Hyaline Cartilage

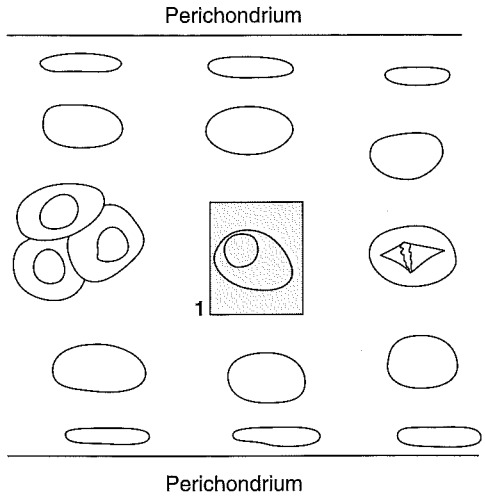
Cartilage is found in regions requiring support in conjunction with tensile strength (fibrocartilage), elasticity (elastic cartilage), and relative rigidity (hyaline cartilage). The functional characteristics of cartilage are carried out primarily by the fibers and proteoglycans of the extracellular matrix. The cells that synthesize these matrix components, **chondrocytes** (C, micrograph 1), have well-developed Golgi (G, micrograph 1) and rough endoplasmic reticulum (r, micrograph 1) characteristic of protein-secreting cells.

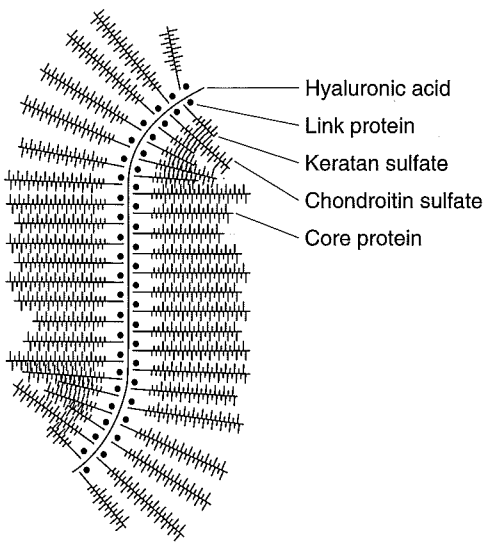
In both hyaline and elastic cartilage, chondrocytes develop from progenitor cells located in a surrounding connective tissue perichondrium (appositional growth). Even as differentiated cells are surrounded by matrix, they retain their ability to undergo division (interstitial growth).

In routine electron micrographs, collagen (arrows, micrograph 1) is the only obvious component of the extracellular matrix. **Type II collagen,** characteristic of hyaline and elastic cartilage, is composed of thin (20–30 nm), faintly striated fibrils. The fibrils are not grouped together into fibers and therefore not typically observed in the light microscope.

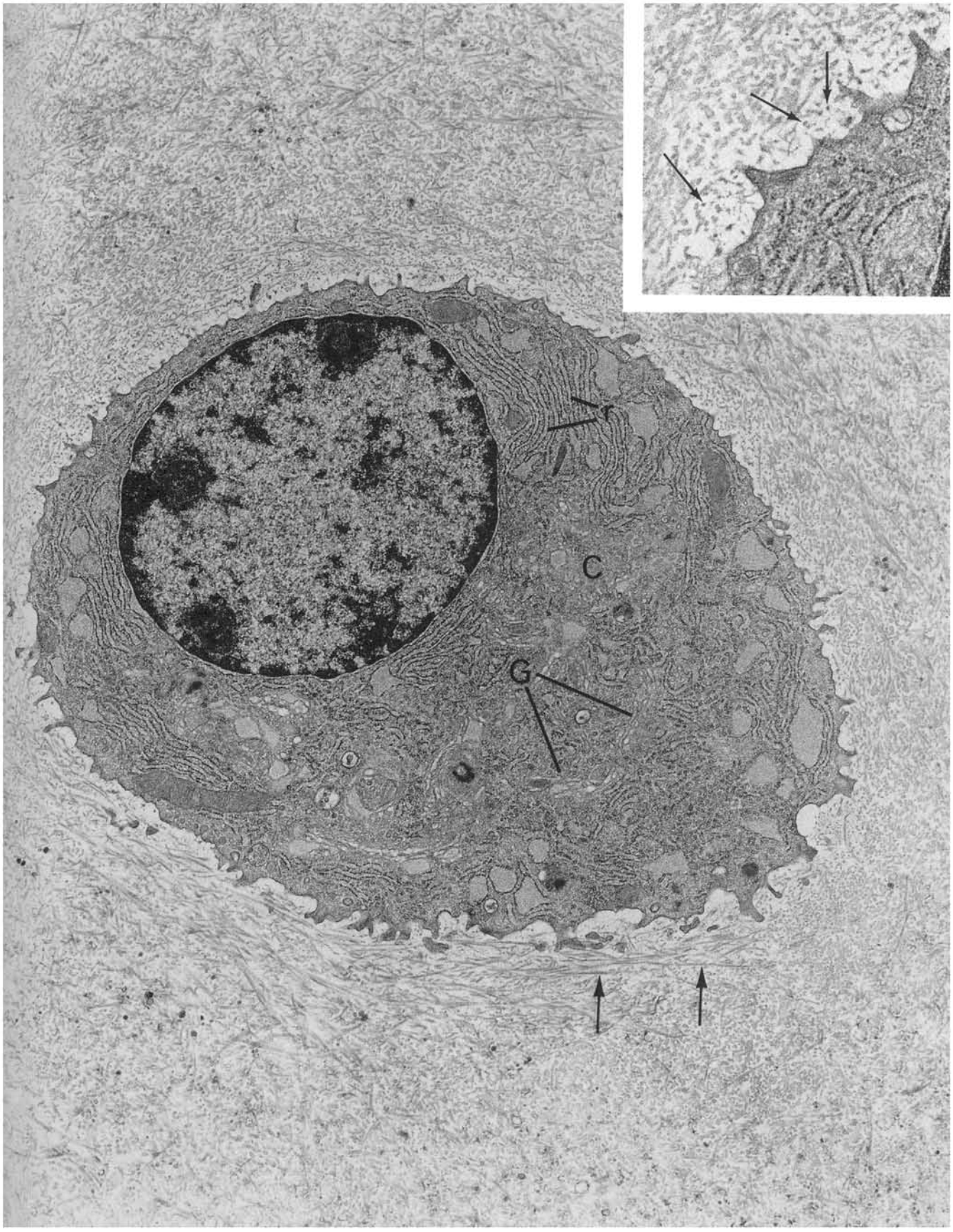
Proteoglycans, an important component of all types of cartilage, are particularly prominent in hyaline cartilage (10% of the wet weight of the tissue). The **glycosaminoglycans** (GAGs) chondroitin sulfate and keratan sulfate are covalently linked to a **protein core,** forming the basic proteoglycan unit. In cartilage, large numbers of these units form **aggregates with hyaluronic acid** (an extremely large GAG) to form molecular structures 1200 nm long. The proteoglycans, like collagen, are synthesized via rough ER and Golgi. Hyaluronic acid, however, is synthesized at the plasma membrane, with the growing chain passing to the cell exterior. The final assembly into the aggregates of proteoglycans and hyaluronic acid occurs outside of the cell. Each hyaluronic acid molecule binds up to 100 proteoglycan molecules. Following isolation, hyaluronic acid aggregates appear as shown in the diagram; however, in routine electron micrographs they form 70-nm particles (arrows, inset).

The rigidity of cartilage is a result of swelling due to the high concentration of water associated with the anionic sites on the GAGs. The hydrated GAGs can be displaced, resulting in some deformability, but the large proteoglycan aggregates, "bound" between collagen fibrils, return to their original location, drawing water and providing resiliency. Bound water functions also as the medium for the transport of nutrients and wastes in this avascular tissue.





Modified from L. Stryer, *Biochemistry,* Freeman, New York, 1988.

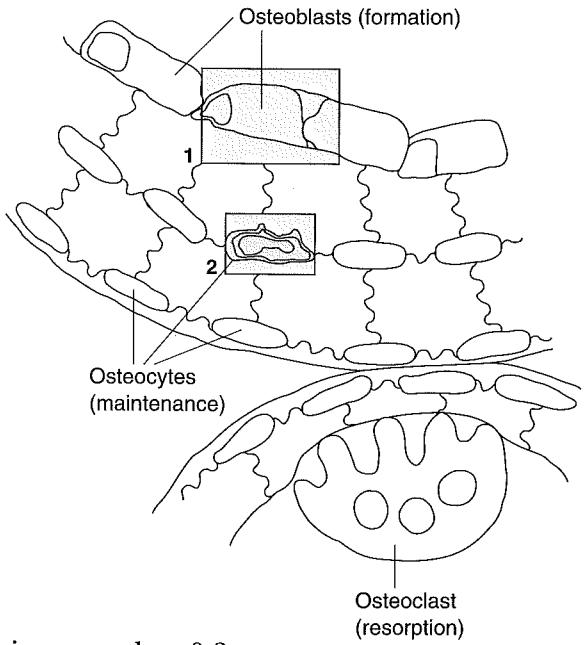

C
G

BONE: Osteoblasts and Osteocytes

Bone, the skeletal support system of the body, is a dynamic tissue that is continually being formed by osteoblasts, removed by osteoclasts, and maintained by osteocytes.

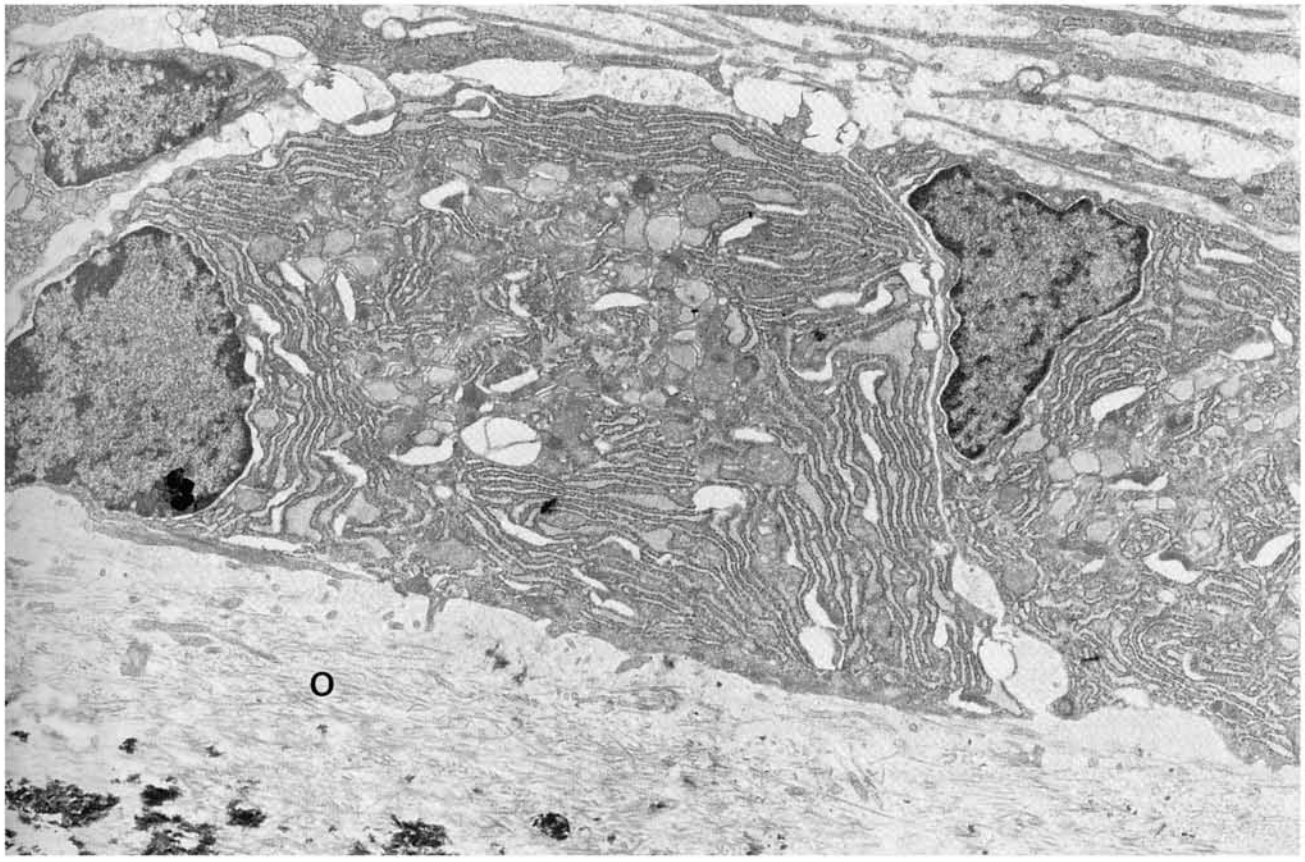
Osteoblasts (micrograph 1) synthesize the organic matrix of bone and regulate its mineralization. They are highly polarized cells with eccentric nuclei and a cytoplasm bulging with organelles synthesizing and sorting proteins. The collagen and proteoglycan matrix secreted by these cells is not mineralized initially and is referred to as **osteoid** (o, micrograph 1). Areas of mineralization can be seen as black regions in the lower left corner of micrograph 1.

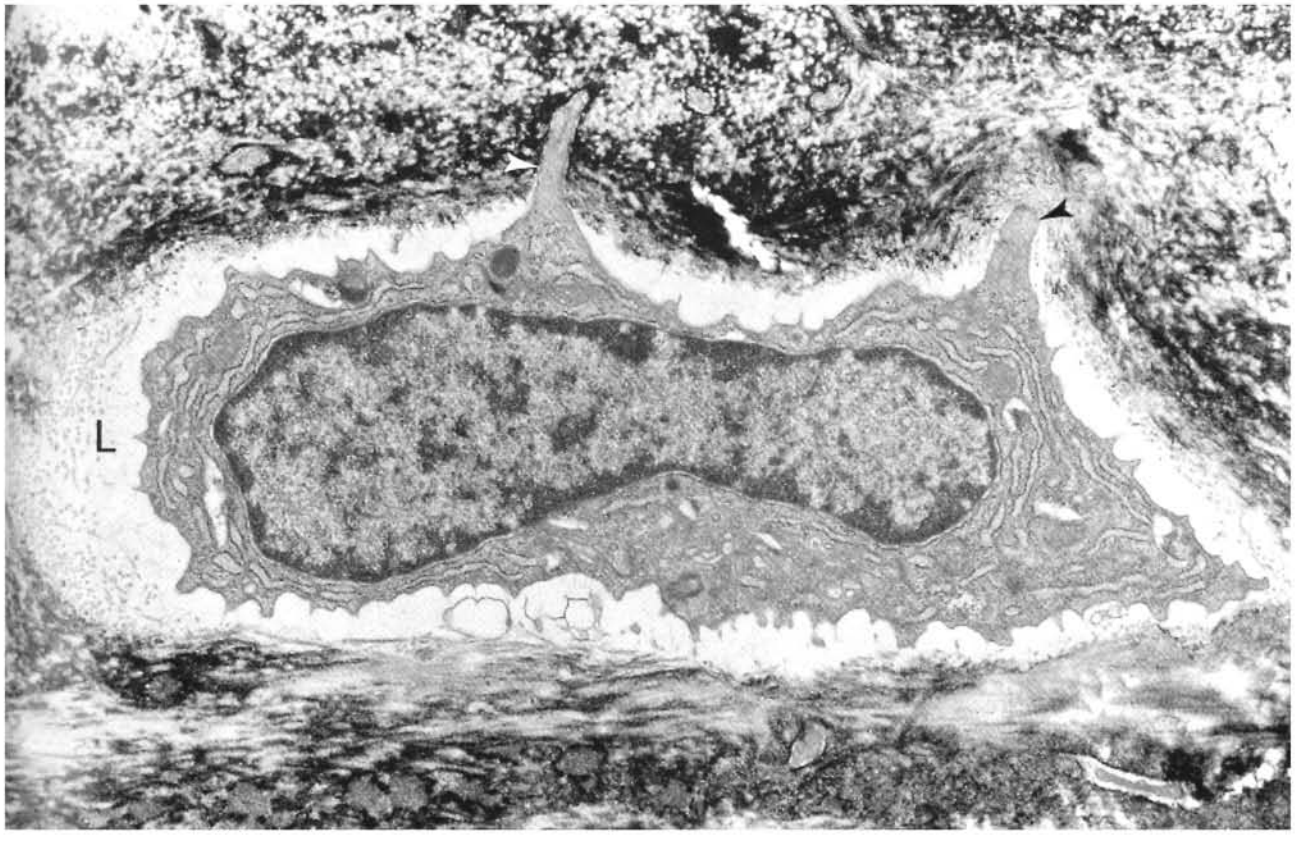
Osteoblasts originate from sheetlike mesenchymal cells lining the outer (periosteal) and inner (endosteal) bone surfaces. They form layers adjacent to the bone, attached to each other by gap junctions. At some point osteoblasts lose their polarity, secrete matrix around their circumference, and become trapped in spaces called lacunae (L, micrograph 2). Once enclosed by bone, these cells, now known as **osteocytes** (micrograph 2), become smaller and less active. Osteocytes are not responsible for a net increase in bone matrix; however, they are essential to the maintenance and routine turnover of the matrix.



Cellular processes of osteocytes (arrowheads, micrograph 2) remain attached to each other and to osteoblasts through channels in the bone referred to as **canaliculi.** Nutrients from blood vessels outside the bone matrix diffuse to the osteocytes both through the canaliculi surrounding the cell processes and through the cells themselves via gap junctions. Since the effective diffusion distance is limited, osteocytes cannot survive more than 0.2 mm away from a blood vessel. This limitation defines the size of spongy bone and the basic structural unit of compact bone, the Haversian system.

> Defining osteoblasts as "bone forming" is a correct but simplistic view of these cells. Bone formation and bone resorption are intimately tied together, so much so that one normally does not occur without the other. Considerable evidence suggests that osteoblasts are essential for both. In culture, osteoclasts fail to resorb bone in the absence of osteoblasts. In addition, receptors for parathyroid hormone, which increases bone resorption and osteoclast activity in vivo, are found on osteoblasts but not osteoclasts.


O

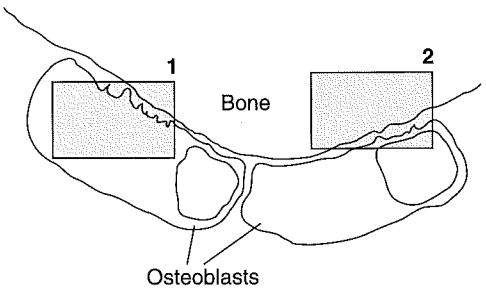

L

BONE: Extracellular Matrix

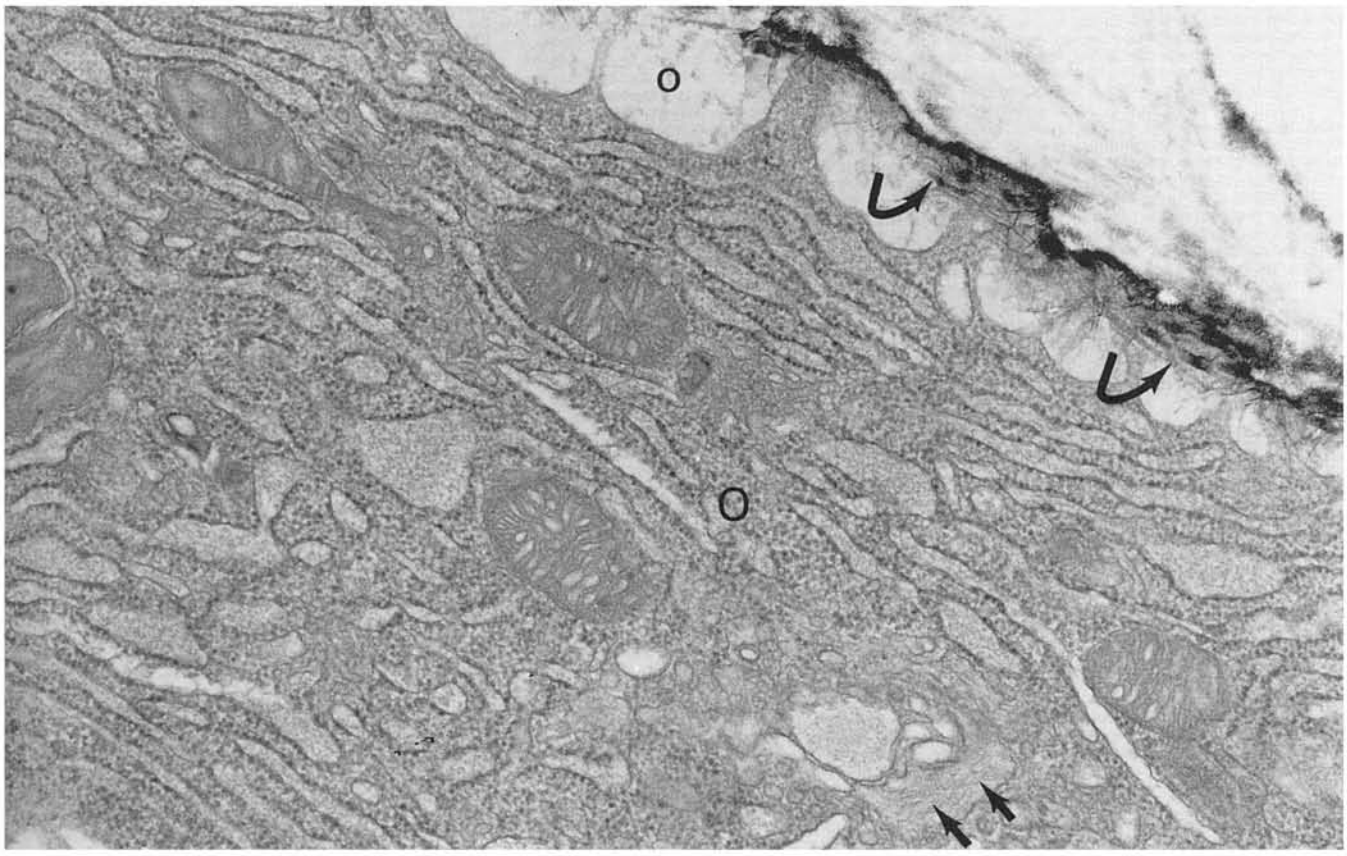
Extracellular matrix accounts for 90% of the total weight of compact bone. The inorganic component of extracellular matrix, **microcrystalline hydroxyapatite,** contributes 60% of total bone weight, and the organic component, primarily proteins, contributes 30%.

Nearly all of the organic matrix of bone is **Type I collagen,** and much of the activity observed in **osteoblasts** (O, micrographs 1 and 2) is directed toward its synthesis. The fine threads (straight arrows, micrograph 1) within Golgi saccules are the rigid triple helices of procollagen that form prior to secretion.

In this preparation, the nonmineralized osteoid (o, micrographs 1 and 2) is clearly differentiated from the region where mineralization and hydroxyapatite crystal deposition have begun (curved arrows, micrographs 1 and 2). In the area of mineralization, matrix vesicles (v, micrograph 2), believed to originate from osteoblasts, accumulate calcium, and contain enzymes (e.g., alkaline phosphatase) that could bring about its precipitation. The first crystals of hydroxyapatite are formed within the 35-nm gaps between tropocollagen molecules.



Osteoblasts synthesize and secrete several noncollagenous proteins, including osteopontin and osteocalcin, that appear to have central roles in matrix organization and calcium homeostasis. Osteopontin, a phosphorylated glycoprotein, is concentrated in areas where cartilage is transforming into bone, in particular where osteoclasts attach to the bone surface. It has been suggested that osteopontin binds to osteoclasts and facilitates their association with calcified matrix. Osteocalcin, a small protein that contains the modified amino acid γ-carboxyglutamic acid, has also been implicated in osteoclast activity. This protein, bound to bone mineral crystals, attracts and activates osteoclasts and is important in bone turnover. The severe bone disorders that resulted as a side effect of the clinical use of the anticoagulant warfarin were found to result from interference in the carboxylation of glutamic acid residues. This led, as expected, to interference with the synthesis of certain clotting proteins but also had the detrimental effect of interfering with the synthesis of osteocalcin.


o
o

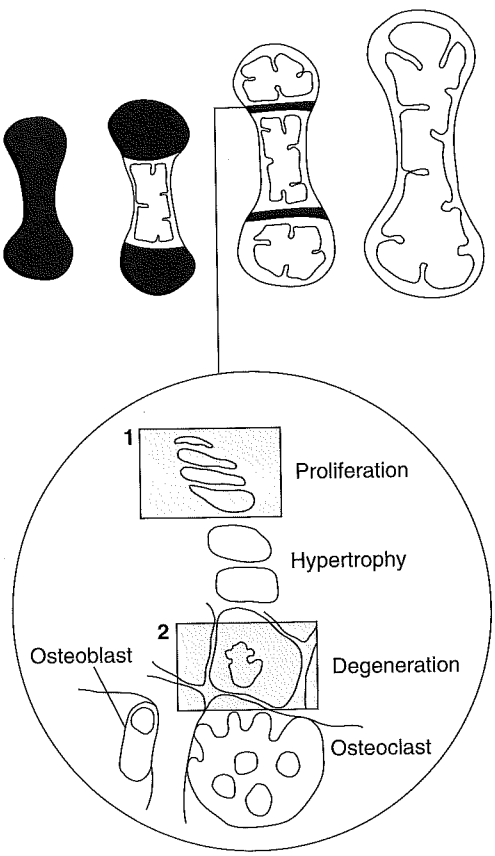

v
o
o

BONE: Long Bone Growth

All long bones are initially composed of **hyaline cartilage** (black regions in the diagram). Beginning in the fourth week of fetal development, this cartilage is gradually replaced by bone. Even though bone replaces cartilage and strengthens the skeletal framework, cartilage remains in defined regions **(epiphyseal plates)** at the ends of growing long bones as a source of dividing cells. The division and development of cartilage in this region is responsible for the growth in length of long bones. Epiphyseal cartilage is replaced by bone as growth terminates.

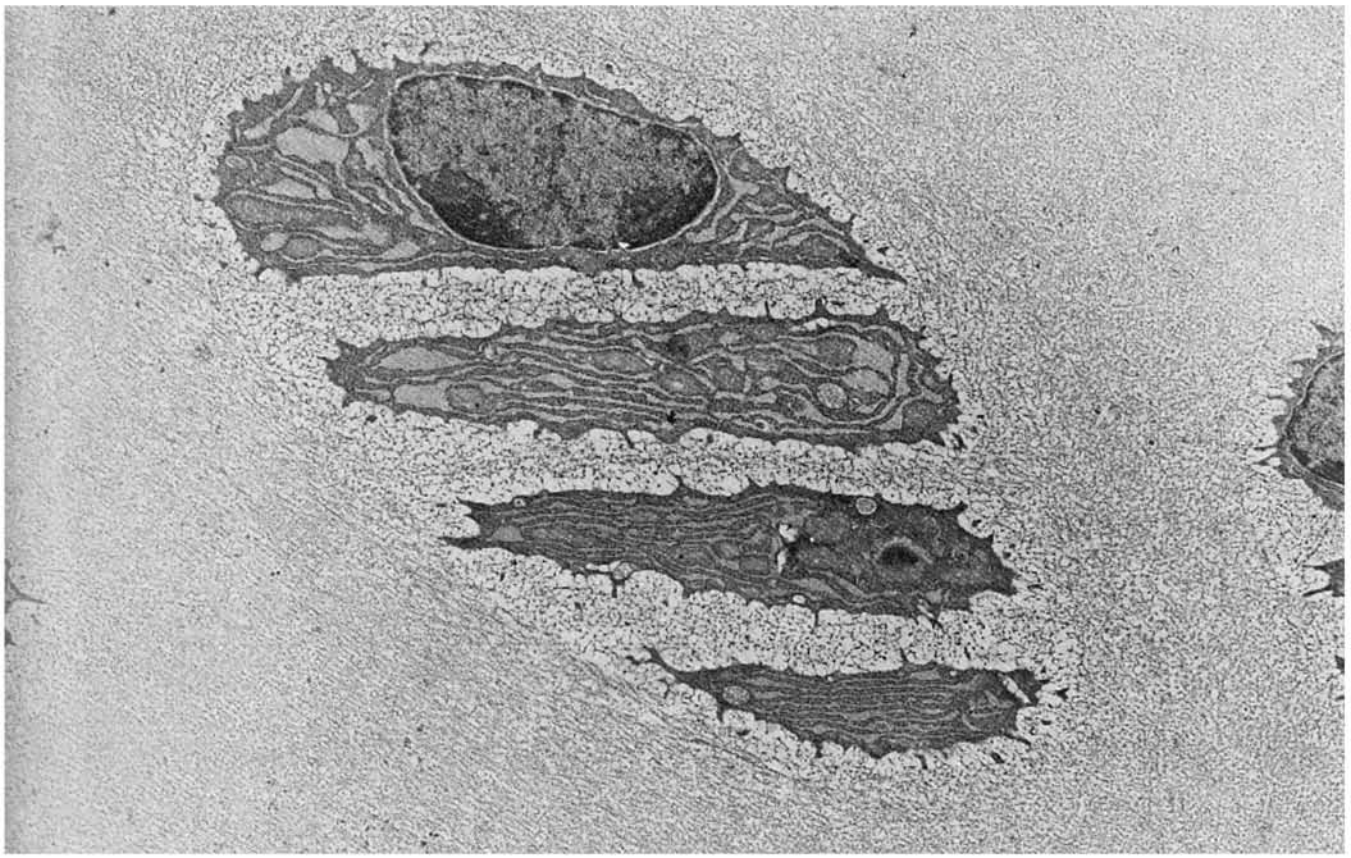
The progression of events in the epiphyseal plate is highly ordered, with a **proliferative, hypertrophic,** and **degenerative** sequence in the programmed cell death of chondrocytes. The chondrocytes in micrograph 1 are recent products of division of a single cell in the proliferative stage. They are characteristically arranged in a row and still close enough together to be in a common matrix region (territorial matrix) containing a high concentration of proteoglycans.

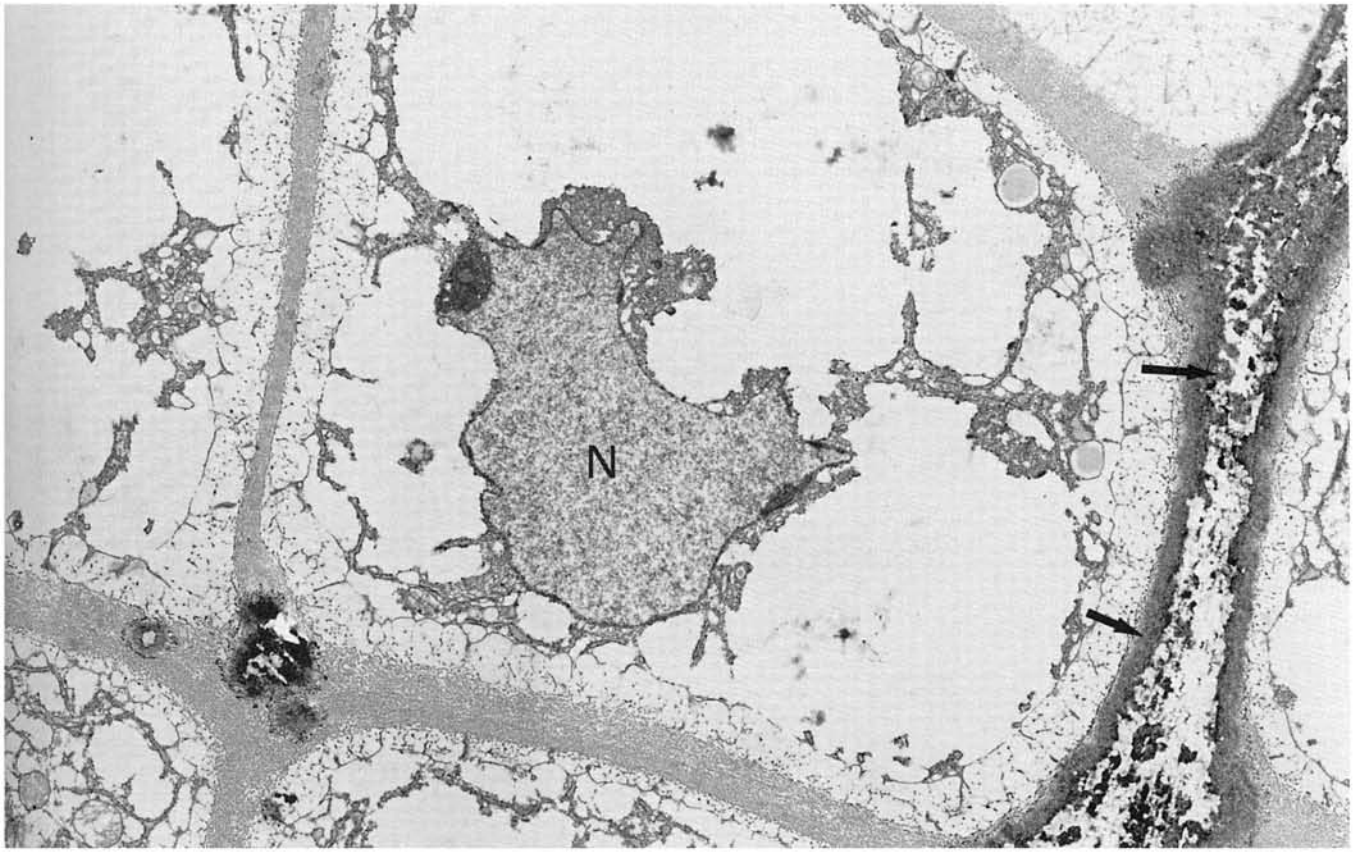
Each chondrocyte becomes separated from its neighbor in the developing row as it grows in size (hypertrophy) and forms more matrix. At this stage of development the matrix in the longitudinal septa (arrows, micrograph 2) between rows of cells calcifies. The resulting isolation of these cells from their nutrient supply facilitates chondrocyte death, leaving remnants of cells (N, nucleus of a degenerating chondrocyte, micrograph 2) within lacunae. The spaces once occupied by chondrocytes are remodeled in the metaphysis region by the removal of matrix and cells by osteoclasts and the addition of bone by osteoblasts.



The proliferation of chondrocytes in the epiphyseal plate is regulated by growth hormone from the anterior pituitary. Evidence indicates that this hormone acts directly on chondrocytes, which in turn synthesize somatomedins, peptide growth factors that promote clonal expansion in an autocrine manner.

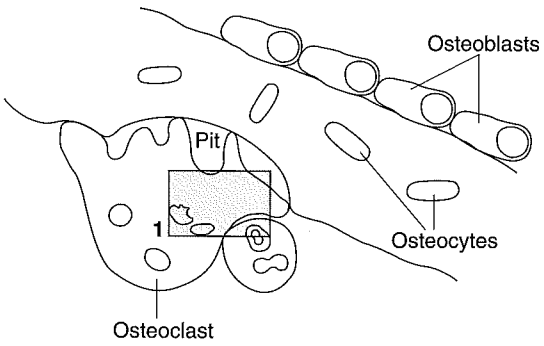
Vitamin D is essential for the normal mineralization of bone. In children with rickets, a disease caused by vitamin D deficiency, the cartilage in the epiphyseal regions fail to calcify, resulting in the prolonged life of chondrocytes and thickening of the epiphyseal plate. This increased amount of cartilage, along with an increase in the proportion of nonmineralized osteoid, leads to skeletal weakness and deformity.




N

Osteoclasts (micrograph 1) are giant (100-μm) **multinucleate** cells that originate from the fusion of several monocytes. These cells resorb bone by attaching to the bony surface (upper right, micrographs 1 and 2), sliding back and forth, and dissolving the matrix components in a pit underneath the cell. The osteoclast membrane adjacent to the bone forms a **ruffled border** of deeply infolded membranes (arrows, micrographs 1 and 2).

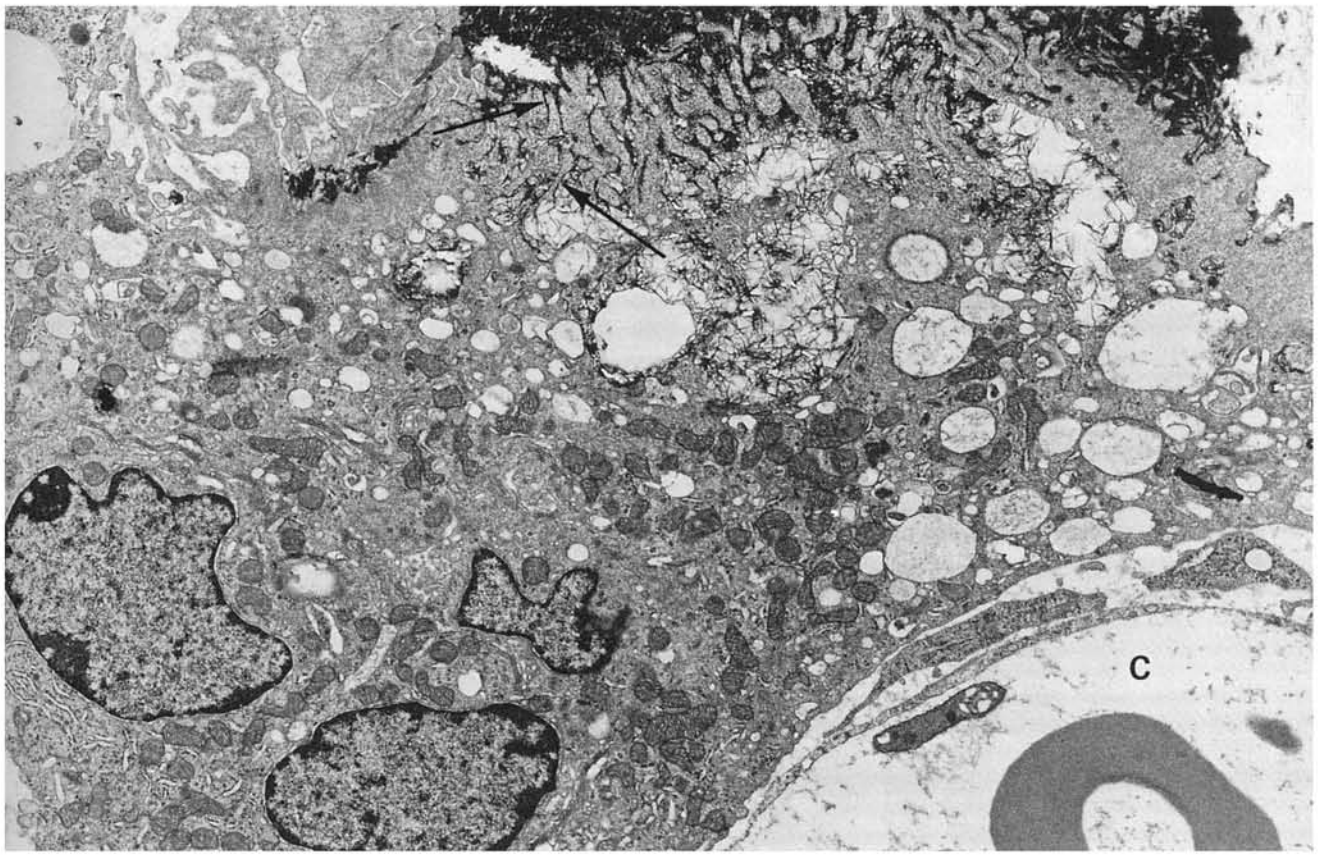
The **extracellular pit** between the osteoclast and the bone is the site of resorption. A proton pump localized in the ruffled border in this region maintains high concentrations of hydrogen ion in the pit. The resulting low pH dissolves the inorganic component, hydroxyapatite. Lysosomal enzymes released into the pit via exocytosis are activated by the low pH. These enzymes degrade the organic components such as collagen and proteoglycans. The extra surface area provided by the ruffled border and the large number of associated mitochondria (particularly evident in micrograph 1) are typically found in regions of cells involved in ion pump activity.



The extracellular pit is analogous to a secondary lysosome in other cell types. In both, digestion occurs in a low pH maintained by a membrane pump. What is unique in the action of the osteoclast is the exocytosis of lysosomal enzymes and their action outside instead of inside the cell. The large amounts of calcium released during osteoclast digestive activity would be incompatible with intracellular functioning. Inorganic and organic products of osteoclast activity enter capillaries (c, micrograph 1) and are recycled to other locations.

During the growth and continual remodeling of bone, osteoclasts and osteoblasts work together in the balance of resorption and formation. The placement of osteoclasts and osteoblasts determines the shape of bones as they grow. In the developing skull, resorptive activities of the osteoclast on the inside surface are balanced by bone formation by osteoblasts on the outside surface. Such an interaction maintains the thickness of the skull as it is expanding to accommodate the developing brain.

> From age 20 on, the balance between these two events shifts, such that resorption by osteoclasts is not completely repaired by osteoblasts. The resulting reduction in bone mass increases susceptibility to fracture. In women, accelerated bone loss appears to follow the reduction of estrogen levels at menopause. Estrogen replacement at this time increases bone mass. One effect of estrogen is to inhibit the osteoblast synthesis of interleukin-6, a cytokine that stimulates the development of osteoclasts, thus maintaining bone mass by balancing bone synthesis and resorption.


C