closely related to other macrophages. Morphologically, they resemble the Langhans’ giant cells formed by fusion of tissue macrophages (histiocytes) in loose connective tissue.

**BONE FORMATION**

Bone formation is a complex process that is sometimes difficult to understand if an adequate distinction is not made between the development of bone as an organ and the histogenesis of bone tissue.

**The Development of a Bone Is Traditionally Classified as Endochondral or Intramembranous**

The distinction between endochondral and intramembranous formation rests on whether a cartilage model serves as the precursor of the bone (endochondral ossification) or whether the bone is formed by a simpler method, without the intervention of a cartilage precursor (intramembranous ossification). The bones of the extremities and those parts of the axial skeleton that bear weight (e.g., vertebrae) develop by endochondral ossification. The flat bones of the skull and face, the mandible, and the clavicle develop by intramembranous ossification.

The separate labels for types of bone or bone tissue should not be taken to mean existing bone is either membrane bone or endochondral bone. These names refer only to the mechanism by which a bone is initially formed. Because of the remodeling that occurs later, the initial bone tissue that was laid down by endochondral formation or by intramembranous formation is soon replaced. The replacement bone is established on the preexisting bone by appositional growth.
Bone Formation 8.11. As the process continues, the newly organized tissue at the presumptive bone site becomes more vascularized, and the aggregated mesenchymal cells become larger and rounded. The cytoplasm of the mesenchymal cells changes from eosinophilic to basophilic, and a clear Golgi area becomes evident. These cytologic changes result in the differentiated osteoblast, which then secretes the collagen and proteoglycans of the bone matrix (osteoid). The osteoblasts within the bone matrix become increasingly separated from one another as the matrix is produced, but they remain attached by thin cytoplasmic processes. Because of the abundant collagen content, the bone matrix appears more dense than the surrounding mesenchyme in which the intercellular spaces reveal only delicate connective tissue fibers.

**Newly Formed Bone Matrix Appears in Histologic Sections as Small, Irregularly Shaped Spicules and Trabeculae**

With time, the matrix becomes calcified, and the interconnecting cytoplasmic processes of the bone-forming cells, now termed osteocytes, are contained within canaliculi.

**Intramembranous Ossification**

**In Intramembranous Ossification, Bone Is Formed by Differentiation of Mesenchymal Cells Into Osteoblasts**

The first evidence of intramembranous ossification occurs around the eighth week of gestation in the human. Some of the pale-staining elongate mesenchymal cells within the mesenchyme migrate and aggregate in specific areas, the sites where bone is destined to form. This condensation of cells within the mesenchymal tissue is the membrane referred to in the term intramembranous ossification (Fig.

**Figure 8.9.** In c, the cell is a resorptive osteocyte. It contains a substantial amount of rER, a large Golgi (G), mitochondria (M), and lysosomes (L). The pericellular space is devoid of collagen fibrils and may contain some flocculent material. The lacuna containing a resorptive osteocyte is bounded by an osmiophilic lamina (OL).

and is identical in both cases. Although the long bones are classified as forming by endochondral formation, their continued growth involves the histogenesis of endochondral bone and the histogenesis of intramembranous bone, with the latter occurring through the activity of the periosteal (membrane) tissue.

**Figure 8.10.** Photomicrograph of bone tissue where resorption is taking place. Two osteoclasts are evident. Note that each cell exhibits several nuclei. One of the osteoclasts is in close apposition to the bone, and the site of the ruffled border can be seen between the arrows. Also, note the osteocytes in the bone tissue in the region.
Concomitantly, more of the surrounding primitive cells in the membrane proliferate, giving rise to a population of what may now be regarded as osteoprogenitor cells. Some of the osteoprogenitor cells come into apposition with the initially formed spicules, become osteoblasts, and add more matrix. By this method, appositional growth, the spicules enlarge and become joined in a trabecular network having the general shape of the developing bone.

Through continued mitotic activity, the osteoprogenitor cells maintain their numbers and thus provide a constant source of osteoblasts for growth of the bone spicules. The new osteoblasts, in turn, lay down bone matrix in successive layers, giving rise to woven bone. This immature bone, discussed on page 153, is characterized internally by interconnecting spaces occupied by connective tissue and blood vessels. Bone tissue formed by the method just described is referred to as membrane bone or intramembranous bone.

**Endochondral Ossification**

Endochondral ossification, also, begins with the proliferation and aggregation of mesenchymal cells at the site of the future bone. However, the mesenchymal cells differentiate into chondroblasts that, in turn, produce cartilage matrix.

**The Hyaline Cartilage Produced at This Early Stage Acquires the General Shape of the Bone That Will Be Formed; i.e., It Is a Cartilage Model**

The cartilage model, once established, grows by interstitial as well as appositional growth. Most of the increase in length of the cartilage model can be attributed to interstitial growth. The increase in its width is largely by addition of cartilage matrix produced by new chondrocytes that differentiate from the chondrogenic layer of perichondrium surrounding the cartilage mass. Illustrations 1 and 1a of Figure 8.12 show an early cartilage model.

**The First Sign of Ossification Is the Appearance of a Cuff of Bone Around the Cartilage Model**

The perichondrial cells in the midregion of the cartilage model no longer give rise to chondrocytes. Instead, bone-forming cells or osteoblasts are produced. Thus, the connective tissue surrounding the middle of the cartilage is no longer functionally a perichondrium; rather, because of its altered role, it is now called periosteum. Moreover, we may now describe an osteogenic layer within the periosteum because the cells within this layer are differentiating into osteoblasts. As a result of these changes, a thin layer of bone is formed around the cartilage model. We may describe this...
Figure 8.12. Schematic diagram of development of a long bone. Illustrations 1–10 depict longitudinal sections; 1a–4a depict cross sections through the shaft of the bone. The process begins with the formation of a cartilage model (1 and 1a), next, a periosteal (perichondrial) collar of bone forms about the diaphysis (shaft) of the cartilage model (2 and 2a); then, the cartilaginous matrix in the diaphysis begins to calcify (3 and 3a). Blood vessels and connective tissue cells then erode and invade the calcified cartilage (4 and 4a), creating a primitive marrow cavity in which remnant spicules of calcified cartilage remain at the two ends of the cavity. Endochondral bone forms on those spicules of calcified cartilage. The bone at the ends of the developing marrow cavity constitutes the metaphysis. Periosteal bone continues to form: the periosteal bone is intramembranous bone. It can be recognized histologically because it is not accompanied by local cartilage erosion, nor is the bone deposited on spicules of calcified cartilage. Blood vessels and perivascular cells invade the upper epiphyseal cartilage (6), and a secondary center of ossification is established in the upper epiphysis (7). A similar epiphyseal ossification center forms at the lower end of the bone (8), and an epiphyseal disc or plate is thus formed between each epiphysis and the diaphysis. With continued growth of the long bone, the lower epiphyseal plate disappears (9), and finally, with cessation of growth, the upper epiphyseal plate disappears (10). The metaphysis then becomes continuous with the epiphysis. Epiphyseal lines remain where the epiphyseal plate last existed. (From Bloom W, Fawcett DW: A Textbook of Histology, 10th ed. Philadelphia, WB Saunders, 1975, p 266.)
bone as either periosteal bone, because of its location, or as intramembranous bone, because of its method of development. In the case of a long bone, a distinctive cuff of periosteal bone, the bony collar, is established around the cartilage model in what can be described as the diaphyseal portion of the developing bone. This is shown in illustrations 2 and 2a of Figure 8.12.

With the Establishment of the Periosteal Bony Collar, the Chondrocytes in This Midregion of the Cartilage Model Become Hypertrophic

As the chondrocytes enlarge, their surrounding cartilage matrix becomes compressed, forming thin irregular cartilage plates between the hypertrophic cells. The hypertrophic cells begin to synthesize alkaline phosphatase, and concomitantly, the surrounding cartilage matrix undergoes calcification; see illustrations 3 and 3a of Figure 8.12. The calcification of the cartilage matrix is not to be confused with calcification that occurs in bone tissue.

The Calcified Cartilage Matrix Inhibits Diffusion of Nutrients, Causing Death of the Chondrocytes in the Cartilage Model

With the death of the chondrocytes, much of the matrix breaks down, and neighboring lacunae become confluent, producing an increasingly large cavity. While these events are occurring, one or several blood vessels grow through the thin diaphyseal bony collar to vascularize the cavity; see illustrations 4 and 4a of Figure 8.12.

Periosteal Cells Migrate Into the Cavity Along With Growing Blood Vessels

Cells from the periosteum migrate with the penetrating blood vessels and some of the primitive periosteal cells to become osteoprogenitor cells in the cavity. Other primitive cells also gain access to the cavity via the new vasculature, leaving the circulation to give rise to the marrow. As the calcified cartilage breaks down and is partially removed, some remains as irregular spicules. When the osteoprogenitor cells come in apposition to the remaining calcified cartilage spicules, they become osteoblasts and begin to lay down bone (osteoid) on the spicle framework. Thus, the bone formed in this manner is endochondral bone. The combination of the bone, which is initially only a thin layer, and the underlying calcified cartilage is described as a mixed spicule.

Histologically, mixed spicules can be recognized by virtue of their staining characteristics. Calcified cartilage tends to be basophilic, whereas bone is distinctly eosinophilic. Such spicules persist for a short time before the calcified cartilage component is removed. The remaining bone component of the spicule may continue to grow by appositional growth, thus becoming larger and stronger, or it may undergo resorption as new spicules are being formed.

Growth of Endochondral Bone

Endochondral Bone Growth Begins in the Second Trimester of Fetal Life and Continues Into Early Adulthood

The events described above represent the early stage of endochondral bone formation as seen in the fetus, beginning at about the 12th week of gestation. The continuing growth process, which takes place throughout the growing period of the individual into early adulthood, is now described.

The Continued Growth of Long Bones Is Dependent on the Presence of Epiphyseal Cartilage Throughout the Growth Period

As the diaphyseal marrow cavity enlarges (see illustration 5 of Fig. 8.12), a distinct zonation can be recognized in the cartilage at either end of the cavity. This remaining cartilage, referred to as epiphyseal cartilage, exhibits distinct zones as illustrated in Figure 8.13. The zones in the epiphyseal cartilage, beginning with that most distal to the diaphyseal center of ossification and proceeding toward that center, are

- **Zone of reserve cartilage**, which exhibits no cellular proliferation or active matrix production.
- **Zone of proliferation**, which is adjacent to the zone of reserve cartilage in the direction of the diaphysis. In this zone, the cartilage cells undergo division and are organized into distinct columns. These cells are larger than those in the reserve zone and are actively producing matrix.
- **Zone of hypertrophy**, which contains cartilage cells that are greatly enlarged. Their cytoplasm is clear, a reflection of the glycogen that they normally accumulate (and that is lost during fixation), and the matrix is compressed into linear bands between the columns of hypertrophied cartilage cells.
- **Zone of calcified cartilage**, in which the enlarged cells begin to degenerate and the matrix becomes calcified.
- **Zone of resorption**, which is the zone nearest the diaphysis. The cartilage here is in direct contact with the connective tissue of the marrow cavity.

In the zone of resorption, small blood vessels and accompanying connective tissue invade the region occupied by the dying chondrocytes. They form a series of spearheads, leaving the calcified cartilage as longitudinal spicules, at least as seen in a longitudinal section of the bone. Actually, in a cross section of the bone the cartilage would appear as a honeycomb because the invading vessels and connective tissue migrate into the sites previously occupied by the cartilage cells.
Bone Deposition Occurs on the Cartilage Spicules in the Same Manner as Described for the Formation of the Initial Ossification Center

As bone is laid down on the calcified spicules, the cartilage is resorbed, ultimately leaving a primary spongy bone. This spongy bone undergoes reorganization through osteoclastic activity and addition of new bone tissue, thus accommodating to the continued growth and physical stresses placed on the bone.

Shortly after birth, a secondary ossification center develops in the upper epiphysis. The cartilage cells hypertrophy and degenerate. As in the diaphysis, calcification of the matrix occurs, and blood vessels and osteogenic cells from the perichondrium invade the region, creating a new marrow cavity; see illustrations 6 and 7 of Figure 8.12. Later, a similar epiphyseal ossification center forms at the lower end of the bone; see illustration 8 of Figure 8.12. This, too, is regarded as a secondary ossification center, although it develops later. With the development of the secondary ossification centers, the only cartilage that remains from the original model is the articular cartilage at the ends of the bone and a transverse disc, known as the epiphyseal plate, that separates the epiphyseal and diaphyseal cavities.
Figure 8.14. Diagram of external remodeling of a long bone, showing two periods during the growth of the bone. The younger bone profile is shown on the right; the older, on the left. Superimposed on the left side of the figure is the shape of the bone (left half only) as it appeared at the earlier time. The bone is now longer, but it has retained its general shape. To grow in length and retain the general shape of the particular bone, bone resorption occurs on some surfaces, and bone deposition occurs on other surfaces, as indicated in the diagram. (Based on Ham AW: The Journal of Bone and Joint Surgery 34A:701, 1952.)

The Cartilage of the Epiphyseal Plate Is Responsible for Maintaining the Growth Process

For a bone to retain proper proportions and its unique shape, external as well as internal remodeling must occur as the bone grows in length. The proliferative zone of the epiphyseal plate gives rise to the cartilage on which bone is later laid down.

- The thickness of the epiphyseal plate remains relatively constant during growth.
- The amount of new cartilage produced (zone of proliferation) equals the amount resorbed (zone of resorption).
- The resorbed cartilage is, of course, replaced by spongy bone.

In reviewing the growth process, it is important to realize that

- Actual lengthening of the bone occurs when new cartilage matrix is produced at the epiphyseal plate. This has the effect of pushing the epiphysis away from the diaphysis, thus causing elongation of the bone. The events that follow this incremental growth, namely, hypertrophy, calcification, resorption, and ossification, simply involve the mechanism by which the newly formed cartilage is replaced by bone tissue during development.
- Increase in width or diameter of the bone occurs when appositional growth of new bone occurs between the cortical lamellae and the periosteum. The marrow cavity then enlarges by resorption of bone on the endosteal surface of the cortex of the bone.

As Bones Elongate, Remodeling Is Required

Remodeling consists of preferential resorption of bone in some areas and deposition of bone in other areas, as described above and outlined in Figure 8.14. The histologic features of intramembranous and endochondral bone formation are considered further in Plates 19–23, pages 176–185.
Cessation of Growth

When an Individual Achieves Maximal Growth, Proliferation of New Cartilage Within the Growing Bones Terminates

When proliferation of new cartilage ceases, the cartilage that has already been produced in the epiphyseal plate continues to undergo the changes that lead to the deposition of new bone until, finally, there is no remaining cartilage. At this point, the epiphyseal and diaphyseal marrow cavities become confluent. The elimination of the epiphyseal plate is referred to as **epiphyseal closure**. In illustration 9 of Figure 8.12, the lower epiphyseal cartilage is no longer present, and in illustration 10, both epiphyseal cartilages are gone. Growth is now complete, and the only remaining cartilage is found on the articular surfaces of the bone. Vestigial evidence of the site of the epiphyseal plate is reflected by an **epiphyseal line**, consisting of bone tissue (see Fig. 8.2).

### NUTRITIONAL FACTORS IN BONE FORMATION

Both nutritional and hormonal factors affect the degree of bone mineralization. It has long been known that calcium deficiency during growth causes **rickets**, a condition in which the bone matrix does not calcify normally. Rickets may be due to insufficient amounts of dietary calcium or to insufficient vitamin D (a steroid prohormone), which is needed for absorption of calcium by the intestines. In the adult, the same nutritional or vitamin deficiency leads to **osteomalacia**.

Although rickets and osteomalacia are no longer major problems where nutrition is adequate, another form of insufficient bone mineralization is regularly seen in the condition known as **osteoporosis**. In this condition, bone tissue (both mineral and matrix) is diminished, presumably because resorption by osteoclasts exceeds deposition by osteoblasts. Osteoporosis develops as a consequence of immobilization (as in a bedridden patient) and in postmenopausal women. The factors that bring on the imbalance in cellular activity are not known, although some relief appears to result from maintaining hormonal (estrogen) levels and dietary fluoride levels.

In addition to its influence on intestinal absorption of calcium, vitamin D is also needed for normal calcification. Other vitamins long known to affect bone are A and C. Vitamin A deficiency results in a suppression of endochondral growth of bone; vitamin A excess leads to fragility and subsequent fractures of long bones. Vitamin C is essential for syntheses of collagen, and its deficiency leads to scurvy. The matrix produced in scurvy is not calcifiable.

### Development of the Osteonal System (Haversian System)

**Osteons Typically Develop in Preexisting Compact Bone**

The compact bone might have formed from fetal spongy bone by continued deposition of bone on the spongy bone spicules, it might have been deposited directly as adult compact bone, e.g., the circumferential lamellae of an adult bone, or it might be older compact bone consisting of osteons and interstitial lamellae.

**In the Development of New Osteons, a Tunnel Is Bored Through the Compact Bone by Osteoclasts**

When the osteoclasts have produced an appropriately sized cylindrical tunnel by resorption of compact bone, blood vessels and their surrounding connective tissue occupy the tunnel. As the tunnel is occupied, new bone deposition on its wall begins almost immediately. These two aspects of cellular activity, namely, osteoclast resorption and osteoblast synthesis, constitute a **bone-remodeling** unit. There are two distinct parts to the bone-remodeling unit: an advancing **cutting cone** (also called a resorption canal) and a **closing cone**. The cutting cone consists of active osteoclasts followed by an advancing capillary loop and periosteocytes. It also contains numerous cells in mitosis. These give rise to osteoblasts, additional periosteocytes, and endothelial cells. (Recall that the osteoclasts derive from blood-borne monocytes.) The osteoclasts cut a canal about 200 μm in diameter. This canal establishes the diameter of the future osteonal (Haversian) system. The cutting cone constitutes only a small fraction of the length of the bone-remodeling unit; thus, it is seen much less frequently than the closing cone of the developing osteon.

After the diameter of the future Haversian system is established, osteoblasts begin to deposit the organic matrix (osteoid) of bone on the walls of the canal in successive lamellae. With time, the bone matrix in each of the lamellae becomes mineralized. As the successive lamellae of bone are deposited, **from the periphery inward**, the canal ultimately attains the relatively narrow diameter of the adult osteonal canal. This process in which new osteons are formed is referred to as **internal remodeling**.

**Compact Adult Bone Contains Haversian Systems of Varying Age and Size**

If a ground section of bone is examined microradiographically, it can be seen that younger Haversian systems are less completely mineralized than older systems (Fig. 8.15). They undergo a progressive secondary mineralization that continues (up to a point) even after the osteon has been fully formed. Figure 8.15 also illustrates the dynamic internal remodeling of compact bone. In the adult, deposition balances resorption. In the aged, resorption often exceeds deposition. If this imbalance becomes excessive, osteoporosis develops (see Nutritional Factors in Bone Formation, page 166).