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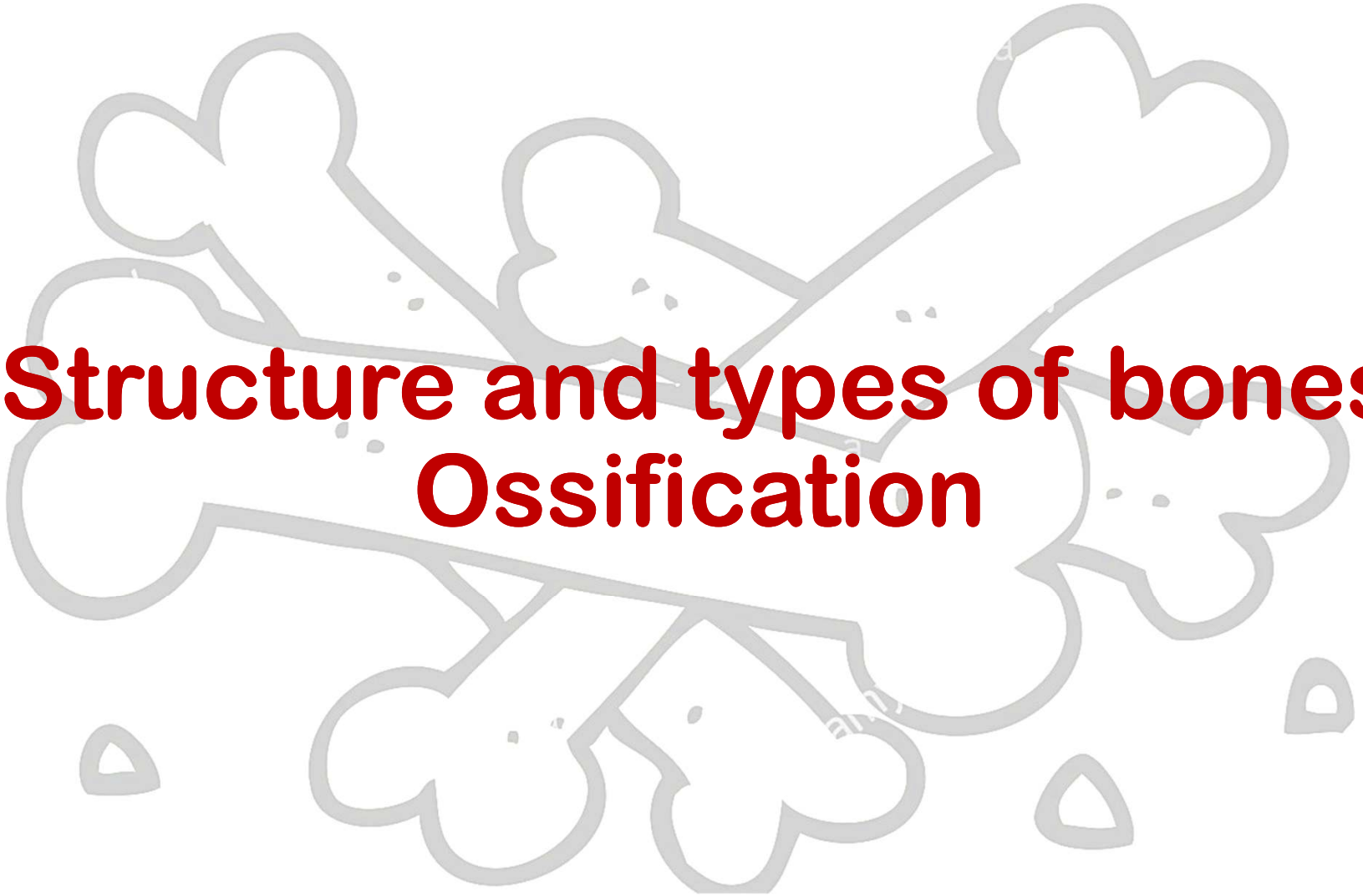

Structure and types of bones
Ossification

Bone

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- 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.**
- 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.
- Bone tissue is **highly vascularized** and **metabolically very active.**
- 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.**
- 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.**

Structure of Bone

Periosteum & Endosteum

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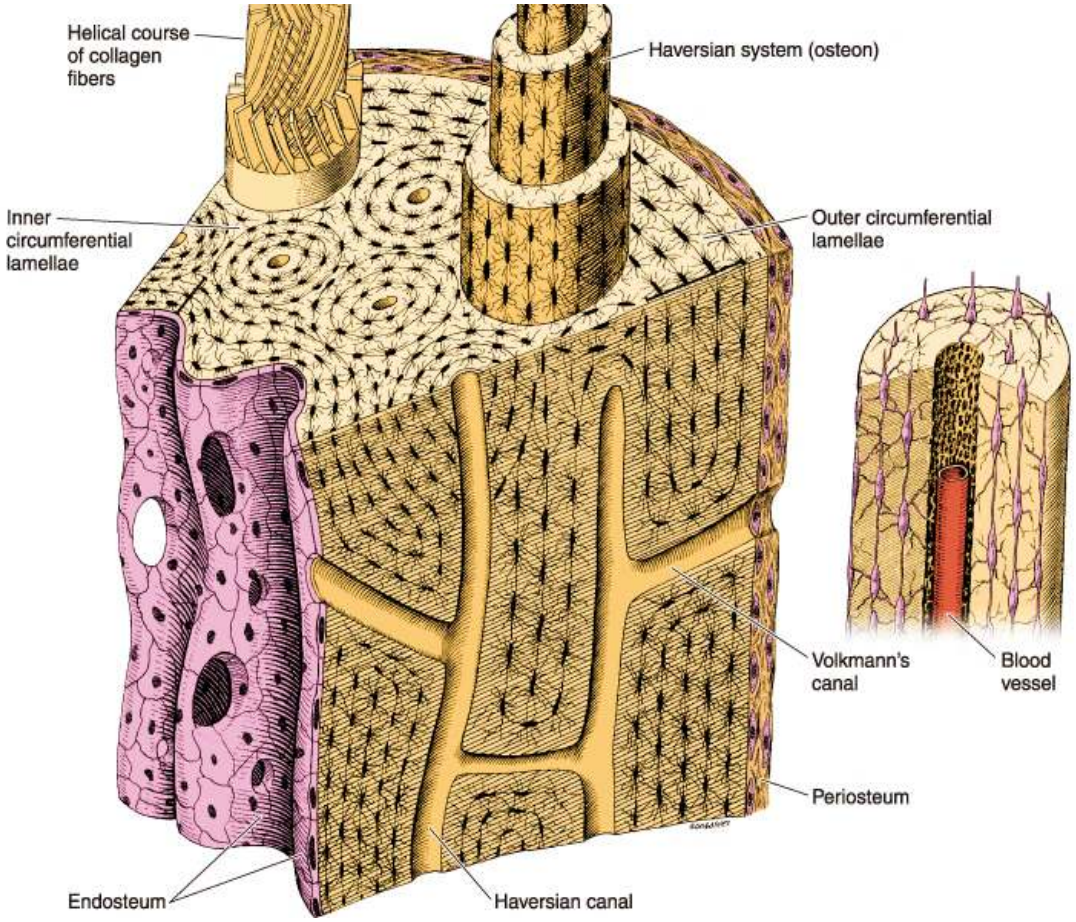

- **External and internal surfaces** of bone are covered by **layers of bone-forming cells and connective tissue called periosteum and endosteum.**
- The **periosteum consists of an outer layer of collagen fibers and fibroblasts.** Bundles of periosteal collagen fibers, called **Sharpey's fibers,** penetrate 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.**
- 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 Cells

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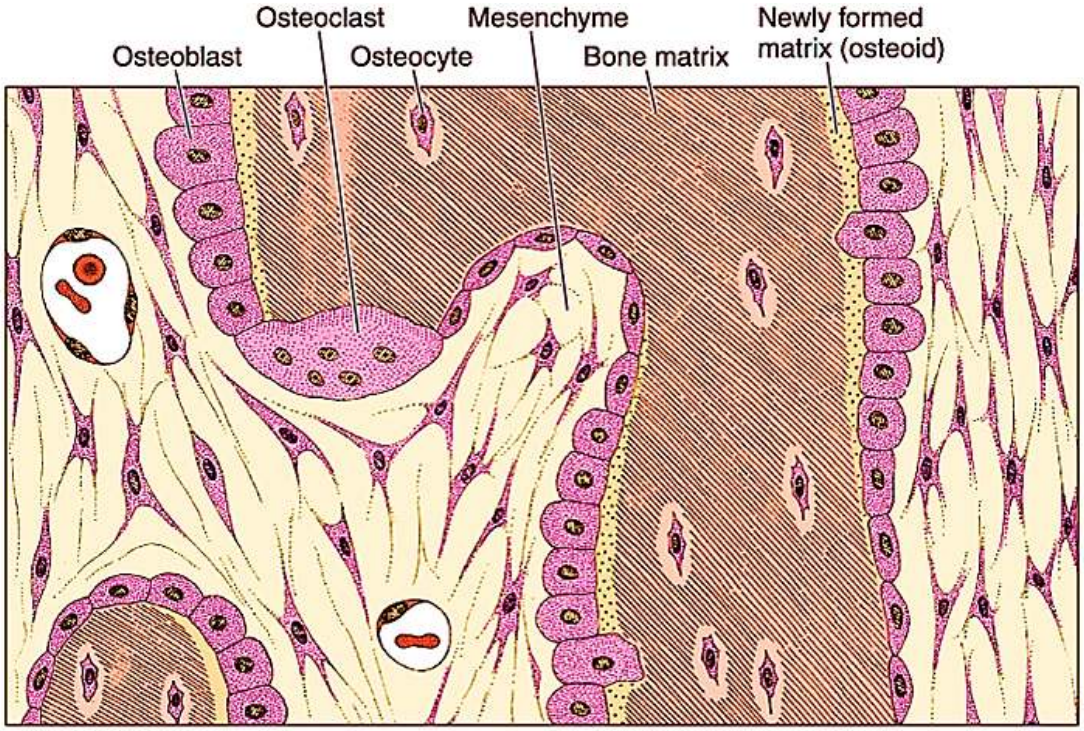

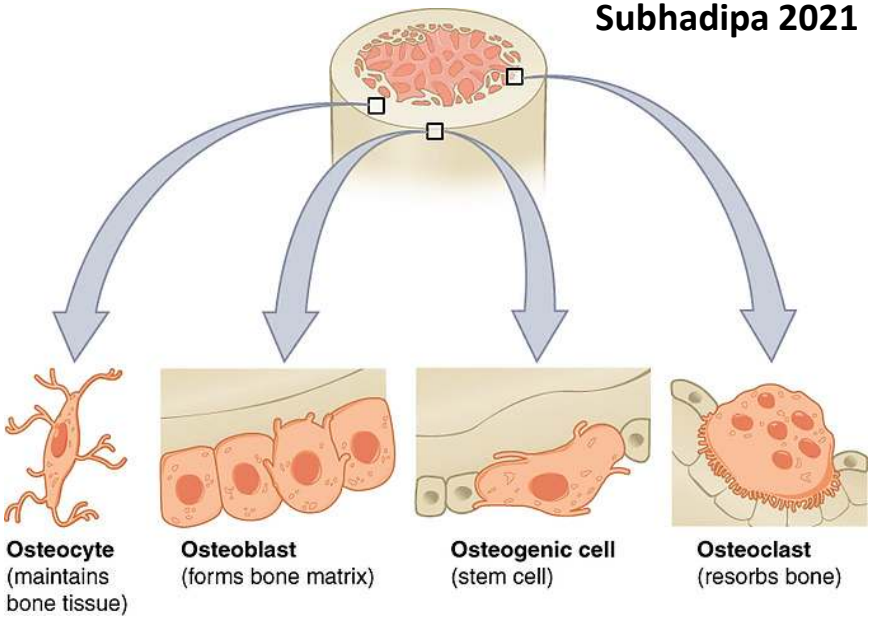

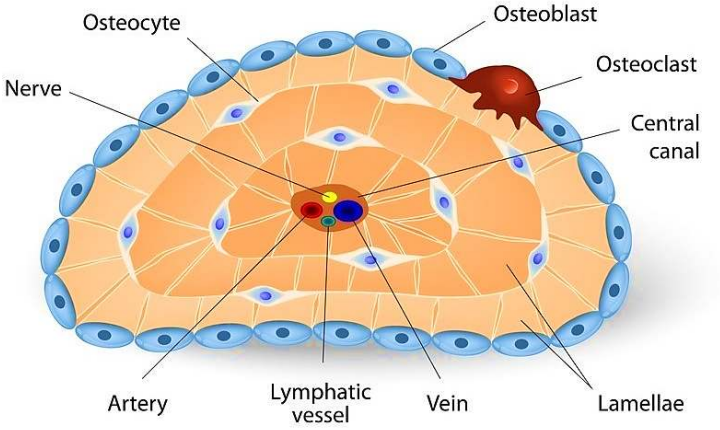

Osteoblasts

- Osteoblasts are responsible for the **synthesis of the organic components of bone matrix (type I collagen, proteoglycans, and glycoproteins).**
- 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.**
- 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 noncalcified matrix.**
- **During matrix synthesis**, **osteoblast**s 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.

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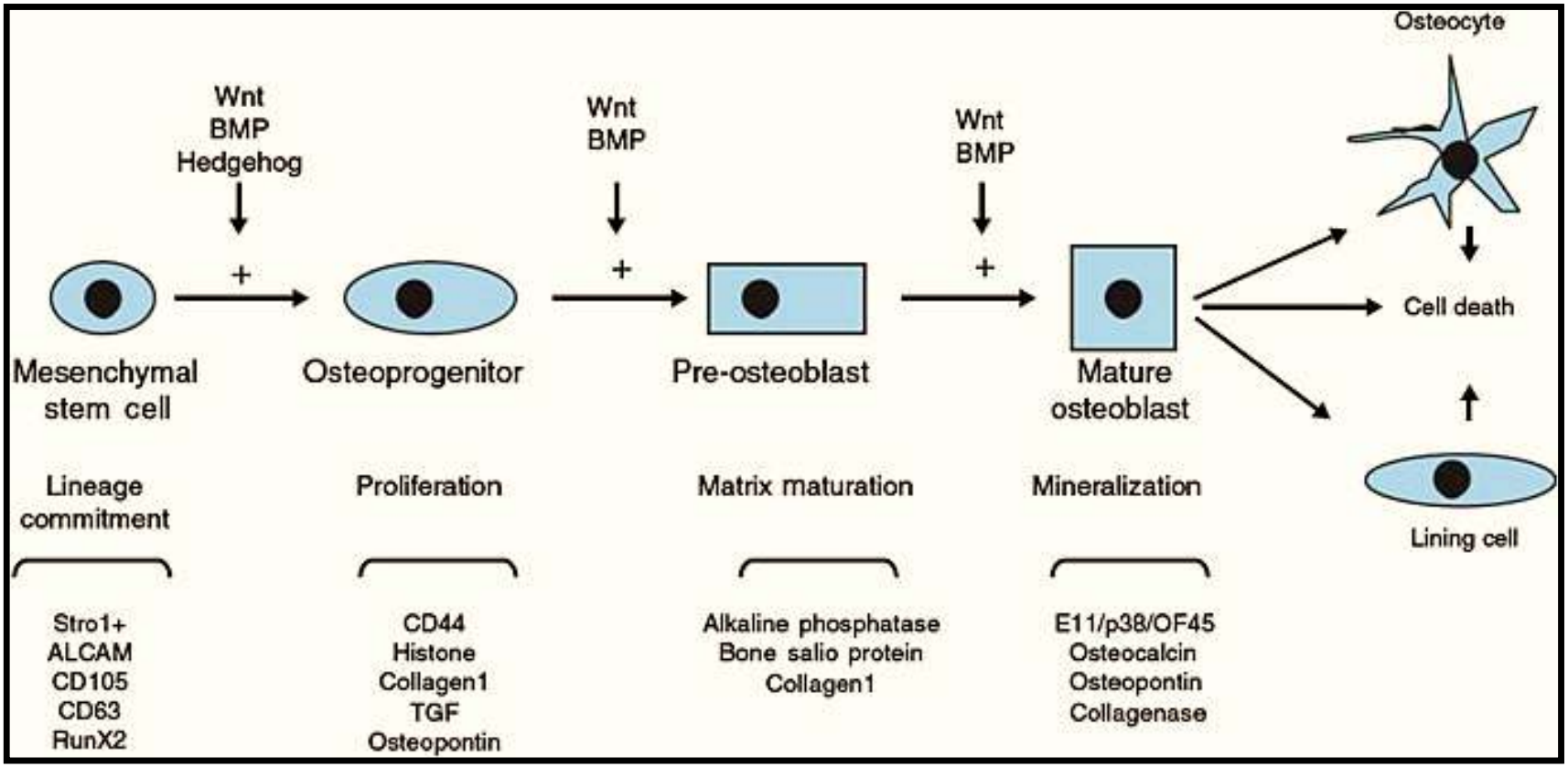
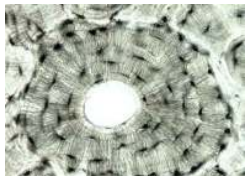
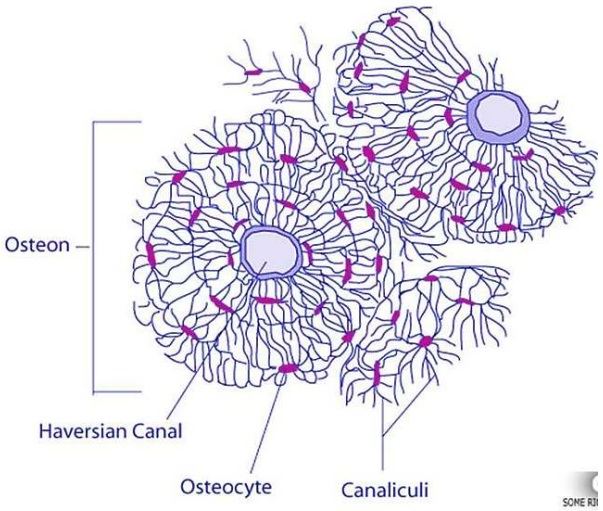
Formation of Osteoblast (Osteoblastogenesis)

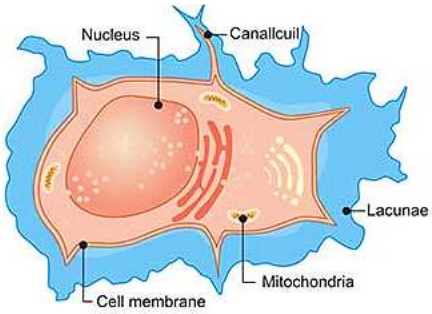


Osteocytes

- **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.







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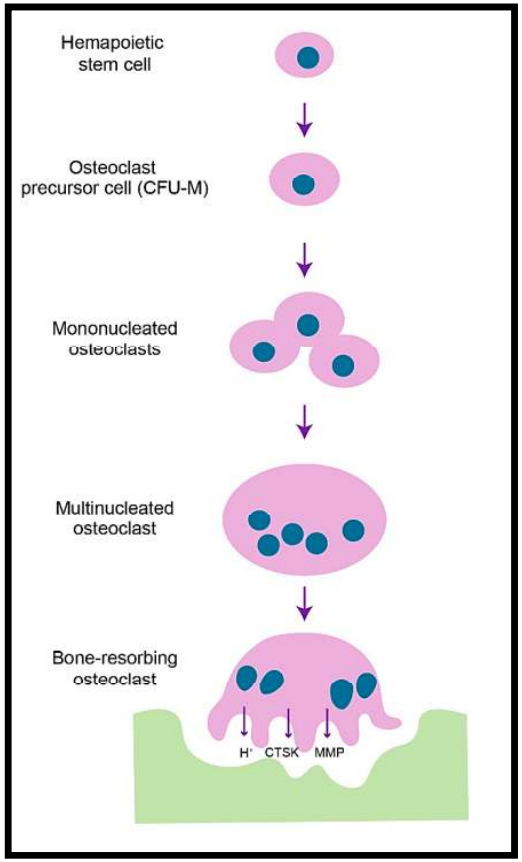
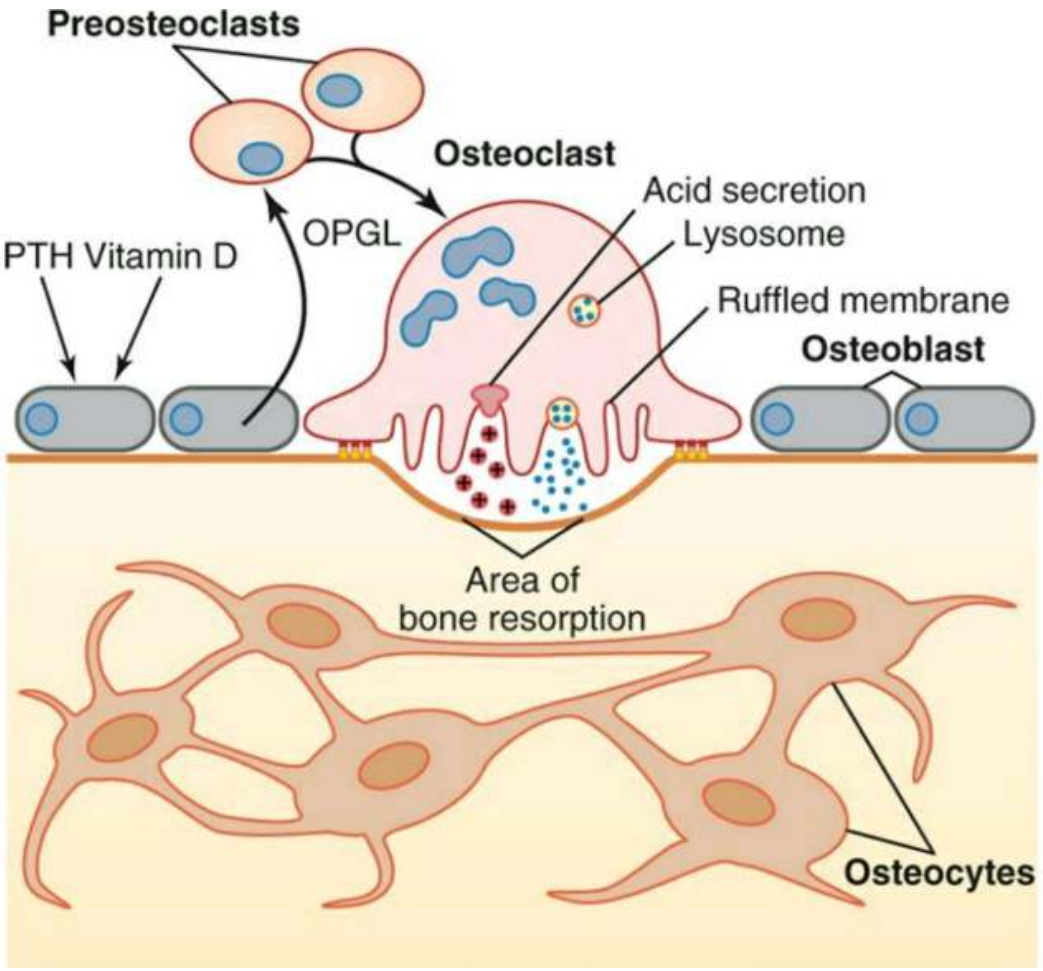
Osteoclasts

- Osteoclasts are very **large, branched motile 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**.
- 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 a subcellular pocket (the microenvironment referred to above), 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.**



Bone Resorption



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.
- Significant quantities of **amorphous (noncrystalline) calcium phosphate are also present**.
- In electron micrographs, **hydroxyapatite crystals of bone appear as plates that lie alongside the collagen fibrils but are surrounded by ground substance.**
- The **surface ions of hydroxyapatite are hydrated**, and a layer of water and ions forms around the crystal.
- This layer, the hydration shell, facilitates the exchange of ions between the crystal and the body fluids.

Types of Bones

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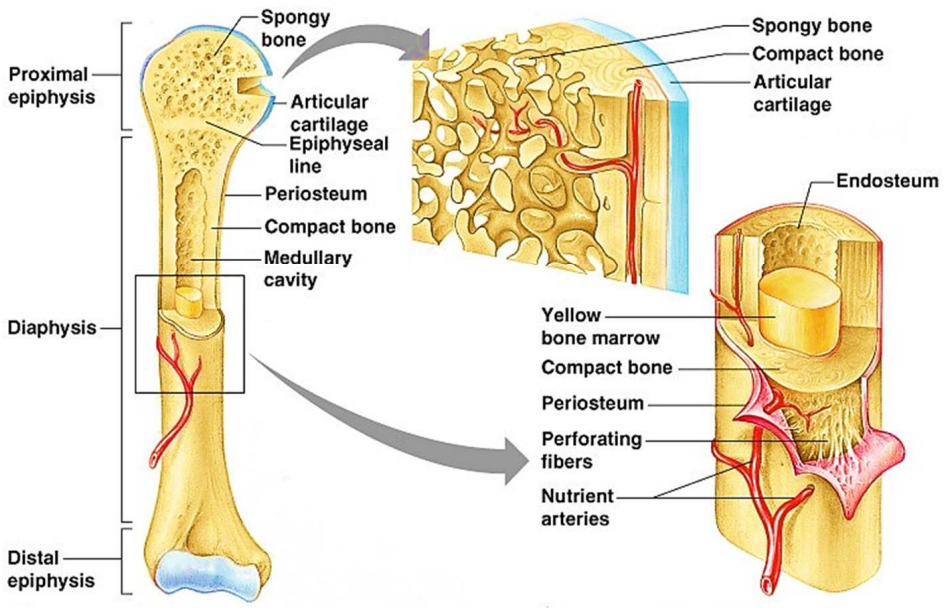
- Gross observation of bone in cross section shows **dense areas without cavities corresponding to compact bone** and areas with **numerous interconnecting cavities corresponding to cancellous (spongy) bone.**
- Under the microscope, however, both compact bone and the trabeculae separating the cavities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

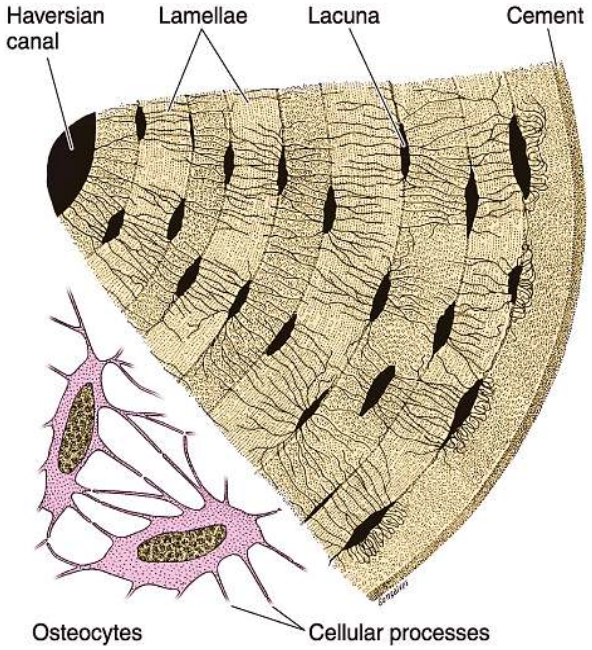
Primary Bone Tissue

- Primary bone tissue is usually **temporary** and**, except in a very few places in the body (eg, 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.**
- 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.**

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



Bone ossification

- 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

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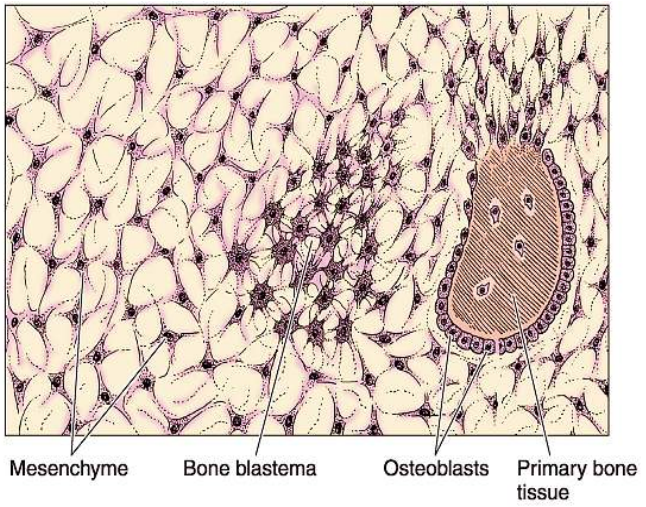
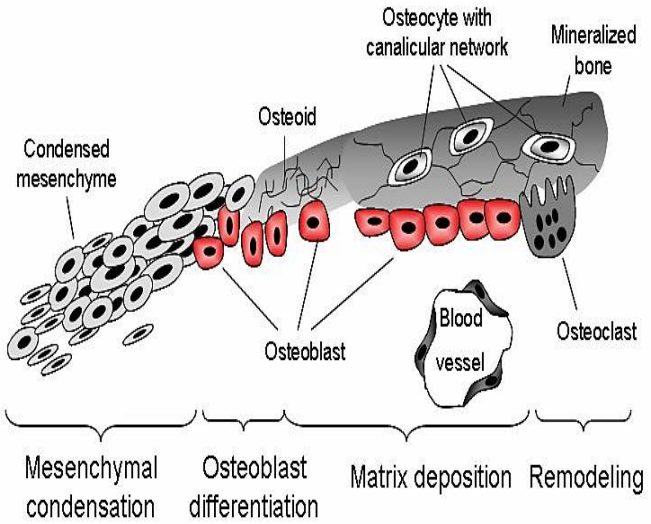
- Intramembranous ossification, the source of most of the flat bones, is so called because it **takes place within condensations of mesenchymal tissue.** **The frontal and parietal bones of the skull** as well **as parts of the occipital and temporal bones and the mandible and maxilla** are formed by 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.
- The **ossification centers of a bone grow radially and finally fuse together**, replacing the original connective tissue. The fontanelles of newborn infants, for example, are soft areas in the skull that correspond to parts of the connective tissue that are not yet ossified.
- In cranial flat bones there is a marked predominance of bone formation over bone resorption at both the internal and external surfaces. Thus**, two layers of compact bone (internal and external plates) arise, whereas the central portion (diploÃ«) maintains its spongy nature.**
- The portion of the connective tissue layer that **does not undergo ossification gives rise to the endosteum and the periosteum of intramembranous bone.**



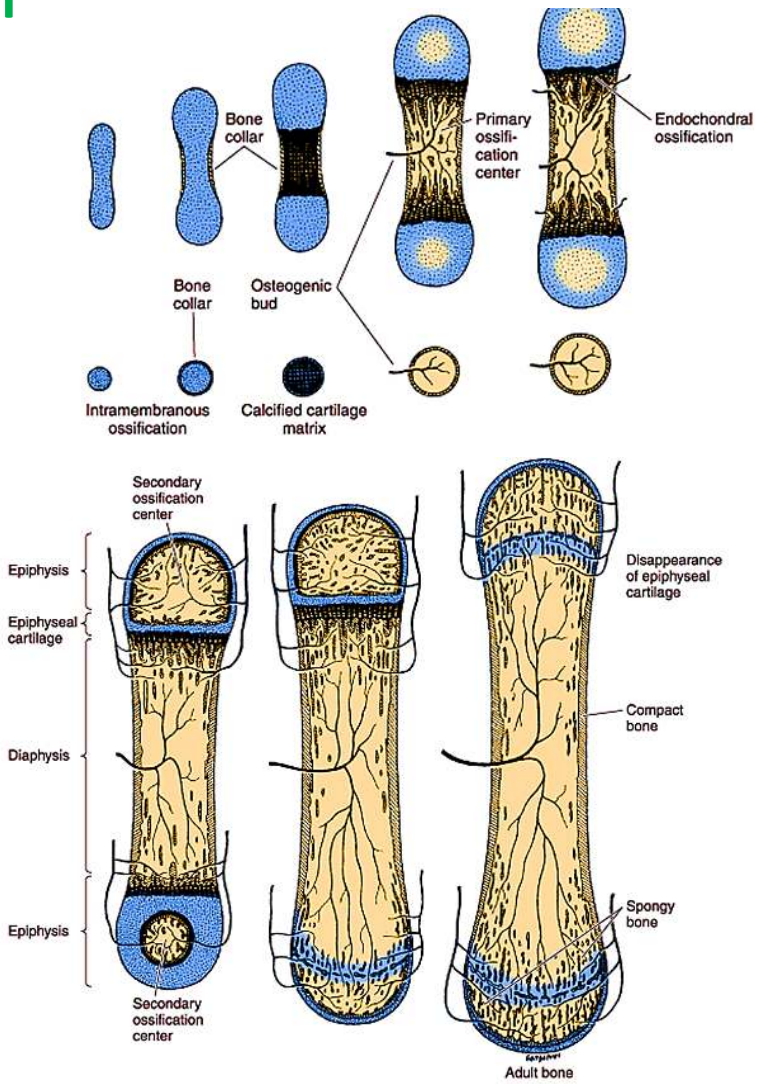


Endochondral Ossification

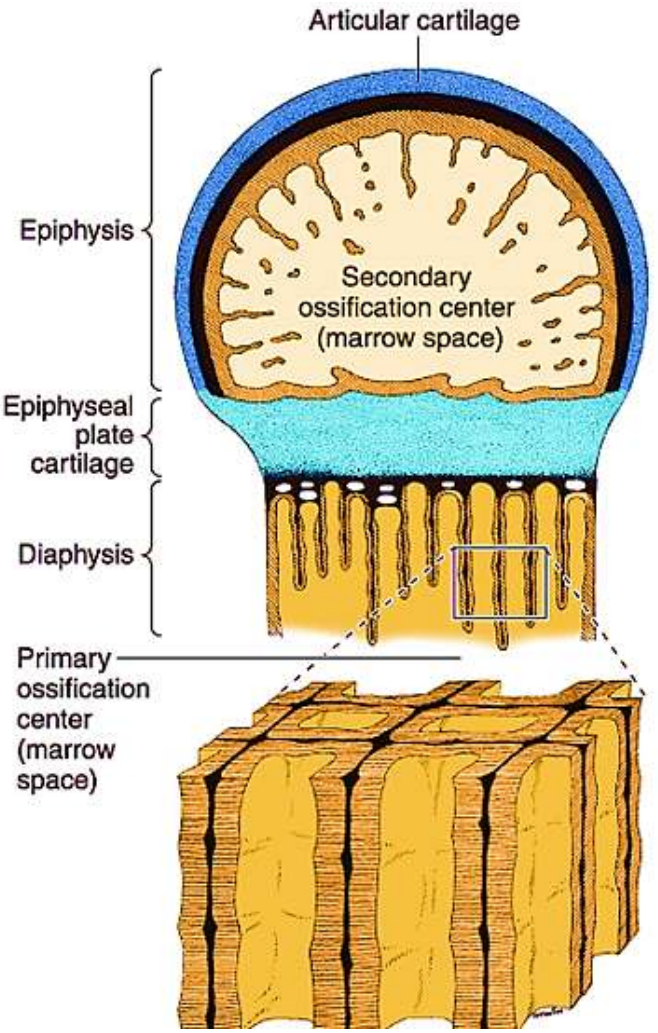
Endochondral (Gr. *endon,* within, + *chondros,* cartilage) ossification **takes place within a piece of hyaline cartilage whose shape resembles a small version, or model, of the bone to be formed**. This type of ossification) is **principally responsible for the formation of short and long bones.**

Endochondral ossification of a long bone consists of the following sequence of events:

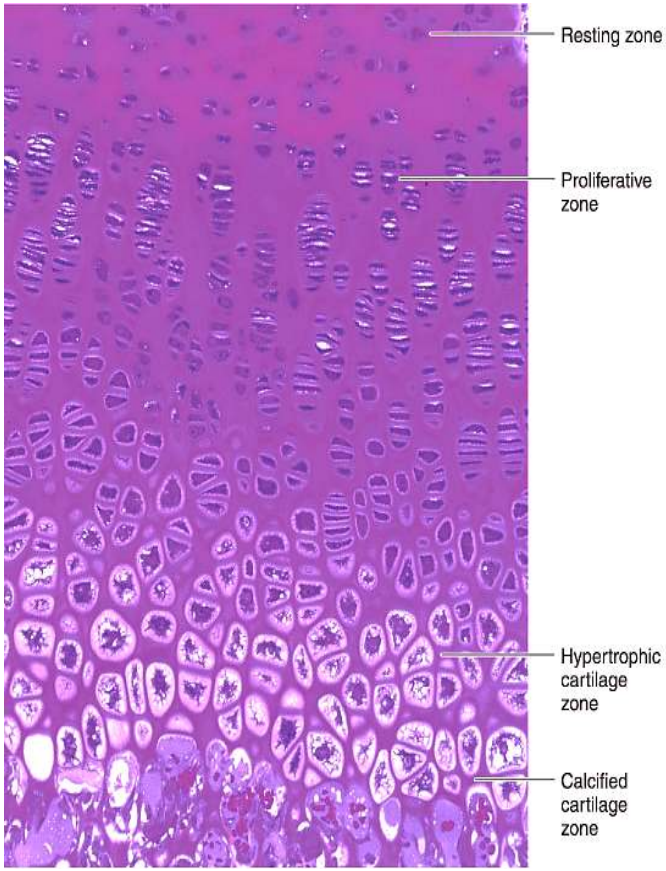
- Initially, the **first bone tissue appears as a hollow bone cylinder** that surrounds the mid portion of the cartilage model. This structure, the **bone collar,** is produced by **intramembranous ossification within the local perichondrium.**
- In the next step, the local cartilage **undergoes a degenerative 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.
- The **closure of the epiphyses follows a chronological order according to each bone and is complete at about 20 years of age**. Through x-ray examination of the growing skeleton, it is possible to determine the "bone age" of a young person, noting which epiphyses are open and which are closed. Once the epiphyses have closed, growth in length of bones becomes impossible, although widening may still occur.



- 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.



Mechanisms of Calcification

- There is still no generally accepted hypothesis to explain the events occurring during calcium phosphate deposition on bone matrix.
- It is known that c**alcification begins by the deposition of calcium salts on collagen fibrils, a process induced by proteoglycans and high-affinity calcium-binding glycoproteins**. The deposition of calcium salts is probably **accelerated by the ability of osteoblasts to concentrate them in intracytoplasmic vesicles and to release these vesicles, when necessary, to the extracellular medium (matrix vesicles).**
- Calcification is aided, in some unknown way, by alkaline phosphatase, which is produced by osteoblasts and is present at ossification sites.