Bone Biology

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Part I: Structure, Blood Supply, Cells, Matrix, and Mineralization*

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Knowledgeable treatment of diseases, deformities, and injuries of the musculoskeletal system requires an understanding of the complex, constantly changing nature of bone. Current orthopaedic practice relies on the ability of surgeons to drill, cut, ream, and realign bone; to fix one piece to another with screws, plates, wires, or rods; and to obtain union between bone and plastic or metal implants. The success of many of these procedures is often attributed to the technical skill of the surgeon or to the design of an implant or instrument, but it is actually the remarkable properties of bone that make these operations successful. Failure to consider these properties can compromise the most technically expert orthopaedic treatment, whereas understanding them well can help to solve the most complicated clinical problems.

The mechanical properties of bone are readily apparent[30,41,87]. While the tensile strength of bone is nearly equal to that of cast iron, bone is three times lighter and ten times more flexible. However, bone is not a homogeneous, inert material like iron or the plastics and metals from which most orthopaedic implants are made. Its matrix consists of organic and inorganic components, and its internal and external surfaces are covered by cells and cell processes. An elaborate system of lacunae (canals or tunnels containing cells and cell processes, blood vessels, lymphatic vessels, and nerves) permeates the matrix, and a variety of specialized cell populations responsible for maintaining the tissue lie within the lacunae of the matrix and on the surfaces of the bone. In most people, bone appears to remain unchanged for decades, but this appearance is deceptive. Bone is constantly changing in response to mechanical and hormonal signals. Recent advances in the understanding of how bone responds to these signals have formed the foundation for new methods of stimulating or suppressing the formation of bone and of changing the organization of bone to treat musculoskeletal disorders. Advances in the treatment of injuries, diseases, and deformities of the musculoskeletal system will increasingly rely on an understanding of the structure of bone; its constituent parts; its blood supply, cells, and matrix; the mechanisms of the formation of bone; and the control of bone-cell function.

Structure of Bone

On the basis of general shape, bones can be classified into three groups: short, flat, and long or tubular. Short bones, such as the tarsals, carpals, and vertebral bodies, measure approximately the same in all directions and are trapezoidal, cuboidal, cuneiform, or irregular in shape. These bones have relatively thin cortices. Flat and tubular bones have one dimension that is much shorter or longer than the other two. The larger flat bones form the cranial vault, the scapula, and the wing of the ileum. The lamina of a vertebra is an example of a smaller flat bone. Long or tubular bones, such as the femur, tibia, humerus, metacarpals, metatarsals, and phalanges, have an expanded metaphysis and an epiphysis at either end of a thick-walled tubular diaphysis.

Mature bones consist of a central fatty or hematopoietic marrow that is supported and surrounded by bone tissue and periosteum (Fig. 1). Although these three component tissues differ in composition, structure, and function, they are not independent. Marrow can serve as a source of bone cells, the blood vessels in marrow form a critical part of the circulatory system in bone, and disorders or mechanical disruption of the marrow can affect the activities of bone and periosteal cells. However, most diseases, deformities, and injuries of the skeleton and most orthopaedic treatments primarily affect the bone tissue and the periosteum.

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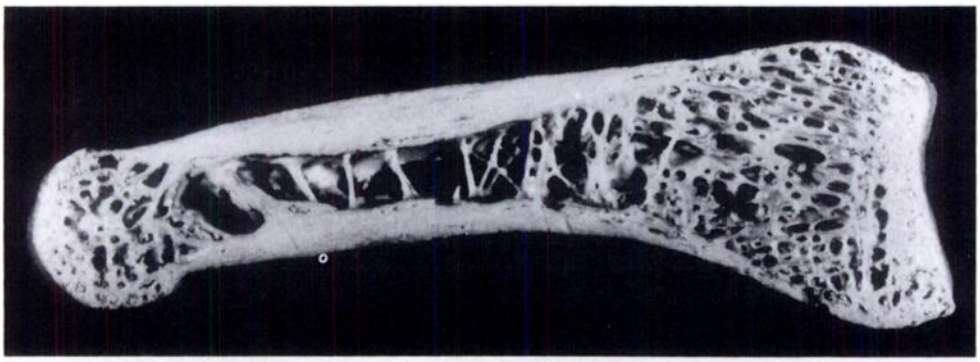

FIG. 1

Longitudinal section of a human phalanx, showing the central marrow space and the distribution of cortical and cancellous bone in a typical tubular (long) bone. The metaphyses contain most of the cancellous bone. The cortical bone decreases in thickness from the mid-part of the diaphysis to the metaphyses, where the plates of cancellous bone are arranged to support the subchondral bone. Large long bones, such as the femur, tibia, and humerus, have the same structural pattern. (Reprinted, with permission, from: Buckwalter, J. A., and Cooper, R. R.: Bone structure and function. In Instructional Course Lectures, The American Academy of Orthopaedic Surgeons. Vol. 36, p. 29. Park Ridge, Illinois, The American Academy of Orthopaedic Surgeons, 1987.)

Cortical and Cancellous Bone

Gross inspection shows that there are two forms of bone tissue: cortical (compact) bone and cancellous (trabecular) bone[17,30,68,87,88,104] (Fig. 1). Cortical and cancellous bone have the same matrix composition and structure, but the mass of the cortical bone matrix per unit of volume is much greater — that is, cortical bone has greater density or less porosity (approximately 10 per cent porosity) than cancellous bone (50 to 90 per cent porosity). As the compressive strength of bone is proportional to the square of its density, the modulus of elasticity and the ultimate compressive strength of cortical bone may be as much as ten times greater than those of a similar volume of cancellous bone[29,30,40,96].

Cortical bone forms approximately 80 per cent of the mature skeleton[87] and surrounds the marrow and the cancellous bone plates. In long or tubular bones, dense cortical bone forms the diaphysis, and there is little or no cancellous bone in this region. The thick cortical walls of the diaphysis become thinner and increase in diameter as they form the metaphysis, where plates of cancellous bone orient themselves to provide support for a thin shell of subchondral bone that underlies the articular cartilage. Short bones, such as the tarsals, carpals, and vertebral bodies, and flat bones, such as the skull and pelvis, generally have thinner cortices than do the diaphyses of long bones and contain more cancellous bone. Thus, the vertebral bodies, the pelvic bones, and the metaphyses of long bones contain most of the cancellous bone, surrounded by a relatively thin layer of cortical bone, and the diaphyses of long bones consist primarily of thick cortical bone.

Although cortical and cancellous bone have the same composition and material properties, differences in distribution and arrangement are responsible for the differences in the mechanical properties of specific bones and parts of bones[17,29-31,40,68,101]. In long bones, the thick, dense, tubular cortical bone of the diaphysis provides maximum resistance to torsion and bending. In the metaphyses and epiphyses, the thinner cortices and subchondral bone supported by cancellous bone allow greater deformation to occur under the same load. Thus, the complex formed by the subchondral bone and epiphyseal-metaphyseal trabeculae and cortices not only broadens the bone to form an articular surface, it also helps to absorb impact loads applied across synovial joints[30,50], thereby protecting the articular cartilage and subchondral bone from damage. Replacement of this epiphyseal-metaphyseal osseous complex and articular cartilage with a joint prosthesis inserted and fixed to bone with polymethylmethacrylate eliminates its impact-absorbing effects and thereby dramatically increases the peak force transmitted across the joint by an impact load[50].

The structure of both cancellous and cortical bone changes in response to applied loads, immobilization, hormonal influences, and other factors[29-31,40,86,96]. Cancellous bone has approximately twenty times more surface area per unit of volume than does cortical bone, and its cells lie primarily between lamellae or on the surface of the trabeculae, where they can be directly influenced by adjacent bone-marrow cells[17,68,101]. In contrast, a higher proportion of the cell population of cortical bone is completely surrounded by bone matrix[17,68,101]. Perhaps at least in part because of this difference in organization — that is, in the extent of cell-covered surface area and in the proximity of blood vessels in the marrow — cancellous bone usually has a higher rate of metabolic activity and remodeling and appears to respond more rapidly to changes in mechanical loads than does cortical bone. This difference can be observed on radiographs of the long bones of an immobilized limb. A decrease in the density of the cancellous bone, caused by resorption of trabeculae, usually can be seen before there is a visible increase in the porosity of the cortical bone, caused by the formation of resorption cavities.

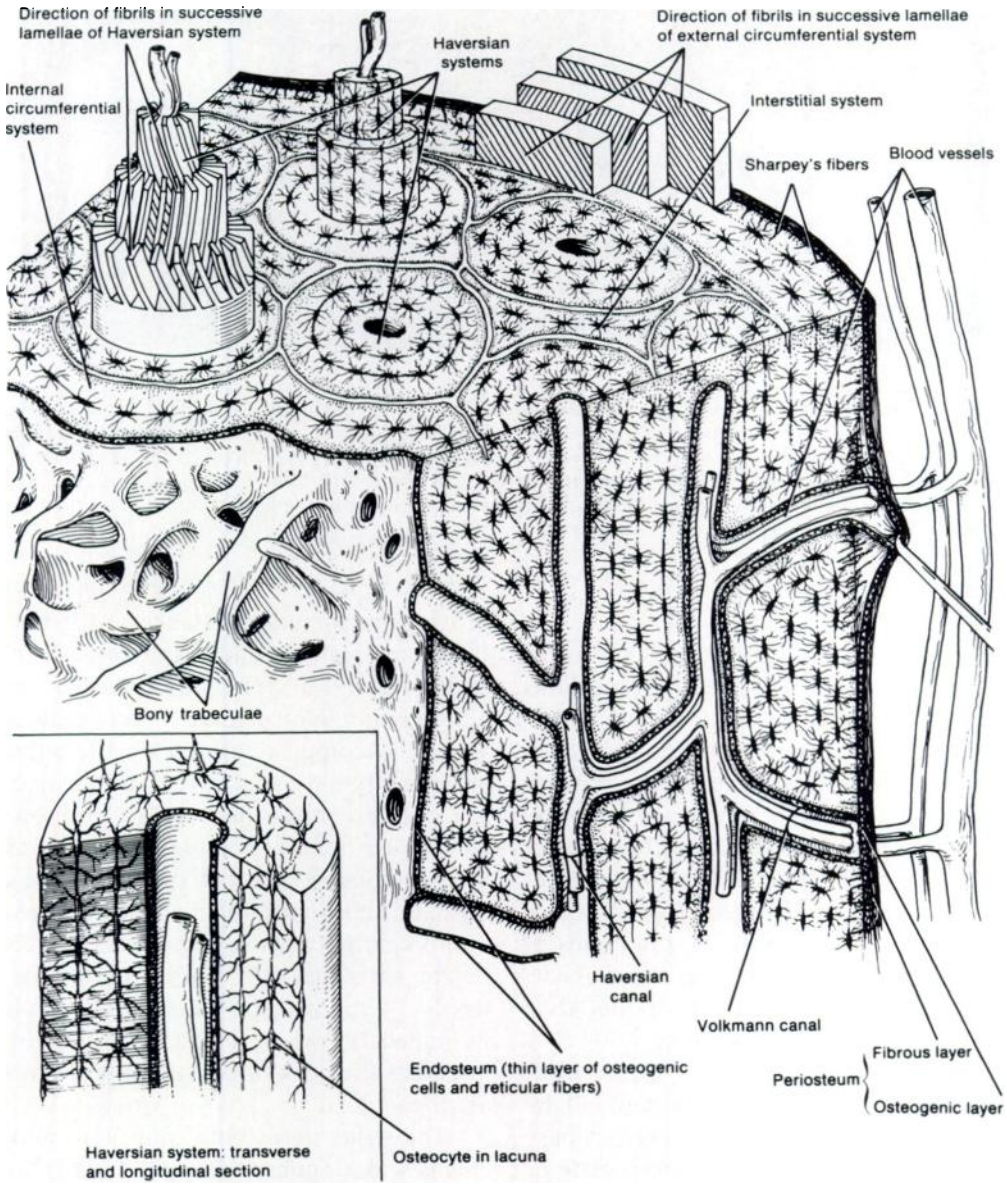

FIG. 2

Diagram of the structure of cortical bone, showing the types of cortical lamellar bone: the internal circumferential system, interstitial system, osteonal lamellae, and outer circumferential system. The diagram also shows the intraosseous vascular system that serves the osteocytes and connects the periosteal and medullary blood vessels. The haversian canals run primarily longitudinally through the cortex, while the Volkmann canals create oblique connections between the haversian canals. Cement lines separate each osteon from the surrounding bone. Periosteum covers the external surface of the bone and consists of two layers: an osteogenic (inner) cellular layer and a fibrous (outer) layer. (Reprinted, with permission, from: Kessel, R. G., and Kardon, R. H.: Tissues and Organs: a Text-Atlas of Scanning Microscopy, p. 25. New York, W. H. Freeman, 1979.)

Woven and Lamellar Bone

Cortical or cancellous bone may consist of woven (fiber or primary) or lamellar (secondary) bone[16,30,101]. Woven bone forms the embryonic skeleton and is then resorbed and replaced by mature bone as the skeleton develops. Fracture callus follows the same sequence[18]. Small amounts of woven bone may form permanent parts of tendon and ligament attachments, the suture margins of the cranial bones, and the ossicles of the ear, and woven bone is formed in the growth plates. With these exceptions, woven bone rarely is present in the normal human skeleton after the age of four or five years. It can, however, appear at any age in response to osseous or soft-tissue injury, treatments that stimulate the formation of bone, metabolic and neoplastic diseases, or inflammation.

Woven and lamellar bone differ with regard to their formation, composition, organization, and mechanical properties. Woven bone has a rapid rate of deposition and turnover, whereas lamellar bone is usually less active. Compared with lamellar bone, woven bone has an irregular, almost random, pattern of collagen fibrils consistent with its name, and it contains approximately four times as many osteocytes per unit of volume. The os-

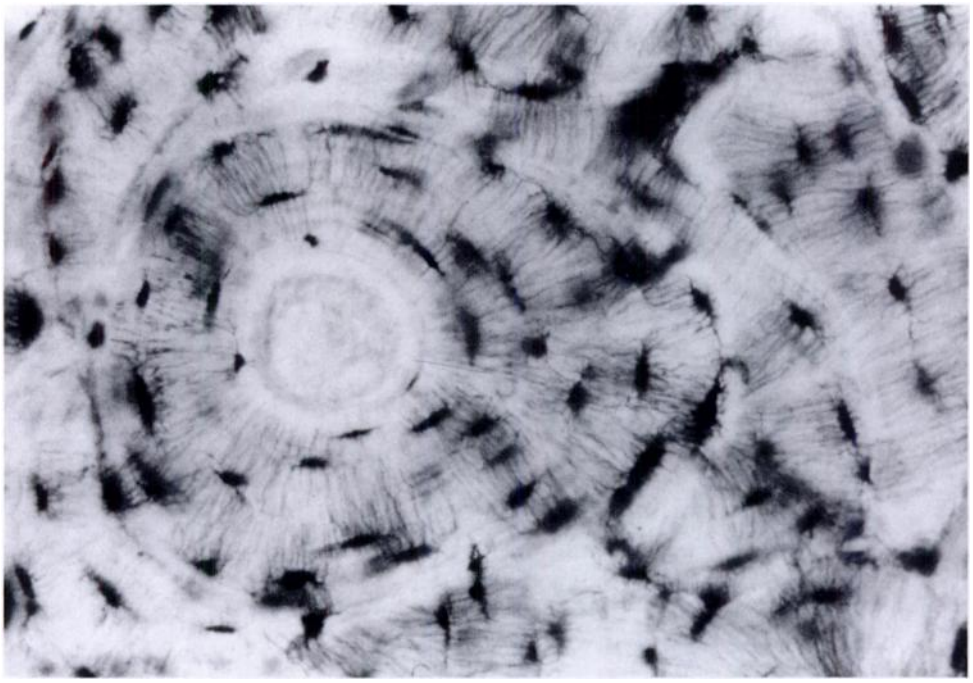

FIG. 3

Light micrograph of a transverse section of an osteon. Note the central canal of the osteon and the dark elliptical or lens-shaped osteocyte cell bodies. Each osteocyte sends fine, thread-like cell processes through the matrix to come into contact with cell processes from other osteocytes. The osteon forms a distinct structural and functional unit within the cortex. Few, if any, cell processes penetrate the cement line that marks the boundary of the osteon (× 950). (Reprinted, with permission, from: Buckwalter, J. A., and Cooper, R. R.: Bone structure and function. In Instructional Course Lectures, The American Academy of Orthopaedic Surgeons. Vol. 36, p. 31. Park Ridge, Illinois, The American Academy of Orthopaedic Surgeons, 1987.)

teocytes of woven bone vary in size, orientation, and distribution, while those of lamellar bone are relatively uniform in size with their principal axis oriented parallel to that of other cells and to the collagen fibrils of the matrix. The mineralization of woven bone follows an irregular pattern; mineral deposits vary in size and in their relationship to collagen fibrils. This pattern of mineralization, combined with the frequent patchwork formation of woven bone, creates an irregular radiographic appearance. In contrast, the collagen fibrils of lamellar bone vary less in diameter and lie in tightly organized parallel sheets, forming distinct lamellae four to twelve micrometers thick with almost uniform distribution of mineral within the matrix[28,30,31,68]. This organization gives cortical lamellar bone a homogeneous radiographic appearance that is distinctly different from that of woven bone.

Because of its irregular collagen-fibril orientation, relatively high cell and water content, and irregular pattern of mineralization, woven bone is more flexible, more easily deformed, and weaker than lamellar bone[109]. The almost random alignment of the collagen fibrils makes the mechanical properties of woven bone similar regardless of the orientation of the applied forces — that is, it behaves isotropically when loaded. In contrast, lamellar bone behaves anisotropically: its mechanical properties differ depending on the orientation of the applied forces. For these reasons, restoration of normal mechanical properties to bone tissue at the site of a healing fracture requires the eventual replacement of the woven bone of the fracture callus with mature lamellar bone[18].

Forms of Lamellar Bone

There are four general forms of lamellar bone: the trabecular lamellae of cancellous bone, the inner and outer circumferential lamellae of cortical bone, the interstitial lamellae of cortical bone, and the lamellae of osteons[16,17] (Fig. 2). Each lamella consists of highly oriented, densely packed collagen fibrils. The fibrils and adjacent lamellae run in different directions, similar to the alternating directions of the wood grain in plywood. The collagen fibrils frequently interconnect not only within but also between lamellae, thereby increasing the strength of the bone.

Osteons form the bulk of the diaphyseal cortex of the mature human skeleton[16,17,27,70,101] (Fig. 3). They consist of irregularly branching and anastomosing, longitudinally running cylinders that spiral around the diaphysis[25]; they are formed from concentric lamellae of bone surrounding central canals[28]. The central canals of osteons, referred to as haversian canals, contain blood vessels, lymphatic vessels, and occasionally nerves (Fig. 4). Canaliculi containing the cell processes of osteocytes extend in a radial pattern from the central canal like the spokes of a wheel[16,17,101] (Fig. 5). These canaliculi connect the central canal to osteocytes and pass from osteocyte to osteocyte. Since the diffusion of nutrients through

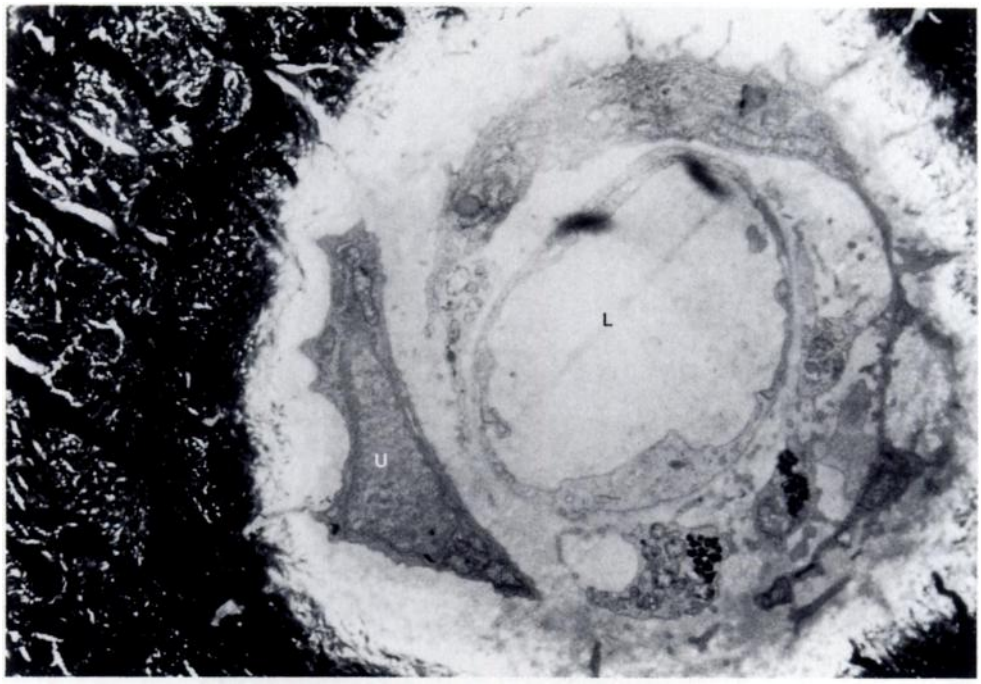

FIG. 4

Electron micrograph showing the central canal of an osteon. The peripheral darkly stained mineralized bone matrix surrounds the canal. An undifferentiated cell (u) and a blood vessel lie within the canal. L = lumen of the blood vessel (× 4500). (Reprinted, with permission, from: Buckwalter, J. A., and Cooper, R. R.: Bone structure and function. In Instructional Course Lectures, The American Academy of Orthopaedic Surgeons. Vol. 36, p. 32. Park Ridge, Illinois, The American Academy of Orthopaedic Surgeons, 1987.)

mineralized bone matrix is limited, the cells depend primarily on the canaliculi for the delivery of their metabolic requirements. The longitudinal orientation of the osteons (Fig. 2) may explain why diaphyseal cortical bone is stronger in both tension and compression when it is loaded parallel rather than perpendicular to its long axis.

Cement lines define the outer boundary of each osteon[16,17,28,30]. These thin layers of organic matrix, the composition of which is similar to that of osteoid, mark sites where the resorption of bone stopped and new-bone formation began. They separate rather than bind or cement adjacent matrix lamellae. Although they appear as lines on histological sections, they actually cover the entire outer surface of the osteon and therefore might more accurately be considered as sheaths or lamellae. In general, the cell processes of canaliculi and the collagen fibrils of osteons do not cross cement lines, so each osteon is left isolated from adjacent ones. For this reason, cracks in the bone matrix tend to follow cement lines rather than to cross osteons[30,41]. This deflection of crack propagation may prevent fatigue cracks from extending rapidly across a bone, allowing the bone cells to repair the cracks before a complete fracture occurs.

A complex internal network of canals, lacunae, and canaliculi forms one of the most remarkable structural features of mature lamellar cortical bone. Throughout even the most dense cortical bone, osteonal central canals branch and anastomose and join obliquely oriented vascular canals, referred to as Volkmann canals[28] (Fig. 2). This elaborate network of intraosseous canals not only runs through the length of the bone but also connects the periosteal surface with the endosteal surface, thereby creating potential channels of communication extending from the marrow to the periosteum. Within osteons and the other three forms of lamellar bone (interstitial lamellae and inner and outer circumferential lamellae), osteocyte lacunae and canaliculi-containing osteocyte cell processes create an even larger surface area. Taken together, the surface area of the vascular canals and osteocyte lacunae and canaliculi is roughly 100 times larger than the combined periosteal and endosteal surface area of mature cortical bone[17].

The network of canals and lacunae within bone forms an extensive extravascular space where ions and fluid can flow freely, directly adjacent to the mineralized matrix[67], and deformation of bone by applied loads causes fluid and ion flows that generate electrical potentials[45,59,84,105,118]. Evidence that bone cells respond to electrical signals[11-13,45] suggests that the electrical potentials generated by loading of the bone have a role in regulating bone-cell function.

Periosteum

The periosteum has received relatively little attention from investigators interested in bone biology, yet it has considerable mechanical and biological importance. It covers the external surfaces of bones except in the regions immediately around or within synovial joints,

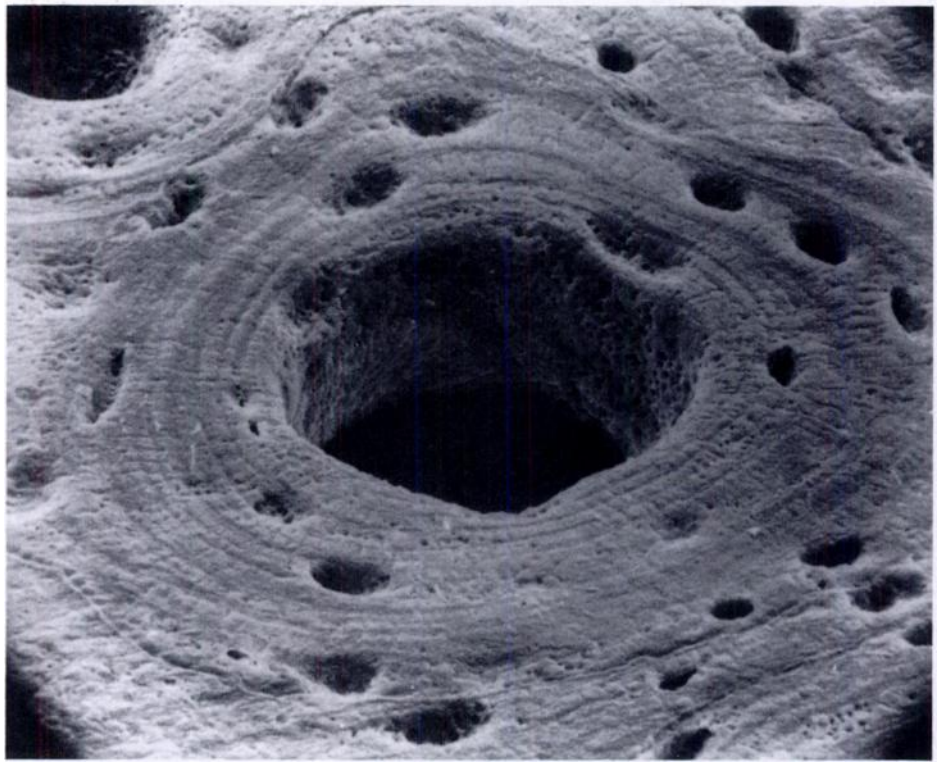

FIG. 5

Scanning electron micrograph of an osteon. The grooves in the bone matrix forming concentric circles around the central canal separate adjacent lamellae. Lacunae, the spaces occupied by osteocytes in living cells, appear as depressions or holes in the matrix. The canaliculi that contain osteocyte cell processes in living cells appear as fine grooves radiating from the central canal (× 1050). Note the canaliculi that open into the central canal. (Reprinted, with permission, from: Kessel, R. G., and Kardon, R. H.: Tissues and Organs: a Text-Atlas of Scanning Microscopy, p. 28. New York, W. H. Freeman, 1979.)

such as the femoral neck, and at the sites of tendon, ligament, and interosseous membrane insertions. In most regions, the periosteum can be easily stripped from the bone, but near the articular surfaces and at the site of muscle, tendon, and intraosseous membrane insertions, the periosteum joins with the insertion so firmly that sharp dissection is frequently needed to separate it as a distinct layer. The periosteum contributes an important part of the blood supply to the bone. Periosteal cells can resorb and form bone in response to local or systemic stimuli and may have important roles in bone metabolism.

The periosteum consists of two layers: an outer layer that is dense and fibrous and an inner layer that is looser and more vascular and cellular[16,87]. The inner layer contains cells that are capable of becoming osteoblasts and thus is referred to as the cambium or osteogenic layer. The cells of this layer can also form hyaline cartilage under appropriate circumstances, and they help to form extraosseous callus during fracture-healing[18]. During bone growth, they secrete the organic matrix that enlarges the diameter of the bone. The outer, fibrous layer has fewer cells and more collagen. It continues into the joint capsule and thereby connects one bone to the next. In addition, some tendons and ligaments insert primarily into this layer of the periosteum[17,27].

The periosteum changes with age[119]. The thick cellular cambium layer in children readily forms new bone. This capacity is demonstrated when the diaphysis of a bone in a child is destroyed by osteomyelitis or trauma and, under favorable conditions, the remaining periosteum forms a new diaphysis (an involucrum)[32] (Figs. 6-A and 6-B). With increasing age, the periosteum becomes thinner and its osteogenic capacity decreases. At skeletal maturity, the cambium layer has almost completely disappeared, and the more superficial fibrous layer thins and becomes less cellular[119]. Despite these changes, periosteal cells continue to form new bone throughout life, as demonstrated by the increasing diameter of the diaphysis of long bones with increasing age.

Blood Supply to the Bone

Mature bones have an elaborate vascular system that supplies the cells of the marrow, bone tissue, and periosteum. Even within dense cortical bone tissue, the organization of vascular canals ensures that no cell lies more than 300 micrometers from a blood vessel[3,15,17,28,56,57,70,91,101,115,119] (Fig. 2). Disruptions of the blood supply to the bone due to disease, injury, or operative procedures can cause necrosis and impair healing. Thus, effective treatment of musculoskeletal injuries and good operative planning require an understanding of the blood supply to the involved bones. This is particularly important in such regions as the talus, scaphoid, tibial

diaphysis, femoral head, and other epiphyses, where trauma or an operation can easily disrupt the blood supply. The rate of blood flow varies among bones[63], and the anatomy of the blood supply for each bone has some unique characteristics. Despite these differences, all long bones have the same general pattern of blood vessels, consisting of two circulatory systems: the periosteal-diaphyseal-metaphyseal system and the epiphyseal-physeal system. These two systems form anastomoses of variable extent on and within the periosteum and across the physes[119].

Periosteal-Diaphyseal-Metaphyseal Blood Supply

The diaphyses and metaphyses of long bones have three sources of blood supply: nutrient arteries, arteries that penetrate the epiphysis and metaphysis, and periosteal arteries[3,15,56,79,101,110,119]. Nutrient arteries pass through the diaphysis and branch proximally and distally within the medullary cavity. Proximal and distal branches of the nutrient arteries join multiple fine branches from the periosteal and metaphyseal arteries to form the medullary arterial system. Under normal circumstances, this medullary vascular system supplies most of the periosteum-covered bone; therefore, the primary direction of blood flow through the cortex is centrifugal[15,91,110]. This pattern changes where dense fascial structures, such as tendons, ligaments, or interosseous membranes, insert into the bone[91]. In these areas, periosteal vessels usually supply the outer third of the cortex.

The periosteum also has an elaborate vascular system[26,79,91,108,119-121,125]. A vascular plexus lies over the sur-

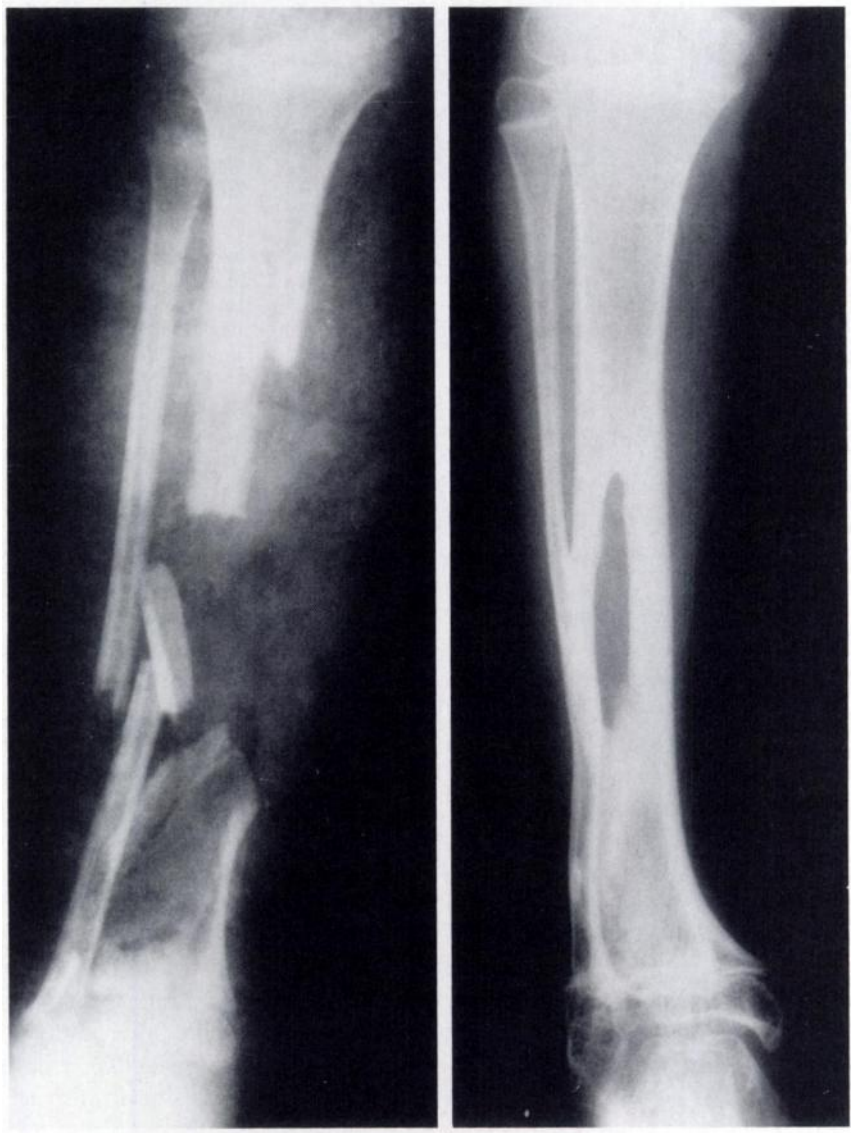

FIG. 6-A FIG. 6-B

Figs. 6-A and 6-B: Radiographs of the leg of an eleven-year-old child. The leg was caught in the blades of a mowing machine.

Fig. 6-A: Immediately after the injury, there was segmental loss of the tibia. The soft tissues were also severely damaged, leaving only a few shreds of periosteum. The wound was debrided, and closure of the remnants of the tibial periosteal sleeve was attempted.

Fig. 6-B: Eighteen months after the injury, there was regeneration of an imperfect but functional tibial diaphysis.

face of the outer, fibrous layer and anastomoses with the vessels in skeletal muscle and with the network of vessels in the cambium layer of the periosteum. In children, the vessels of the cambium layer form a well developed vascular plexus and also penetrate bone to join with the intraosseous vessels. With increasing age, the number of periosteal vessels diminishes, and their contribution to the osseous blood supply may decline[119]. Nonetheless, the periosteal vascular network remains an important part of osseous circulation throughout life.

Under certain circumstances, the periosteal vessels may also be an important source of blood supply to skeletal muscle[119,120]. When the blood supply to a muscle has been decreased or interrupted by damage to its nutrient artery or by a crush injury, separation of the muscle from the underlying periosteum further increases the probability of necrosis of the muscle[119,120]. If the nutrient artery to a muscle is severed but the muscle-periosteal vascular connections are left intact, blood flow to the muscle does not markedly decrease[119,120]. Therefore, crush injuries of muscle are less likely to cause ischemic damage if the muscle-periosteal vascular connections remain intact. The blood supply to the outer, fibrous layer and the inner, cambium layer of the periosteum depends on vascular connections with skeletal muscle. This concept is supported by the observation that periosteum can survive and form bone after it has been stripped from the bone, as long as the vascular connections between the muscle and the periosteum remain intact[58] (Figs. 6-A and 6-B). Thus, disruption of the vascular connections between the periosteum and muscle may adversely influence both the collateral blood supply to the muscle and the ability of the periosteum to form new bone. While subperiosteal dissection preserves these vascular connections, it can also be harmful because the bone is deprived of its usual route of venous drainage and its collateral arterial supply when the periosteum is stripped from it[119-122].

The anastomosis between the medullary vascular system and the periosteal system, including the arteries penetrating the metaphysis, gives the diaphysis and metaphysis a dual blood supply[15-17,79,108,119-121]. This may be important after bone or soft-tissue injuries or after operative procedures[108,112,119-122]. Limited circumferential stripping of the periosteum does not decrease blood flow in the middle layers of diaphyseal cortical bone. Similarly, reaming of the medullary canal and thereby destruction of the medullary vascular system also does not substantially decrease blood flow in these layers. These observations as well as the complex anatomy of the vascular supply to cortical bone (Fig. 2) suggest that the diaphysis can receive a major portion of its blood supply from either the periosteal or the medullary system and that either system can provide venous drainage[108,119-122].

This dual circulatory system explains why diaphyseal and metaphyseal bone can remain viable and fractures

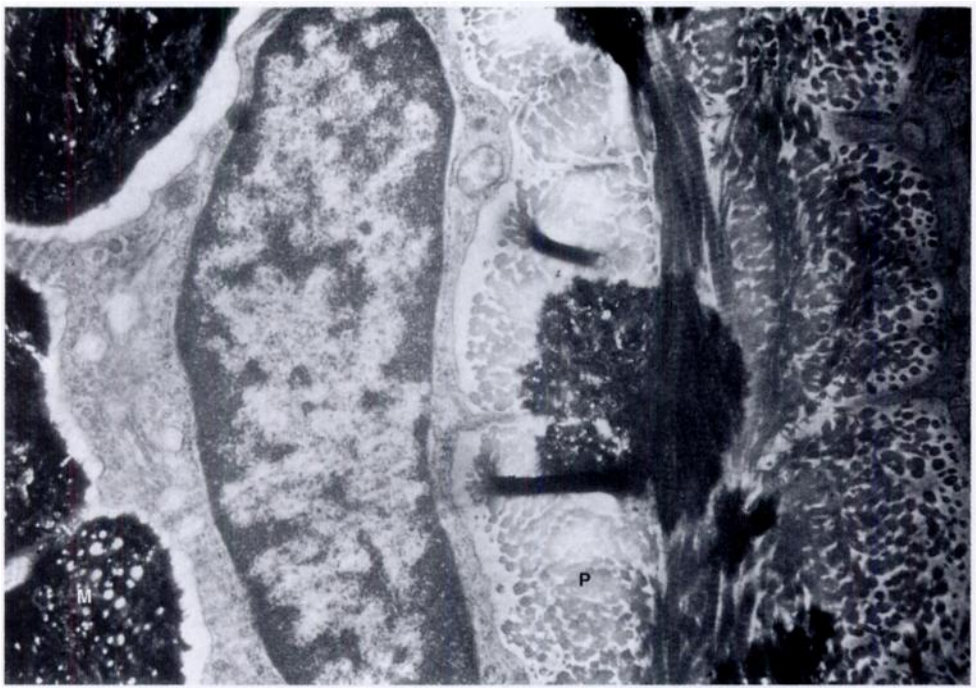

FIG. 7

Transmission electron micrograph showing an osteoblast becoming an osteocyte. The cell has surrounded itself with matrix that has become mineralized on one side of the cell (M) and partially mineralized (P) on the other. Cell processes extend from the cell into the mineralized matrix (× 7000). (Reprinted, with permission, from: Buckwalter, J. A., and Cooper, R. R.: Bone structure and function. In Instructional Course Lectures, The American Academy of Orthopaedic Surgeons. Vol. 36, p. 39. Park Ridge, Illinois, The American Academy of Orthopaedic Surgeons, 1987.)

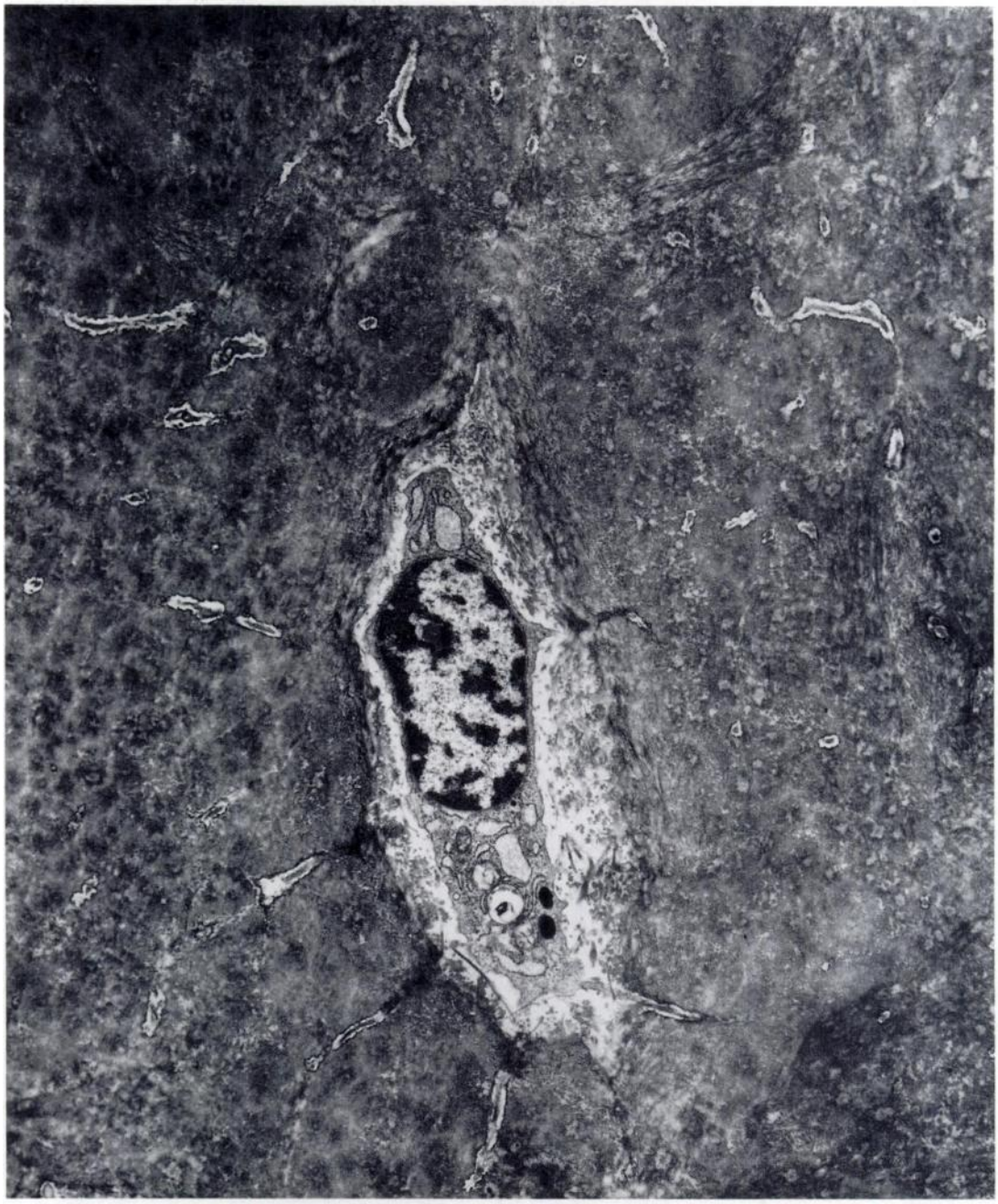

FIG. 8

Transmission electron micrograph showing an osteocyte surrounded by matrix that was demineralized during preparation of the specimens. Note the lens-shaped body of the cell and the large number of cell processes extending into the matrix surrounding the cell (× 6000).

can heal after either medullary reaming or periosteal stripping. It also explains, in part, why segmental fractures that disrupt the medullary vascular supply in a limb that also has extensive soft-tissue injury or periosteal stripping may result in delayed union or non-union and why it is best to avoid operative procedures that may damage both the medullary and the periosteal blood supply.

Epiphyseal-Physeal Blood Supply

The blood supply to most epiphyses is more precarious than the supply to the periosteum, diaphysis, or metaphysis[24,74,91,113,114,119]. During skeletal growth, few if any blood vessels cross the cartilaginous portion of the physis, leaving the epiphysis and the epiphyseal pole of the physis dependent on blood vessels that penetrate the epiphysis. After closure of the physis, vascular channels penetrate the physeal scar, but their functional importance remains uncertain[24,113,114,119]. In particular, they may not be sufficient to supply the entire epiphysis after damage to the penetrating epiphyseal arteries. This arrangement appears to be at least partially responsible for the frequency of necrosis of the capital femoral epiphysis, the epiphysis of the second metatarsal, and

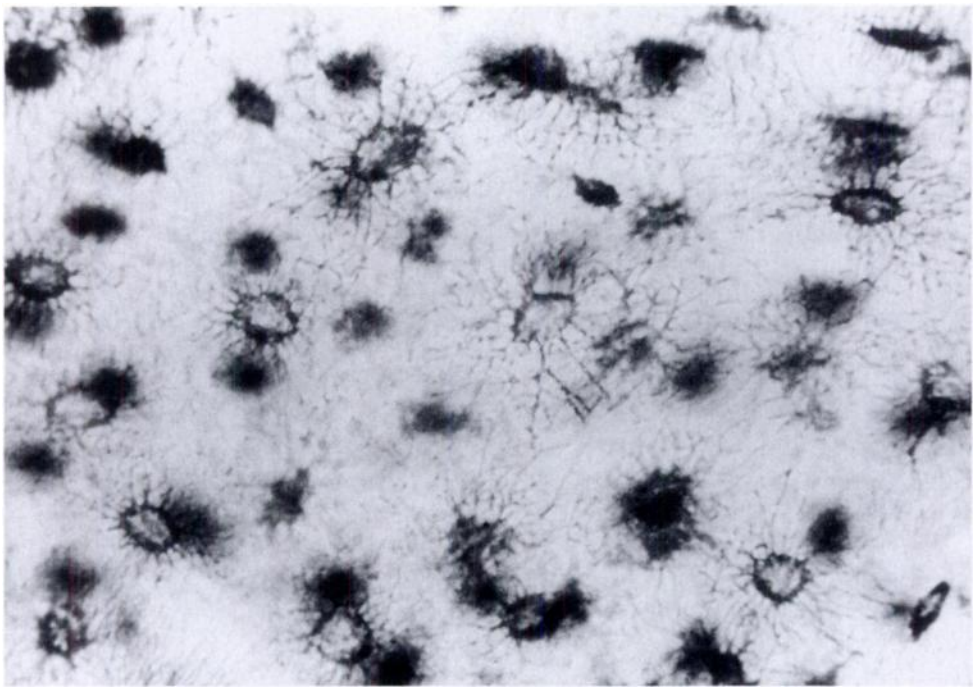

FIG. 9

Light micrograph of osteocytes, showing their cell processes. The cell processes extend radially from the cell bodies and form complex branching patterns (× 2500).

the epiphysis of the humeral capitellum. The capital femoral epiphysis is especially vulnerable to loss of its blood supply since its penetrating vessels must pass along the femoral neck, where they are easily injured by dislocation of the hip, fracture of the femoral neck, or operative dissection.

Bone Cells

To carry out the diverse functions of the formation of bone, resorption of bone, mineral homeostasis, and repair of bone, bone cells assume specialized forms distinguished by morphology, function, and characteristic location. They originate from two cell lines: a mesenchymal stem-cell line and a hematopoietic stem-cell line. The mesenchymal stem-cell line consists of undifferentiated cells or preosteoblasts, osteoblasts, bone-lining cells, and osteocytes. The hematopoietic stem-cell line consists of circulating or marrow monocytes, preosteoclasts, and osteoclasts.

Undifferentiated Mesenchymal Cells

Undifferentiated mesenchymal cells that have the potential to become osteoblasts (also referred to as preosteoblasts) reside in the bone canals, endosteum, periosteum, and marrow[5,16,17,28,42,47,76,77,81,101] (Fig. 4). They may also enter the bone by migrating from surrounding tissues or from the blood. Vascular pericytes provide another source of preosteoblasts[14,33]. Undifferentiated cells have an irregular form, a single nucleus, minimum cytoplasm, and few organelles (Fig. 4). They remain in their undifferentiated state until they are stimulated to proliferate and differentiate into osteoblasts. For example, after a fracture, a sequence of events (including the release of a variety of growth factors) stimulates migration, proliferation, and differentiation of these undifferentiated cells into the osteoblasts responsible for fracture-healing[18].

Osteoblasts

Osteoblasts line the surfaces of bone and pack tightly against adjacent osteoblasts[16,17,28,87,93,101]. When active, they have a rounded, oval, polyhedral form and an osteoid seam separates them from mineralized matrix. When synthesizing new matrix, they contain abundant endoplasmic reticulum, Golgi membranes, and mitochondria. The surface of the cell applied to the newly formed organic matrix contains primarily endoplasmic reticulum while the nucleus lies in the pole of the cell opposite the endoplasmic reticulum. The cytoplasmic processes of osteoblasts extend through the osteoid matrix to come into contact with osteocytes within the mineralized matrix (Fig. 7). These specialized cell contacts may help to coordinate the activities of these two types of cells.

The most apparent function of osteoblasts is the synthesis and secretion of the organic matrix of bone, but these cells may also have a role in controlling electrolyte fluxes between the extracellular fluid and the osseous fluid and they may influence the mineralization of bone matrix through the synthesis of organic matrix components of bone and the production of matrix vesicles[85,86,97]. In addition, systemic hormones, in-

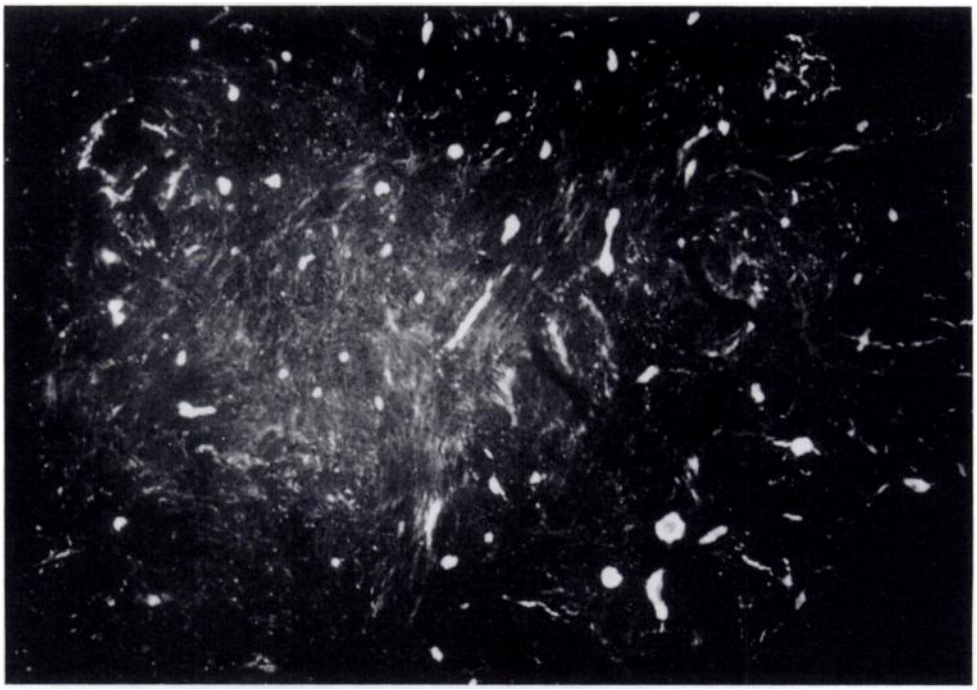

FIG. 10-A

Figs. 10-A and 10-B: Transmission electron micrographs of dense, mineralized cortical bone, showing canaliculi containing osteocyte cell processes in densely mineralized bone matrix.

Fig. 10-A: Multiple canaliculi extending throughout the matrix (× 5000).

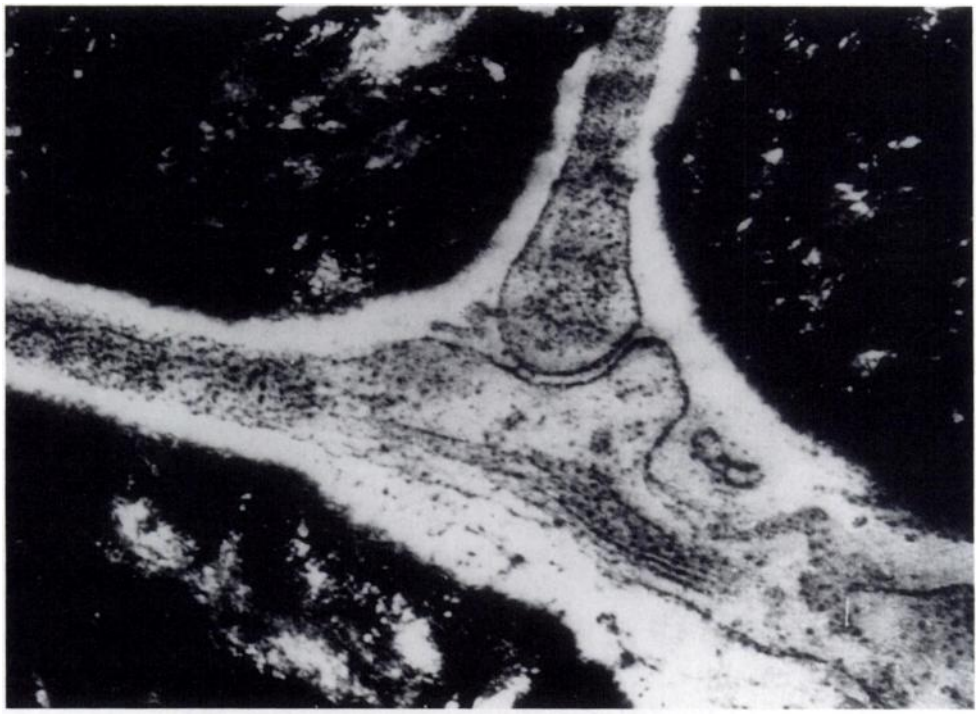

FIG. 10-B

Higher-magnification micrograph showing two osteocyte cell processes making contact at a distance from the cell bodies (× 16,000).

cluding parathyroid hormone and local cytokines, may stimulate osteoblasts to release mediators that activate osteoclasts[94].

Active osteoblasts may follow one of three courses. They may remain on the surface of the bone, decrease their synthetic activity, and assume the flatter form of bone-lining cells; they may surround themselves with matrix and become osteocytes (Fig. 7); or they may disappear from the site of bone formation[87]. In adults, many more osteoblasts appear at the site of bone for-

mation, including the site of bone-remodeling and that of fracture-healing, than survive as osteocytes or bone-lining cells after the completion of the bone formation. Therefore, a large number of osteoblasts must be removed by some as yet unknown mechanism[87].

Bone-Lining Cells

Bone-lining cells lie directly against the bone matrix and have an elongated or flattened form and cytoplasmic extensions that penetrate the bone matrix to come into contact with the cytoplasmic extensions of osteocytes[70,87]. They are sometimes referred to as resting osteoblasts or surface osteocytes. Both lining cells and osteocytes have less cytoplasm and fewer organelles than do active osteoblasts. When exposed to parathyroid hormone, lining cells contract and secrete enzymes that remove the thin layer of osteoid that covers the mineralized matrix[87]. These actions appear to be the first steps in permitting osteoclasts to attach to the surface of the bone and to begin resorption of bone. Through these actions and possibly others, lining cells may have a role in attracting osteoclasts to specific sites and in stimulating them to resorb bone.

Osteocytes

More than 90 per cent of the bone cells in the mature human skeleton are osteocytes[17,28,70,88,101]. They surround themselves with an organic matrix that can mineralize (Fig. 8) and, together with the periosteal and endosteal cells, they cover the matrix. Osteocytes have a single nucleus and their cytoplasm varies in organelle content and volume with their activity. Long, branching cytoplasmic processes project from their ellipsoidal or lens-shaped bodies (Fig. 9) through canaliculi that extend throughout the mineralized bone matrix to come into contact with cytoplasmic processes from other cells (Figs. 10-A and 10-B). The total surface area of the bone matrix that is covered by osteocyte cell bodies and their cell processes exceeds the combined area covered by periosteal and endosteal cells by roughly two orders of magnitude[17].

The large, complex network of cells covering the internal and external surfaces of bone may be extremely sensitive to stresses on the bone and also may be able to control the movement of ions in and out of the mineralized matrix. In particular, this arrangement may be critical in allowing the cell-mediated exchange of mineral to take place between the fluid in the bone and the blood. The interconnections between osteocytes, osteoblasts, and bone-lining cells may allow this cell network to sense deformation of bone and streaming potentials occurring therein and to coordinate the formation and resorption of bone and the flow of mineral ions between the bone matrix and the extravascular fluid spaces of the bone.

Osteoclasts

Unlike the other bone cells, osteoclasts appear to share a hematopoietic stem-cell precursor with cells of the monocyte family[48,54,75,86,93,117]. Specific hormones and growth factors influence the stem cells to develop into osteoclast precursors. These osteoclast precursor cells may be found in the marrow or the circulating blood. When stimulated, the mononuclear osteoclast precursors proliferate and then fuse to form large multinucleated osteoclasts. Typically, osteoclasts have three to twenty nuclei and large numbers of mitochondria and lysosomes. They are rarely found in normal bone but

FIG. 11

Scanning electron micrograph showing two osteoclasts that have created resorption cavities. Note the irregular shapes of the cells (×5500). (Reprinted, with permission, from: Kessel, R. G., and Kardon, R. H.: Tissues and Organs: a Text-Atlas of Scanning Microscopy, p. 31. New York, W. H. Freeman, 1979.)

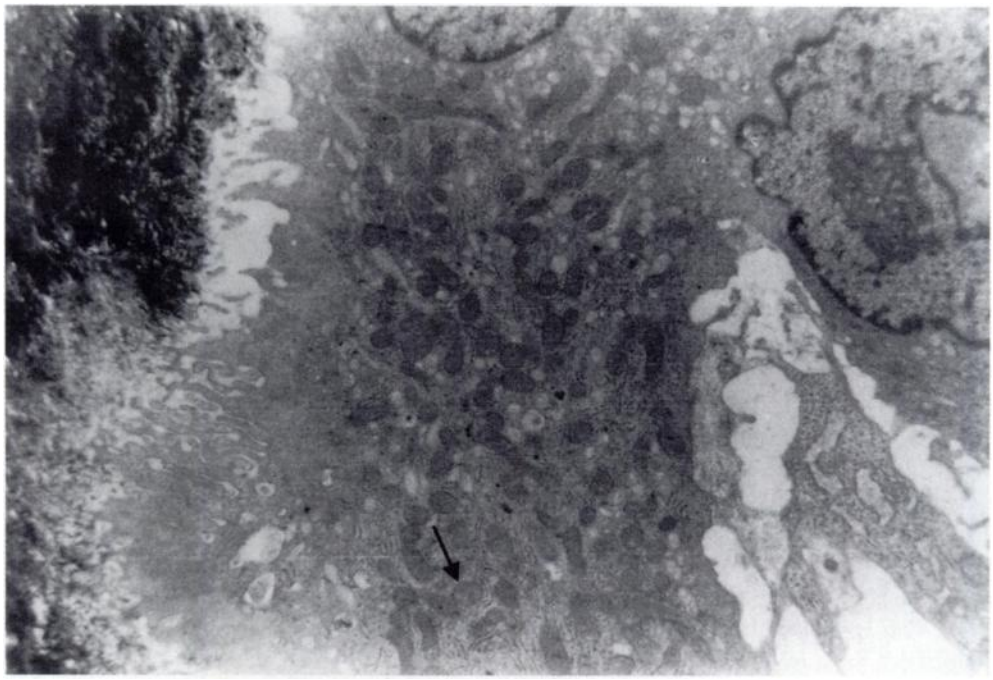

FIG. 12-A

Figs. 12-A, 12-B, and 12-C: Transmission electron micrographs of osteoclasts. (Reprinted, with permission, from: Buckwalter, J. A., and Cooper, R. R.: Bone structure and function. In Instructional Course Lectures, The American Academy of Orthopaedic Surgeons. Vol. 36, pp. 42-44. Park Ridge, Illinois, The American Academy of Orthopaedic Surgeons, 1987.)

Fig. 12-A: The osteoclast has applied itself to the surface of the mineralized bone matrix. Note the multiple nuclei, the large number of mitochondria (oval or ellipsoidal membrane-bound organelles that serve as the cells' principal source of energy) (arrow), and the formation of a ruffled cell border in the region of resorption (× 6000).

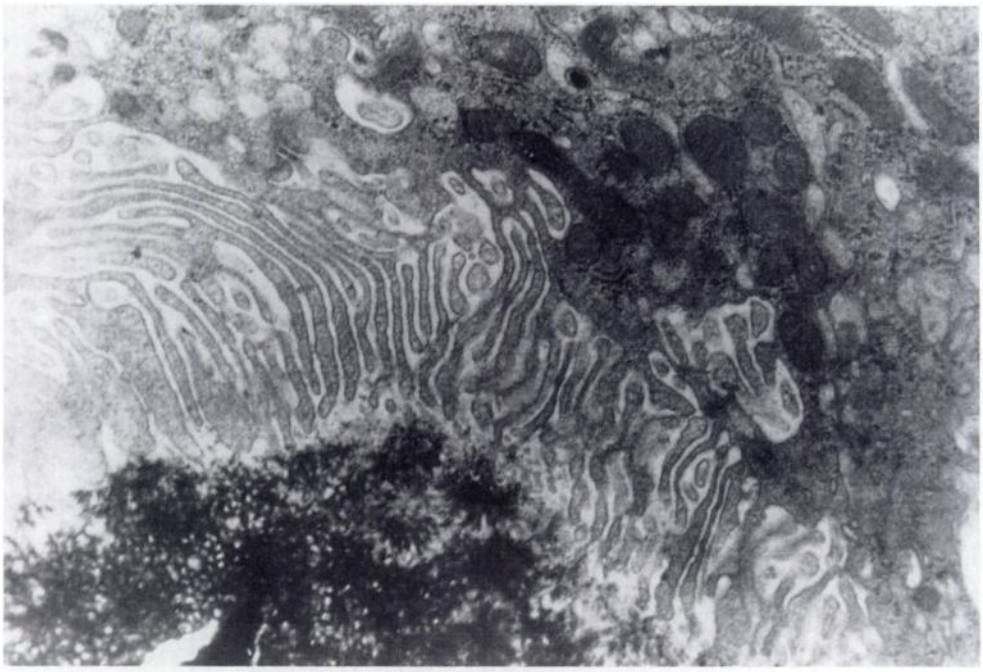

FIG. 12-B

An osteoclast brush border. Note the complex folding of the cell membrane and the direct application of the brush border to the mineralized matrix (× 8500).

occasionally can be found on the surface of endosteal, haversian, and periosteal bone. On cancellous or periosteal surfaces, they create a characteristic depression referred to as a Howship lacuna. In dense cortical bone, they lead osteonal cutting cones that tunnel through the bone, creating resorption cavities (Fig. 11).

When osteoclasts are active, mitochondria fill much of their cytoplasm to supply the great amount of energy

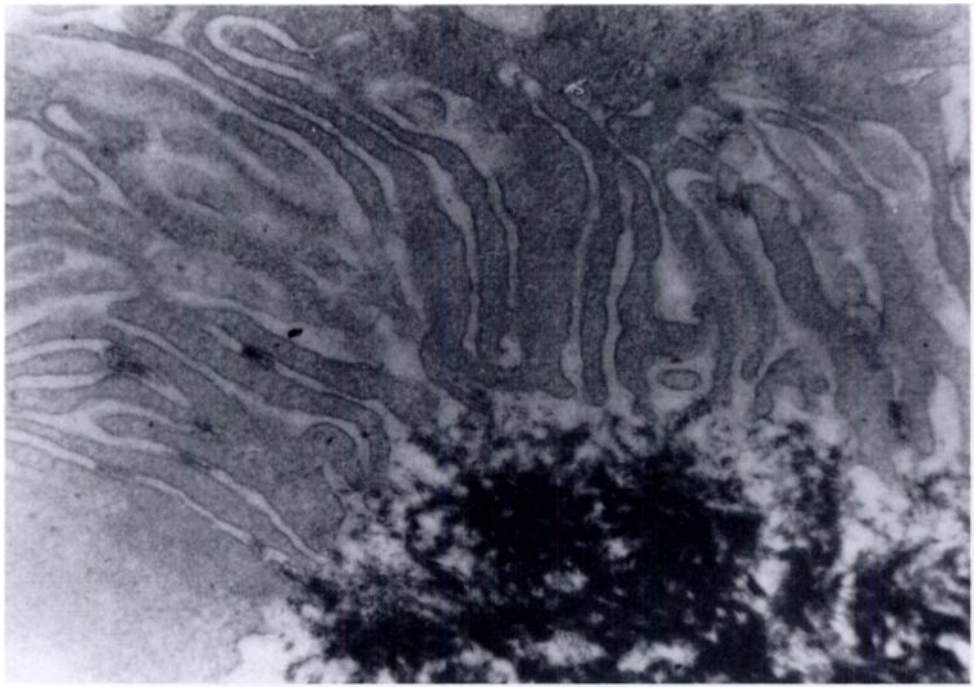

FIG. 12-C

High-magnification micrograph of the brush border applied to the mineralized matrix (× 12,000).

that is required to resorb bone (Figs. 12-A, 12-B, and 12-C). Perhaps their most distinctive feature is the complex folding of the cytoplasmic membrane where it is applied to the site of resorption of the bone matrix (Figs. 12-A, 12-B, and 12-C). This ruffled or brush border appears to play a critical part in the degradation of the matrix. A region of cytoplasm that is free of organelles, referred to as the clear zone, surrounds the brush border. The clear zone may allow the brush border to move along the surface of the bone matrix or help the cell to bind to the matrix and seal off a region for resorption. A region containing membrane-bound vesicles and vacuoles lies within the cell, deep to the ruffled border. These structures may be invaginations or infoldings of the brush border and may contain fragments of degraded bone matrix.

Osteoclasts have an extremely efficient method for destroying bone matrix[4,6,117]. They first bind themselves to the surface of the bone, creating a sealed space between the cell and the bone matrix. Endosomes containing membrane-bound proton pumps move to the region of the cell nearest the bone and insert into the cell membrane, the brush border forms, and the pumps transport protons into the sealed space, decreasing the pH from approximately 7 to approximately 4[4,6]. The acid pH solubilizes the bone mineral. To degrade the remaining organic matrix, the cell secretes acid proteases[6,86]. In addition, the osteoclast may phagocytize some matrix fragments and degrade them within cytoplasmic vacuoles. At least in areas of cancellous bone, an osteoclast may move from one site of resorption to another. Once an osteoclast has completed its resorptive activity, it may divide into mononuclear cells that can be reactivated to form new osteoclasts.

Osteopetrosis demonstrates the adverse effects of inadequate or ineffective osteoclast function[123]. In this disorder, the bone is extremely dense, hard, and white. A mixture of calcified cartilage, bone, and fibrous tissue occupies most of the marrow, leaving little normal marrow space. Loss of normal marrow elements, as a result of failure of the osteoclasts to resorb calcified cartilage and bone, may lead to severe anemia, infection, and death. Although a number of abnormalities may contribute to this condition, the principal defect is that the osteoclasts fail to resorb calcified cartilage and bone. This observation and those concerning the origin of osteoclasts have led to the successful treatment of human and animal forms of osteopetrosis by transplantation of bone marrow containing monocytes that differentiate into bone-resorbing cells and remove excessive bone and calcified cartilage[123].

Bone Matrix

Bone matrix has such great durability and stability that it can remain essentially unchanged and retain much of its strength for centuries after death[100]. Examination of lamellar bone shows that the matrix usually makes up more than 90 per cent of the volume of the tissue, with the remainder made up mostly of cells, cell processes, and blood vessels[111] (Fig. 3). Bone matrix is a composite material consisting of an organic and an inorganic component[36,111]. The inorganic component contributes approximately 65 per cent of the wet weight of the bone. The organic component usually contributes a

little more than 20 per cent of the wet weight, and water contributes approximately 10 per cent[49,111]. The organic component, primarily collagen, gives bone its form and contributes to its ability to resist tension, while the inorganic, or mineral, component primarily resists compression. Bones that have been demineralized or have had their organic matrix removed readily demonstrate the different contributions of the two matrix components. Either of these procedures leaves the bone with its original form and size but greatly alters its mechanical properties. Demineralized bone, like a tendon or ligament, is flexible, pliable, and resistant to fracture. A demineralized long bone such as the fibula can be bent or even tied in a knot without fracturing. In contrast, removal of the organic matrix makes bone rigid and brittle; even slight deformation fractures it, and a sharp blow shatters it.

Organic Matrix

The organic matrix of bone resembles the matrix of dense fibrous tissues such as tendons, ligaments, and joint capsules. Collagens, predominantly type I along with small amounts of types V and XII, make up approximately 90 per cent of the organic matrix. The other 10 per cent consists of non-collagenous glycoproteins and bone-specific proteoglycans[111]. Type-I collagen is distinguished from other collagens by its unique amino-acid content, the relatively large diameter of its fibrils, and its presence in tissues subjected to large tensile loads, including tendon and ligament.

Heritable abnormalities of type-I collagen lead to a heterogeneous group of conditions recognized as osteogenesis imperfecta[95]. Thus far, all kindreds of patients who have osteogenesis imperfecta have been shown to have collagen defects due to amino acid substitutions, but different kindreds may have different substitutions[7,95,102,103,107]. Patients who have these abnormalities of bone type-I collagen have increased fragility of bone associated with either a decreased amount of normal type-I collagen or an abnormality of the structure of the type-I collagen.

Bone also contains a variety of non-collagenous proteins that may influence the organization of the matrix, the mineralization of the bone, and the behavior of the bone cells. These proteins include osteocalcin, osteonectin, bone sialoprotein, bone phosphoproteins, and small proteoglycans[9,21,23,35,51,52,55,64,65,71,78,80,99,111,124]. The specific functions of these molecules remain uncertain, but at least some of them may influence mineralization[9,34,53]. For example, phosphoprotein complexes may help to initiate calcification[36]. Bone matrix also contains growth factors that can influence the function of bone cells. Growth factors that have been identified in bone include the transforming growth factor-β family, insulin-like growth factor-1, insulin-like growth factor-2, bone morphogenic proteins, platelet-derived growth factors, interleukin-1, interleukin-6, and colony-stimulating factors[19,20,22,34,39,44,46,73,83,86,97,106,116]. It is not certain whether all of these proteins are synthesized by bone cells or whether they are synthesized by cells outside of the bone and then incorporated into the bone matrix. However, their presence within bone and their potential to affect the activity of bone cells strongly suggest that they have an important role in controlling bone-cell function.

Inorganic Matrix

The inorganic matrix, or mineral phase, of bone performs two essential functions: it serves as an ion reservoir, and it gives bone most of its stiffness and strength[36]. Approximately 99 per cent of the body calcium, approximately 85 per cent of the phosphorus, and between 40 and 60 per cent of the total body sodium and magnesium are associated with the bone mineral crystals, the major source of these ions to and from the extracellular fluid[36]. By serving as a reservoir for these ions, the inorganic matrix of bone helps to maintain their extracellular fluid concentrations within the ranges necessary for critical physiological functions, including nerve conduction and muscle contraction as well as most of the important biochemical reactions. In addition, the rock-like calcium-phosphate crystals within the organic matrix of bone create a rigid material with the mechanical properties necessary to withstand the forces imposed by normal activity.

Recent studies[36,89,90] of the bone mineral crystals have shown that they are not pure hydroxyapatite ($Ca_{10}[PO_4][OH_2]_2$). Instead, they contain both carbonate ions and acid phosphate groups (HPO_4^{-2}). The acid phosphate groups in bone crystals have been shown to be unique to these biological crystals and are not the same acid phosphate groups found in other calcium crystals, such as brushite ($CaHPO_4{}^{-2}2H_2O$). In addition, unlike pure hydroxyapatite, bone crystals do not contain OH groups. Therefore, bone mineral crystals should be classified as apatite rather than hydroxyapatite. Large numbers of the carbonate and acid phosphate groups are very labile and probably play important roles in the interaction of the crystals with the surrounding extracellular fluid and with the organic components of the matrix.

Bone mineral crystals undergo important changes in composition, especially in the concentrations of carbonate and acid phosphate groups as a function of the age of the crystal. Thus, the biological functions of the crystals, including their role as ion reservoirs and their effects on cell function[38], and possibly their contributions to the mechanical properties of bone, depend not only on the amount of mineral present but also on the age of the mineral crystals. Recent advances in phosphorus-31 nuclear magnetic resonance spectroscopy and imaging[1] have made it possible to detect the kind and concentration of acid phosphate groups in intact bone and to obtain simultaneous three-dimensional images of the bone mineral in selected regions of bone. In the future,

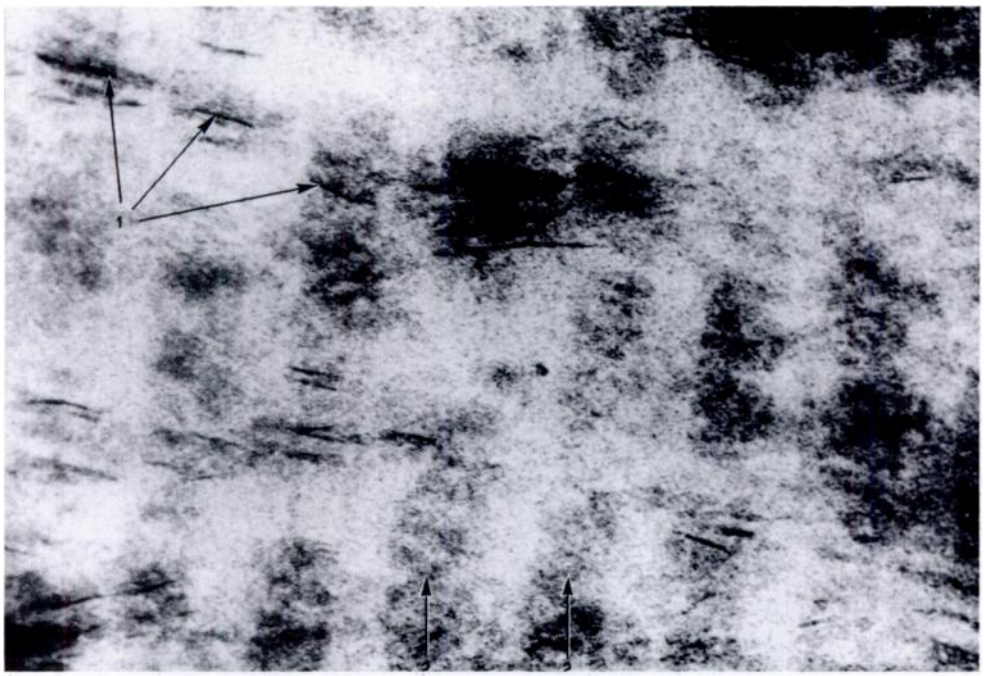

FIG. 13

Electron micrograph showing a very early stage of mineralization. The deposition of mineral occurs in a highly ordered fashion with an axial repeating pattern of approximately 700 angstroms. Individual, plate-like crystals appear as dense lines when viewed on edge (1). Because the crystals are quite thin, when viewed lying flat they appear as less dense outlines (2). (Reprinted, with permission, from: Glimcher, M. J., and Krane, S. M.: The organization and structure of bone, and the mechanism of calcification. In Treatise on Collagen, edited by B. S. Gould. Vol. 2. New York, Academic Press, 1968.)

these techniques will make it possible to assess the local structure and metabolism of bone in metabolic bone diseases and during fracture-healing.

Mineralization

Mineralization of bone, the formation of solid calcium phosphate from soluble calcium and phosphate in the organic matrix of bone, does not occur as a result of a chemical reaction; it represents a phase transformation, a process exemplified by the formation of ice from water[36]. Solid calcium phosphate in bone first appears as a poorly crystalline apatite[36,92]. With time, the crystallinity of the apatite increases, but it never approaches the highly crystalline state of naturally occurring geological hydroxyapatite or synthetic hydroxyapatite made by the precipitation of calcium phosphate *in vitro*[36,69].

Mineralization of bone collagen fibrils occurs in an organized fashion[36,60,61,92] (Fig. 13); mineral first appears in specific hole zone regions of the collagen fibrils. Unmineralized regions of the fibrils initially separate the mineralized hole zone regions. Progressive mineralization of the matrix occurs as mineral appears in an increasing number of hole zone regions within collagen fibrils and as the growth of the crystals extends to include the zone of the collagen fibrils between hole zones so that mineral deposits eventually occupy all of the available space within the fibrils (Fig. 14). During the mineralization of bone, granules of calcium phosphate appear in osteoblast mitochondria and matrix vesicles, extracellular membrane-bound structures formed from the osteoblast plasma membrane[2,9,10,36]. Although it has been suggested that mitochondria and matrix vesicles may influence mineralization directly or indirectly by concentrating and releasing calcium phosphate[2,8,62], their exact role has not been established clearly[36].

Mineralization usually proceeds quite rapidly once it begins; 60 per cent or more of the ultimate mineral forms within hours. After this initial phase, mineral continues to accumulate over a prolonged time, gradually increasing the density of the bone. Although the changes in the composition of the organic matrix that occur during mineralization remain poorly understood, it is clear that, as mineralization proceeds, the water and probably the non-collagenous protein concentrations decrease as the mineral concentration increases, but the collagen concentration and organization remain relatively unchanged[111]. Changes in specific non-collagenous matrix components during mineralization may be important in controlling the process, but these possible effects have not been well defined.

With increasing mineralization and organization of the matrix, maturation of bone crystals, and replacement of woven bone by lamellar bone, the stiffness of bone increases[109]. These maturational changes in bone matrix help to explain why the bones of children often differ from those of adults with regard to patterns of fracture[17,18,66,98]. When subjected to excessive force, normal adult bone usually breaks rather than deforms.

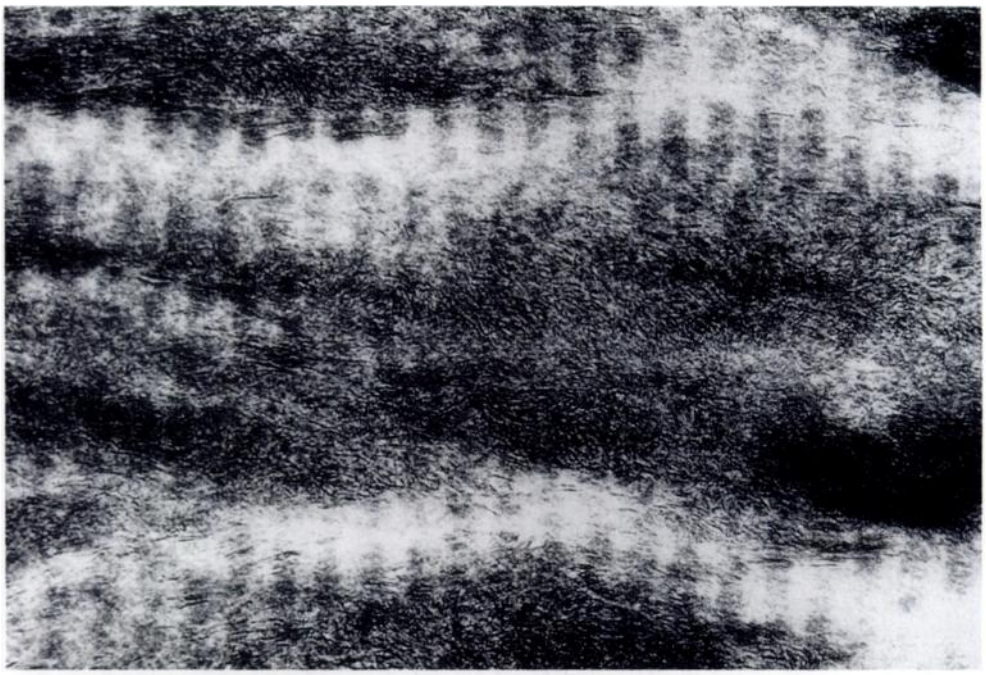

FIG. 14

Electron micrograph of undecalcified, unstained, well mineralized bone. Note the regular axial repeating pattern (approximately 700 angstroms) of mineral densities along the longitudinal axes of the collagen fibrils. In some areas, dense crystals obscure the collagen fibrils. (Reprinted, with permission, from: Glimcher, M. J., and Krane, S. M.: The organization and structure of bone, and the mechanism of calcification. In Treatise on Collagen, edited by B. S. Gould. Vol. 2. New York, Academic Press, 1968.)

In contrast, the bones of children may bow or buckle rather than break. Bowing, or plastic deformation, of the bones of children is most commonly seen when an injury deforms the ulna, the radius, or the fibula beyond its ability to resume its original shape. In torus fractures in children, a compression load buckles the bone, usually in the metaphysis, rather than breaking it. Children also sustain greenstick fractures in which a bending force fractures the cortex and ruptures the periosteum on the side of the bone loaded in tension but leaves the cortex and periosteum intact on the opposite side. As a child matures and the mineralization of the bone increases and the mineral phase matures, these fracture patterns become less common.

The abnormal mechanical properties of bone in patients who have osteomalacia or rickets[82] illustrate the importance of mineralization for normal skeletal function. In these patients, osteoblasts synthesize and secrete osteoid but the osteoid fails to mineralize. As the proportion of the unmineralized matrix increases relative to the mineralized matrix, the bone weakens and skeletal deformities or pathological fractures may develop.

References

1. **Ackerman, J. L.; Raleigh, D. P.;** and **Glimcher, M. J.:** Phosphorus-31 magnetic resonance imaging of hydroxyapatite: a model for bone imaging. *Magnet. Reson. Med.*, 25: 1-11, 1992.
2. **Anderson, H. C.:** Calcium-accumulating vesicles in the intercellular matrix of bone. In *Ciba Foundation Symposium on Hard Tissue Growth, Repair and Remineralization*, pp. 213-246. Edited by K. Elliot and D. W. Fitzsimmons. Amsterdam, Elsevier, 1973.
3. **Arlet, J.; Ficat, R. P.;** and **Hungerford, D. S.** [editors]: *Bone Circulation.* Baltimore, Williams and Wilkins, 1984.
4. **Baron, R.; Neff, L.; Louvard, D.;** and **Courtoy, P. J.:** Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein in the osteoclast ruffled border. *J. Cell Biol.*, 101: 2210-2222, 1985.
5. **Beresford, J. N.:** Osteogenic stem cells and the stromal system of bone and marrow. *Clin. Orthop.*, 240: 270-280, 1989.
6. **Blair, H. C.; Teitelbaum, S. L.; Ghiselli, R.;** and **Gluck, S.:** Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science*, 245: 855-857, 1989.
7. **Bonadio, J.,** and **Byers, P. H.:** Subtle structural alterations in the chains of type I procollagen produce osteogenesis imperfecta type II. *Nature*, 316: 363-366, 1985.
8. **Bonucci, E.:** The locus of initial calcification in cartilage and bone. *Clin. Orthop.*, 78: 108-139, 1971.
9. **Boskey, A. L.:** Noncollagenous matrix proteins and their role in mineralization. *Bone and Min.*, 6: 111-123, 1989.
10. **Boskey, A. L.:** Mineral-matrix interactions in bone and cartilage. *Clin. Orthop.*, 281: 244-274, 1992.
11. **Brighton, C. T.,** and **Hunt, R. M.:** Ultrastructure of electrically induced osteogenesis in the rabbit medullary canal. *J. Orthop. Res.*, 4: 27-36, 1986.
12. **Brighton, C. T.,** and **McCluskey, W. P.:** Response of cultured bone cells to a capacitively coupled electrical field: inhibition of cAMP response to parathyroid hormone. *J. Orthop. Res.*, 6: 567-571, 1988.

13. **Brighton, C. T.; Okereke, E.; Pollock, S. R.;** and **Clark, C. C.:** In vitro bone-cell response to a capacitively coupled electrical field. The role of field strength, pulse pattern, and duty cycle. *Clin. Orthop.*, 285: 255-262, 1992.
14. **Brighton, C. T.; Lorich, D. B.; Kupcha, R.; Reilly, T. M.; Jones, A. R.;** and **Woodbury, R. A., II:** The pericyte as a possible osteoblast progenitor cell. *Clin. Orthop.*, 275: 287-299, 1992.
15. **Brooks, M.:** *The Blood Supply of Bone.* London, Butterworth, 1971.
16. **Buckwalter, J. A.:** Musculoskeletal tissues and the musculoskeletal system. In *Turek's Orthopaedics: Principles and Their Application*, edited by S. L. Weinstein and J. A. Buckwalter. Ed. 5, pp. 13-67. Philadelphia, J. B. Lippincott, 1994.
17. **Buckwalter, J. A.,** and **Cooper, R. R.:** Bone structure and function. In *Instructional Course Lectures, The American Academy of Orthopaedic Surgeons.* Vol. 36, pp. 27-48. Park Ridge, Illinois, The American Academy of Orthopaedic Surgeons, 1987.
18. **Buckwalter, J. A.,** and **Cruess, R.:** Healing of musculoskeletal tissues. In *Rockwood and Green's Fractures in Adults*, pp. 181-222. Edited by C. A. Rockwood, Jr.; D. P. Green; and R. W. Bucholz. Philadelphia, J. B. Lippincott, 1991.
19. **Canalis, E.,** and **Lian, J. B.:** Effects of bone associated growth factors on DNA collagen and osteocalcin synthesis in cultured fetal rat calvariae. *Bone*, 9: 243-246, 1988.
20. **Canalis, E.; McCarthy, T.;** and **Centrella, M.:** Growth factors and the regulation of bone remodeling. *J. Clin. Invest.*, 81: 277-281, 1988.
21. **Carlson, C. S.; Tulli, H. M.; Jayo, M. J.; Loeser, R. F.; Tracy, R. P.; Mann, K. G.;** and **Adams, M. R.:** Immunolocalization of noncollagenous bone matrix proteins in lumbar vertebrae from intact and surgically menopausal cynomolgus monkeys. *J. Bone and Min. Res.*, 8: 71-81, 1993.
22. **Centrella, M.; McCarthy, T. L.;** and **Canalis, E.:** Current concepts review. Transforming growth factor-beta and remodeling of bone. *J. Bone and Joint Surg.*, 73-A: 1418-1428, Oct. 1991.
23. **Chen, J.; Zhang, Q.; McCulloch, C. A.;** and **Sodek, J.:** Immunohistochemical localization of bone sialoprotein in foetal porcine bone tissues: comparisons with secreted phosphoprotein 1 (SPP-1, osteopontin) and SPARC (osteonectin). *Histochem. J.*, 23: 281-289, 1991.
24. **Chung, S. M. K.:** The arterial supply of the developing proximal end of the human femur. *J. Bone and Joint Surg.*, 58-A: 961-970, Oct. 1976.
25. **Cohen, J.,** and **Harris, W. H.:** The three-dimensional anatomy of haversian systems. *J. Bone and Joint Surg.*, 40-A: 419-434, April 1958.
26. **Cooper, R. C., Jr.,** and **Cawley, A. J.:** Blood supply to the periosteum of the canine tibia. *Am. J. Veter. Res.*, 49: 1419-1423, 1988.
27. **Cooper, R. R.,** and **Misol, S.:** Tendon and ligament insertion. A light and electron microscopic study. *J. Bone and Joint Surg.*, 52-A: 1-20, Jan. 1970.
28. **Cooper, R. R.; Milgram, J. W.;** and **Robinson, R. A.:** Morphology of the osteon. An electron microscopic study. *J. Bone and Joint Surg.*, 48-A: 1239-1271, Oct. 1966.
29. **Cowin, S. C.:** Properties of cortical bone and theory of bone remodeling. In *Biomechanics of Diarthrodial Joints*, edited by V. C. Mow, A. Ratcliffe, and S. L.-Y. Woo. Vol. 2, pp. 119-153. New York, Springer, 1990.
30. **Currey, J. D.:** *The Mechanical Adaptations of Bones.* Princeton, New Jersey, Princeton University Press, 1984.
31. **Currey, J. D.:** Function and form of bone. In *Biomechanics of Diarthrodial Joints*, edited by V. C. Mow, A. Ratcliffe, and S. L.-Y. Woo. Vol. 2, pp. 3-30. New York, Springer, 1990.
32. **Daoud, A.,** and **Saighi-Bouaouina, A.:** Treatment of sequestra, pseudarthroses, and defects in the long bones of children who have chronic hematogenous osteomyelitis. *J. Bone and Joint Surg.*, 71-A: 1448-1468, Dec. 1989.
33. **Diaz-Flores, L.; Gutierrez, R.; Lopez-Alonso, A.; Gonzalez, R.;** and **Varela, H.:** Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis. *Clin. Orthop.*, 275: 280-286, 1992.
34. **Fisher, L. W.,** and **Termine, J. D.:** Noncollagenous proteins influencing the local mechanisms of calcification. *Clin. Orthop.*, 200: 362-385, 1985.
35. **Gernstenfeld, L. C.; Gotoh, Y.; McKee, M. D.; Nanci, A.; Landis, W. J.;** and **Glimcher, M. J.:** Expression and ultrastructural immunolocalization of a major 66 kDa phosphoprotein synthesized by chicken osteoblasts during mineralization in vitro. *Anat. Rec.*, 228: 93-103, 1990.
36. **Glimcher, M. J.:** The nature of the mineral component of bone and the mechanisms of calcification. In *Disorders of Bone and Mineral Metabolism*, pp. 265-286. Edited by F. L. Coe and M. J. Favus. New York, Raven Press, 1992.
37. **Glimcher, M. J.,** and **Krane, S. M.:** The organization and structure of bone, and the mechanism of calcification. In *Treatise on Collagen*, edited by B. S. Gould. Vol. 2, pp. 67-251. New York, Academic Press, 1968.
38. **Glowacki, J.; Rey, C.; Glimcher, M. J.; Cox, K. A.;** and **Lian, J.:** A role for osteocalcin in osteoclast differentiation. *J. Cell Biochem.*, 45: 292-302, 1991.
39. **Goldring, M. B.,** and **Goldring, S. R.:** Skeletal tissue response to cytokines. *Clin. Orthop.*, 258: 245-278, 1990.
40. **Goldstein, S. A.; Hollister, S. J.; Kuhn, J. L.;** and **Kikuchi, N.:** The mechanical and remodeling properties of trabecular bone. In *Biomechanics of Diarthrodial Joints*, edited by V. C. Mow, A. Ratcliffe, and S. L.-Y. Woo. Vol. 2, pp. 61-81. New York, Springer, 1990.
41. **Gordon, J. E.:** Some stiff biological materials: wood, bone, and antlers. In *The Science of Structures and Materials*, edited by J. E. Gordon. New York, Scientific American Library, 1988.
42. **Goshima, J.; Goldberg, V. M.;** and **Caplan, A. I.:** The osteogenic potential of culture-expanded rat marrow mesenchymal cells assayed in vivo in calcium phosphate ceramic blocks. *Clin. Orthop.*, 262: 298-311, 1991.
43. **Gowen, M.,** and **Mundy, G. R.:** Actions of recombinant interleukin 1, interleukin 2 and interferon-gamma on bone resorption in vitro. *J. Immunol.*, 136: 2478-2482, 1986.
44. **Gowen, M.; Wood, D. D.; Ihrie, E. J.; McGuire, M. K.;** and **Russell, R. G.:** An interleukin 1 like factor stimulates bone resorption in vitro. *Nature*, 306: 378-380, 1983.
45. **Hastings, G. W.,** and **Mahmud, F. A.:** Electrical effects in bone. *J. Biomed. Eng.*, 10: 515-521, 1988.
46. **Hauschka, P. V.; Mavrakos, A. E.; Iafrati, M. D.; Doleman, S. E.;** and **Klagsbrun, M.:** Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-Sepharose. *J. Biol. Chem.*, 261: 12665-12674, 1986.
47. **Haynesworth, S. E.; Goshima, J.; Goldberg, V. M.;** and **Caplan, A. I.:** Characterization of cells with osteogenic potential from human marrow. *Bone*, 13: 81-88, 1992.
48. **Helfrich, M. H.; Mieremet, R. H.;** and **Thesingh, C. W.:** Osteoclast formation in vitro from progenitor cells present in the adult mouse circulation. *J. Bone and Min. Res.*, 4: 325-334, 1989.
49. **Herring, G. M.:** Methods for the study of glycoproteins and proteoglycans of bone using bacterial collagenase. Determination of bone sialoprotein and chondroitin sulfate. *Calcif. Tissue Res.*, 24: 29-36, 1977.

50. **Hoshino, A.,** and **Wallace, W. A.:** Impact-absorbing properties of the human knee. *J. Bone and Joint Surg.*, 69-B(5): 807-811, 1987.
51. **Hosoda, K.; Kanzaki, S.; Eguchi, H.; Kyoki, M.; Yamaji, T.; Koshihara, Y.; Shiraki, M.;** and **Seino, Y.:** Secretion of osteocalcin and its propeptide from human osteoblastic cells: dissociation of the secretory patterns of osteocalcin and its propeptide. *J. Bone and Min. Res.*, 8: 553-565, 1993.
52. **Hulth, A.; Johnell, O.; Lindberg, L.;** and **Heinegard, D.:** Sequential appearance of macromolecules in bone induction in the rat. *J. Orthop. Res.*, 11: 367-378, 1993.
53. **Ibaraki, K.; Termine, J. D.; Whitson, S. W.;** and **Young, M. F.:** Bone matrix mRNA expression in differentiating fetal bovine osteoblasts. *J. Bone and Min. Res.*, 7: 743-754, 1992.
54. **Ibbotson, K. J.; Roodman, G. D.; McManus, L. M.;** and **Mundy, G. R.:** Identification and characterization of osteoclast-like cells and their progenitors in cultures of feline marrow mononuclear cells. *J. Cell Biol.*, 99: 471-480, 1984.
55. **Jingushi, S.; Joyce, M. E.;** and **Bolander, M. E.:** Genetic expression of extracellular matrix proteins correlates with histologic changes during fracture repair. *J. Bone and Min. Res.*, 7: 1045-1055, 1992.
56. **Kelly, P. J.:** Anatomy, physiology, and pathology of the blood supply of bones. *J. Bone and Joint Surg.*, 50-A: 766-783, June 1968.
57. **Kelly, P. J.; Montgomery, R. J.;** and **Bronk, J. T.:** Reaction of the circulatory system to injury and regeneration. *Clin. Orthop.*, 254: 275-288, 1990.
58. **King, K. F.:** Periosteal pedicle grafting in dogs. *J. Bone and Joint Surg.*, 58-B(1): 117-121, 1976.
59. **Kowalchuck, R. M.,** and **Pollack, S. R.:** Stress-generated potentials in bone: effects of bone fluid composition and kinetics. *J. Orthop. Res.*, 11: 874-883, 1993.
60. **Landis, W. J.,** and **Geraudie, J.:** Organization and development of the mineral phase during early ontogenesis of the bony fin rays of the trout Oncorhynchus mykiss. *Anat. Rec.*, 228: 383-391, 1990.
61. **Lee, D. D.,** and **Glimcher, M. J.:** Three-dimensional spatial relationship between the collagen fibrils and the inorganic calcium phosphate crystals of pickerel (Americanus americanus) and herring (Clupea harengus) bone. *J. Molec. Biol.*, 217: 487-501, 1991.
62. **Lehninger, A. L.:** Mitochondria and calcium ion transport. *Biochem. J.*, 119: 129-138, 1970.
63. **Li, G.; Bronk, J. T.;** and **Kelly, P. J.:** Canine bone blood flow estimated with microspheres. *J. Orthop. Res.*, 7: 61-67, 1989.
64. **Lian, J. B.,** and **Gundberg, C. M.:** Osteocalcin. Biochemical considerations and clinical applications. *Clin. Orthop.*, 226: 267-291, 1988.
65. **McKee, M. D.; Farach-Carson, M. C.; Butler, W. T.; Hauschka, P. V.;** and **Nanci, A.:** Ultrastructural immunolocalization of noncollagenous (osteopontin and osteocalcin) and plasma (albumin and alpha 2HS-glycoprotein) proteins in rat bone. *J. Bone and Min. Res.*, 8: 485-496, 1993.
66. **Mabrey, J. D.,** and **Fitch, R. D.:** Plastic deformation of pediatric fractures: mechanism and treatment. *J. Pediat. Orthop.*, 9: 310-314, 1989.
67. **Mak, A. F. T.:** Streaming potential in bone. In *Biomechanics of Diarthrodial Joints,* edited by V. C. Mow, A. Ratcliffe, and S. L.-Y. Woo. Vol. 2, pp. 175-194. New York, Springer, 1990.
68. **Martin, R. B.,** and **Burr, D. B.:** *Structure, Function and Adaptation of Compact Bone.* New York, Raven Press, 1989.
69. **Matsushima, N.,** and **Hikichi, K.:** Age changes in the crystallinity of bone mineral and in the disorder of its crystal. *Biochim. Biophys. Acta,* 992: 155-159, 1989.
70. **Matthews, J. L.:** Bone structure and ultrastructure. In *Fundamental and Clinical Bone Physiology,* pp. 4-44. Edited by M. R. Urist. Philadelphia, J. B. Lippincott, 1980.
71. **Mbuyi-Muamba, J. M.; Dequeker, J.;** and **Gevers, G.:** Collagen and non-collagenous proteins in different mineralization stages of human femur. *Acta Anat.*, 134: 265-268, 1989.
72. **Menck, J.; Bertram, C.;** and **Lierse, W.:** Sectorial angioarchitecture of the human tibia. *Acta Anat.*, 143: 67-73, 1992.
73. **Mohan, S.,** and **Baylink, D. J.:** Bone growth factors. *Clin. Orthop.*, 263: 30-48, 1991.
74. **Morgan, J. D.:** Blood supply of the growing rabbit's tibia. *J. Bone and Joint Surg.*, 41-B(1): 185-203, 1959.
75. **Mundy, G. R.,** and **Rodman, G. D.:** Osteoclast ontogeny and function. In *Bone and Mineral Research,* pp. 209-280. Edited by W. A. Peck. New York, Elsevier, 1987.
76. **Nakahara, H.; Goldberg, V. M.;** and **Caplan, A. I.:** Culture-expanded human periosteal-derived cells exhibit potential in vivo. *J. Orthop. Res.*, 9: 465-476, 1991.
77. **Nakahara, H.; Bruder, S. P.; Goldberg, V. M.;** and **Caplan, A. I.:** In vivo osteochondrogenic potential of cultured cells derived from the periosteum. *Clin. Orthop.*, 259: 223-232, 1990.
78. **Ninomiya, J. T.; Tracy, R. P.; Calore, J. D.; Gendreau, M. A.; Kelm, R. J.;** and **Mann, K. G.:** Heterogeneity of human bone. *J. Bone and Min. Res.*, 5: 933-938, 1990.
79. **Oni, O. O.,** and **Gregg, P. J.:** The relative contribution of individual osseous circulations to diaphyseal cortical blood supply. *J. Orthop. Trauma,* 4: 441-448, 1990.
80. **Pacifici, M.; Oshima, O.; Fisher, L. W.; Young, M. F.; Shapiro, I. M.;** and **Leboy, P. S.:** Changes in osteonectin distribution and levels are associated with mineralization of the chicken tibial growth cartilage. *Calcif. Tissue Internat.*, 47: 51-61, 1990.
81. **Paley, D.; Young, M. C.; Wiley, A. M.; Fornasier, V. L.;** and **Jackson, R. W.:** Percutaneous bone marrow grafting of fractures and bony defects. An experimental study in rabbits. *Clin. Orthop.*, 208: 300-312, 1986.
82. **Parfitt, A. M.:** Osteomalacia and related disorders. In *Metabolic Bone Disease and Clinically Related Disorders,* edited by L. V. Avioli and S. M. Krane. Ed. 2, pp. 329-396. Philadelphia, W. B. Saunders, 1990.
83. **Pfeilschifter, J.; Seyedin, S. M.;** and **Mundy, G. R.:** Transforming growth factor beta inhibits bone resorption in fetal rat long bone cultures. *J. Clin. Invest.*, 82: 680-685, 1988.
84. **Pienkowski, D.,** and **Pollack, S. R.:** The origin of stress-generated potentials in fluid-saturated bone. *J. Orthop. Res.*, 1: 30-41, 1983.
85. **Raisz, L. G.,** and **Kream, B. E.:** Regulation of bone formation. *New England J. Med.*, 309: 29-35, 1983.
86. **Raisz, L. G.,** and **Rodan, G. A.:** Cellular basis of bone turnover. In *Metabolic Bone Disease and Clinically Related Disorders,* edited by L. V. Avioli and S. M. Krane. Ed. 2, pp. 1-41. Philadelphia, W. B. Saunders, 1990.
87. **Recker, R. R.:** Embryology, anatomy, and microstructure of bone. In *Disorders of Bone and Mineral Metabolism,* pp. 219-240. Edited by F. L. Coe and M. J. Favus. New York, Raven Press, 1992.
88. **Revell, P. A.:** *Pathology of Bone,* pp. 1-34. New York, Springer, 1986.
89. **Rey, C.; Collins, B.; Goehl, T.; Dickson, I. R.;** and **Glimcher, M. J.:** The carbonate environment in bone mineral: a resolution-enhanced Fourier transform infrared spectroscopy study. *Calcif. Tissue Internat.*, 45: 157-164, 1989.

90. **Rey, C.; Lian, J.; Grynpas, M.; Shapiro, F.; Zylerberg, L.;** and **Glimcher, M. J.:** Non-apatitic environments in bone mineral: FT-IR detection, biological properties and changes in several disease states. *Connect. Tissue Res.*, 21: 267-273, 1989.
91. **Rhinelander, F. W.:** Circulation in bone. In *The Biochemistry and Physiology of Bone*, edited by G. H. Bourne. Ed. 2, vol. 2, pp. 2-77. New York, Academic Press, 1972.
92. **Roberts, J. E.; Bonar, L. C.; Griffin, R. G.;** and **Glimcher, M. J.:** Characterization of very young mineral phases of bone by solid state 31phosphorus magic angle sample spinning nuclear magnetic resonance and x-ray diffraction. *Calcif. Tissue Internat.*, 50: 42-48, 1992.
93. **Rodan, G. A.:** Introduction to bone biology. *Bone*, 13 (Supplement 1): S3-S6, 1992.
94. **Rodan, G. A.,** and **Martin, T. J.:** Role of osteoblasts in hormonal control of bone resorption — a hypothesis. *Calcif. Tissue Internat.*, 33: 349-351, 1981.
95. **Rowe, D. W.,** and **Shapiro, J. R.:** Osteogenesis imperfecta. In *Metabolic Bone Disease and Clinically Related Disorders*, edited by L. V. Avioli and S. M. Krane. Ed. 2, pp. 659-701. Philadelphia, W. B. Saunders, 1990.
96. **Rubin, C. T.,** and **McLeod, K. J.:** Biologic modulation of mechanical influences in bone remodeling. In *Biomechanics of Diarthrodial Joints*, edited by V. C. Mow, A. Ratcliffe, and S. L.-Y. Woo. Vol. 2, pp. 97-118. New York, Springer, 1990.
97. **Russell, R. G. G.; Bunning, R. A. D.; Hughes, D. E.;** and **Gowen, M.:** Humoral and local factors affecting bone formation and resorption. In *New Techniques in Metabolic Bone Disease*, pp. 1-20. Edited by J. C. Stevenson. London, Butterworth, 1990.
98. **Sanders, W. E.,** and **Heckman, J. D.:** Traumatic plastic deformation of the radius and ulna. A closed method of correction of deformity. *Clin. Orthop.*, 188: 58-67, 1984.
99. **Sato, S.; Rahemtulla, F.; Prince, C. W.; Tomana, M.;** and **Butler, W. T.:** Proteoglycans of adult bovine compact bone. *Connect. Tissue Res.*, 14: 65-75, 1985.
100. **Schaedler, J. M.; Krook, L.; Wootton, J. A.; Hover, B.; Brodsky, B.; Naresh, M. D.; Gillette, D. D.; Madsen, D. B.; Horn, R. H.;** and **Minor, R. R.:** Studies of collagen in bone and dentin matrix of a Columbian mammoth (late Pleistocene) of central Utah. *Matrix*, 12: 297-307, 1992.
101. **Sevitt, S.:** *Bone Repair and Fracture Healing in Man*, pp. 1-24. New York, Churchill Livingstone, 1981.
102. **Sillence, D.:** Osteogenesis imperfecta: an expanding panorama of variants. *Clin. Orthop.*, 159: 11-25, 1981.
103. **Sillence, D. O.; Senn, A.;** and **Danks, D. M.:** Genetic heterogeneity in osteogenesis imperfecta. *J. Med. Genet.*, 16: 101-116, 1979.
104. **Singh, I.:** The architecture of cancellous bone. *J. Anat.*, 127: 305-310, 1978.
105. **Singh, S.,** and **Saha, S.:** Electrical properties of bone. *Clin. Orthop.*, 196: 249-271, 1984.
106. **Sporn, M. B.; Roberts, A. B.; Wakefield, L. M.;** and **Assoian, R. K.:** Transforming growth factor-beta: biological function and chemical structure. *Science*, 233: 532-534, 1986.
107. **Steinmann, B.; Nicholls, A.;** and **Pope, F. M.:** Clinical variability of osteogenesis imperfecta reflecting molecular heterogeneity: cysteine substitutions in the alpha 1(I) collagen chain producing lethal and mild forms. *J. Biol. Chem.*, 261: 8958-8964, 1986.
108. **Strachan, R. K.; McCarthy, I.; Fleming, R.;** and **Hughes, S. P. F.:** The role of the tibial nutrient artery. Microsphere estimation of blood flow in the osteotomised canine tibia. *J. Bone and Joint Surg.*, 72-B(3): 391-394, 1990.
109. **Torzilli, P. A.; Burstein, A. H.; Takebe, K.; Zika, J. C.;** and **Heiple, K. G.:** The mechanical and structural properties of maturing bone. In *Mechanical Properties of Bone*, pp. 145-161. Edited by S. C. Cowen. New York, American Society of Mechanical Engineers, 1981.
110. **Trias, A.,** and **Fery, A.:** Cortical circulation of long bones. *J. Bone and Joint Surg.*, 61-A: 1052-1059, Oct. 1979.
111. **Triffit, J. T.:** The organic matrix of bone tissue. In *Fundamental and Clinical Bone Physiology*, pp. 45-82. Edited by M. R. Urist. Philadelphia, J. B. Lippincott, 1980.
112. **Triffitt, P. D.; Cieslak, C. A.;** and **Gregg, P. J.:** A quantitative study of the routes of blood flow to the tibial diaphysis after an osteotomy. *J. Orthop. Res.*, 11: 49-57, 1993.
113. **Trueta, J.:** The normal vascular anatomy of the human femoral head during growth. *J. Bone and Joint Surg.*, 39-B(2): 358-394, 1957.
114. **Trueta, J.:** The role of the vessels in osteogenesis. *J. Bone and Joint Surg.*, 45-B(2): 402-418, 1963.
115. **Trueta, J.,** and **Caladias, A. X.:** A study of the blood supply of the long bones. *Surg., Gynec. and Obstet.*, 118: 485-498, 1964.
116. **Urist, M. R.; DeLange, R. J.;** and **Finerman, G. A.:** Bone cell differentiation and growth factors. *Science*, 220: 680-686, 1983.
117. **Vaes, G.:** Cellular biology and biochemical mechanism of bone resorption. A review of recent developments on the formation, activation, and mode of action of osteoclasts. *Clin. Orthop.*, 231: 239-271, 1988.
118. **Walsh, W. R.,** and **Guzelsu, N.:** Ion concentration effects on bone streaming potentials and zeta potentials. *Biomaterials*, 14: 331-336, 1993.
119. **Whiteside, L.:** Circulation in bone. In *Surgery of the Musculoskeletal System*, pp. 1:51-1:63. Edited by C. McC. Evarts. New York, Churchill Livingstone, 1983.
120. **Whiteside, L. A.,** and **Lesker, P. A.:** The effects of extraperiosteal and subperiosteal dissection. I. On blood flow in muscle. *J. Bone and Joint Surg.*, 60-A: 23-26, Jan. 1978.
121. **Whiteside, L. A.,** and **Lesker, P. A.:** The effects of extraperiosteal and subperiosteal dissection. II. On fracture healing. *J. Bone and Joint Surg.*, 60-A: 26-30, Jan. 1978.
122. **Whiteside, L. A.; Ogata, K.; Lesker, P.;** and **Reynolds, F. C.:** The acute effects of periosteal stripping and medullary reaming on regional blood flow. *Clin. Orthop.*, 131: 266-272, 1978.
123. **Whyte, M. P.,** and **Murphy, W. A:** Osteopetrosis and other sclerosing bone disorders. In *Metabolic Bone Disease and Clinically Related Disorders*, edited by L. V. Avioli and S. M. Krane. Ed. 2, pp. 616-658. Philadelphia, W. B. Saunders, 1990.
124. **Young, M. F.; Kerr, J. M.; Ibaraki, K.; Heegaard, A.-M.;** and **Robey, P. G.:** Structure, expression, and regulation of the major non-collagenous matrix proteins of bone. *Clin. Orthop.*, 281: 275-294, 1992.
125. **Zucman, J.:** Studies on the vascular connexions between periosteum, bone, and muscle. Part one. *British J. Surg.*, 48: 324-328, 1960.