodeling sometimes occurs in one or more bones. This is called a *regional acceleratory phenomenon*, or RAP. The cause is usually trauma to the bone. When this happens, a transient osteoporosis occurs because there is a lag between resorption and formation in each BMU. The osteoporosis will resolve itself over a remodeling period (about 4 months) as the additional BMUs complete their refilling.

2.9 Fracture Healing

Fracture is the most obvious sign that the mechanical functional capacity of the skeleton has been exceeded. It may be argued that much of skeletal physiology is directed at preventing fracture. Even so, fractures are relatively common, and usually occur because of trauma: an accidental over-

load substantially exceeds the normal range of loading to which the bone has adapted during its growth and development.

Pathologic fractures are caused by normal loading of a bone weakened by disease. The most common pathologic fractures are caused by osteoporosis in the elderly. Osteoporotic fractures are more common in women than men. Bone tumors are another important cause of pathologic fractures. The tissue in a bone tumor is variable in its mechanical properties, but it will almost always be different than the surrounding bone and therefore serve as a stress concentrator. Removal of a bone tumor obviously also places the patient at risk for fracture. Predicting and preventing pathologic fractures is an important issue in orthopaedic medicine.

Once a fracture occurs, how does it heal? Today, people seek medical attention for the simplest of fractures, but the basic process of fracture healing obviously evolved as a natural phenomenon. Healed fractures are commonly seen in archaeological skeletons of prehistoric people, and in wild animals. Therefore, fracture healing must be a self-controlling process that ordinarily proceeds on its own to reunite the broken bone. The remainder of this chapter is devoted to describing this process.

Basic Concepts

As we have seen, in the embryo and growing child bone is formed directly by osteoblasts (*intramembranous bone formation*) and indirectly by the calcification of cartilage and replacement by bone (*endochondral ossification*). In the healthy adult, the latter process is absent, and bone is formed only by osteoblasts on existing bone surfaces. When a fracture occurs, however, adults as well as children are able to make bone by both intramembranous and endochondral osteogenesis, leading to much more rapid repair of the damaged bone. Unlike skin and other soft tissues, wound healing in bone does not produce a permanent scar. Eventually, the initial repair tissues are replaced by normal lamellar bone. Therefore, while the woven bone and calcified cartilage in the healing fracture can be considered "scar tissue," they are temporary, and the tissues in the healed bone are ultimately indistinguishable from those in the original bone.

A fracture that fails to heal is called a *non-union*. Frequently, non-unions produce a jointlike cartilaginous structure called a *pseudoarthrosis* (literally, 'false joint'); these pathologic joints are lubricated with synovial fluid. A fracture that does not heal in the expected length of time, but which is still progressing, is called a *delayed union*. The length of time required to heal a particular fracture depends primarily on its anatomical site. Ribs, clavicles, and other bones of the torso heal in a few weeks; appendicular bones generally require several months (Table 2.4).

TABLE 2.4. Typical healing times for common fractures

Bone	Typical healing time, weeks
Distal radius	6
Humeral shaft	12
Tibial shaft	18
Femoral neck	24

Important Tissues in Fracture Healing

Two tissues provide the cells responsible for the healing of a typical fracture: the periosteum and the marrow of the injured bone. The periosteum has two layers: a fibrous outer layer, and a highly cellular *cambium* or inner layer. Ordinarily, the osteoblasts of the adult periosteum produce lamellar bone at a relatively slow rate, so that long bones expand very slowly with age. However, even a slight amount of trauma stimulates the periosteum to produce more osteoblasts, which manufacture large amounts of woven bone in a short amount of time. In the case of a fracture, this periosteal woven bone takes the form of trabeculae, which arch over the fracture line and fuse to bridge the gap and make what is known as the *periosteal callus* (Fig. 2.22).

The endosteal surfaces of bones are not actually covered by a membrane. Instead, they are lined with bone lining cells; this is true whether or not the endosteal space contains trabecular bone. In places, the endosteal surface may be experiencing ordinary remodeling, but most of the adult endosteal surface is quiescent. The bone lining cells may be stimulated to

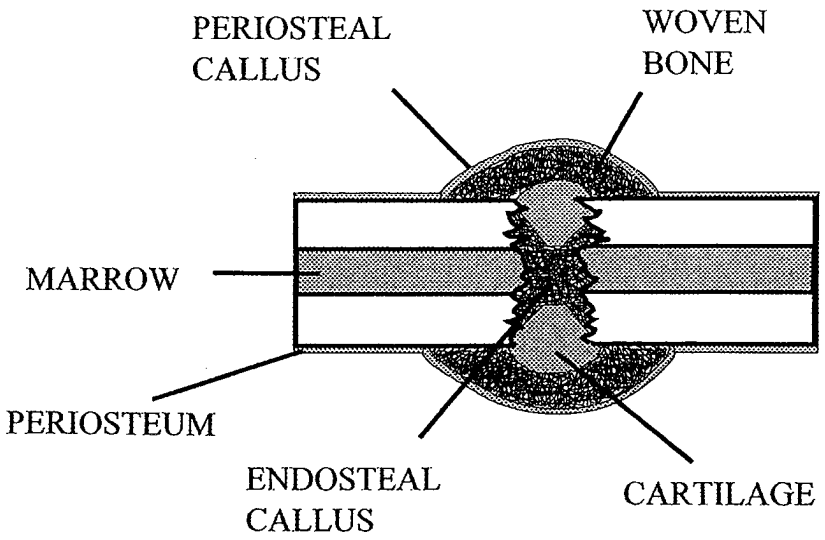


FIGURE 2.22. Diagram of a healing fracture.

initiate bone remodeling by various factors, including mechanical ones, but such remodeling does not play a role in the initial fracture healing response. Instead, trauma to the **marrow** stimulates the formation of the medullary version of the periosteal callus. It has been shown that, even without fracture of the cortex, mechanical disruption of the marrow activates the differentiation of osteoblasts and the formation of woven bone within the medullary canal (Amsel et al., 1969). (This bone formation is a prerequisite to the regeneration of the hematopoietic function of the marrow, and so serves a dual role when fracture occurs.) The periosteal callus tends to be more obvious, but the *medullary callus* is usually considered more important in achieving **initial** union.

Obviously, the jagged ends of the broken bone, as well as other trauma to the fracture site, can be expected to break many blood vessels. The resulting hematoma was once thought to be the primary source of the cells responsible for fracture healing. It is now thought that most of these cells come from the marrow and periosteum.

Three Biological Phases

Healing of a fracture may be divided into inflammatory, reparative, and remodeling phases which, taken together, form a self-driven sequence that leads to a healed fracture.

Inflammatory Phase

This initial phase serves to immobilize the fractured bone and activate the cells responsible for repair. Immobilization is promoted by pain and by swelling, which in many cases serves to hydrostatically splint the fracture. In addition to hematoma formation, this phase is characterized by vasodilation, serum exudation, and infiltration by inflammatory cells. It lasts 3–7 days.

It is important to realize that fracture repair is not accomplished by existing osteoblasts and chondroblasts[5] but depends on the creation of an entirely new work force of cells from a relatively small population of stem cells. The mobilization of this work force requires a variety of chemical mediators and ancillary cell types that are poorly understood. However, it is clear that this process is similar to other biologic cascades. Once set in motion, each step is predicated on what happened earlier, and if a mistake is made, the process may make a wrong turn rather than stopping, with no way to recover the conditions at the point of the error. It follows that many if not most non-unions or delayed unions are caused by a defect in the initial mobilization of the repair process, and once the problem becomes apparent, there is no way to correct it by manipulating the current group of cells. This is consistent with the fact that the most effective treatment for non-unions is to replace the tissues in the

[5]Chondroblasts are chondrocytes capable of producing cartilage at a rapid rate.

fracture site with a bone graft, which essentially initiates an entirely new repair process. Alternatively, electrical signals (see following) may be able to start a new cascade.

Interruption of the normal vascular supply to the bone by the fracture also results in death of the osteocytes in the bone matrix. The significance of this is not clear, but it may initiate the remodeling activity that is important in the last stage of fracture healing.

Reparative Phase

In this phase of healing, which lasts about 1 month, the periosteal and medullary calluses are formed by osteoblasts from the periosteum and marrow. The stem cells for medullary osteoblasts are thought to be pluripotential mesenchymal cells in the marrow stroma. These cells are also stem cells for fibroblasts, chondroblasts, and some marrow cells. Mesenchymal cell proliferation and differentiation are accompanied by intense vascular proliferation. The resulting osteoblasts produce woven bone at a high rate.

The fracture site exhibits some degree of endochondral ossification as well as direct bone formation by osteoblasts. This is thought to be related to inadequate blood supply in some portions of the fracture region (see Carter et al., 1988, for citations and discussion). In the absence of adequate vascularization, stem cells are thought to differentiate into chondroblasts rather than osteoblasts. An important contributing factor to inadequate vascularization can be insufficient immobilization of the fracture fragments. Alternatively, the nature of the stresses at a site within the callus may affect the differentiation pathway (Carter et al., 1988). In any event, the chondrogenic regions of the callus, which tend to be near the fracture line, usually calcify and are replaced by lamellar bone, just as the woven bone regions are. If this does not happen, a pseudoarthrosis may develop.

Regardless of how much of the callus is cartilaginous, it takes a week or two for the woven bone or cartilage to accumulate substantial mineral. This early, rather compliant callus is known as the *provisional callus*. As calcification proceeds, the callus material becomes increasingly rigid; it is then referred to as the *bony callus*. The rigidity of the callus is enhanced by the fact that it has a larger cross-sectional area (and moment of inertia, see Section 4.2) than the original bone cortex. This means that even though the **material** in the bony callus is less rigid (and strong) than cortical bone, the rigidity (and strength) of the callus **structure** can equal or exceed that of the intact bone. When this occurs, *bony union* has been achieved, completing the reparative phase.

Remodeling Phase

Once bony union has been achieved, the broken bone is approximately as strong as the intact bone was, but often it has greater mass than the original bone, and is therefore less mechanically efficient. Modeling and remodeling of the fracture site gradually restore the original contour and internal

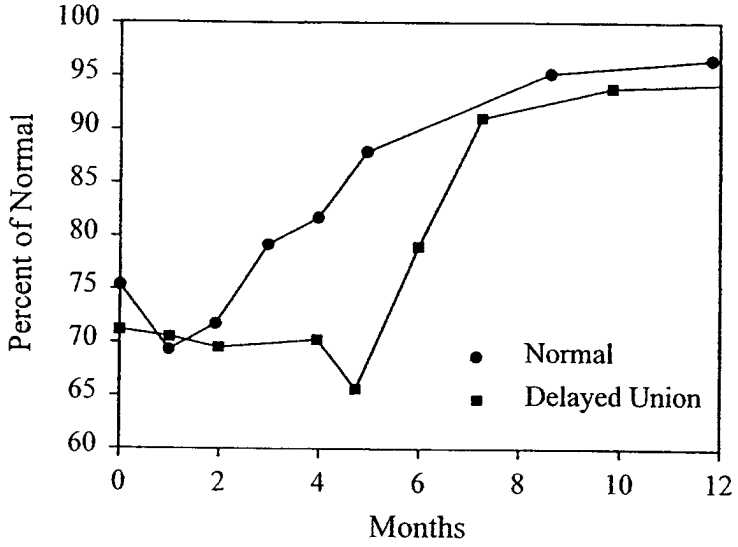
structure of the bone, although radiographic evidence of the fracture may persist for many years. As the bone remodels, it is able to maintain its strength with less material than in the earlier phases of healing, thereby increasing its mechanical efficiency. The medullary and periosteal calluses are removed, and the remaining material (woven bone or calcified cartilage) is replaced by secondary lamellar bone (cortical or trabecular, as the site dictates). If the fracture has healed with incorrect angulation, this may be partially corrected as well by modeling drifts. In general, modeling and remodeling proceed much more rapidly in children than in adults, and this applies to the remodeling phase of fracture healing as well.

In considering the capacity of a malaligned healed fracture to correct itself, there are two important geometric principles to remember. First, an angulation in the plane of motion of the adjacent joint corrects itself better than one out of the joint plane. Second, rotational errors do not correct themselves very well.

Box 2.2 Technical Note
Clinical Assessment of Healing Fractures

It is very important for the physician treating a fracture patient to be able to judge the strength and stiffness of the healing bone. If the cast is removed before the fracture is mechanically competent to bear the loads and moments applied to it, there is risk of refracture or malunion. On the other hand, if the cast is left on too long, there is risk of excessive disuse osteoporosis and contracture of soft tissues about the adjacent joints, not to mention the inconvenience and expense of needless immobilization. The usual clinical methods to assess fracture healing are inspection of radiographs and manipulation to see how stiff the healing fracture feels; both of these are subjective and imprecise. Several groups of investigators have experimented with methods to assess fracture healing in a more quantitative way.

If the fracture has been fixed with an external fixation frame, application of a strain gauge to the fixation frame can allow bone stiffness to be estimated (Burny et al., 1984; Cunningham et al., 1990). A second method for measuring the mechanical characteristics of a healing fracture involves the propagation of sound or stress waves through the tissues. The velocity of sound in an elastic bar is $c = (E/\rho)^{1/2}$ where E is the elastic modulus and ρ is the density of the tissue. The resonant frequency of a vibration in an elastic bar depends on the ratio of c to the length of the bar. When a vibration traveling along the bar reaches a segment having different material properties, a portion of the wave is reflected backward. The magnitudes of the transmitted and reflected waves depend on the ratio of the acoustic impedance, $z = c\,\rho$, of the two materials. These dependencies on the elastic modulus of the tissues are potential means for probing the stiffness of the fracture callus. For example, the velocity of an ultrasonic wave across the fracture site can be measured (Gerlanc et al., 1975). As the fracture heals, the wave velocity increases; healing can be assessed by comparison with the contralateral, uninjured bone (see figure). Alternatively, the resonant frequency can be determined by tapping the fractured bone with a hammer (Collier and Donarski, 1987), or vibrating it over a range of frequencies with a shaker. Another approach is to measure the attenuation of the impulse as it crosses



Sound velocity, normalized by the values in the contralateral bone, measured through the tissues in a healing fracture is plotted against healing time. Examples shown represent human tibias with normal (*circles*) and delayed (*squares*) healing. (Replotted from data by Gerlanc et al., 1975.)

the fracture site. These methods are most easily applied to the ulna and tibia, which are subcutaneous along at least one margin. Bones that are fully enveloped in muscle (e.g., the femur) are too inaccessible for these vibration methods.

Although each of these methods is capable of probing the mechanical attributes of a healing fracture, in practice they are too cumbersome and imprecise to have come into general clinical use and their applications have been limited to research. A fast, noninvasive, and easy method to measure bone stiffness in vivo remains to be invented.

Four Biomechanical Stages

A standard way to study fracture healing in experimental animals is to mechanically test the healing bone in torsion and compare the result with the stiffness of an intact bone. When this was done with rabbit tibial fractures (White et al., 1977), four stages of healing were identified (Fig. 2.23).

Stage I: Virtually no stiffness is seen, and failure occurs through the original fracture line. This stage corresponds to the inflammatory and early reparative (i.e., provisional callus) phases of healing. (Days 1–26 in the rabbit tibia.)

Stage II: Substantial stiffness is now encountered, but failure still occurs through the original fracture line. This stage corresponds to the middle of the reparative phase when the callus has become bony but still is not so large or calcified as it will become. (Days 27–49 in the rabbit tibia.)

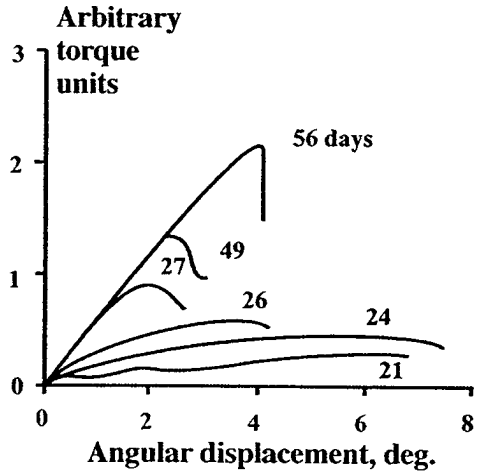


FIGURE 2.23. Torsional stiffness of healing rabbit fractures as a function of days of healing. (After White et al., 1977.)

Stage III: There is no further increase in stiffness, but failure is now partially through the original fracture line and partially through intact bone. (Days 49–56 in the rabbit tibia.)

Stage IV: Failure occurs through the intact bone rather than the callus at the fracture site. (After day 56 in the rabbit tibia.)

Stages III and IV correspond to the later portion of the reparative phase and the early remodeling phase, when the full bony callus exists but its mineral content and strength are still increasing. Another interesting observation from these mechanical studies of healing fractures is that there is typically a slight reduction in the strength of the fracture callus during stage IV of healing. It is reasonable to think that this corresponds to the beginning of the remodeling phase, when the resorptive portion of BMU activity has begun but substantial refilling of the osteonal cavities has yet to occur.

Stability of Fixation

The importance of achieving good stability or immobilization of a fracture depends very much on the anatomical site. For example, ribs heal well even with a great deal of motion—rib non-unions are unknown. On the other hand, tibias and other large bones are very prone to non-union as the result of excessive motion of the fragments. This knowledge must be balanced against the fact that **some** motion is beneficial: there seems to be an inverse relationship between the amount of motion and callus size, up to a point that has not been defined. In the same vein, applying loads across the fracture (i.e., weight-bearing) serves to strengthen the callus, but obviously too much weight can lead to refracture or excessive motion and non-union. There are no clear rules about how much weight-bearin or motion are safe.

Torsional loading best satisfied these requirements. The investigators chose a pendulum system to generate the torsional loads because it could be easily designed to produce a loading rate similar to that estimated for common traumatic fractures (see figure). The pendulum traverses the bottom 30° of its swing in 75 milliseconds (ms), similar to the load duration in fractures caused by skiing or motor vehicle accidents. The pendulum has a mass of 14 kg and a length of 23 cm. The pendulum's energy greatly exceeds the energy required to fracture most bones, so that minimal changes in pendulum velocity or loading rate occur during the fracture event (less than 2% in the case of rabbit bones). Measurement instrumentation consists of a reaction torque sensor, a precision angular position transducer, and an oscilloscope to capture and display the voltage outputs from the two transducers.

To conduct a test, the bone is rigidly mounted in alignment with the rotational axis of the device. The pendulum is dropped from a set height and at the bottom of its swing engages a cam on the load shaft; this imparts an angular deformation to the specimen that is measured by the angular position sensor. The resistive torque in the specimen is simultaneously measured by the torque transducer at the opposite end of the bone. A torque-vs.-angular deformation curve is then constructed, which represents the whole bone's failure characteristics under typical traumatic loading conditions. Common variables derived from the curve include the failure torque and deformation and the energy absorption to failure. Because bones are not perfectly straight cylinders, the derivation of material properties from these data is not usually attempted.

The advent of more advanced material testing machines has reduced the popularity of the Burstein–Frankel apparatus, but, thanks to its superb design, it remains a very legitimate means of testing the structural integrity of whole bones.

Primary Union

If a fracture is rigidly fixed so that the bone ends are closely approximated, healing may occur by osteonal remodeling across the fracture line (Fig. 2.24). This is known as *primary fracture healing*. (Therefore, *secondary healing* is ordinary fracture healing as previously described.) Usually the only way that primary healing can happen is when a plate or screw is used very effectively to internally "fix" (rigidly fasten together the fragments of) the fracture. In these cases, there is almost no periosteal callus.

Posttraumatic Osteoporosis

It is commonly observed clinically that the intact portions of the fractured bone become osteoporotic as healing occurs. This generalized osteopenia of the intact regions, called *posttraumatic osteoporosis* or *posttraumatic bone atrophy*, is caused by two factors. First, in addition to the healing response, the fracture causes a remodeling RAP throughout the bone, so that osteonal BMUs riddle the entire cortex with resorption cavities. The second factor is the removal of mechanical loading from the fractured bone. As is seen later, disuse also activates remodeling. If the fracture is well fixed and sufficient load-bearing is resumed, the resorption spaces will refill and the osteopenia will be transient. To the degree that the bone does not resume load-bearing, the atrophy persists.

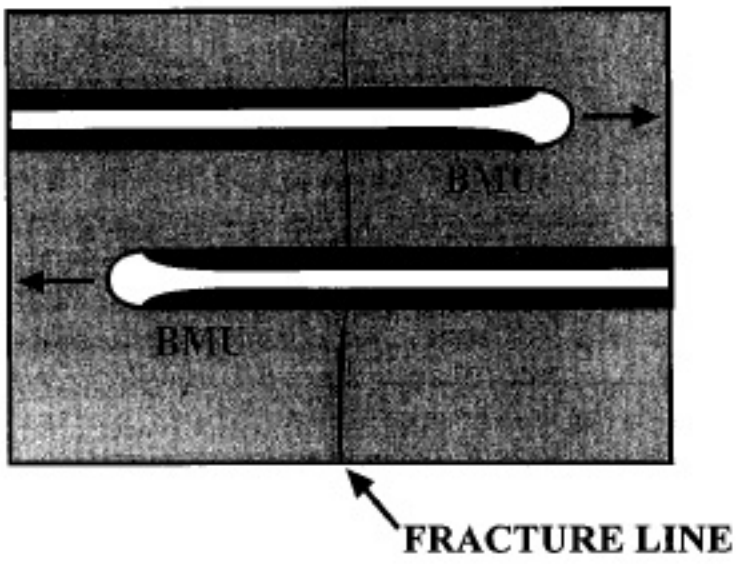


FIGURE 2.24. Sketch illustrating how osteonal BMUs may tunnel across a tightly fixed fracture line or osteotomy (saw cut) interface in cortical bone. This is known as primary healing.

Electrical Phenomena and Fracture Healing

When bones are mechanically deformed, they become electrically polarized via two mechanisms: (1) the ionic fluids in the calcified matrix are forced to move about, creating *streaming potentials* (Eriksson, 1974); and (2) the collagen molecules in the bone tissue are *piezoelectric*, so that they exhibit a dipole moment when strained (Fukada and Yasuda, 1957). The relative roles of these two phenomena in producing *stress-generated potentials* in bone is unclear, but it is well established, for example, that when a bone is bent, the concave surface becomes negatively charged and the convex surface becomes positively charged. Furthermore, when a bone is fractured, the fracture site becomes negatively charged with respect to the remainder of the bone. Experiments have shown that bone formation is enhanced in the vicinity of a cathode, and the optimal current for this effect is about 20 microamperes (μA). For a review of electrical phenomena in bone, see Chapter 4 in Martin and Burr (1989).

Since the 1970s, electrical stimulation has been used as a treatment for non-unions. There are two basic methods of producing such currents. Brighton and Pollack (1985) placed thin wires into the fracture site to carry direct current, with an anode pad on the skin nearby. Bassett and co-worker (1981) passed currents through external coils to induce currents in the tissues noninvasively. Both methods seem to be about 70%–85% successful. They require good immobilization, however, and they do not work in cases of true synovial pseudoarthrosis.

2.10 Summary and Further Reading

This chapter has introduced bone's tissue structure, composition, and cells. We have also described the structure and biology of cartilage, both in regards to articular surfaces and to the growth plate. The physiology of modeling and remodeling were then described. Finally, we described the processes by which broken bones heal. Together with Chapter 1, this chapter lays the foundation for the rest of the book: we now know why the skeleton must support high loads (because of the limitations inherent in muscles) and have a picture of the basic structure and biology of the tissues in the skeleton. In Chapters 3 through 7 we explore more quantitatively the interactions between biology, structure, and function in bones and joints.

Several excellent works can be suggested for further reading. These include *Principles of Bone Biology* (Bilezikian et al., 1996), *The Scientific Basis of Orthopaedics* (Albright and Brand, 1987), *Orthopaedic Basic Science* (Simon, 1994), *Intermediary Organization of the Skeleton* (Frost, 1986), *Bone Formation and Repair* (Brighton et al., 1994), and *Osteoporosis* (Marcus et al., 1996). For more in-depth reading, one may turn to B. K. Hall's series of books titled *Bone*, and an older series by G. F. Bourne titled *The Biochemistry and Physiology of Bone.*

2.11 Exercises

Unlike the exercises at the end of Chapter 1, these are not intended to give you practice in solving problems similar to those illustrated in the text. Instead, they are meant to start you thinking quantitatively about the biological material introduced in this chapter, in preparation for the analytical chapter to follow.

2.1. If a BMU tunnels through cortical bone at a rate of 40 μm/day, how long would it take to tunnel 2 cm through your tibia?

2.2. Estimate the average rate of addition of bone on the diaphyseal surface of your tibia, in micrometers/year, as you grew to skeletal maturity. Palpate your tibia to estimate its present diameter, and assume its diameter was 0.8 cm when you were born. Assume that your tibia's periosteal apposition rate essentially stopped at age 14 if you are a woman or age 16 if you are a man, ignoring any further increase in the diameter of the tibia that occurred after closure of the physes.

2.3. Typically, a newborn infant's tibia is about 6.8 cm long (Jeanty et al., 1984). Estimate the present length of your tibia, then estimate the average rate of growth in the tibial growth plates, in millimeters/year and in micrometers/day. Again, assume that longitudinal growth of the tibia stops at age 14 in females and age 16 in males. How does this

compare with the bone apposition rate estimated in Exercise 2.2 and the BMU tunneling rate in Exercise 2.1?

2.4. The rate of growth of a growth plate is equal to the daily chondrocyte production rate for each column multiplied by the height of the maximally hypertrophied cells (Sissons, 1955). Write a paragraph explaining why this is so in a steady-state situation and stating the necessary assumptions.

2.5. Consider the relationship between the rate of growth in a growth plate, the average number of cells being produced each day, and the average amount of hypertrophy each cell experiences. Assume the following. The growth plate consists of many parallel cell columns, all growing at the same rate. The stem cell at the top of each column divides at a rate r_s (divisions/year), replacing itself and producing one new chondrocyte. Each resulting chondrocyte divides, producing two new chondrocytes, which in turn divide. This mitotic sequence continues until N generations of chondrocytes are produced, all of which are aligned in a single column. Each chondrocyte in the column hypertrophies to an ultimate cell height, h. Using the principle of Exercise 2.4, show that the physeal growth rate is $G = h\, r_s\, 2^N$.

2.6. If it takes 2 months to refill an osteonal resorption cavity that is initially 250 μm in diameter, what is the average apposition rate in micrometers/day? Assume the Haversian canal diameter is 50 μm after refilling is complete. Compare this apposition rate with that in Exercise 2.2.

2.7. Imagine a cross section through your femur. Assume that 5% of the bone in this cross section is replaced annually by osteonal remodeling. The osteons average 250 μm in diameter. What would the osteonal BMU activation rate (new osteons/mm^2 per year) have to be to produce the 5% turnover rate?

2.8. In Exercise 2.7, if each osteonal BMU produced a new Haversian canal that was 50 μm in diameter, what would be the rate of increase in bone porosity (mm^2 of canals/mm^2 of cross section/year)? Assume that the new osteons do not obliterate preexisting Haversian canals.