Bone & Ossification
Lec . 7

Second year

Histology

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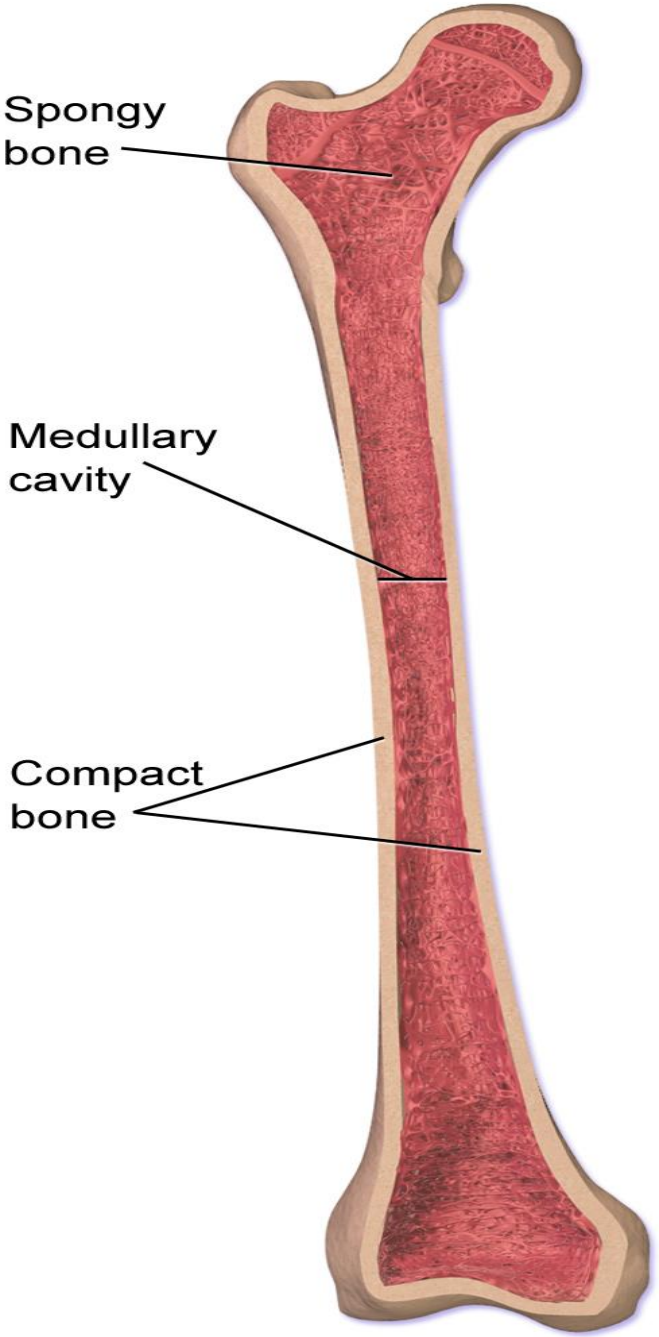
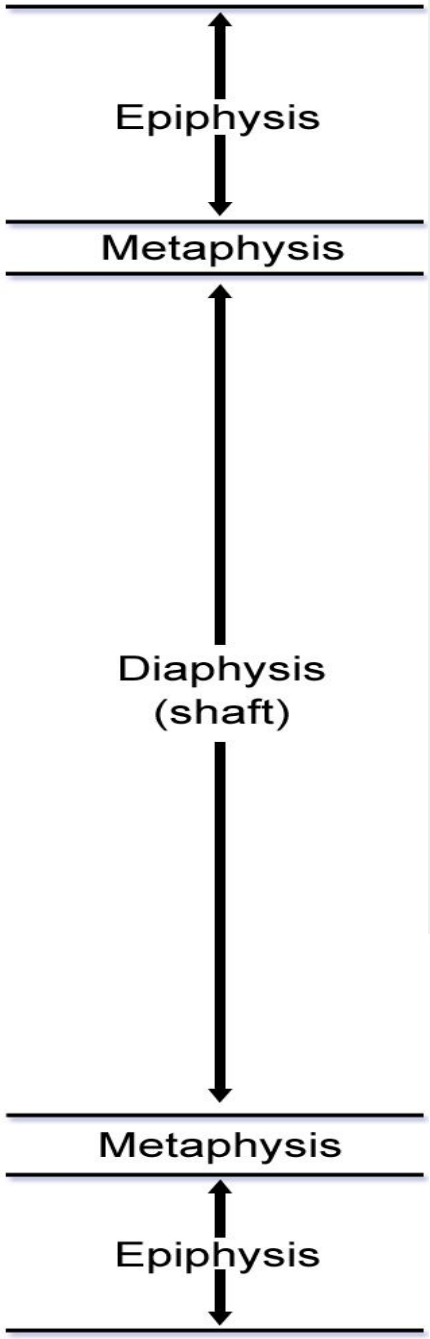
Bone

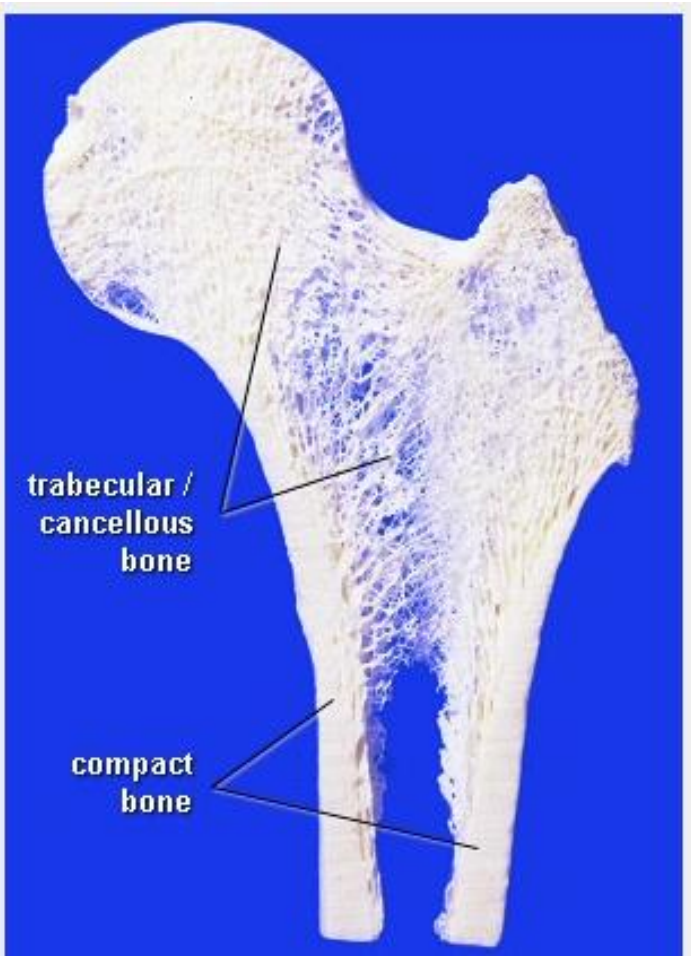
Trabecular bone (also called cancellous or spongy bone) consists of delicate bars and sheets of bone, trabeculae, which branch and intersect to form a sponge like network. The ends of long bones (or epiphyses) consist mainly of trabecular bone.

Compact bone does not have any spaces or hollows in the bone matrix that are visible to the eye. Compact bone forms the thick-walled tube of the shaft (or diaphysis) of long bones, which surrounds the marrow cavity (or medullary cavity). A thin layer of compact bone also covers the epiphyses of long bones.

Structure of a Long Bone

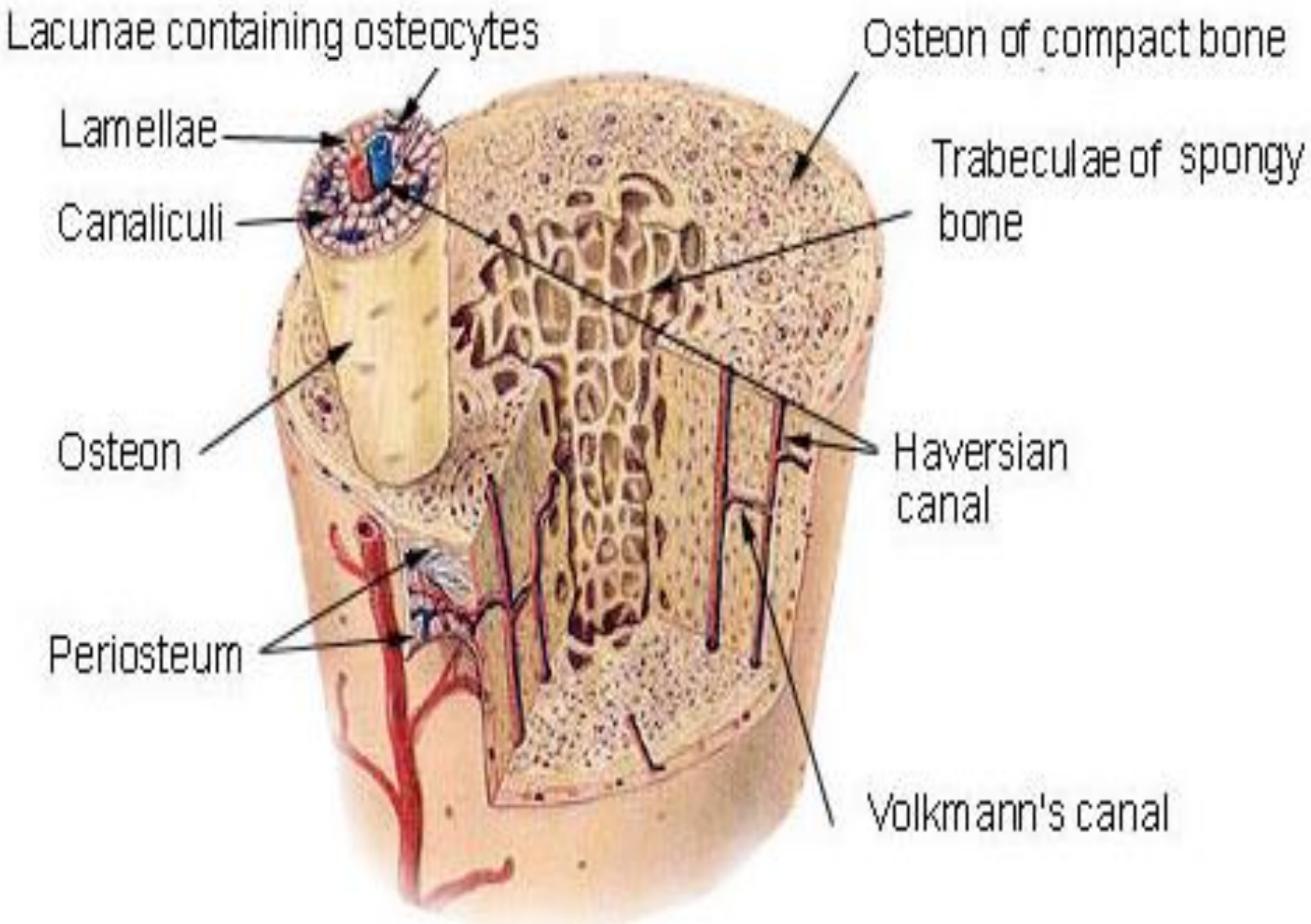






Compact bone consists almost entirely of extracellular substance, the matrix. Osteoblasts deposit the matrix in the form of thin sheets which are called lamellae. Lamellae are microscopical structures. Collagen fibres within each lamella run parallel to each other. Collagen fibres which belong to adjacent lamellae run at oblique angles to each other. Fibre density seems lower at the border between adjacent lamellae, which gives rise to the lamellar appearance of the tissue. Bone which is composed by lamellae when viewed under the microscope is also called lamellar bone.

Compact Bone & Spongy (Cancellous Bone)



Osteoblasts are encased in small hollows within the matrix, the lacunae.

Unlike chondrocytes, osteocytes have several thin processes, which extend from the lacunae into small channels within the bone matrix , the canaliculi. Canaliculi arising from one lacuna may anastomose with those of other lacunae. Canaliculi provide the means for the osteocytes to communicate with each other and to exchange substances by diffusion.

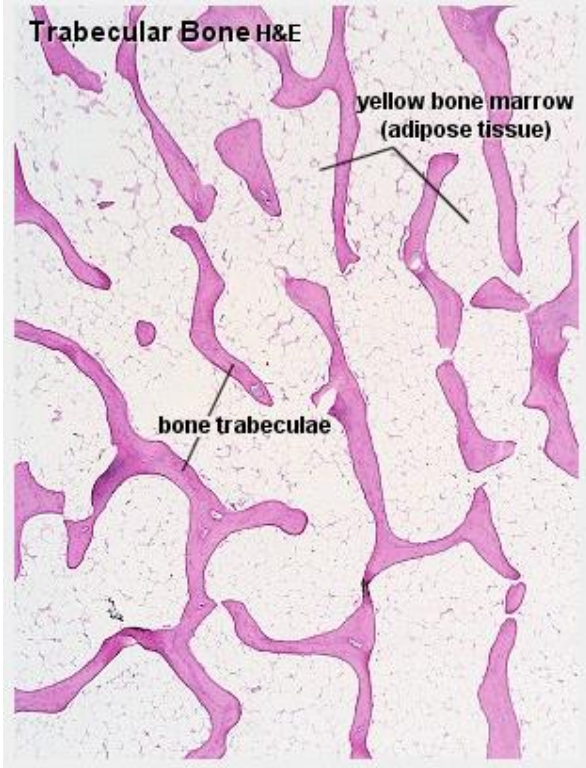
In mature compact bone most of the individual lamellae form concentric rings around larger longitudinal canals (approx. 50 µm in diameter) within the bone tissue. These canals are called **Haversian canals**. Haversian canals typically run parallel to the surface and along the long axis of the bone. The canals and the surrounding lamellae (8-15) are called a Haversian system or an osteon. A Haversian canal generally contains one or two capillaries and nerve fibres.

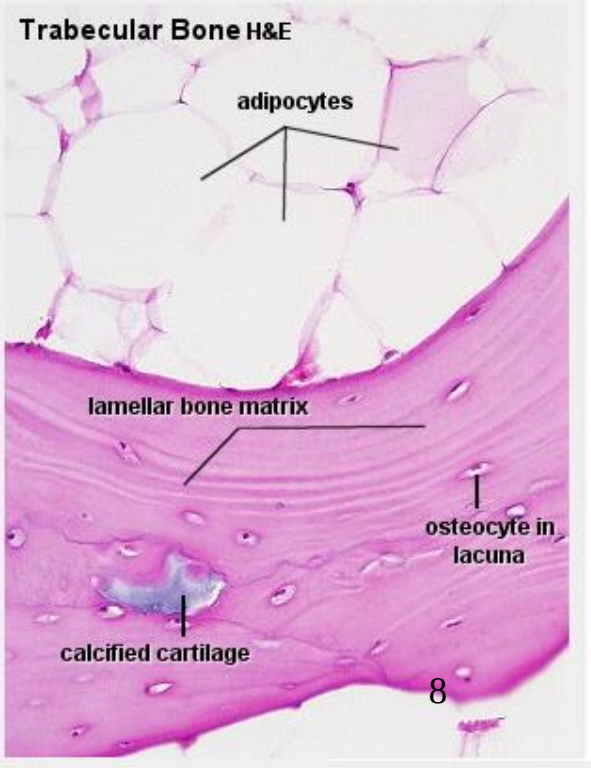
- Irregular areas of **interstitial lamellae**, which apparently do not belong to any Haversian system, are found in between the Haversian systems. Immediately beneath the periosteum and endosteum a few lamella are found which run parallel to the inner and outer surfaces of the bone. They are the circumferential lamellae and endosteal lamellae.
- A second system of canals, called **Volkmann's canals**, penetrates the bone more or less perpendicular to its surface. These canals establish connections of the Haversian canals with the inner and outer surfaces of the bone. Vessels in Volkmann's canals communicate with vessels in the Haversian canals on the one hand and vessels in the endosteum on the other. A few communications also exist with vessels in the periosteum.

Trabecular bone

The matrix of trabecular bone is also deposited in the form of lamellae. In mature bones, trabecular bone will also be lamellar bone. However, lamellae in trabecular bone do not form Haversian systems. Lamellae of trabecular bone are deposited on preexisting trabeculae depending on the local demands on bone rigidity.

Osteocytes, lacunae and canaliculi in trabecular bone resemble those in compact bone.





Bone Matrix

- Bone matrix consists of collagen fibres (about 90% of the organic substance) and ground substance.
- Collagen type I is the dominant collagen form in bone. The hardness of the matrix is due to its content of inorganic salts (hydroxyapatite; about 75% of the dry weight of bone), which become deposited between collagen fibres.
- Calcification begins a few days after the deposition of organic bone substance (or osteoid) by the osteoblasts. Osteoblasts are capable of producing high local concentration of calcium phosphate in the extracellular space, which precipitates on the collagen molecules. About 75% of the hydroxyapatite is deposited in the first few days of the process, but complete calcification may take several months.

Bone Cells

1. **Osteoprogenitor cells (or stem cells of bone)**
   - are located in the periosteum and endosteum. They are very difficult to distinguish from the surrounding connective tissue cells. They differentiate into
2. **Osteoblasts (or bone forming cells).**
   - Osteoblasts may form a low columnar "epitheloid layer" at sites of bone deposition. They contain plenty of rough endoplasmatic reticulum (collagen synthesis) and a large Golgi apparatus. As they become trapped in the forming bone they differentiate into
3. **Osteocytes.**
   - Osteocytes contain less endoplasmatic reticulum and are somewhat smaller than osteoblasts.

4. Osteoclasts

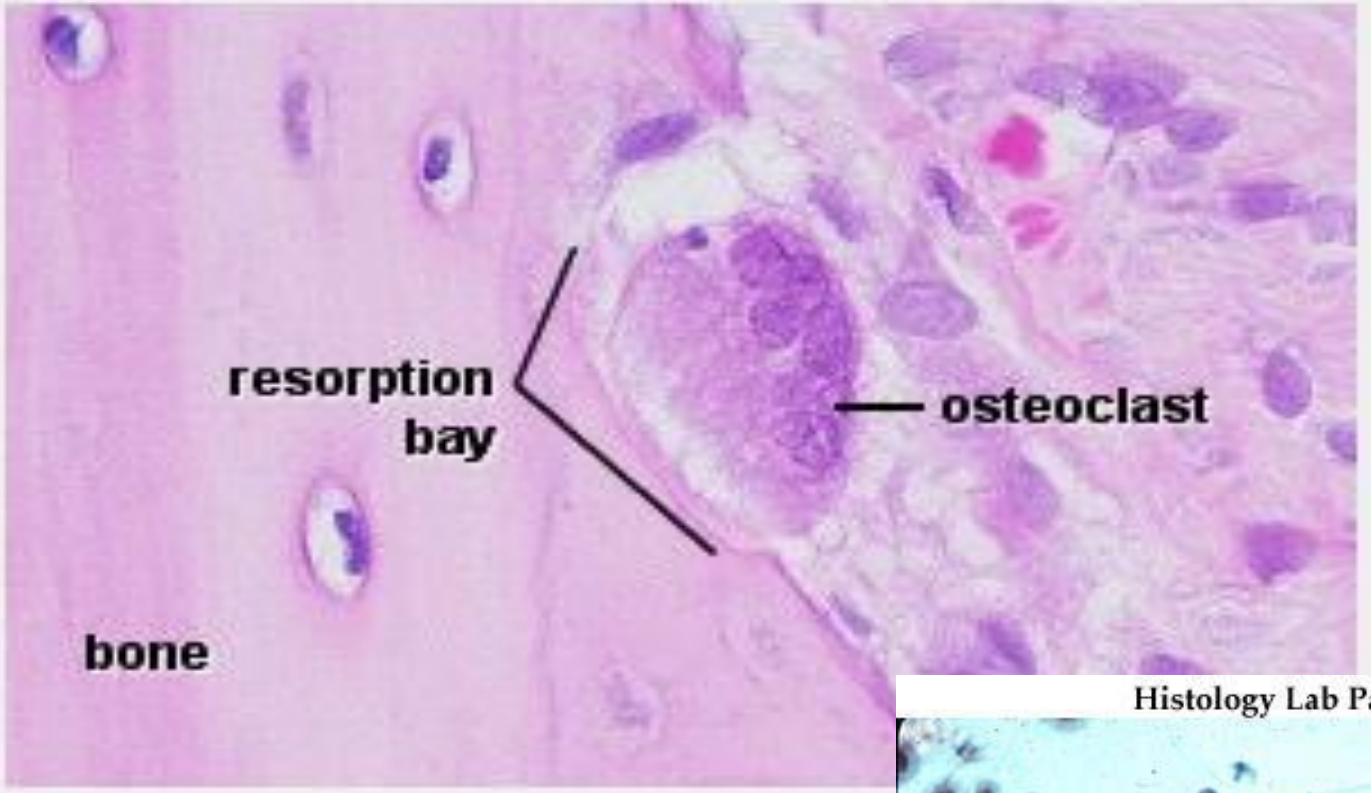
are very large (up to 100 μm), multi-nucleated (about 5-10 visible in a histological section, but up to 50 in the actual cell) bone-resorbing cells.

They arise by the fusion of monocytes (macrophage precursors in the blood) or macrophages.

Osteoclasts attach themselves to the bone matrix and form a tight seal at the rim of the attachment site. The cell membrane opposite the matrix has deep invaginations forming a ruffled border.

Osteoclasts empty the contents of lysosomes into the extracellular space between the ruffled border and the bone matrix. The released enzymes break down the collagen fibres of the matrix.

Osteoclasts are stimulated by parathyroid hormone (produced by the parathyroid gland) and inhibited by calcitonin (produced by specialised cells of the thyroid gland). Osteoclasts are often seen within the indentations of the bone matrix that are formed by their activity (resorption bays or Howship's lacunae).



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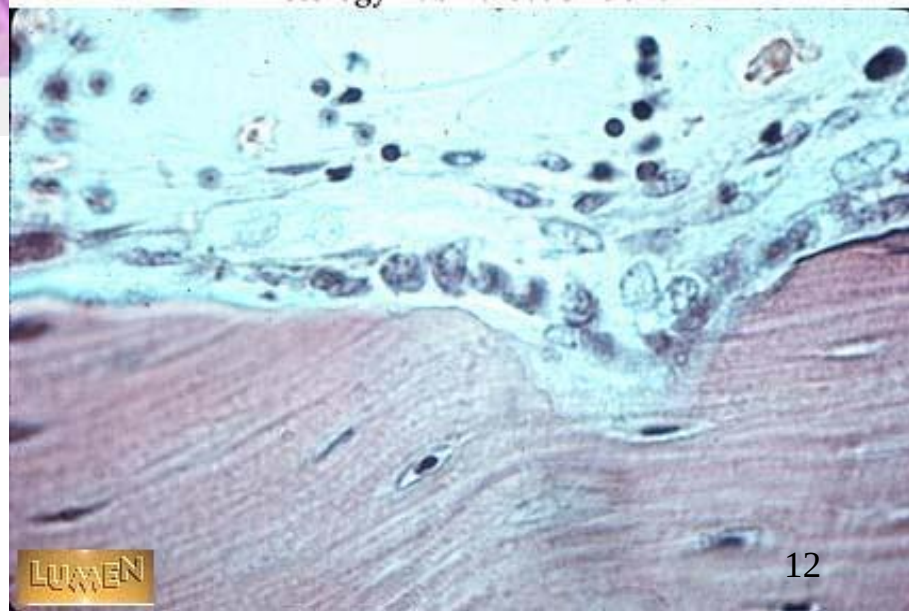
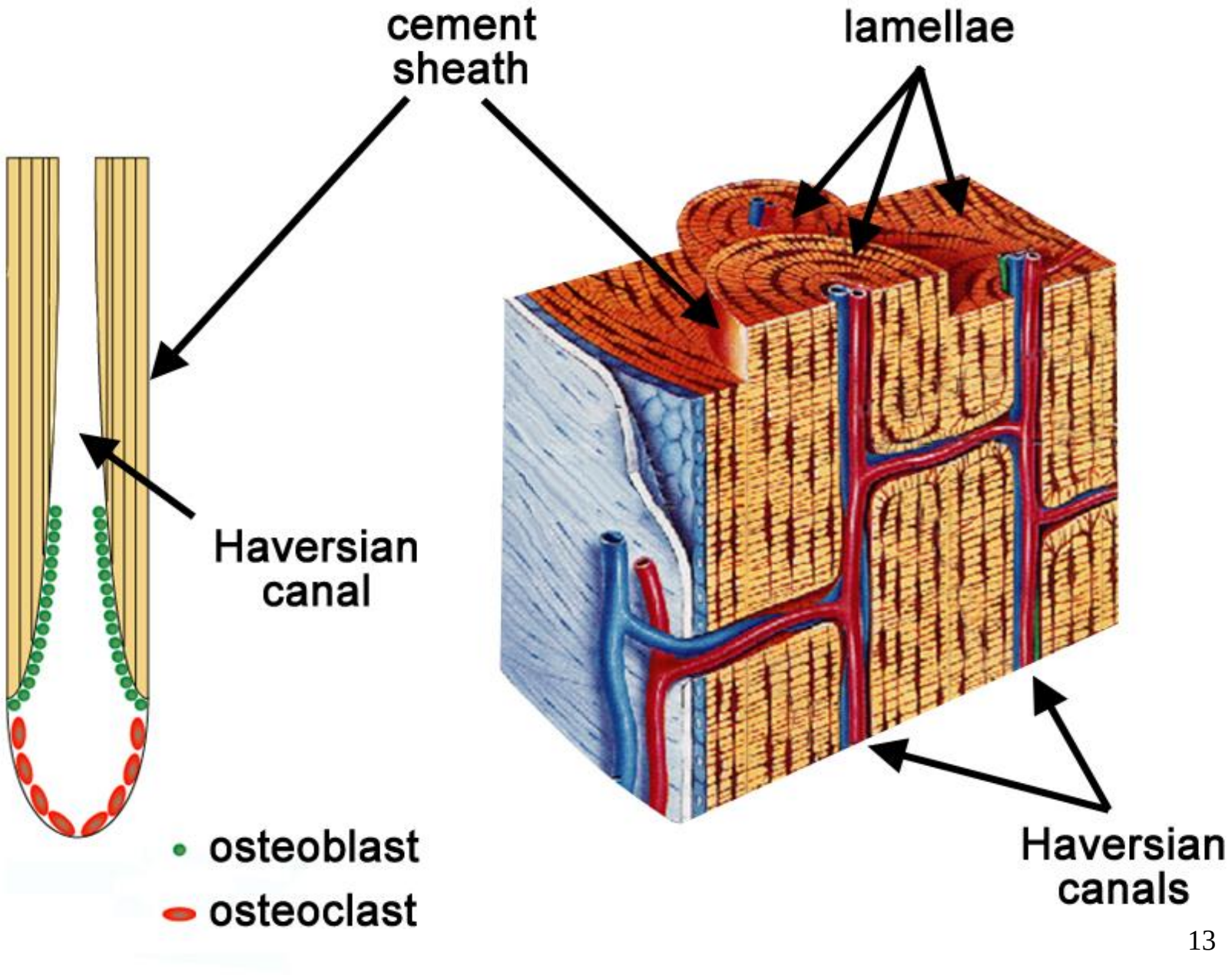
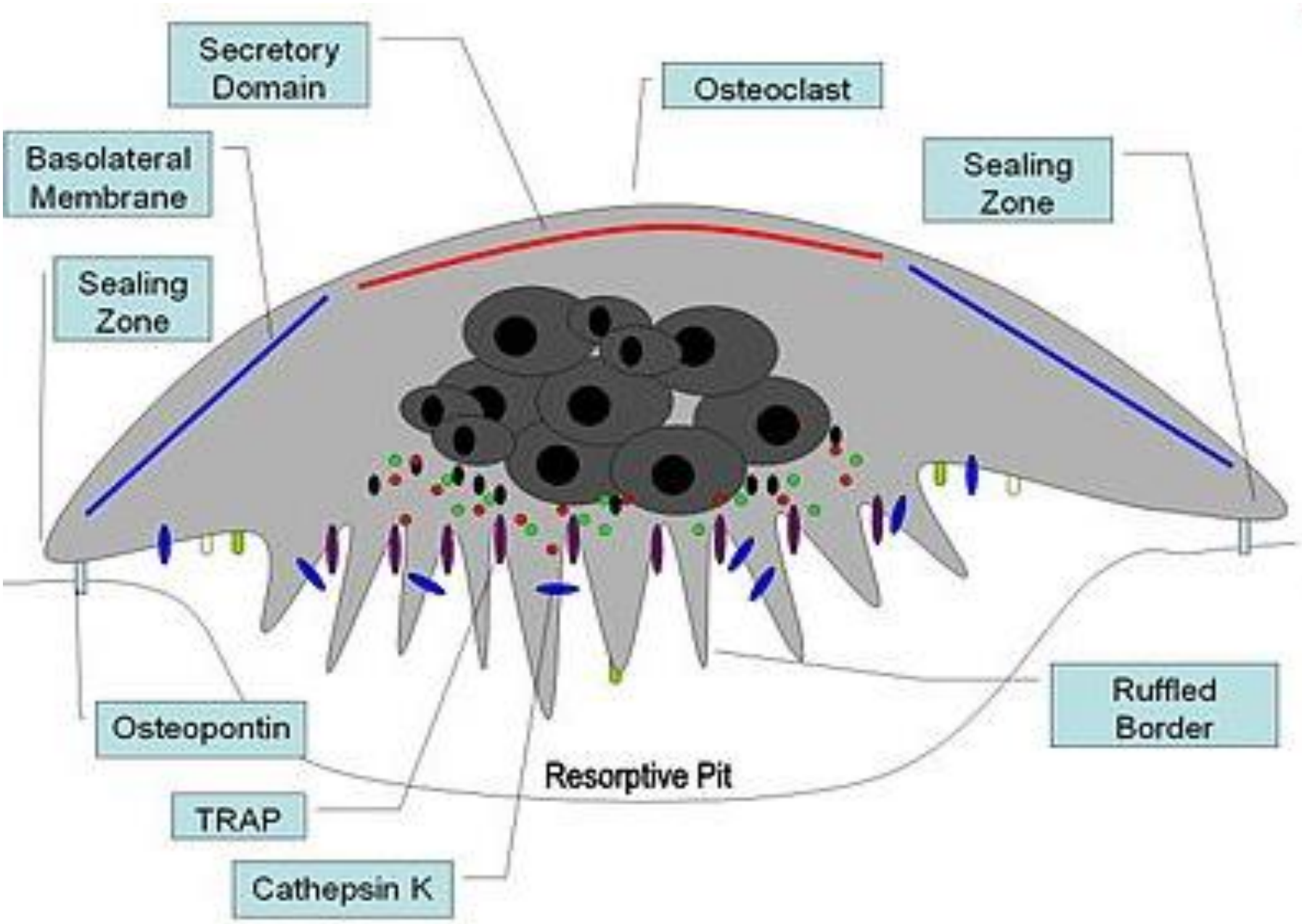
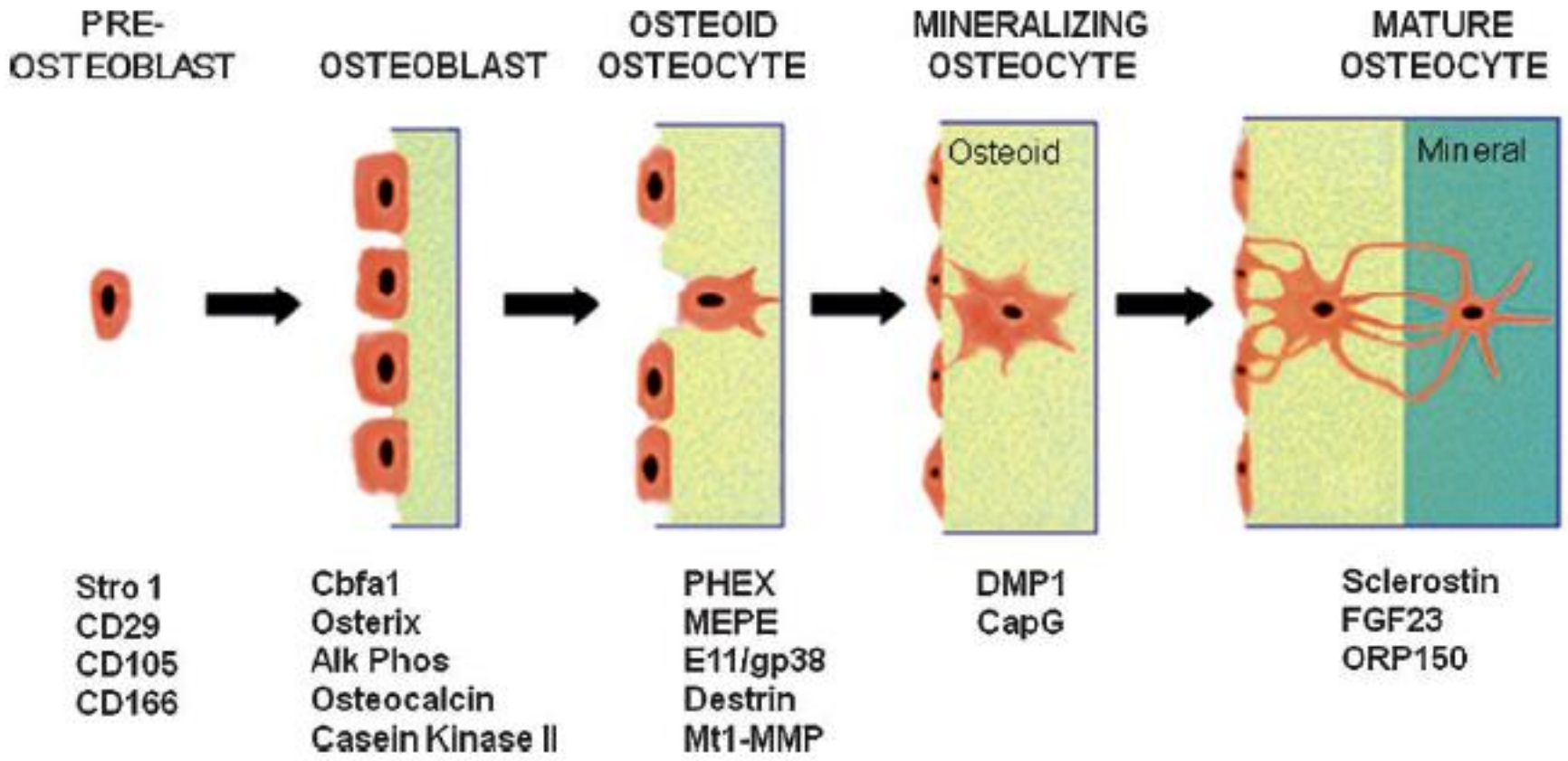



cement sheath
lamellae
Haversian canal
osteoblast
osteoclast
Haversian canals


Secretory Domain
Osteoclast
Basolateral Membrane
Sealing Zone
Sealing Zone
Osteopontin
Ruffled Border
Resorptive Pit
TRAP
Cathepsin K

- Modeling is a process in which bone is sculpted during growth to ultimately achieve its proper shape. Modeling is responsible for the circumferential growth of the bone and expansion of the marrow cavity, modification of the metaphyseal funnel of long bones, and enlargement of the cranial vault curvature.
- Remodeling is a continuous process throughout life, in which damaged bone is repaired, ion homeostasis is maintained, and bone is reinforced for increased stress. In adults, the remodeling rate varies in different types of bones.
- Trabecular bone is remodeled at a higher rate (25% per year) than that of cortical bone (3% per year) in a healthy adult.
- Resorption and deposition are normally balanced, and bone density is maintained.
- A lytic lesion results when resorptive activity exceeds deposition activity in a pathologic state.

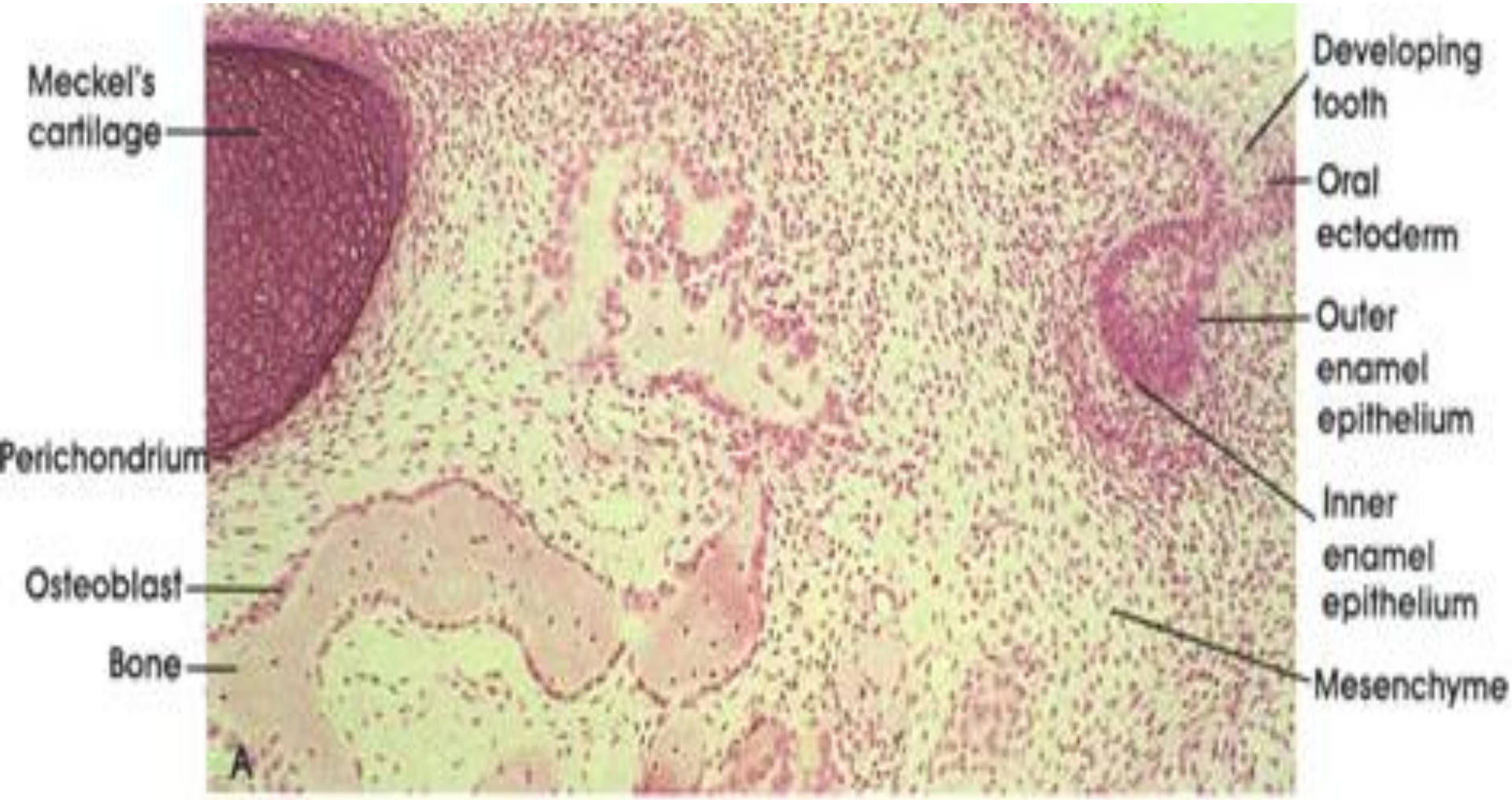

PRE-
OSTEOBLAST
OSTEOBLAST
OSTEOID
OSTEOCYTE
MINERALIZING
OSTEOCYTE
MATURE
OSTEOCYTE
Osteoid
Mineral
Stro 1
CD29
CD105
CD166
Cbfa1
Osterix
Alk Phos
Osteocalcin
Casein Kinase II
PHEX
MEPE
E11/gp38
Destrin
Mt1-MMP
DMP1
CapG
Sclerostin
FGF23
ORP150

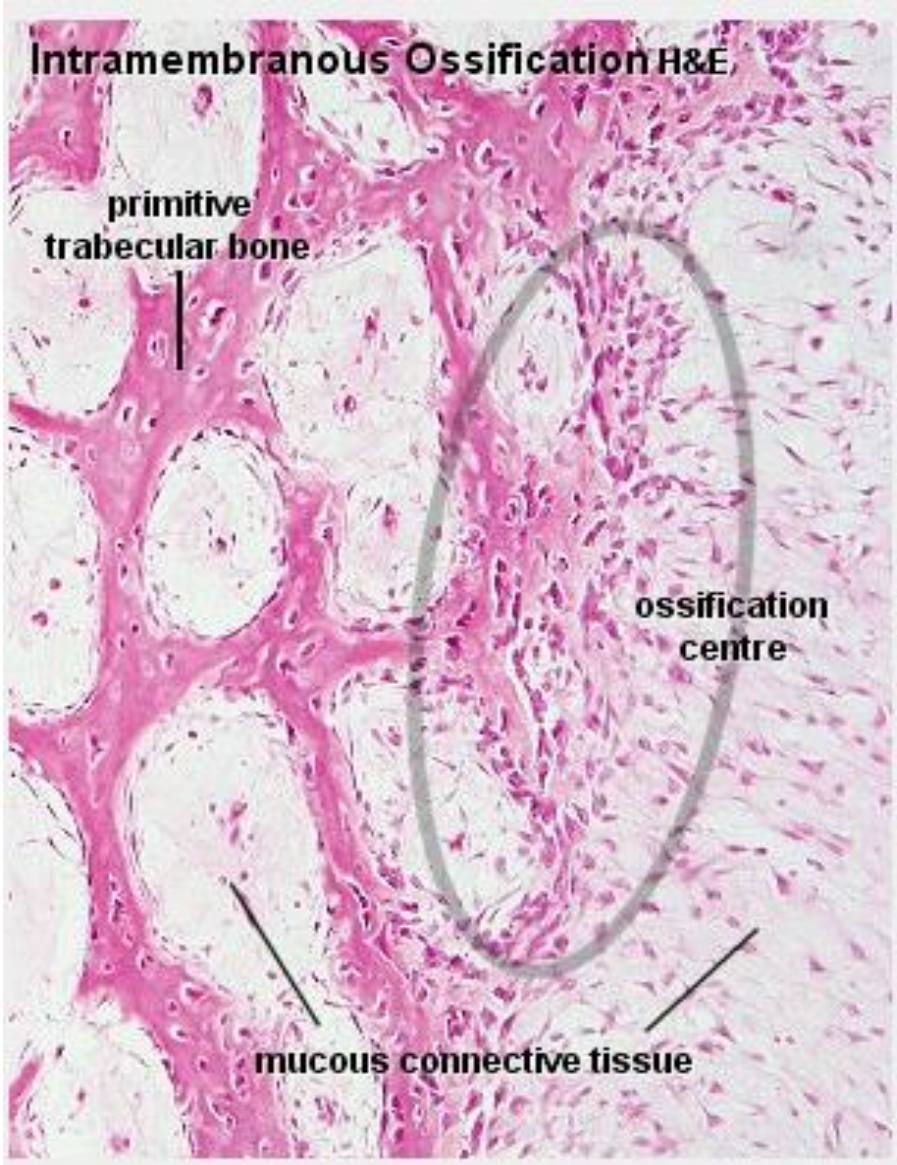
Timetable for human ossification

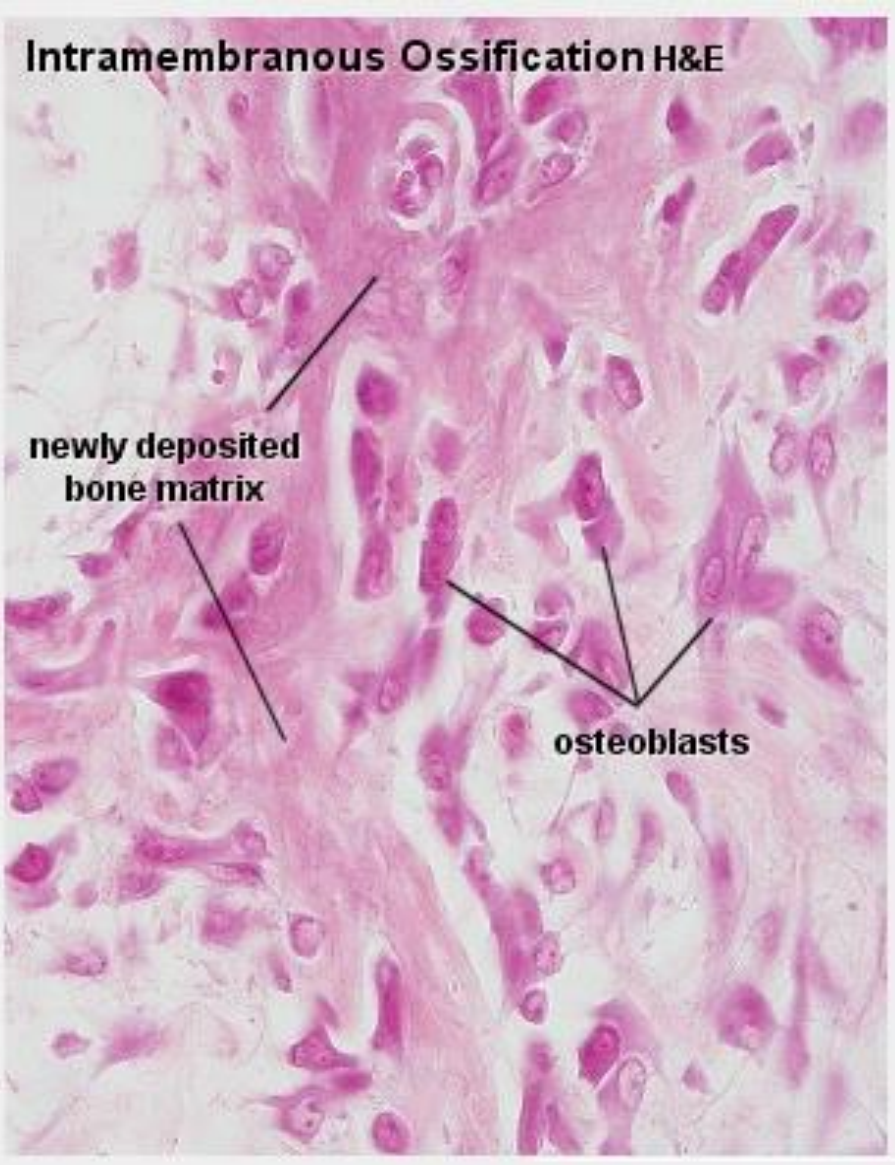
Time period[2]	Bones affected[2]
Third month of embryonic development	Ossification in long bones beginning
Fourth month	Most primary ossification centers have appeared in the diaphyses of bone.
Birth to 5 years	Secondary ossification centers appear in the epiphyses
5 years to 12 years in females, 5 to 14 years in males	Ossification is spreading rapidly from the ossification centers and various bones are becoming ossified
17 to 20 years	Bone of upper limbs and scapulae becoming completely ossified
18 to 23 years	Bone of the lower limbs and os coxae become completely ossified
23 to 25 years	Bone of the sternum, clavicles, and vertebrae become completely ossified
By 25 years	Nearly all bones are completely ossified

Intramembranous Ossification

- Intramembranous ossification occurs within a membranous, condensed plate of mesenchymal cells.
- At the initial site of ossification (ossification centre) mesenchymal cells (osteoprogenitor cells) differentiate into osteoblasts.
- The osteoblasts begin to deposit the organic bone matrix, the osteoid. The matrix separates osteoblasts, which, from now on, are located in lacunae within the matrix.
- The collagen fibres of the osteoid form a woven network without a preferred orientation, and lamellae are not present at this stage.
- Because of the lack of a preferred orientation of the collagen fibres in the matrix, this type of bone is also called woven bone. The osteoid calcifies leading to the formation of primitive trabecular bone.
- Further deposition and calcification of osteoid at sites where compact bone is needed leads to the formation of primitive compact bone.


Meckel's cartilage
Perichondrium
Osteoblast
Bone
A
Developing tooth
Oral ectoderm
Outer enamel epithelium
Inner enamel epithelium
Mesenchyme


Intramembranous Ossification H&E
primitive trabecular bone
ossification centre
mucous connective tissue


Intramembranous Ossification H&E
newly deposited bone matrix
osteoblasts

Endochondral Ossification

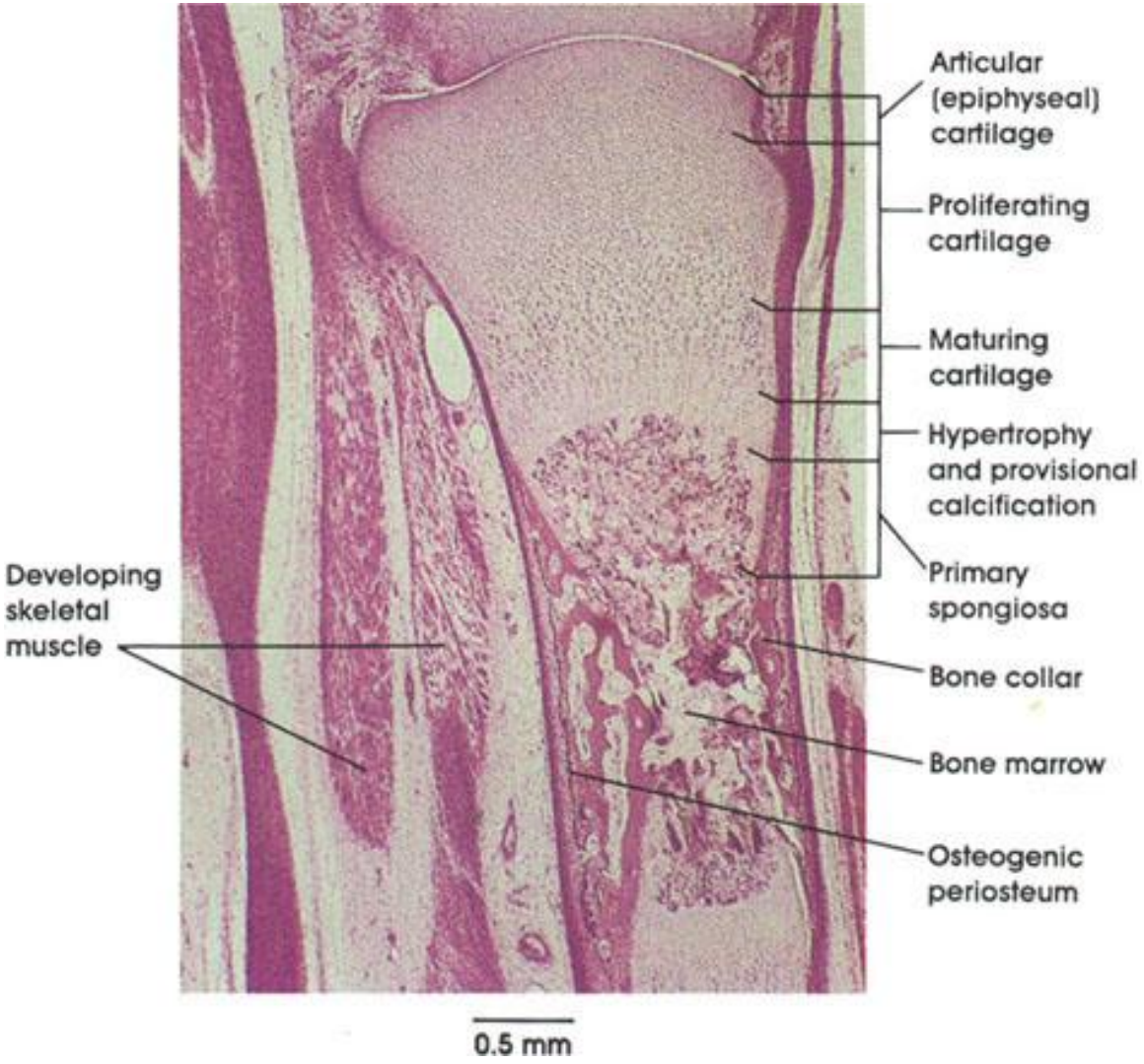
•The bone is formed onto a temporary cartilage model.
•The cartilage model grows (zone of proliferation), then chondrocytes mature (zone of maturation) and hypertropy (zone of hypertrophy), and growing cartilage model starts to calcify.
•As this happens, the chondrocytes are far from blood vessels, and are less able to gain nutrients etc, and the chondrocytes start to die (zone of cartilage degeneration). The fragmented calcified matrix left behind acts as structural framework for bony material.
•Osteoprogenitor cells and blood vessels from periosteum invade this area, proliferate and differentiate into osteoblasts, which start to lay down bone matrix (osteogenic zone).
•In the fetus, the primary ossification centre forms first in the diaphysis. Later on a secondary ossification centre forms in the epiphysis.
•Cartilage is replaced by bone in the epiphysis and diaphysis, except in the epiphyseal plate region. Here the bone continues to grow, until maturity (around 18 years old). The resulting bone is a thick walled cylinder, that encloses a central bone marrow cavity.

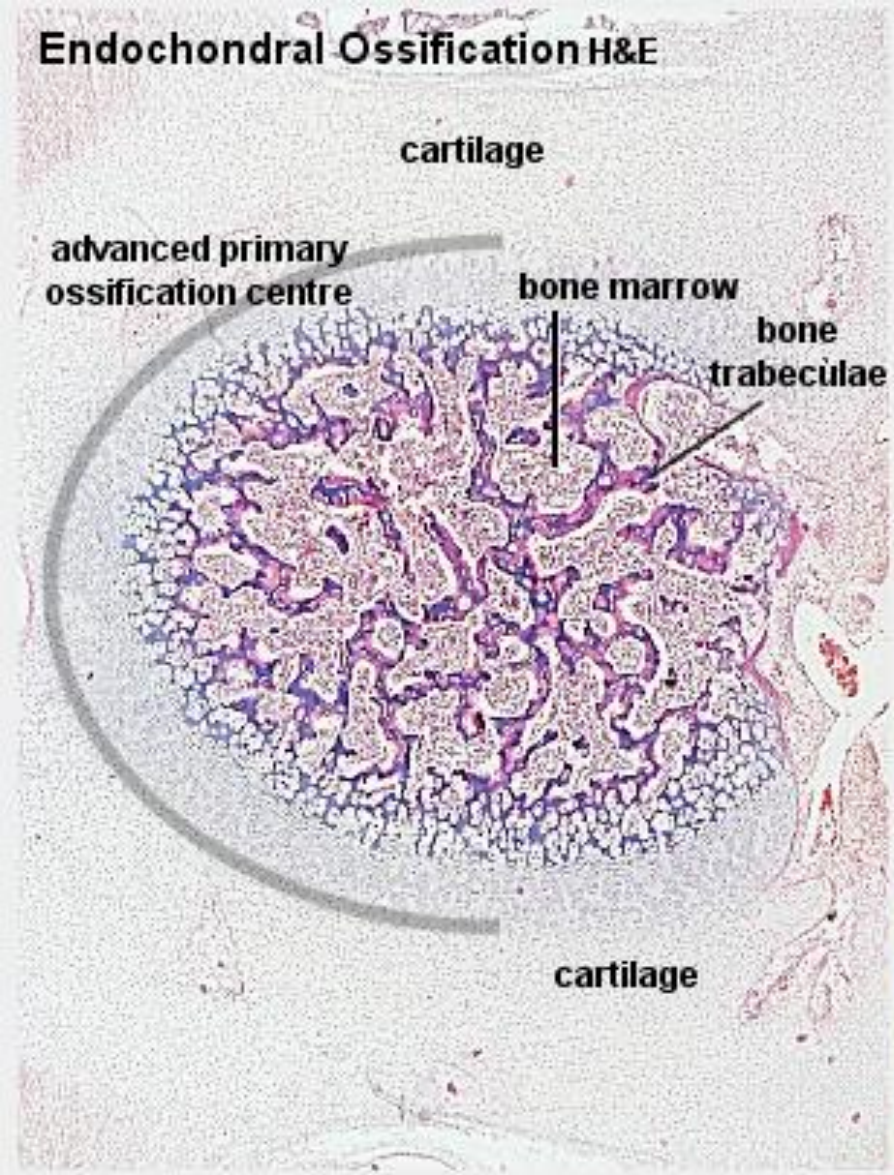
Endochondral Ossification

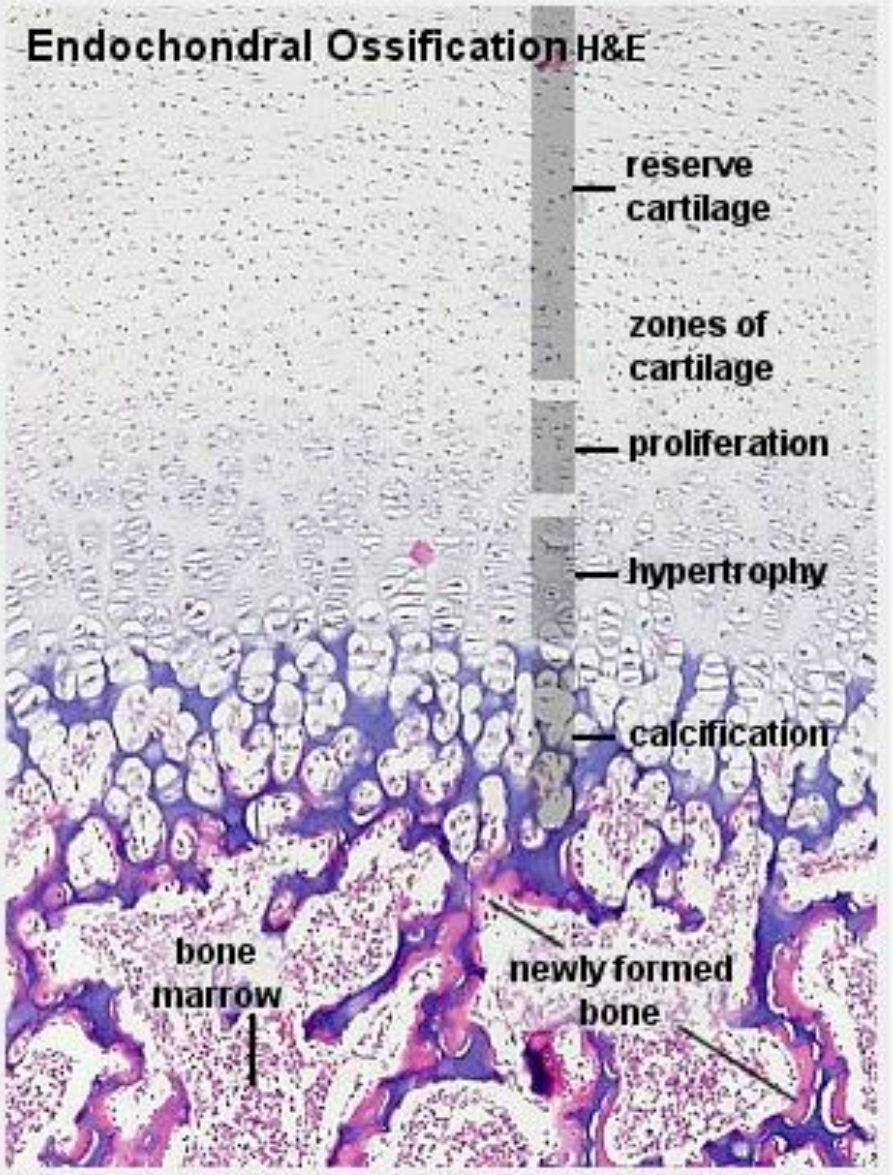
- Most bones are formed by the transformation of cartilage "bone models", a process called endochondral ossification.
- A periosteal bud invades the cartilage model and allows osteoprogenitor cells to enter the cartilage.
- At these sites, the cartilage is in a state of hypertrophy (very large lacunae and chondrocytes) and partial calcification, which eventually leads to the death of the chondrocytes. Invading osteoprogenitor cells mature into osteoblasts, which use the framework of calcified cartilage to deposit new bone.
- The bone deposited onto the cartilage scaffold is lamellar bone.
- The initial site of bone deposition is called a primary ossification centre.
- Secondary ossification centres occur in the future epiphyses of the bone.
  A thin sheet of bone, the periosteal collar, is deposited around the shaft of the cartilage model. The periosteal collar consists of woven bone.

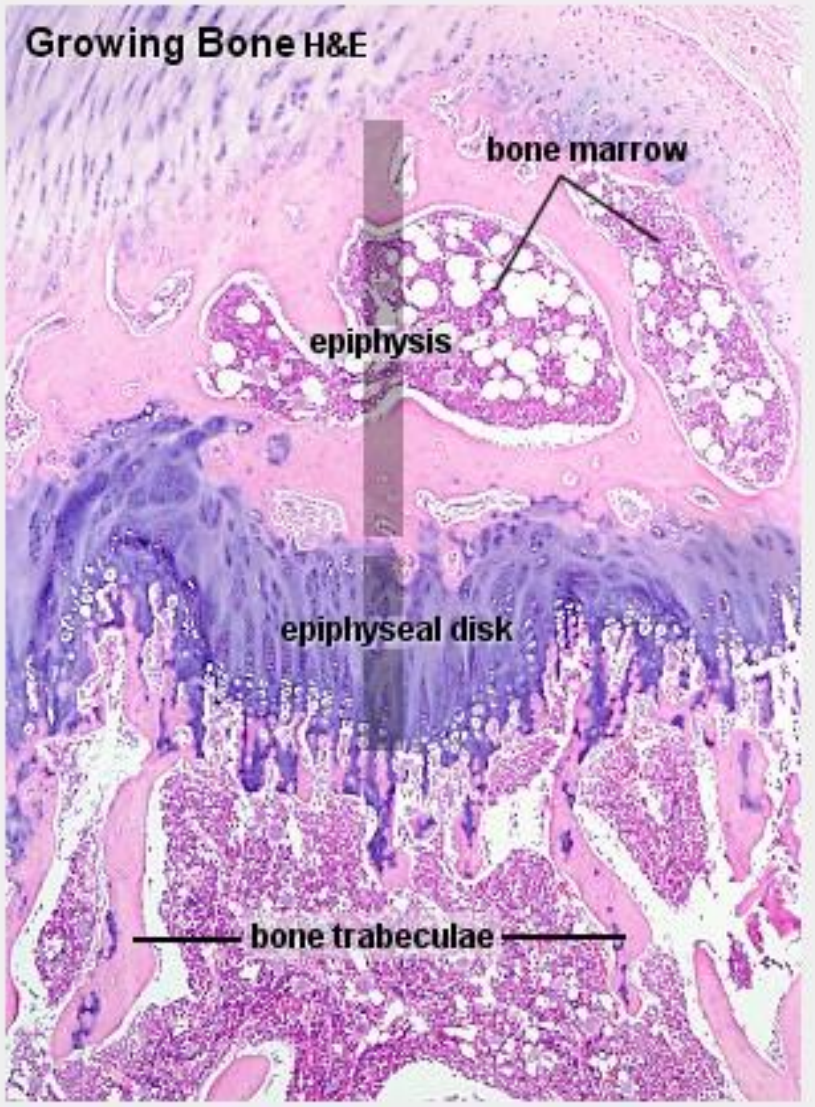
- Primary and secondary ossification centres do not merge before adulthood.
- Between the diaphysis and the epiphyses a thin sheet of cartilage, the epiphyseal plate, is maintained until adulthood.
- By continuing cartilage production, the epiphyseal plate provides the basis for rapid growth in the length of the bone.
- Cartilage production gradually ceases in the epiphyseal plate as maturity is approached.
- The epiphyseal plate is finally removed by the continued production of bone from the diaphyseal side.
- Bone formation and bone resorption go hand in hand during the growth of bone. This first deposited trabecular bone is removed.
- As the zone of ossification moves in the direction of the future epiphyses. This process creates the marrow cavity of the bones.
- Simultaneously, bone is removed from the endosteal surface and deposited on the periosteal surface of the compact bone which forms the diaphysis.
- This results in a growth of the diameter of the bone.

- Close to the zone of ossification, the cartilage can usually be divided into a number of distinct zones :
- Reserve cartilage, furthest away from the zone of ossification, looks like immature hyaline cartilage.
- A zone of chondrocyte proliferation contains longitudinal columns of mitotically active chondrocytes, which grow in size in
- the zone of cartilage maturation and hypertrophy.
- A zone of cartilage calcification forms the border between cartilage and the zone of bone deposition.




Endochondral Ossification H&E
cartilage
advanced primary ossification centre
bone marrow
bone trabeculae
cartilage


Endochondral Ossification H&E
reserve cartilage
zones of cartilage
proliferation
hypertrophy
calcification
bone marrow
newly formed bone



The cartilage model has almost entirely been transformed into bone. The only remaining cartilage is found in the epiphyseal disk. Zones of cartilage proliferation, hypertrophy and calcification are visible at high magnification, but only on one side of the epiphyseal disk - towards the diaphysis, which increases in length as the cartilage generated by the epiphyseal disc is transformed into bone. Osteoclasts may be found on the newly formed trabeculae or associated with parts of the cartilage scaffold.