VBA 204
HISTOLOGY OF CARTILAGE

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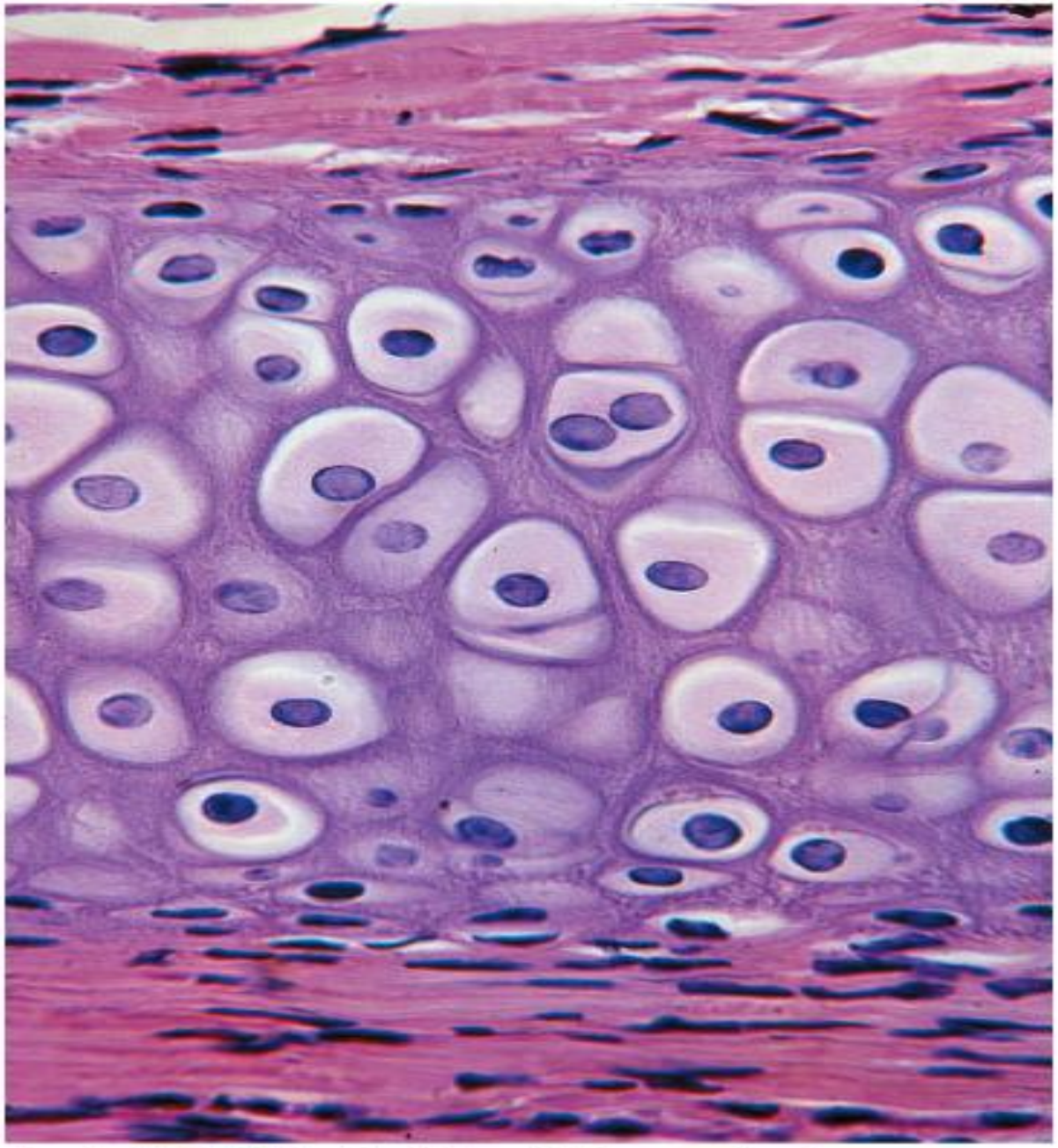
CARTILAGE

- Cartilage is a specialized form of connective tissue in which the firm consistency of the extracellular matrix allows the tissue to bear mechanical stresses without permanent distortion. Another function of cartilage is to support soft tissues. Because it is smooth surfaced and resilient, cartilage is a shock-absorbing and sliding area for joints and facilitates bone movements. Cartilage is also essential for the development and growth of long bones both before and after birth

- Cartilage consists of cells called **chondrocytes** (Gr. *chondros,* cartilage, + *kytos,* cell) and an extensive **extracellular matrix** composed of fibers and ground substance. Chondrocytes synthesize and secrete the extracellular matrix, and the cells themselves are located in matrix cavities called **lacunae.** Collagen, hyaluronic acid, proteoglycans, and small amounts of several glycoproteins are the principal macromolecules present in all types of cartilage matrix. Elastic cartilage, characterized by its great pliability, contains significant amounts of the protein elastin in the matrix.

- As a consequence of various functional requirements, three forms of cartilage have evolved, each exhibiting variations in matrix composition. In the matrix of **hyaline cartilage,** the most common form, type II collagen is the principal collagen type.
- The more pliable and distensible **elastic cartilage** possesses, in addition to collagen type II, an abundance of elastic fibers within its matrix. **Fibrocartilage,** present in regions of the body subjected to pulling forces, is characterized by a matrix containing a dense network of coarse type I collagen fibers



Photomicrograph of hyaline cartilage. Chondrocytes are located in matrix lacunae, and most belong to isogenous groups. The upper and lower parts of the figure show the perichondrium stained pink. Note the gradual differentiation of cells from the perichondrium into chondrocytes. H&E stain. Low magnification

- In all three forms, cartilage is avascular and is nourished by the diffusion of nutrients from capillaries in adjacent connective tissue (perichondrium) or by synovial fluid from joint cavities.
- In some instances, blood vessels traverse cartilage to nourish other tissues, but these vessels do not supply nutrients to the cartilage. As might be expected of cells in an avascular tissue, chondrocytes exhibit low metabolic activity. Cartilage has no lymphatic vessels or nerves.

- The **perichondrium** is a sheath of dense connective tissue that surrounds cartilage in most places, forming an interface between the cartilage and the tissue supported by the cartilage.
- The perichondrium harbors the vascular supply for the avascular cartilage and also contains nerves and lymphatic vessels. Articular cartilage, which covers the surfaces of the bones of movable joints, is devoid of perichondrium and is sustained by the diffusion of oxygen and nutrients from the synovial fluid.

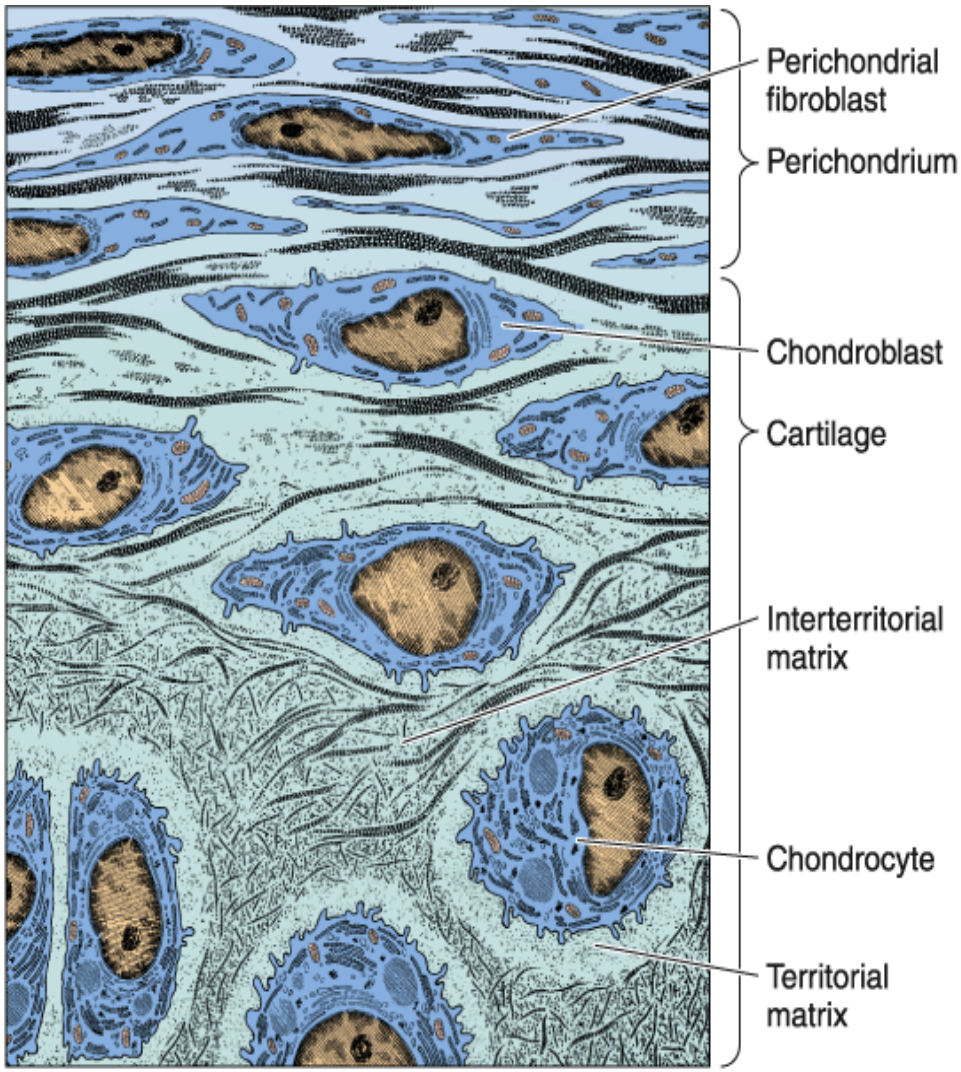


Diagram of the area of transition between the perichondrium and the hyaline cartilage. As perichondrial cells differentiate into chondrocytes, they become round, with an irregular surface. Cartilage (interterritorial) matrix contains numerous fine collagen fibrils except around the periphery of the chondrocytes, where the matrix consists primarily of glycosaminoglycans; this peripheral region is called the territorial, or capsular, matrix.

HYALINE CARTILAGE

- Hyaline cartilage is the most common and best studied of the three forms. Fresh hyaline cartilage is bluish-white and translucent. In the embryo, it serves as a temporary skeleton until it is gradually replaced by bone.
- In adult mammals, hyaline cartilage is located in the articular surfaces of the movable joints, in the walls of larger respiratory passages (nose, larynx, trachea, bronchi), in the ventral ends of ribs, where they articulate with the sternum, and in the **epiphyseal plate,** where it is responsible for the longitudinal growth of bone.

- The cartilage matrix surrounding each chondrocyte is rich in glycosaminoglycan and poor in collagen. This peripheral zone, called the **territorial,** or **capsular,** matrix, stains differently from the rest of the matrix

Perichondrium

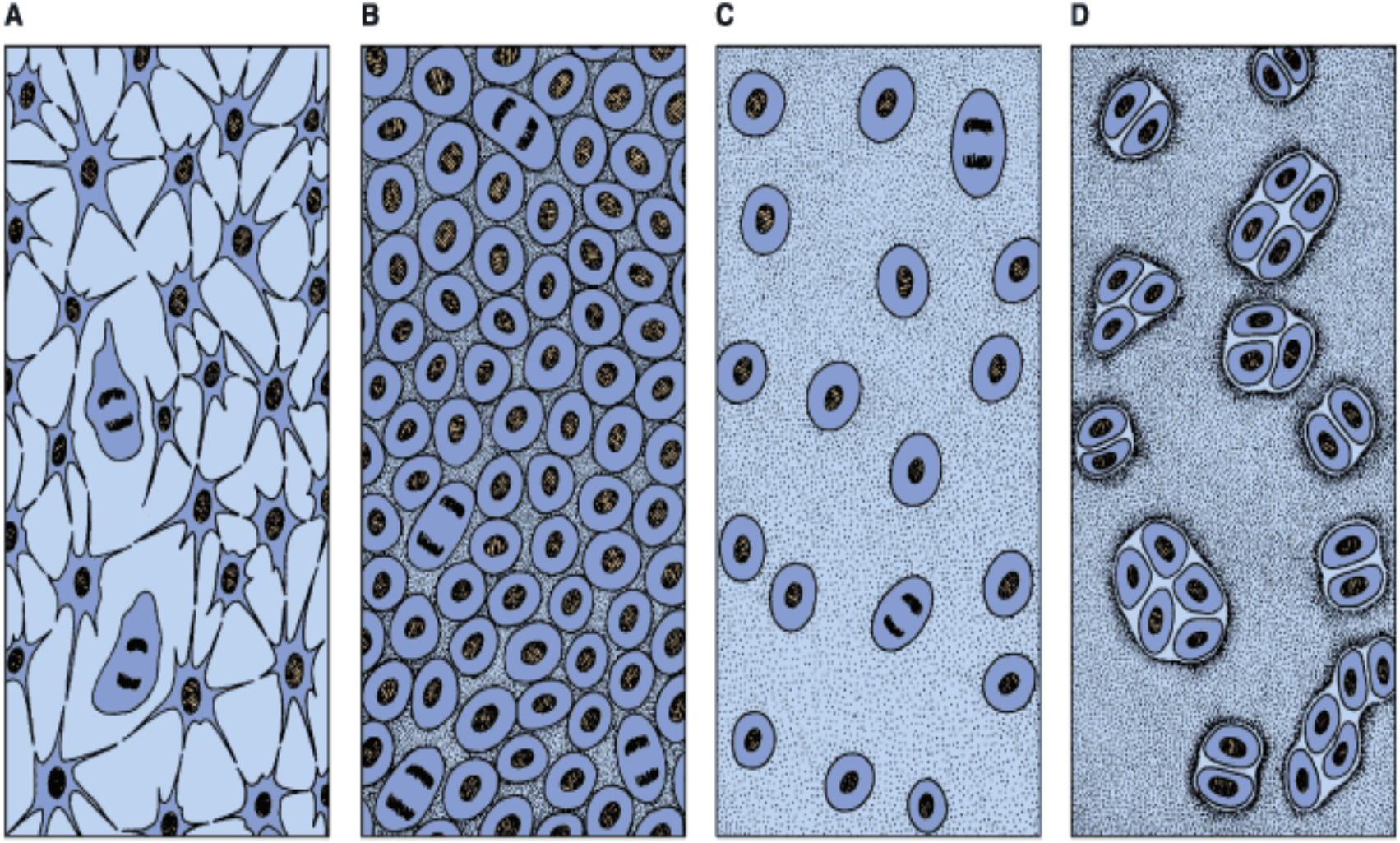
- Except in the articular cartilage of joints, all hyaline cartilage is covered by a layer of dense connective tissue, the perichondrium, which is essential for the growth and maintenance of cartilage. It is rich in collagen type I fibers and contains numerous fibroblasts.
- Although cells in the inner layer of the perichondrium resemble fibroblasts, they are chondroblasts and easily differentiate into chondrocytes.

Chondrocytes

- At the periphery of hyaline cartilage, young chondrocytes have an elliptic shape, with the long axis parallel to the surface. Farther in, they are round and may appear in groups of up to eight cells originating from mitotic divisions of a single chondrocyte. These groups are called **isogenous** (Gr. *isos,* equal, + *genos,* family).
- Cartilage cells and the matrix shrink during routine histological preparation, resulting in both the irregular shape of the chondrocytes and their retraction from the capsule. In living tissue, and in properly prepared sections, the chondrocytes fill the lacunae completely.

HISTOGENESIS

- Cartilage derives from the mesenchyme . The first modification observed is the rounding up of the mesenchymal cells, which retract their extensions, multiply rapidly, and form mesenchymal condensations of chondroblasts. The cells formed by this direct differentiation of mesenchymal cells, now called **chondroblasts,** have a ribosome-rich basophilic cytoplasm. Synthesis and deposition of the matrix then begin to separate the chondroblasts from one another.



Histogenesis of hyaline cartilage. **A:** the mesenchyme is the precursor tissue of all types of cartilage. **B:** Mitotic proliferation of mesenchymal cells gives rise to a highly cellular tissue. **C:** chondroblasts are separated from one another by the formation of a great amount of matrix. **D:** Multiplication of cartilage cells gives rise to isogenous groups, each surrounded by a condensation of territorial (capsular) matrix

- During development, the differentiation of cartilage takes place from the center outward; therefore, the more central cells have the characteristics of chondrocytes, whereas the peripheral cells are typical chondroblasts. The superficial mesenchyme develops into the perichondrium.

GROWTH

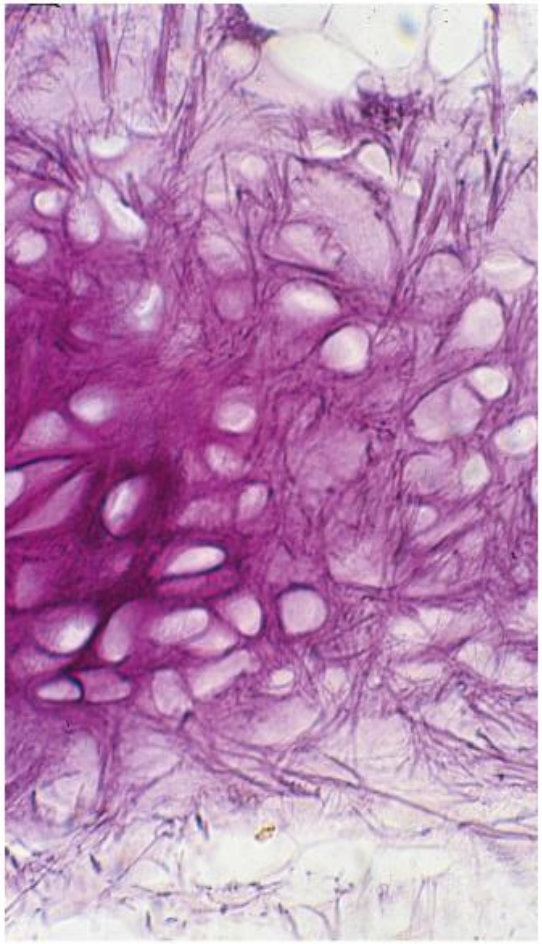
- The growth of cartilage is attributable to two processes: **interstitial growth,** resulting from the mitotic division of preexisting chondrocytes, and **appositional growth,** resulting from the differentiation of perichondrial cells.
- In both cases, the synthesis of matrix contributes to the growth of the cartilage. Interstitial growth is the less important of the two processes. It occurs only during the early phases of cartilage formation, when it increases tissue mass by expanding the cartilage matrix from within. Interstitial growth also occurs in the epiphyseal plates of long bones and within articular cartilage.

- In the epiphyseal plates, interstitial growth is important in increasing the length of long bones and in providing a cartilage model for endochondral bone formation (see Chapter 8: Bone).
- In articular cartilage, as the cells and matrix near the articulating surface are gradually worn away, the cartilage must be replaced from within, since there is no perichondrium there to add cells by apposition.

- In cartilage found elsewhere in the body, interstitial growth becomes less pronounced, as the matrix becomes increasingly rigid from the cross-linking of matrix molecules. Cartilage then grows in girth only by apposition.
- Chondroblasts of the perichondrium proliferate and become chondrocytes once they have surrounded themselves with cartilaginous matrix and are incorporated into the existing cartilage .

ELASTIC CARTILAGE

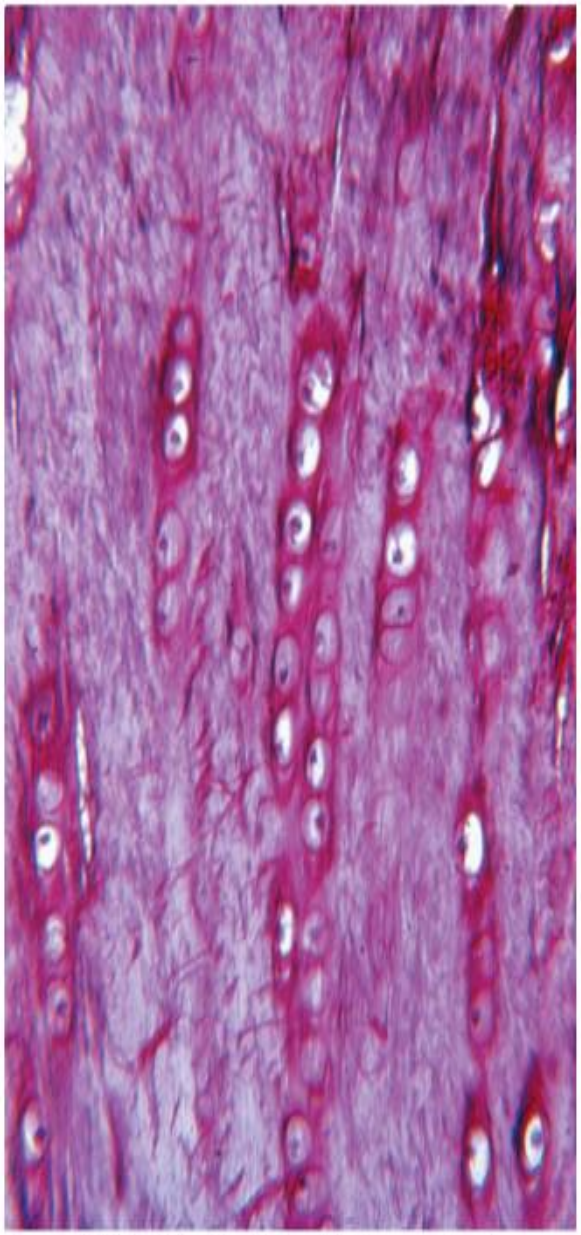
- Elastic cartilage is found in the auricle of the ear, the walls of the external auditory canals, the auditory (eustachian) tubes, the epiglottis, and the cuneiform cartilage in the larynx.
- Elastic cartilage is essentially identical to hyaline cartilage except that it contains an abundant network of fine elastic fibers in addition to collagen type II fibrils. Fresh elastic cartilage has a yellowish color owing to the presence of elastin in the elastic fibers.
- Like hyaline cartilage, elastic cartilage possesses a perichondrium



Photomicrograph of elastic cartilage, stained for elastic fibers. Cells are not stained. This flexible cartilage is present, for example, in the auricle of the ear and in the epiglottis. Resorcin stain. Medium magnification.

FIBROCARTILAGE

- Fibrocartilage is a tissue intermediate between dense connective tissue and hyaline cartilage. It is found in intervertebral disks, in attachments of certain ligaments to the cartilaginous surface of bones, and in the symphysis pubis.
- Fibrocartilage is always associated with dense connective tissue, and the border areas between these two tissues are not clear-cut, showing a gradual transition.



Photomicrograph of fibrocartilage. Note the rows of chondrocytes separated by collagen fibers. Fibrocartilage is frequently found in the insertion of tendons on the epiphyseal hyaline cartilage. Picrosiriusâ€"hematoxylin stain. Medium magnification.

- Fibrocartilage contains chondrocytes, either singly or in isogenous groups, usually arranged in long rows separated by coarse collagen type I fibers. Because it is rich in collagen type I, the fibrocartilage matrix is acidophilic.

- In fibrocartilage, the numerous collagen fibers either form irregular bundles between the groups of chondrocytes or are aligned in a parallel arrangement along the columns of chondrocytes.This orientation depends on the stresses acting on fibrocartilage, since the collagen bundles take up a direction parallel to those stresses. There is no identifiable perichondrium in fibrocartilage.

INTERVERTEBRAL DISKS

- Each intervertebral disk is situated between two vertebrae and is held to them by means of ligaments. The disks have two components: the fibrous annulus fibrosus and the nucleus pulposus.
- The intervertebral disk acts as a lubricated cushion that prevents adjacent vertebrae from being eroded by abrasive forces during movement of the spinal column. The nucleus pulposus serves as a shock absorber to cushion the impact between vertebrae.

- The **annulus fibrosus** has an external layer of dense connective tissue, but it is mainly composed of overlapping laminae of fibrocartilage in which collagen bundles are orthogonally arranged in adjacent layers. The multiple lamellae, with the 90° registration of type I collagen fibers in adjacent layers, provide the disk with unusual resilience that enables it to withstand the pressures generated by impinging vertebrae.

- The **nucleus pulposus** is situated in the center of the annulus fibrosus. It is derived from the embryonic notochord and consists of a few rounded cells embedded in a viscous matrix rich in hyaluronic acid and type II collagen fibrils. In children, the nucleus pulposus is large, but it gradually becomes smaller with age and is partially replaced by fibrocartilage.

MEDICAL APPLICATION

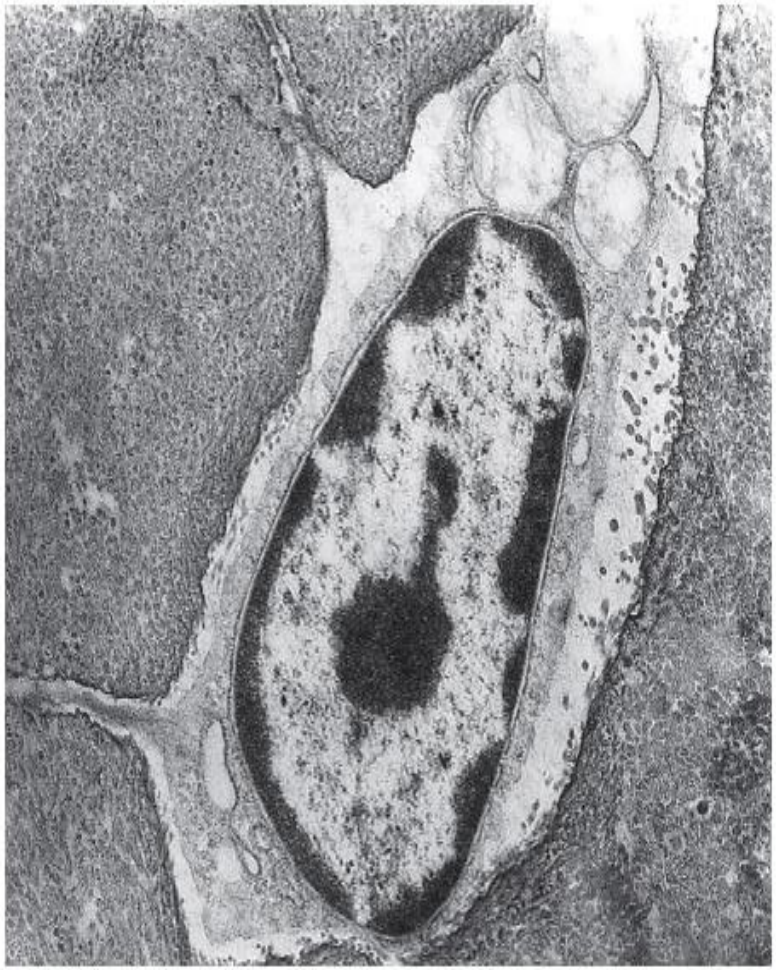
- Rupture of the annulus fibrosus, which most frequently occurs in the posterior region where there are fewer collagen bundles, results in expulsion of the nucleus pulposus and a concomitant flattening of the disk. As a consequence, the disk frequently dislocates or slips from its position between the vertebrae. If it moves toward the spinal cord, it can compress the nerves and result in severe pain and neurological disturbances. The pain accompanying a slipped disk may be perceived in areas innervated by the compressed nerve fibers usually the lower lumbar region

HISTOLOGY OF BONE

BONE: INTRODUCTION

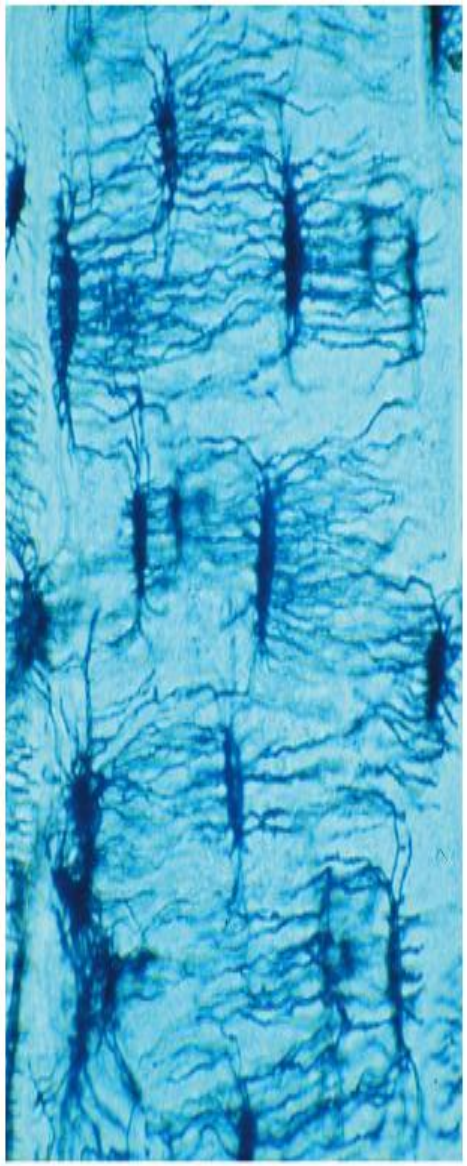
- As the main constituent of the adult skeleton, bone tissue supports fleshy structures, protects vital organs such as those in the cranial and thoracic cavities, and harbors the bone marrow, where blood cells are formed. Bone tissue is highly vascularized and metabolically very active. It serves as a reservoir of calcium, phosphate, and other ions that can be released or stored in a controlled fashion to maintain constant concentrations of these important ions in body fluids.
- In addition, bones form a system of levers that multiplies the forces generated during skeletal muscle contraction and transforms them into bodily movements. This mineralized tissue confers mechanical and metabolic functions to the skeleton.

- Bone is a specialized connective tissue composed of intercellular calcified material, the **bone matrix,** and three cell types:
  - **osteocytes** (Gr. *osteon,* bone, + *kytos,* cell), which are found in cavities (**lacunae**) within the matrix;
  - **osteoblasts** (*osteon* + Gr. *blastos,* germ), which synthesize the organic components of the matrix; and
  - **osteoclasts** (*osteon* + Gr. *klastos,* broken), which are multinucleated giant cells involved in the resorption and remodeling of bone tissue.



Section of bone tissue showing an osteocyte with its cytoplasmic processes surrounded by matrix. The ultrastructure of the cell nucleus and cytoplasm is compatible with a low level of protein synthesis.

- Because metabolites are unable to diffuse through the calcified matrix of bone, the exchanges between osteocytes and blood capillaries depend on communication through the **canaliculi** (L. *canalis,* canal), which are thin, cylindrical spaces that perforate the matrix.



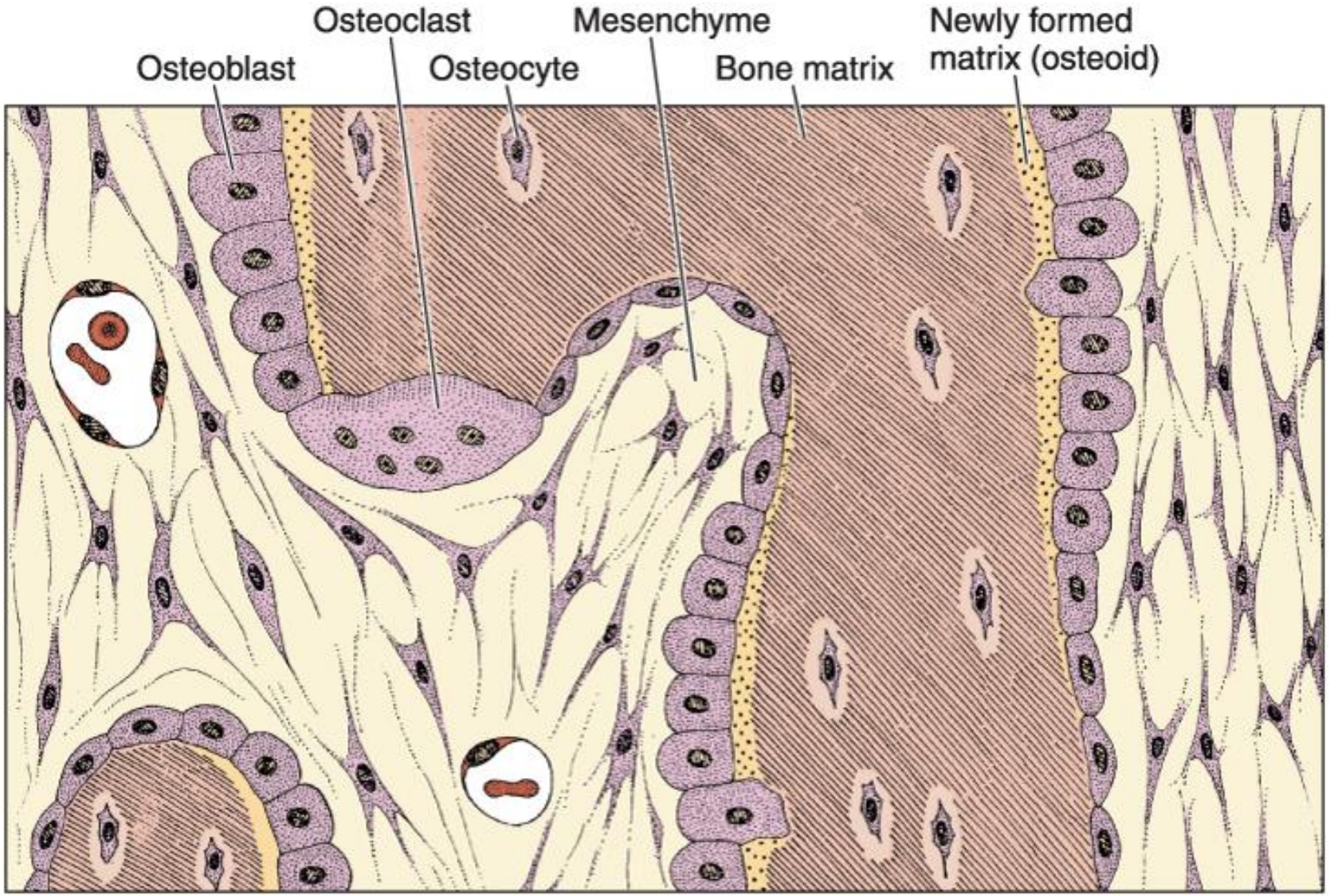
Photomicrograph of dried bone ground very thin. The lacunae and canaliculi filled with air deflect the light and appear dark, showing the communication between these structures through which nutrients derived from blood vessels flow. Medium magnification.

- All bones are lined on both internal and external surfaces by layers of tissue containing osteogenic cells(osteoprogenitor cells) - **endosteum** on the internal surface and **periosteum** on the external surface.
- Because of its hardness, bone is difficult to section with the microtome, and special techniques must be used for its study. A common technique that permits the observation of the cells and organic matrix is based on the decalcification of bone preserved by standard fixatives. The mineral is removed by immersion in a solution containing a calcium-chelating substance (eg, ethylenediaminetetraacetic acid [EDTA]). The decalcified tissue is then embedded, sectioned, and stained.

BONE CELLS

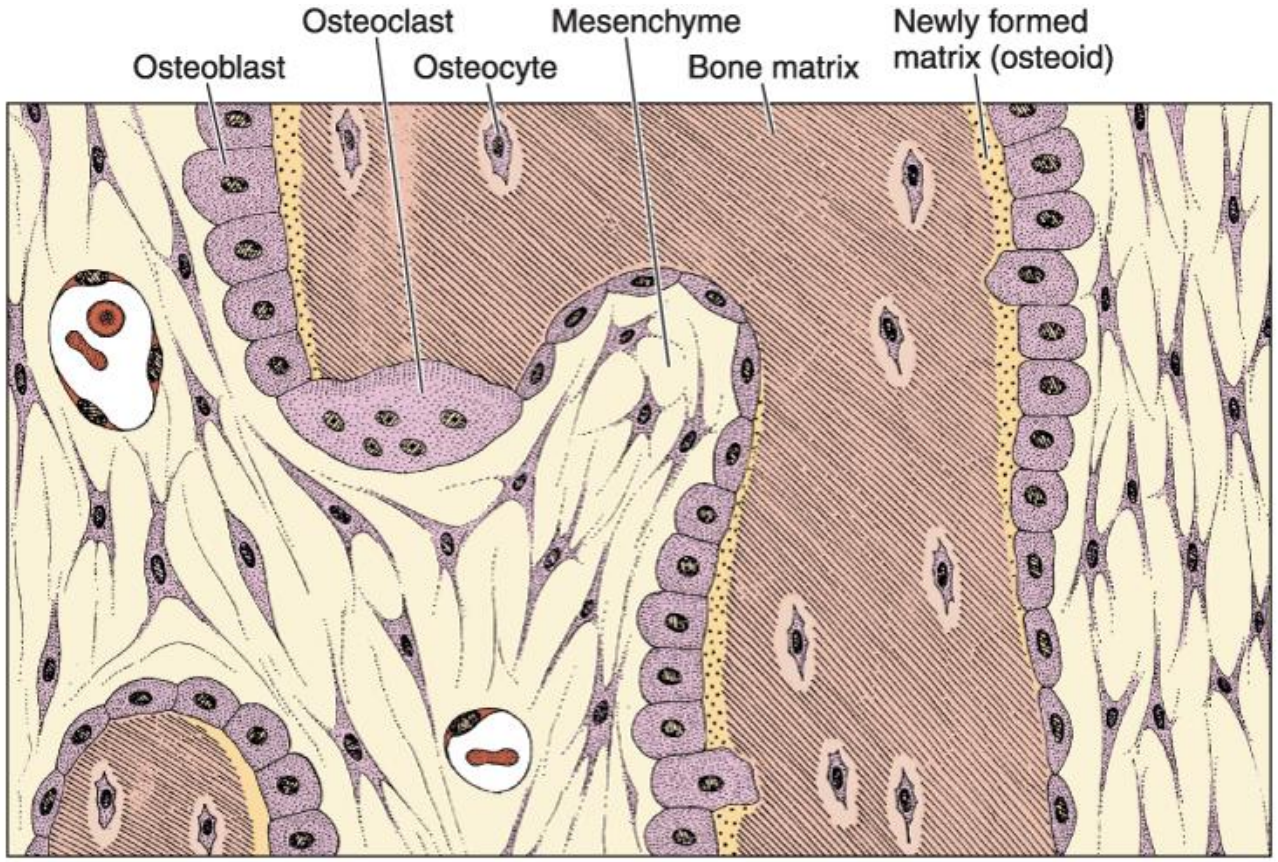
Osteoblasts

- Responsible for the synthesis of the organic components of bone matrix (type I collagen, proteoglycans, and glycoproteins).
- Deposition of the inorganic components of bone also depends on the presence of viable osteoblasts. Osteoblasts are exclusively located at the surfaces of bone tissue, side by side, in a way that resembles simple epithelium.
- When they are actively engaged in matrix synthesis, osteoblasts have a cuboidal to columnar shape and basophilic cytoplasm. When their synthesizing activity declines, they flatten, and cytoplasmic basophilia declines.



Events that occur during intramembranous ossification. Osteoblasts are synthesizing collagen, which forms a strand of matrix that traps cells. As this occurs, the osteoblasts gradually differentiate to become osteocytes. The lower part of the drawing shows an osteoblast being trapped in newly formed bone matrix.

- Some osteoblasts are gradually surrounded by newly formed matrix and become **osteocytes.** During this process a space called a **lacuna** is formed. Lacunae are occupied by osteocytes and their extensions, along with a small amount of extracellular non-calcified matrix.
- During matrix synthesis, osteoblasts have the ultrastructure of cells actively synthesizing proteins for export. Osteoblasts are polarized cells.



- Matrix components are secreted at the cell surface, which is in contact with older bone matrix, producing a layer of new (but not yet calcified) matrix, called **osteoid,** between the osteoblast layer and the previously formed bone.

- This process, **bone apposition,** is completed by subsequent deposition of calcium salts into the newly formed matrix. Quiescent osteoblasts (not producing bone matrix) become flattened. However, they easily revert to the cuboidal shape typical of the active synthesizing state.

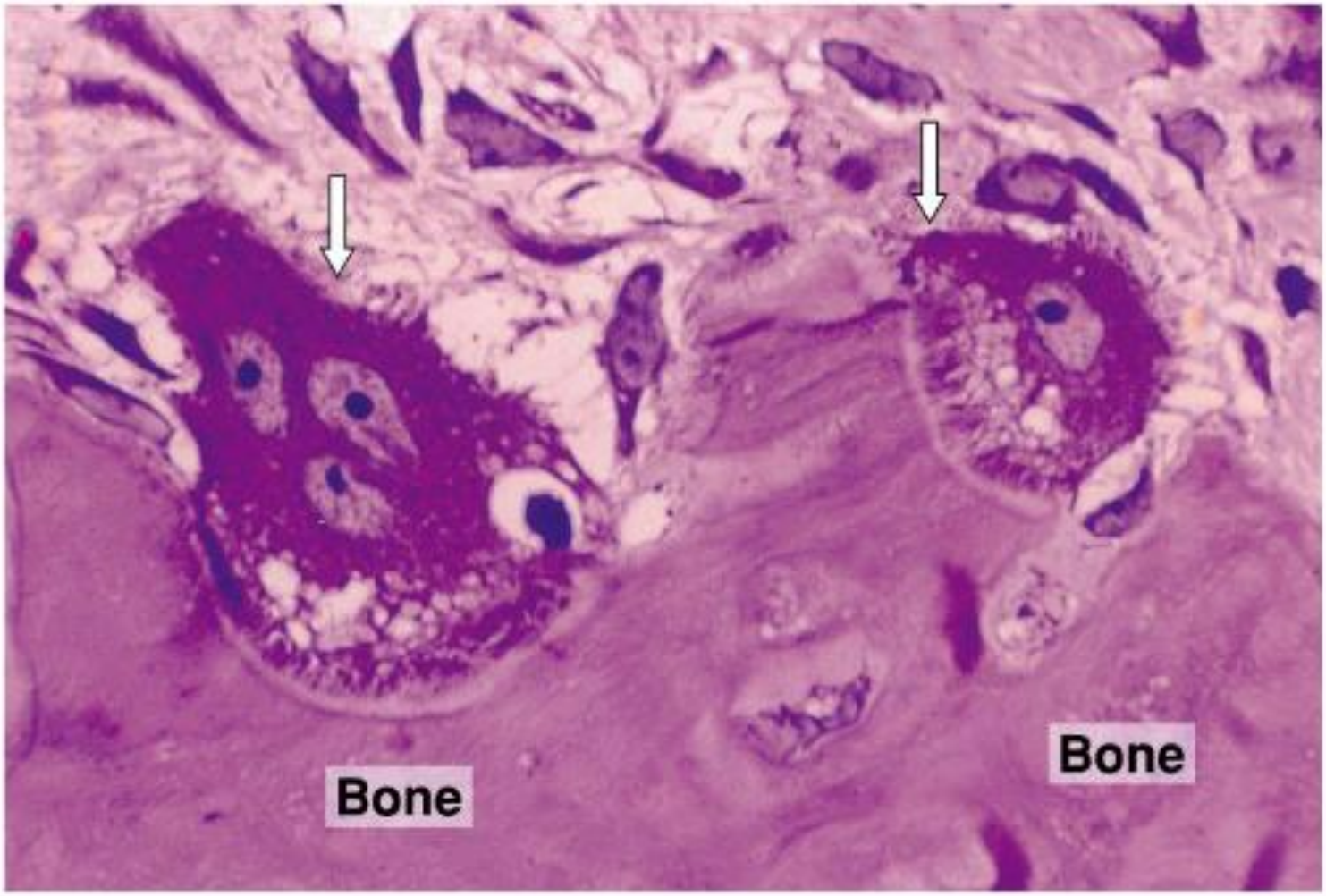
OSTEOCYTES

- Osteocytes, which derive from osteoblasts, lie in the lacunae situated between lamellae (L. diminutive of *lamina,* leaf) of matrix.
- **Only one osteocyte is found in each lacuna**. The thin, cylindrical matrix canaliculi house cytoplasmic processes of osteocytes. Processes of adjacent cells make contact via gap junctions, and molecules are passed via these structures from cell to cell.

- Some molecular exchange between osteocytes and blood vessels also takes place through the small amount of extracellular substance located between osteocytes (and their processes) and the bone matrix. This exchange can provide nourishment for a chain of about 15 cells.
- When compared with osteoblasts, the flat, almond-shaped osteocytes exhibit a significantly reduced rough endoplasmic reticulum and Golgi complex and more condensed nuclear chromatin. ***These cells are actively involved in the maintenance of the bony matrix, and their death is followed by resorption of this matrix***. Osteocytes are long-living cells.

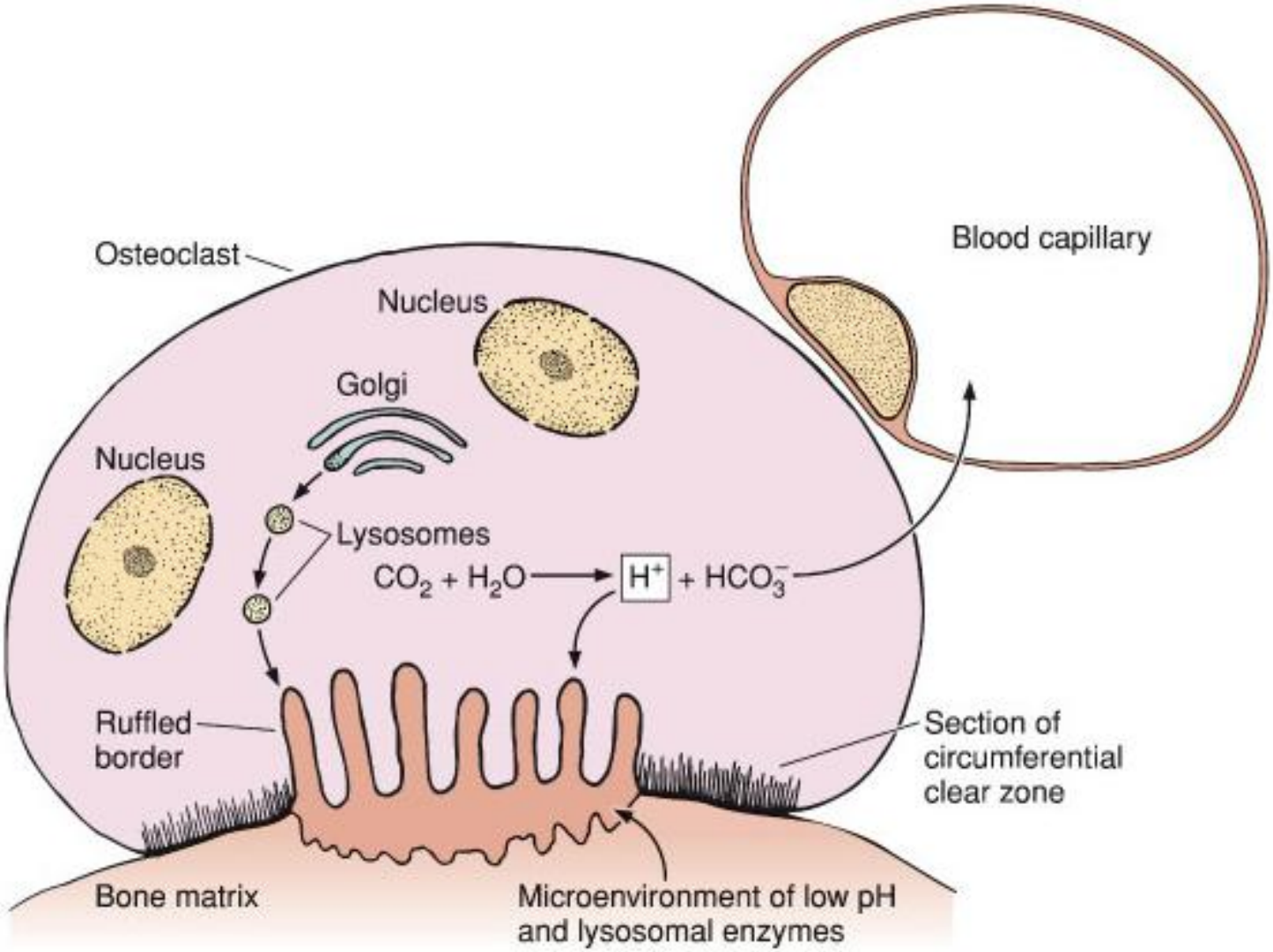
OSTEOCLASTS

- Osteoclasts are very large, branched motile (multinucleate giant) cells. Dilated portions of the cell body contain from 5 to 50 (or more) nuclei.
- In areas of bone undergoing resorption, osteoclasts lie within enzymatically etched depressions in the matrix known as **Howship's lacunae.**
- Osteoclasts are derived from the fusion of bone marrow-derived mononucleated cells.



Section showing three osteoclasts (arrows) digesting bone tissue. The osteoclast is a large cell with several nuclei and a ruffled border close to the bone matrix. Note the clear compartment where the process of bone erosion occurs. This compartment is acidified by a proton pump localized in the osteoclast membrane. It is the place of decalcification and matrix digestion and can be compared to a giant extracellular lysosome. Chondroclasts found in eroded regions of epiphyseal calcified cartilage are similar in shape to osteoclasts.

- In active osteoclasts, the surface-facing bone matrix is folded into irregular, often subdivided projections, forming a **ruffled border.** Surrounding the ruffled border is a cytoplasmic zone - the **clear zone** - that is devoid of organelles, yet rich in actin filaments. This zone is a site of adhesion of the osteoclast to the bone matrix and creates a microenvironment between the cell and the matrix in which bone resorption occurs.



- The osteoclast secretes collagenase and other enzymes and pumps protons into the microenvironment, promoting the localized digestion of collagen and dissolving calcium salt crystals. Osteoclast activity is controlled by cytokines (small signaling proteins that act as local mediators) and hormones.

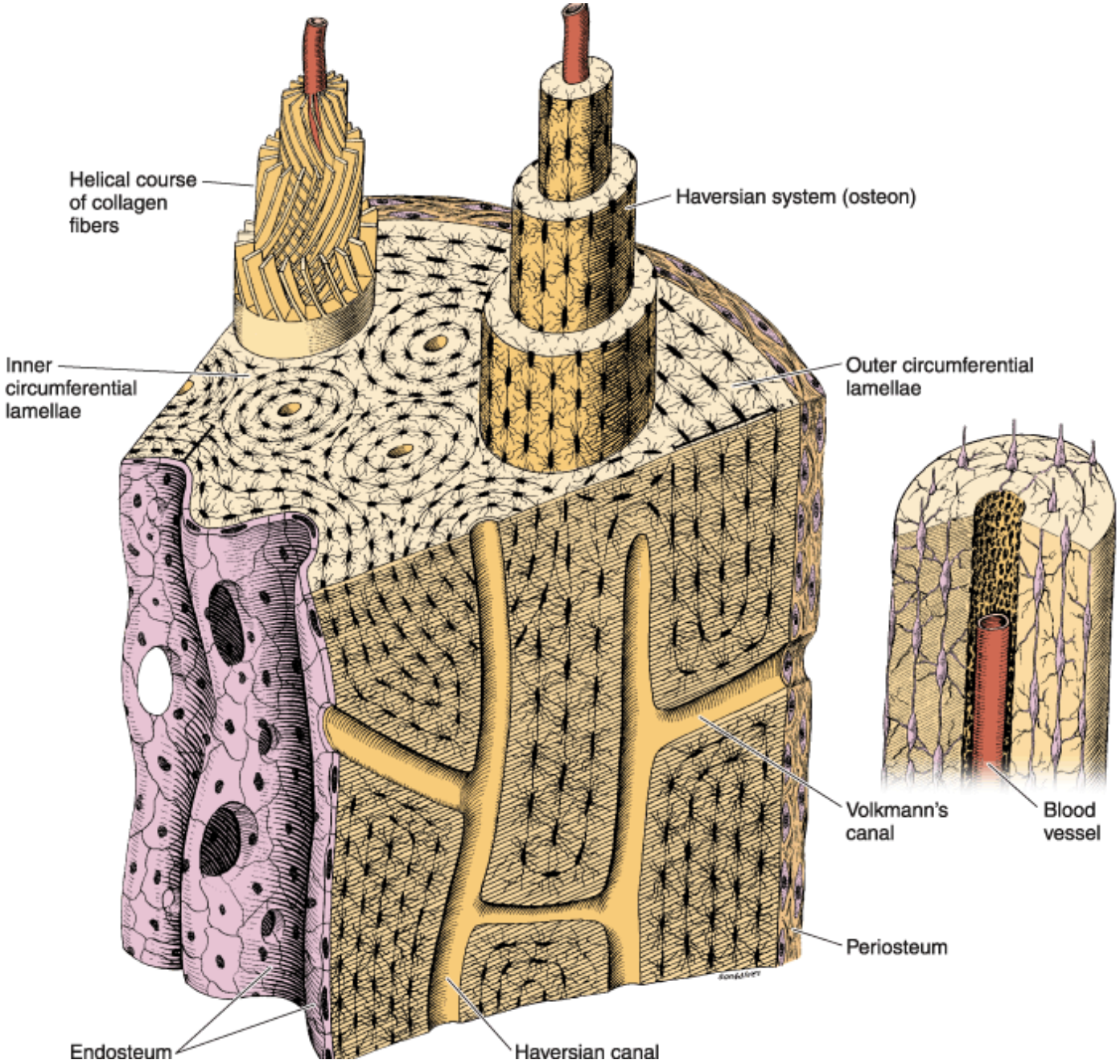
- Osteoclasts have receptors for calcitonin, a thyroid hormone, but not for parathyroid hormone. However, osteoblasts have receptors for parathyroid hormone and, when activated by this hormone, produce a cytokine called osteoclast stimulating factor.
- Ruffled borders are related to the activity of osteoclasts.

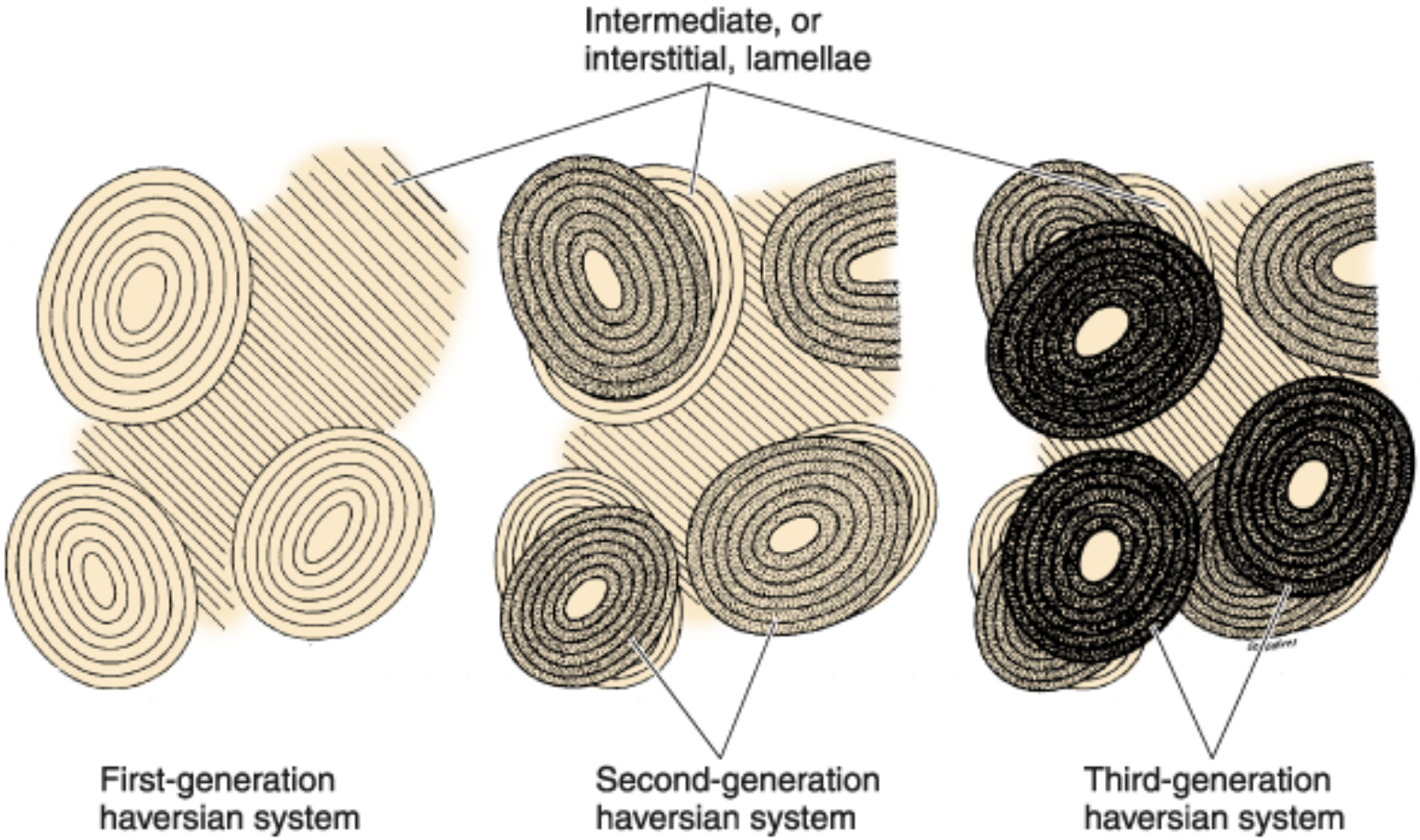
BONE MATRIX

- Inorganic matter represents about 50% of the dry weight of bone matrix. Calcium and phosphorus are especially abundant, but bicarbonate, citrate, magnesium, potassium, and sodium are also found.
- X-ray diffraction studies have shown that calcium and phosphorus form hydroxyapatite crystals with the composition $Ca_{10}(PO_4)_6(OH)_2$. However, these crystals show imperfections and are not identical to the hydroxyapatite found in the rock minerals.

Periosteum & Endosteum

- External and internal surfaces of bone are covered by layers of bone-forming cells (osteoprogenitor cells) and connective tissue called periosteum and endosteum.
- The **periosteum** consists of an outer layer of collagen fibers and fibroblasts. Bundles of periosteal collagen fibers, called **Sharpey's fibers,** penetrate the bone matrix, binding the periosteum to bone. The inner, more cellular layer of the periosteum is composed of fibroblast-like cells called **osteoprogenitor cells,** with the potential to divide by mitosis and differentiate into osteoblasts.
- Osteoprogenitor cells play a prominent role in bone growth and repair.

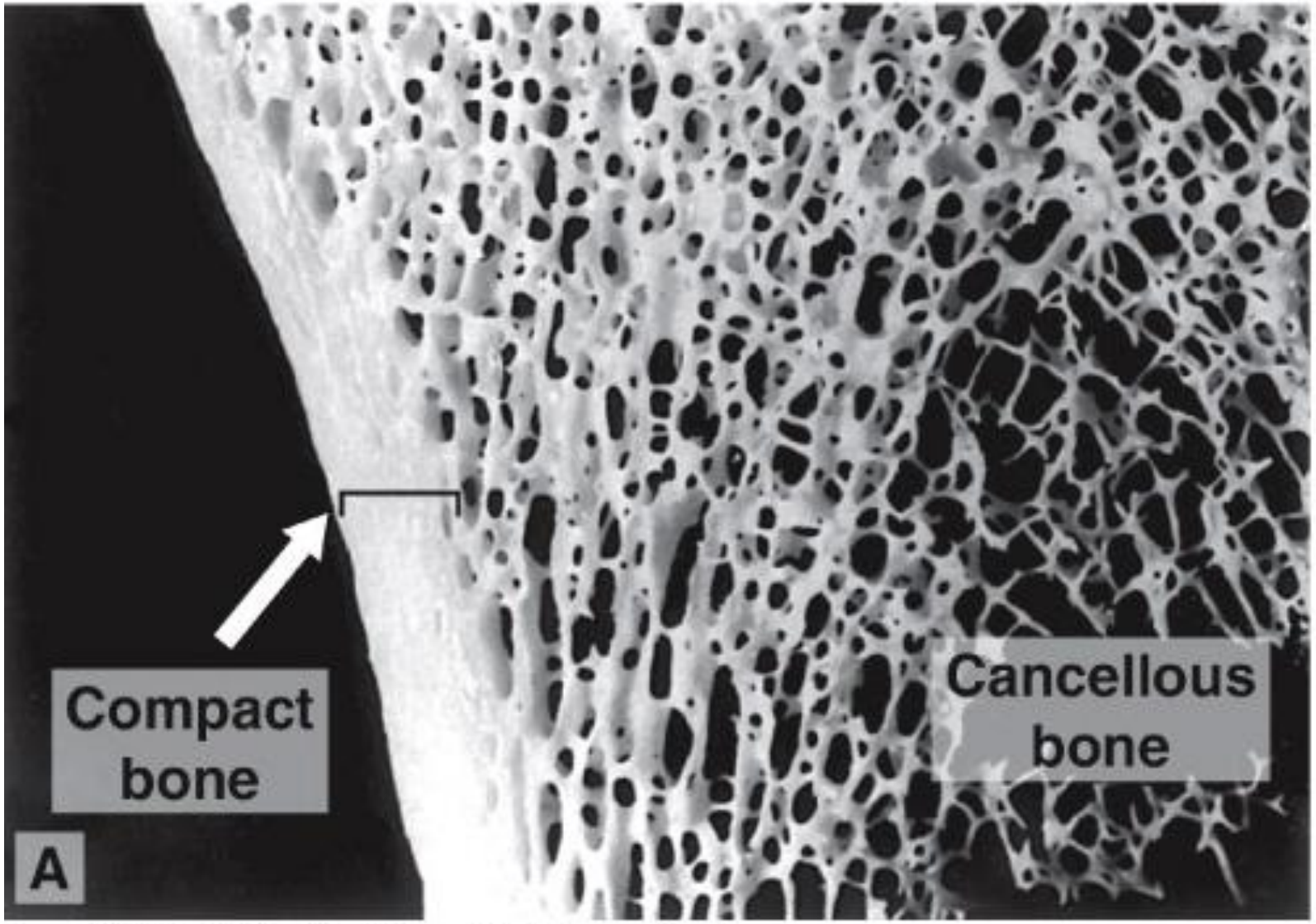

Helical course of collagen fibers
Haversian system (osteon)
Inner circumferential lamellae
Outer circumferential lamellae
Volkmann's canal
Blood vessel
Periosteum
Endosteum
Haversian canal



- The **endosteum** lines all internal cavities within the bone and is composed of a single layer of flattened osteoprogenitor cells and a very small amount of connective tissue. The endosteum is therefore considerably thinner than the periosteum.
- The **principal functions** of periosteum and endosteum are nutrition of osseous tissue and provision of a continuous supply of new osteoblasts for repair or growth of bone.

TYPES OF BONE

- **Gross observation** of bone in cross section shows dense areas without cavities - corresponding to **compact bone** - and areas with numerous interconnecting cavities - corresponding to **cancellous** (**spongy**) **bone.**
- **Under the microscope**, however, both compact bone and the trabeculae separating the cavities of cancellous bone have the same basic histological structure.



- In long bones, the bulbous end - called **epiphyses** (Gr. *epiphysis,* an excrescence) - are composed of spongy bone covered by a thin layer of compact bone.
- The cylindrical part - **diaphysis** (Gr. *diaphysis,* a growing between) - is almost totally composed of compact bone, with a small component of spongy bone on its inner surface around the bone marrow cavity.
- Short bones usually have a core of spongy bone completely surrounded by compact bone. The flat bones that form the calvaria have two layers of compact bone called **plates** (tables), separated by a layer of spongy bone called the **diploÃ«.**

- **Microscopic examination** of bone shows two varieties: **primary, immature,** or **woven bone** and **secondary, mature,** or **lamellar bone.** Primary bone is the first bone tissue to appear in embryonic development and in fracture repair and other repair processes. It is characterized by random disposition of fine collagen fibers, in contrast to the organized lamellar disposition of collagen in secondary bone.

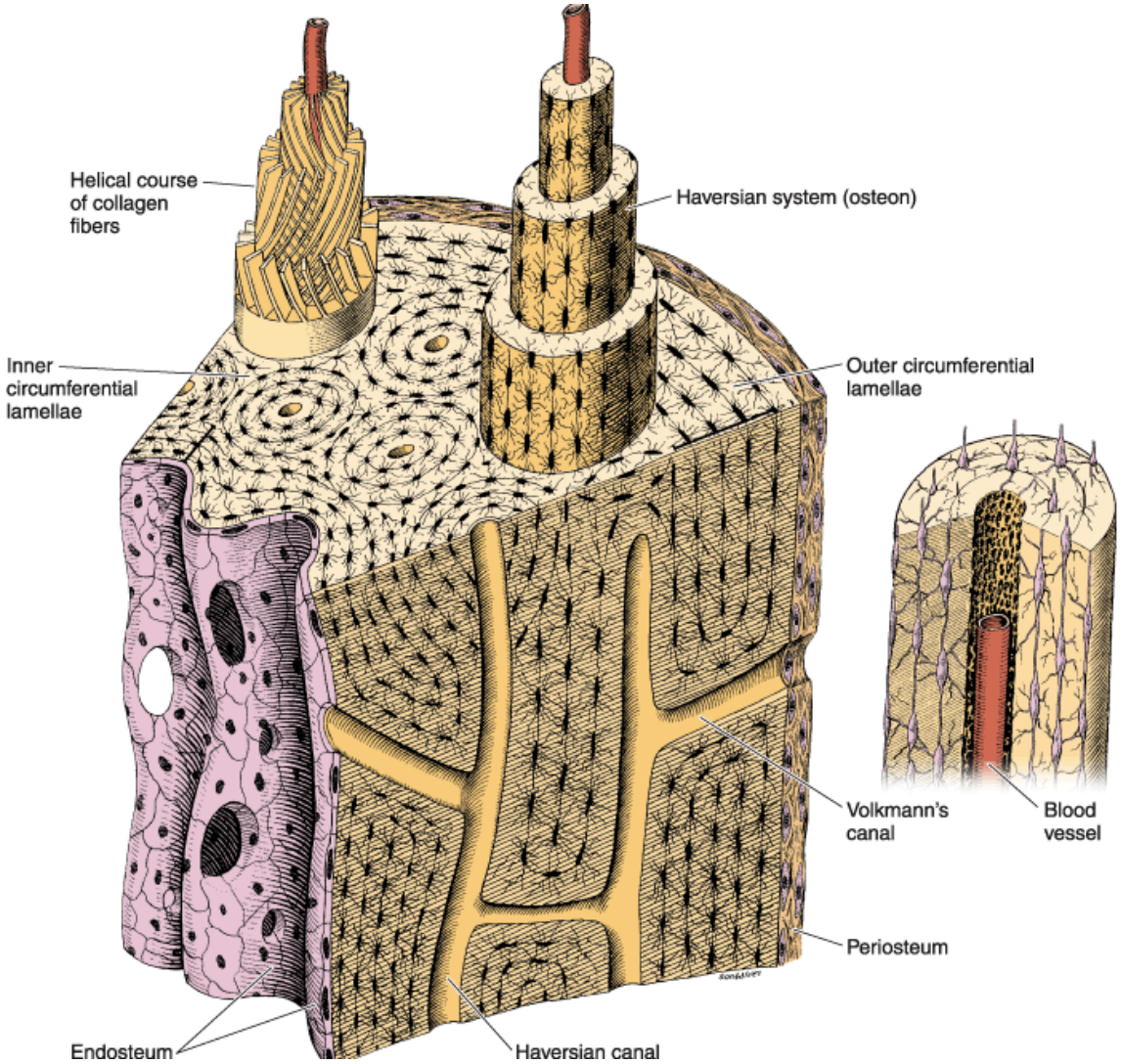
PRIMARY BONE TISSUE

- Primary bone tissue is usually temporary and, except in a very few places in the body (e.g, near the sutures of the flat bones of the skull, in tooth sockets, and in the insertions of some tendons), is replaced in adults by secondary bone tissue.
- In addition to the irregular array of collagen fibers, other characteristics of primary bone tissue are a lower mineral content (it is more easily penetrated by x-rays) and a higher proportion of osteocytes than in secondary bone tissue.

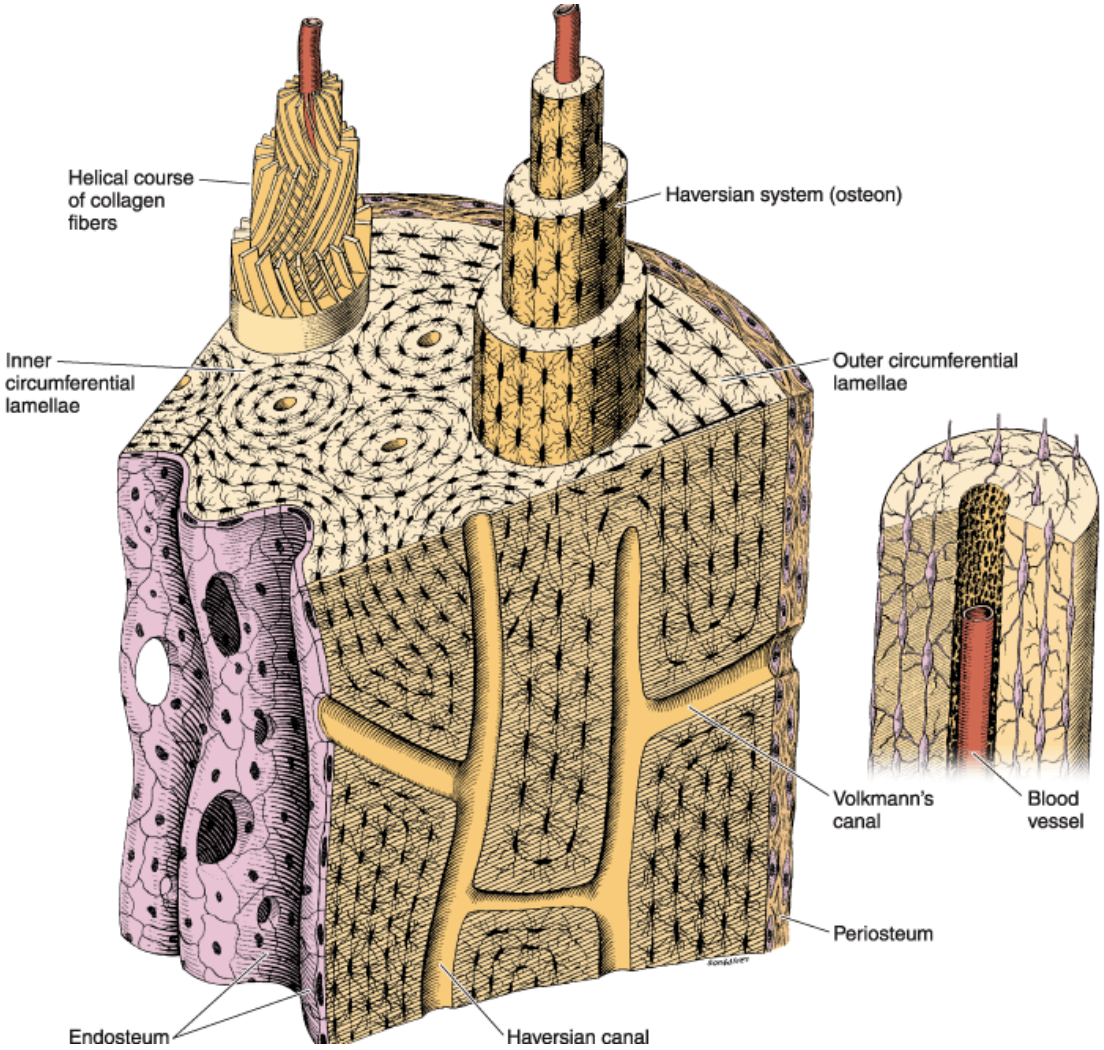
SECONDARY BONE TISSUE

- Secondary bone tissue is usually found in adults. It characteristically shows collagen fibers arranged in lamellae that are parallel to each other or concentrically organized around a vascular canal.

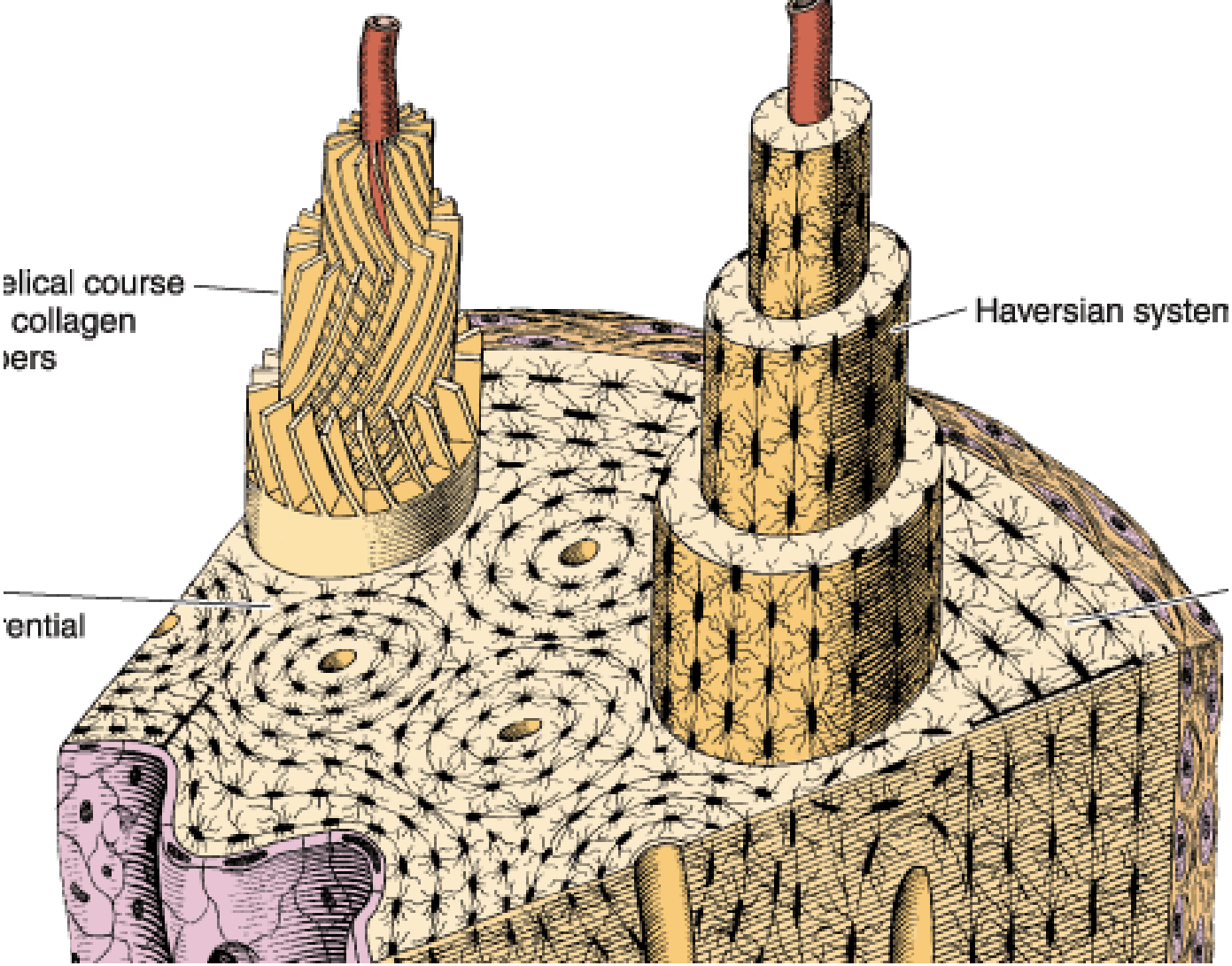
- Mature compact bone is composed of three lamellar arrangements:
  - Osteon (harvesian systems)
  - Circumferential systems
  - Interstitial systems



- In compact bone (e.g., the diaphysis of long bones), the lamellae exhibit a typical organization consisting of **haversian systems, outer circumferential lamellae, inner circumferential lamellae,** and **interstitial lamellae**.

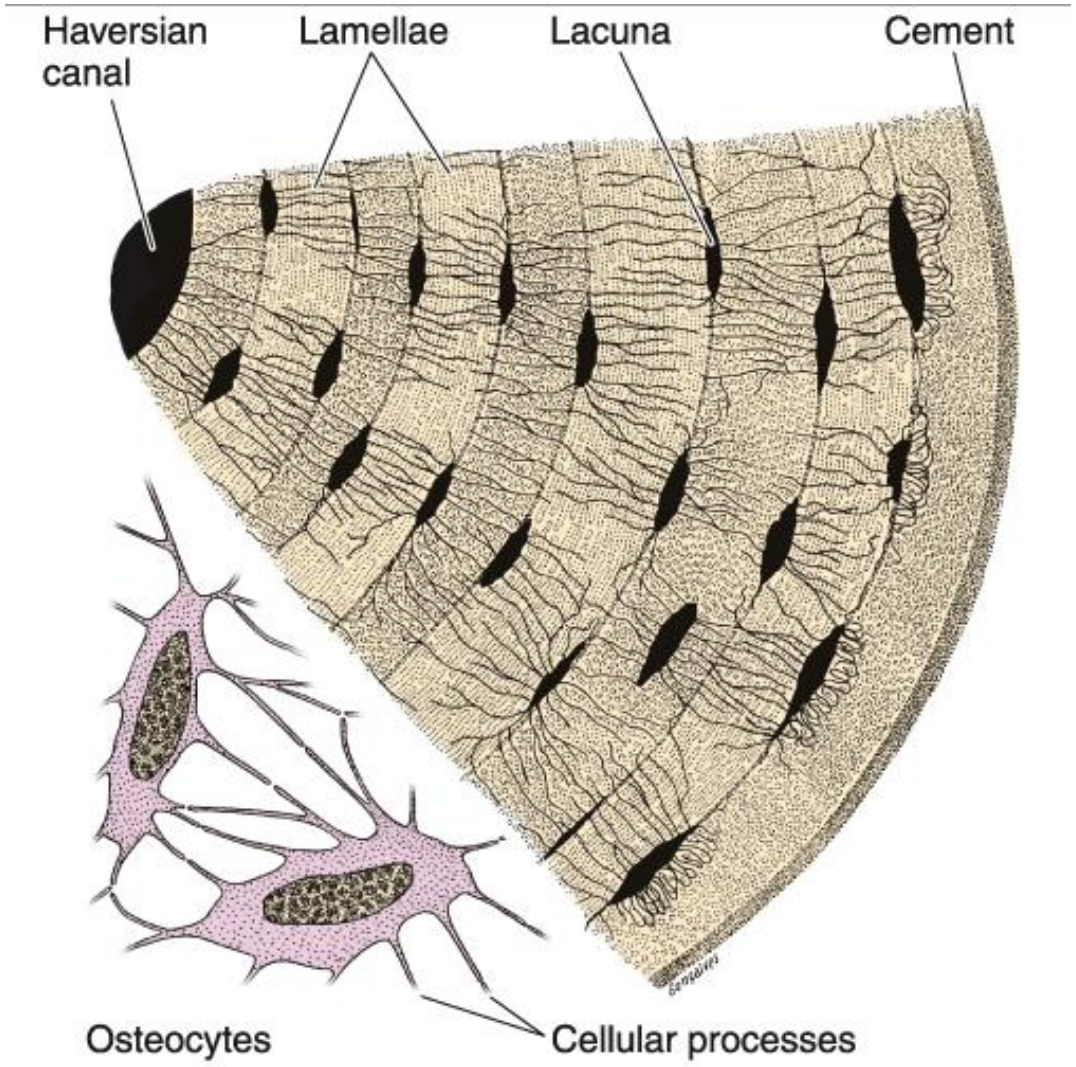


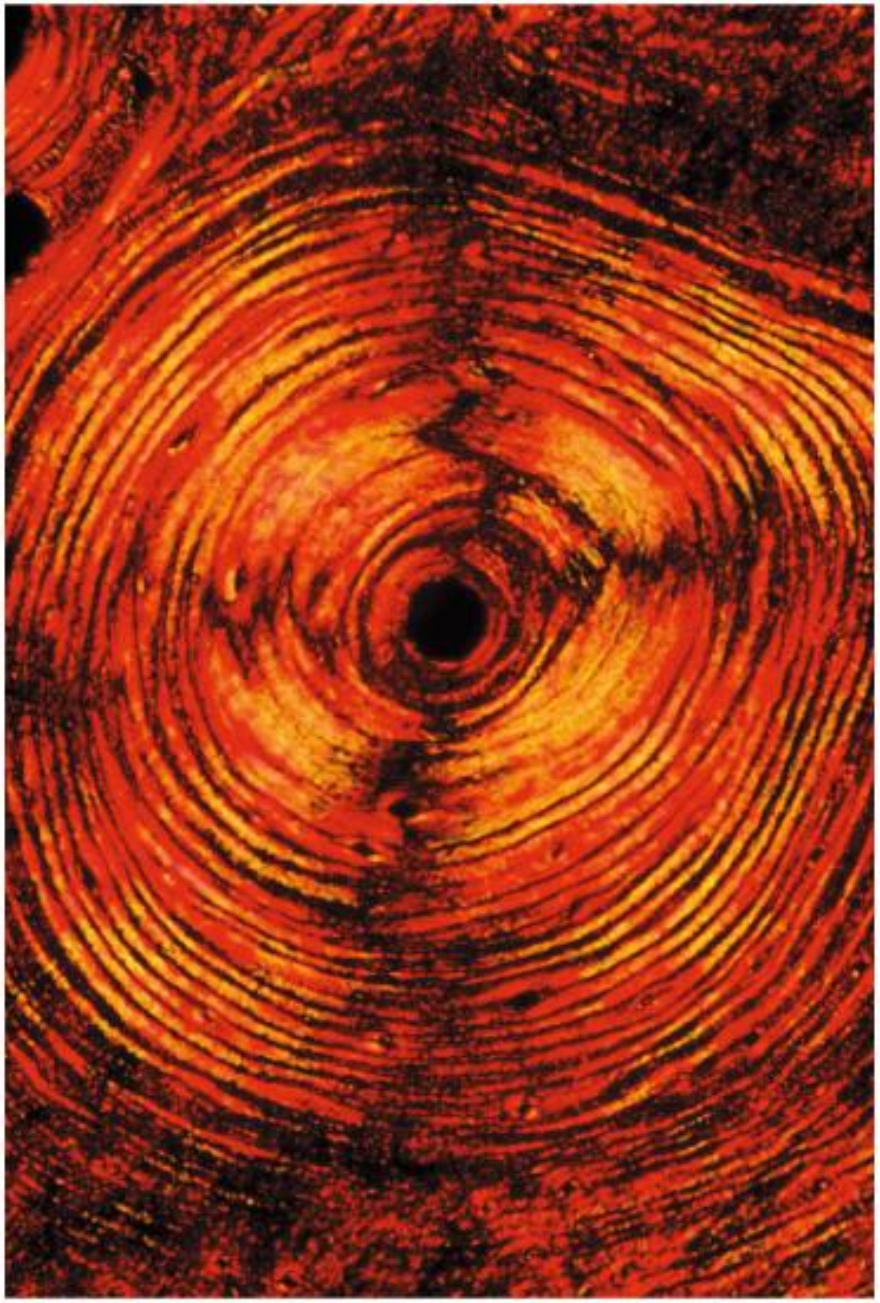
- The whole complex of concentric lamellae of bone surrounding a canal containing blood vessels, nerves, and loose connective tissue is called a **haversian system,** or **osteon**.



- Lacunae containing osteocytes are found between, and occasionally within, the lamellae. In each lamella, collagen fibers are parallel to each other.

- Surrounding each haversian system is a deposit of amorphous material called the **cementing substance** that consists of mineralized matrix with few collagen fibers.





Section of a haversian system, or osteon. Note the alternation of clear and dark circles resulting from the alternation in the direction of the collagen fibers.

- Inner circumferential lamellae are located around the marrow cavity, and outer circumferential lamellae are located immediately beneath the periosteum. There are more outer than inner lamellae.
- Between the two circumferential systems are numerous haversian systems, including triangular or irregularly shaped groups of parallel lamellae called **interstitial** (or **intermediate**) **lamellae.** These structures are lamellae left by haversian systems destroyed during growth and remodeling of bone.
- They are recognised as irregular lamellae structure that lack a central Haversian canal

- Each Haversian system is a long, often bifurcated cylinder parallel to the long axis of the diaphysis. It consists of a central canal surrounded by 4 - 20 concentric lamellae.
- Each endosteum-lined canal contains blood vessels, nerves, and loose connective tissue. The Haversian canals communicate with the marrow cavity, the periosteum, and one another through transverse or oblique Volkmann's canals.
- Volkmann's canals do not have concentric lamellae; instead, they perforate the lamellae. All vascular canals found in bone tissue come into existence when matrix is laid down around preexisting blood vessels.

HISTOGENESIS

- Bone can be formed in two ways:
  - by direct mineralization of matrix secreted by osteoblasts (**intramembranous ossification**) or
  - by deposition of bone matrix on a preexisting cartilage matrix (**endochondral ossification**).
- In both processes, the bone tissue that appears first is primary, or woven.

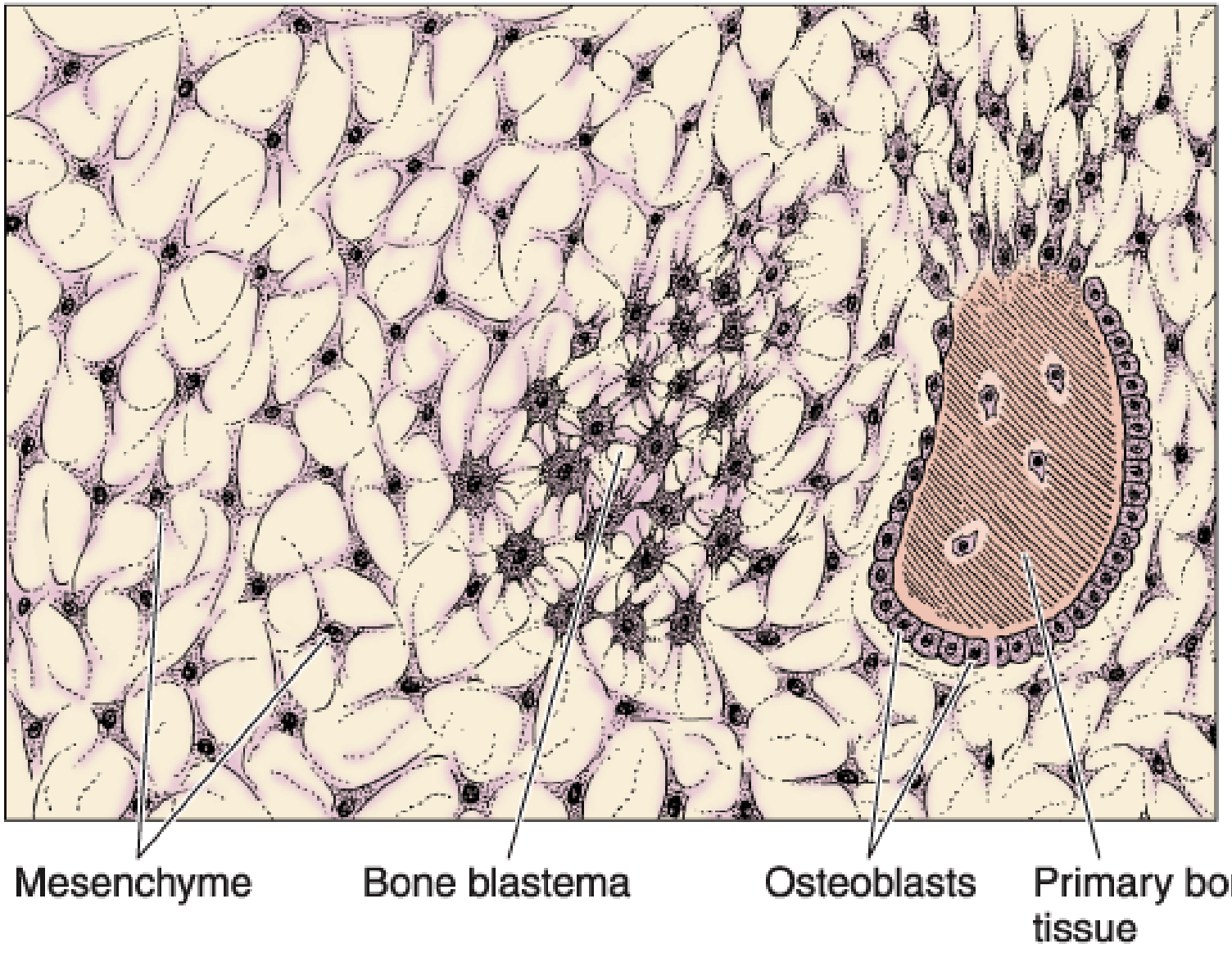
- Primary bone is a temporary tissue and is soon replaced by the definitive lamellar, or secondary, bone. During bone growth, areas of primary bone, areas of resorption, and areas of secondary bone appear side by side. This combination of bone synthesis and removal (**remodeling**) occurs not only in growing bones but also throughout adult life, although its rate of change in adults is considerably slower.

INTRAMEMBRANOUS OSSIFICATION

- **Intramembranous ossification**, the source of most of the flat bones, is so called because it takes place within condensations of mesenchymal tissue. The frontal and parietal bones of the skull - as well as parts of the occipital and temporal bones and the mandible and maxilla - are formed by intramembranous ossification.
- This process also contributes to the growth of short bones and the thickening of long bones.

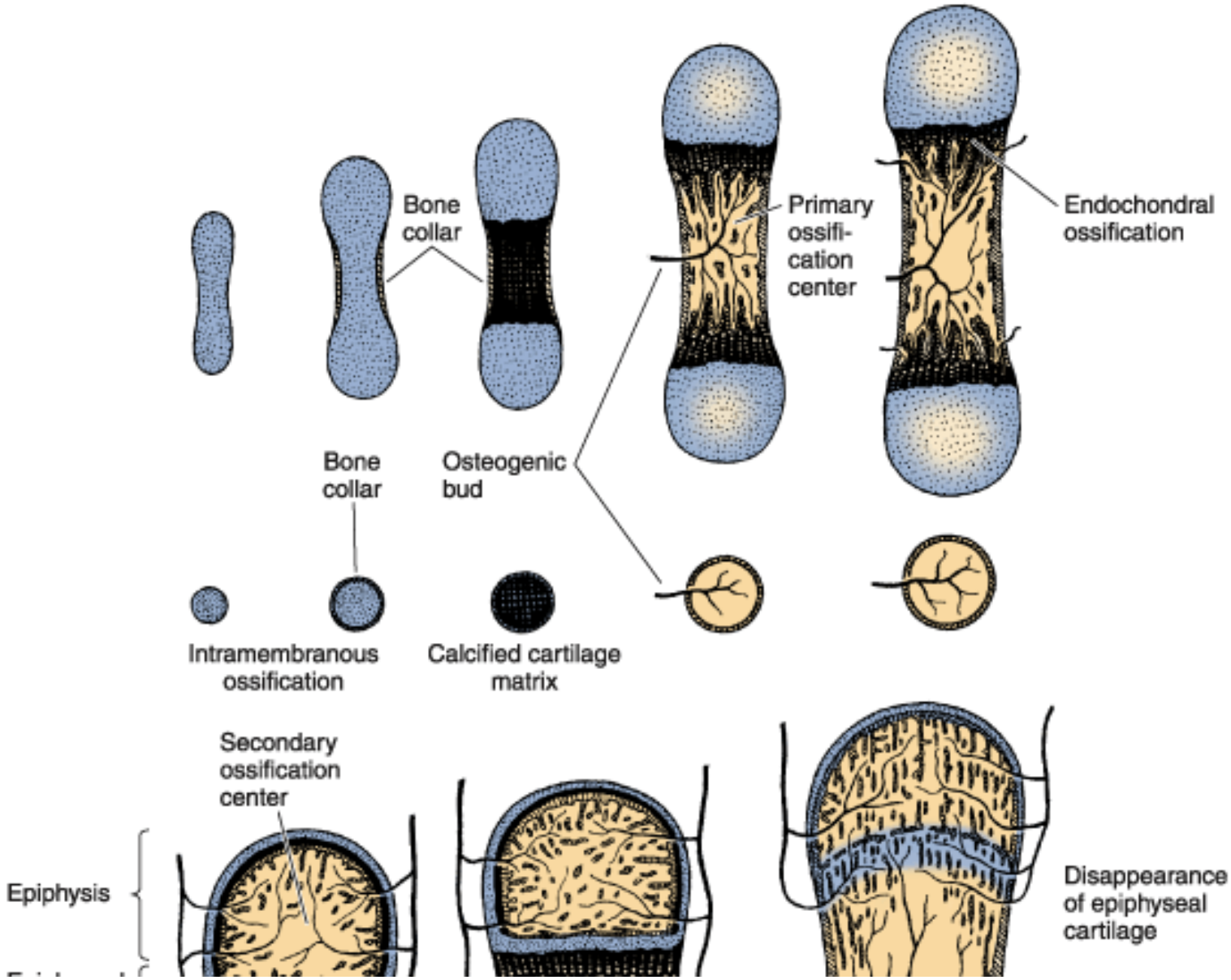
- In the mesenchymal condensation layer, the starting point for ossification is called a **primary ossification center.** The process begins when groups of cells differentiate into osteoblasts. Osteoblasts produce bone matrix and calcification follows, resulting in the encapsulation of some osteoblasts, which then become osteocytes.

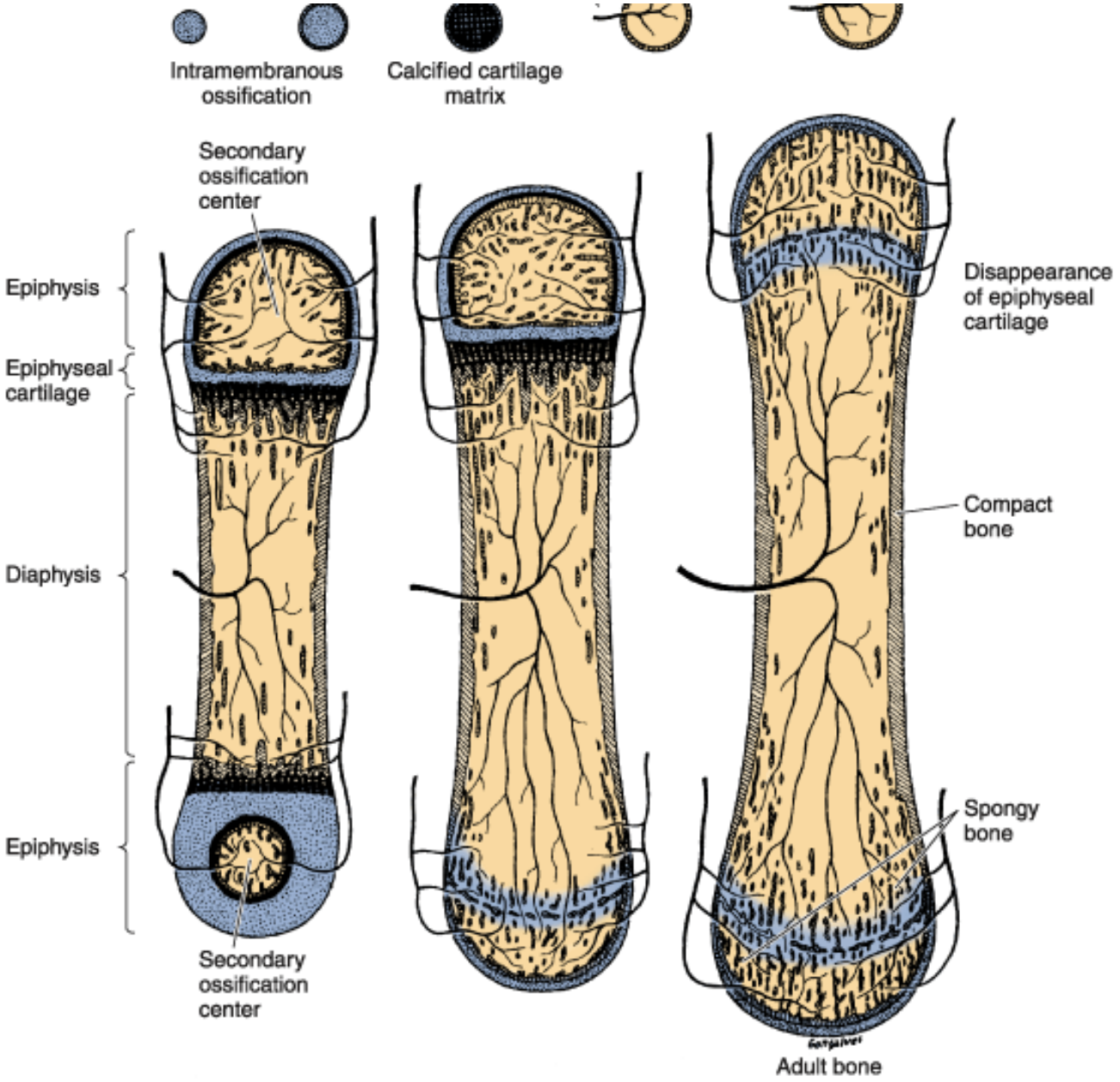
- These islands of developing bone form walls that delineate elongated cavities containing capillaries, bone marrow cells, and undifferentiated cells.
- Several such groups arise almost simultaneously at the ossification center, so that the fusion of the walls gives the bone a spongy structure.
- The connective tissue that remains among the bone walls is penetrated by growing blood vessels and additional undifferentiated mesenchymal cells, giving rise to the bone marrow cells.


Mesenchyme
Bone blastema
Osteoblasts
Primary bone tissue

ENDOCHONDRAL OSSIFICATION

- Endochondral (Gr. *endon,* within, + *chondros,* cartilage) ossification takes place within a piece of hyaline cartilage whose shape resembles a small version, or model, of the bone to be formed.
- This type of ossification is principally responsible for the formation of short and long bones.


Bone collar
Primary ossifi-cation center
Endochondral ossification
Bone collar
Osteogenic bud
Intramembranous ossification
Calcified cartilage matrix
Secondary ossification center
Epiphysis
Disappearance of epiphyseal cartilage

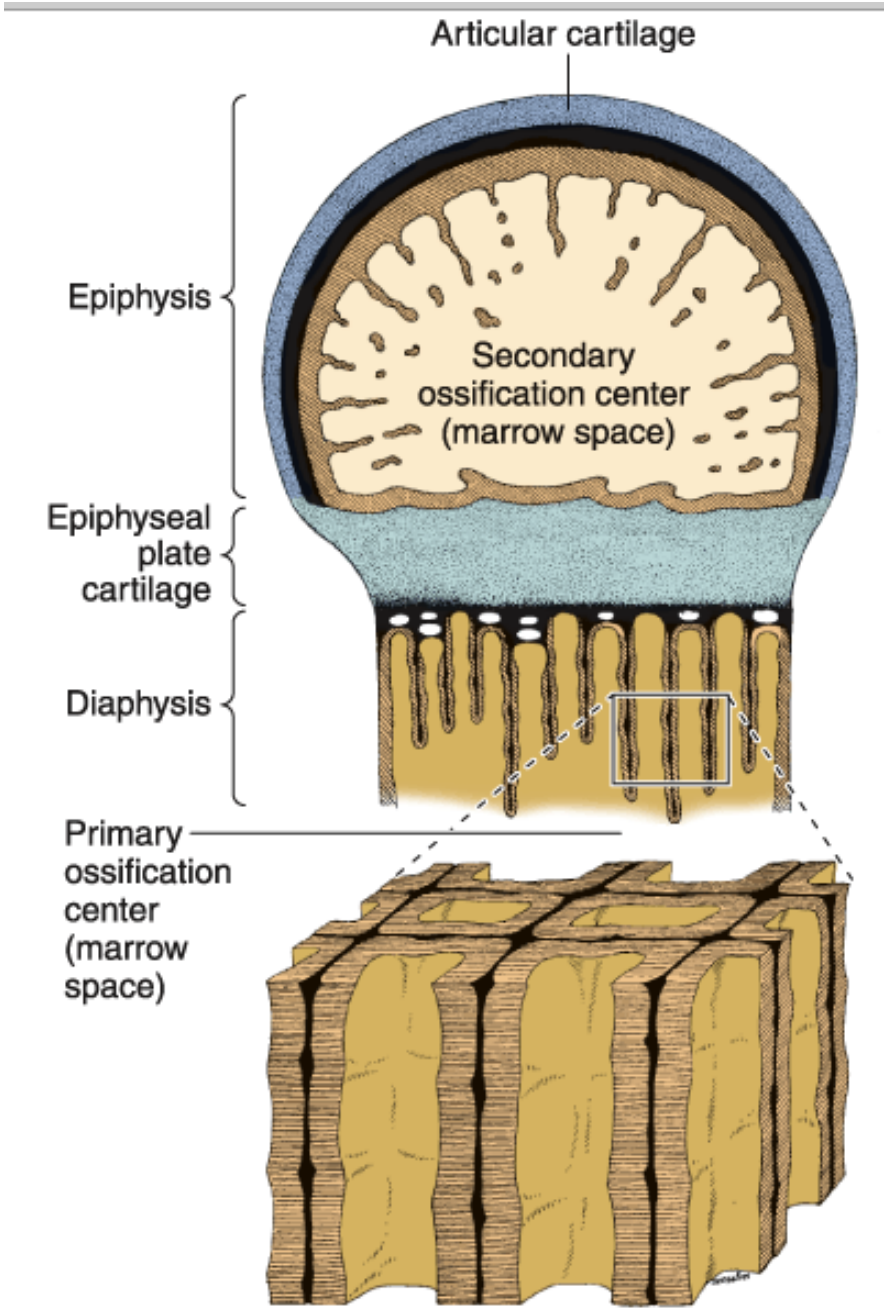


- Endochondral ossification of a long bone consists of the following sequence of events. Initially, the first bone tissue appears as a hollow bone cylinder that surrounds the mid portion of the cartilage model. This structure, the **bone collar,** is produced by intramembranous ossification within the local perichondrium.

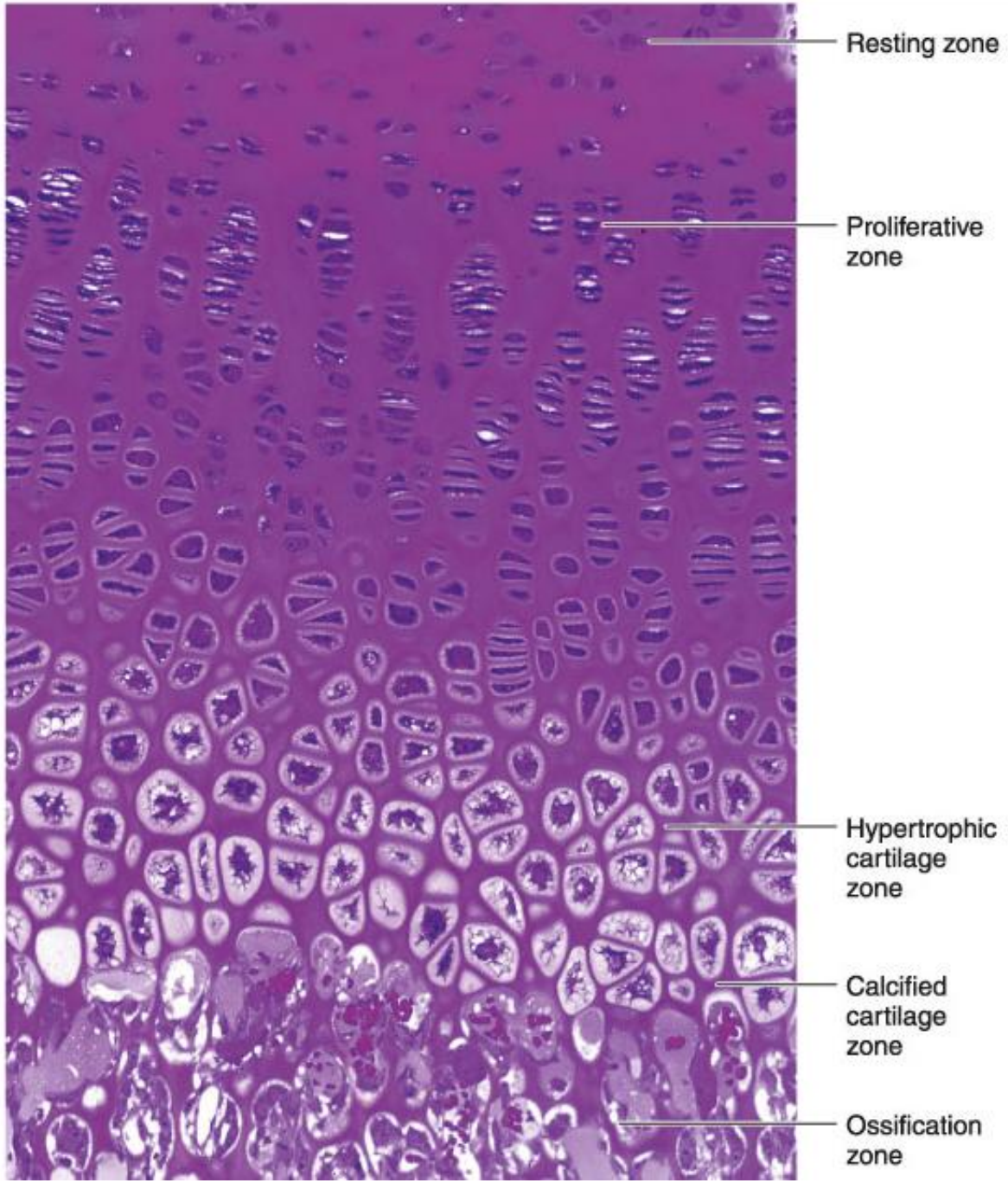
- In the next step, the local cartilage undergoes a degenerative process of programmed cell death with cell enlargement (hypertrophy) and matrix calcification, resulting in a three-dimensional structure formed by the remnants of the calcified cartilage matrix.

- This process begins at the central portion of the cartilage model (diaphysis), where blood vessels penetrate through the bone collar previously perforated by osteoclasts, bringing osteoprogenitor cells to this region.
- Next, osteoblasts adhere to the calcified cartilage matrix and produce continuous layers of primary bone that surround the cartilaginous matrix remnants.

- At this stage, the calcified cartilage appears basophilic, and the primary bone is eosinophilic. In this way the **primary ossification center** is produced.
- Then, **secondary ossification centers** appear at the swellings in the extremities of the cartilage model (epiphyses). During their expansion and remodeling, the primary and secondary ossification centers produce cavities that are gradually filled with bone marrow.



- In the secondary ossification centers, cartilage remains in two regions: the **articular cartilage,** which persists throughout adult life and does not contribute to bone growth in length, and the **epiphyseal cartilage,** also called the **epiphyseal plate,** which connects the two epiphyses to the diaphysis.
- The epiphyseal cartilage is responsible for the growth in length of the bone, and it disappears in adults, which is why bone growth ceases in adulthood.


Resting zone
Proliferative zone
Hypertrophic cartilage zone
Calcified cartilage zone
Ossification zone

- Epiphyseal cartilage is divided into five zones, starting from the epiphyseal side of cartilage:
  - (1) The **resting zone** consists of hyaline cartilage without morphological changes in the cells.
  - (2) In the **proliferative zone,** chondrocytes divide rapidly and form columns of stacked cells parallel to the long axis of the bone.

- (3) The **hypertrophic cartilage zone** contains large chondrocytes whose cytoplasm has accumulated glycogen. The resorbed matrix is reduced to thin septa between the chondrocytes.
- (4) Simultaneous with the death of chondrocytes in the **calcified cartilage zone,** the thin septa of cartilage matrix become calcified by the deposit of hydroxyapatite.
- (5) In the **ossification zone,** endochondral bone tissue appears. Blood capillaries and osteoprogenitor cells formed by mitosis of cells originating from the periosteum invade the cavities left by the chondrocytes. The osteoprogenitor cells form osteoblasts, which are distributed in a discontinuous layer over the septa of calcified cartilage matrix.

- Ultimately, the osteoblasts deposit bone matrix over the three-dimensional calcified cartilage matrix.

- In summary, growth in length of a long bone occurs by proliferation of chondrocytes in the epiphyseal plate adjacent to the epiphysis. At the same time, chondrocytes of the diaphyseal side of the plate hypertrophy; their matrix becomes calcified, and the cells die. Osteoblasts lay down a layer of primary bone on the calcified cartilage matrix.
- Because the rates of these two opposing events (proliferation and destruction) are approximately equal, the epiphyseal plate does not change thickness. Instead, it is displaced away from the middle of the diaphysis, resulting in growth in length of the bone.

BONE GROWTH & REMODELING

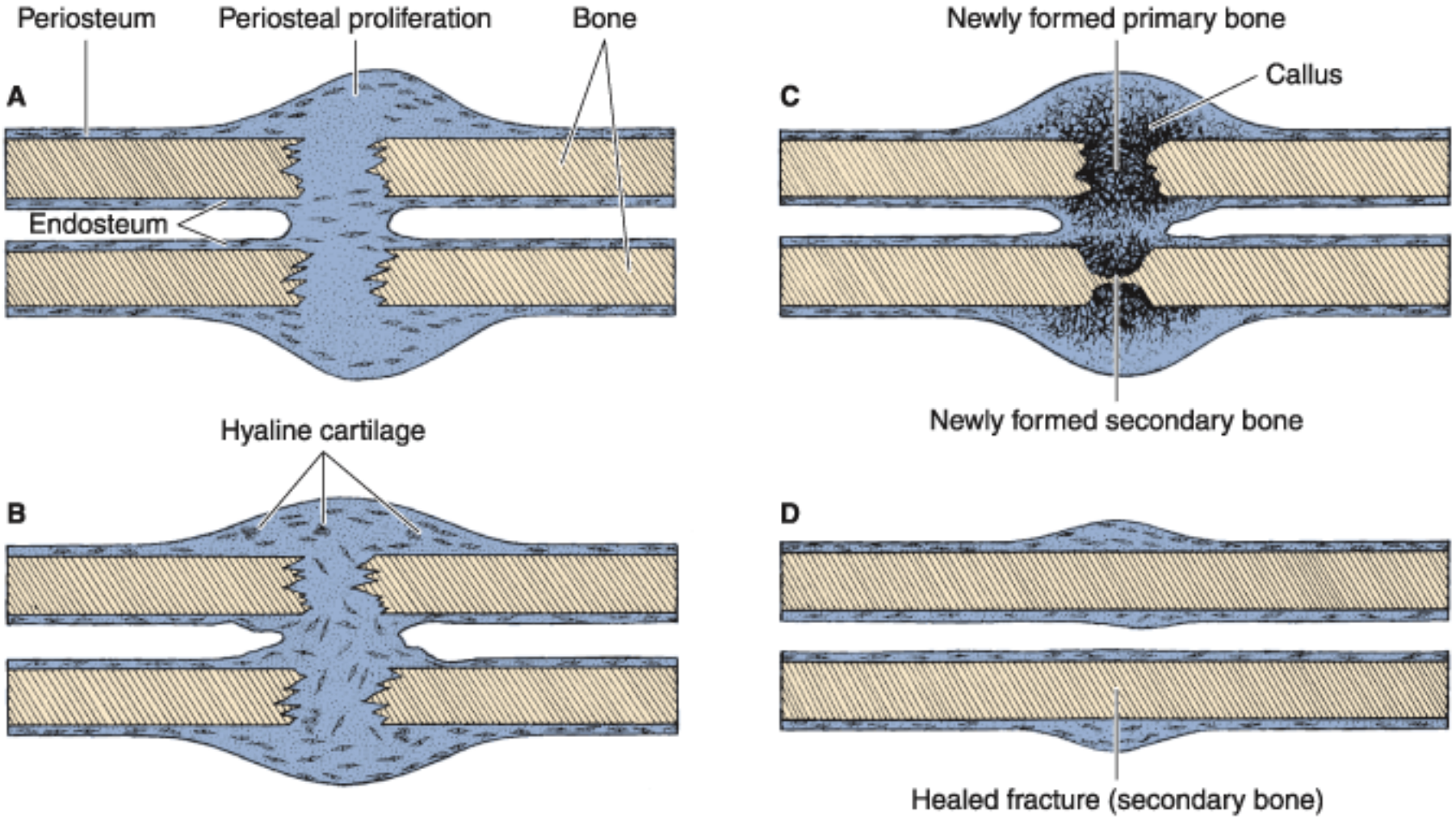
- Bone growth is generally associated with partial resorption of preformed tissue and the simultaneous laying down of new bone (exceeding the rate of bone loss). This process permits the shape of the bone to be maintained while it grows. Bone remodeling (**bone turnover**) is very active in young children, where it can be 200 times faster than the rate in adults. Bone remodeling in adults is a dynamic physiological process that occurs simultaneously in multiple locations of the skeleton, not related to bone growth.

- Cranial bones grow mainly because of the formation of bone tissue by the periosteum between the sutures and on the external bone surface. At the same time, resorption takes place on the internal surface.
- Because bone is an extremely plastic tissue, it responds to the growth of the brain and forms a skull of adequate size. The skull will be small if the brain does not develop completely and will be larger than normal in a person suffering from hydrocephalus, a disorder characterized by abnormal accumulation of spinal fluid and dilatation of the cerebral ventricles.

FRACTURE REPAIR

- When a bone is fractured, bone matrix is destroyed and bone cells adjoining the fracture die. The damaged blood vessels produce a localized hemorrhage and form a blood clot.
- During repair, the blood clot, cells, and damaged bone matrix are removed by macrophages. The periosteum and the endosteum around the fracture respond with intense proliferation producing a tissue that surrounds the fracture and penetrates between the extremities of the fractured bone

- Primary bone is then formed by endochondral and intramembranous ossification, both processes contributing simultaneously to the healing of fractures. Repair progresses in such a way that irregularly formed trabeculae of primary bone temporarily unite the extremities of the fractured bone, forming a **bone callus**.
- Stresses imposed on the bone during repair and during the patient's gradual return to activity serve to remodel the bone callus. If these stresses are identical to those that occurred during the growth of the bone - and therefore influence its structure - the primary bone tissue of the callus is gradually resorbed and replaced by secondary tissue, remodeling the bone and restoring its original structure. Unlike other connective tissues, bone tissue heals without forming a scar.



Repair of a fractured bone by formation of new bone tissue through periosteal and endosteal cell proliferation.

INTERNAL STRUCTURE OF BONES

- Despite its hardness, bone is capable of changes in its internal structure in response to the various stresses to which it is subjected. For example, the positions of the teeth in the jawbone can be modified by lateral pressures produced by orthodontic appliances.
- Bone is formed on the side where traction is applied and is resorbed where pressure is exerted (on the opposite side). In this way, teeth move within the jawbone while the alveolar bone is being remodeled.

METABOLIC ROLE OF BONE TISSUE

- The skeleton contains 99% of the total calcium of the body and acts as a reservoir of calcium and phosphate ions. The concentration of calcium ions in the blood and tissues is quite stable because of a continuous interchange between blood calcium and bone calcium.
- Bone calcium is mobilized by two mechanisms, one rapid and the other slow. The first is the simple transfer of ions from hydroxyapatite crystals to interstitial fluid - from which, in turn, calcium passes into the blood. This purely physical mechanism takes place mainly in spongy bone.

- The younger, slightly calcified lamellae that exist even in adult bone (because of continuous remodeling) receive and lose calcium more readily. These lamellae are more important for the maintenance of calcium concentration in the blood than are the older, greatly calcified lamellae, whose role is mainly that of support and protection.

- The second mechanism for controlling blood calcium level depends on the action of hormones on bone. **Parathyroid hormone** promotes osteoclastic resorption of the bone matrix with the consequent liberation of calcium. This hormone acts primarily on osteoblast receptors. The activated osteoblasts stop producing bone and start the secretion of an **osteoclast-stimulating factor.**
- Another hormone, **calcitonin,** which is synthesized mainly by the parafollicular cells of the thyroid gland, inhibits matrix resorption. Calcitonin has an inhibitory effect on osteoclast activity.