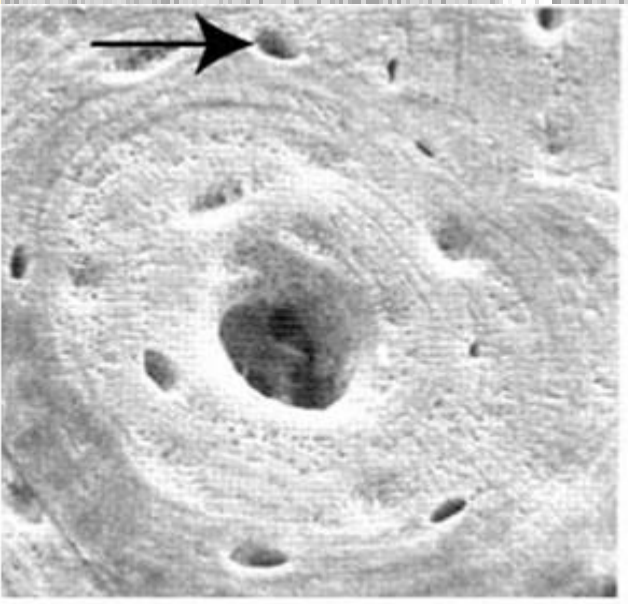
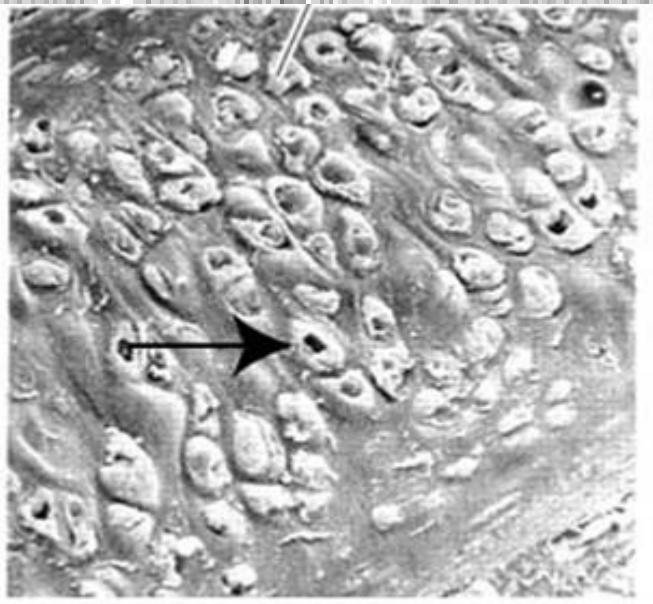
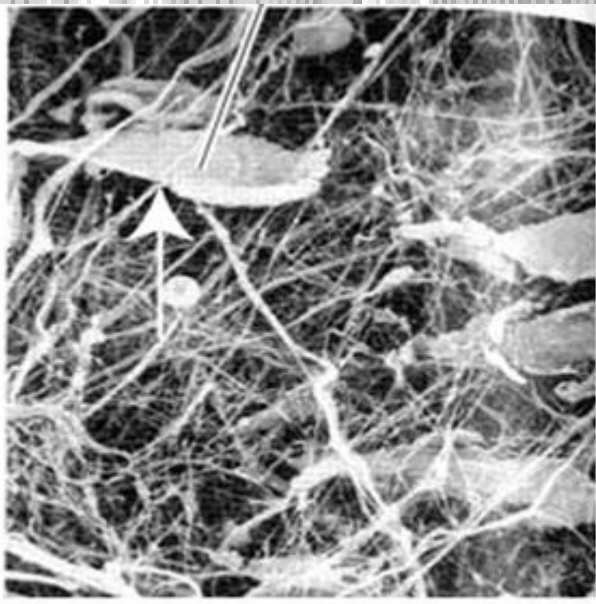
CARTILAGE and BONE



Bone Tissue



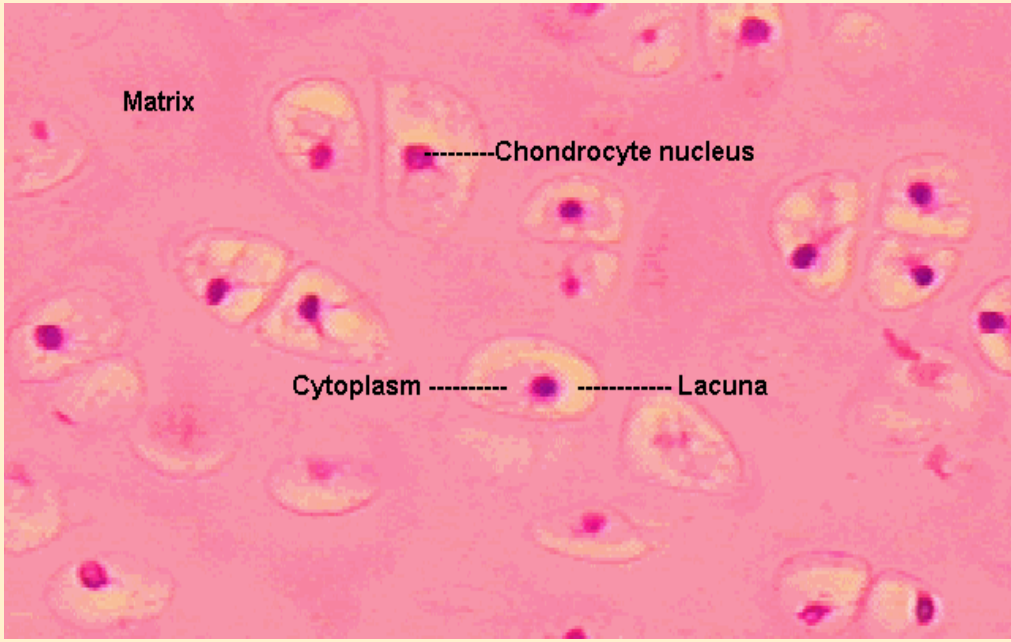
Cartilege

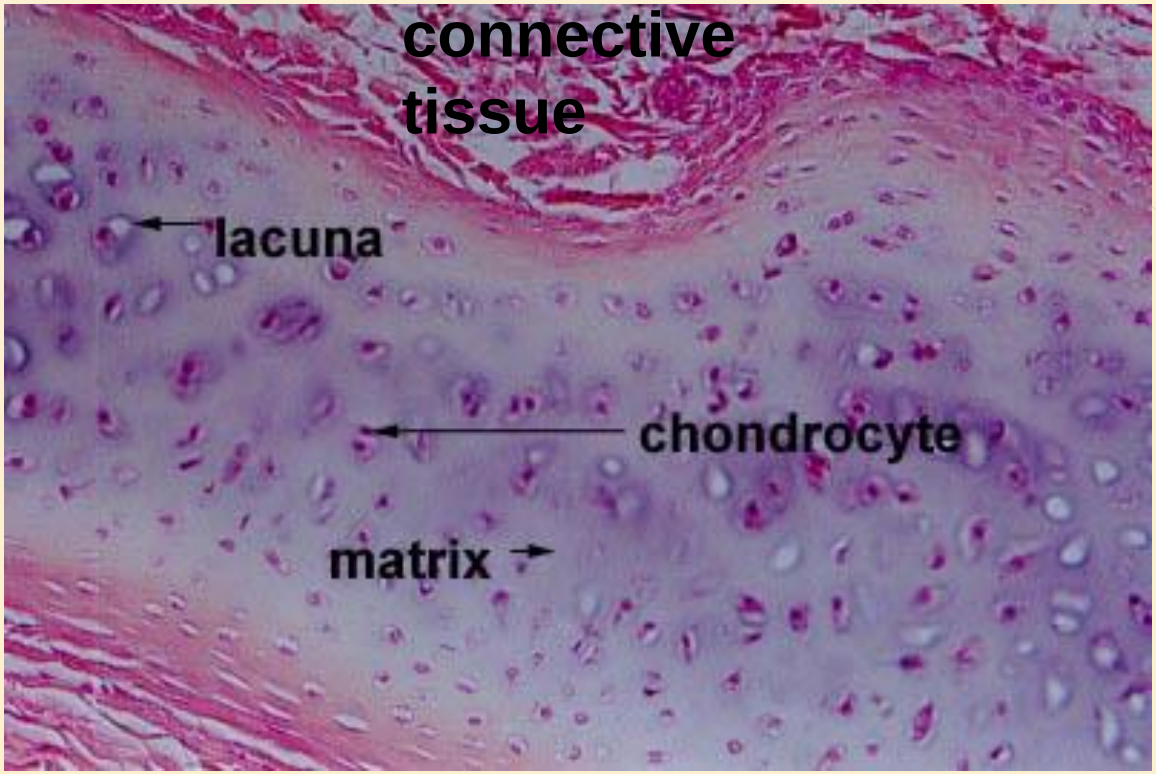


Connective Tissue

Structure of cartilage

- **cells – chondrocytes (in lacunae)**
- **extracellular matrix**

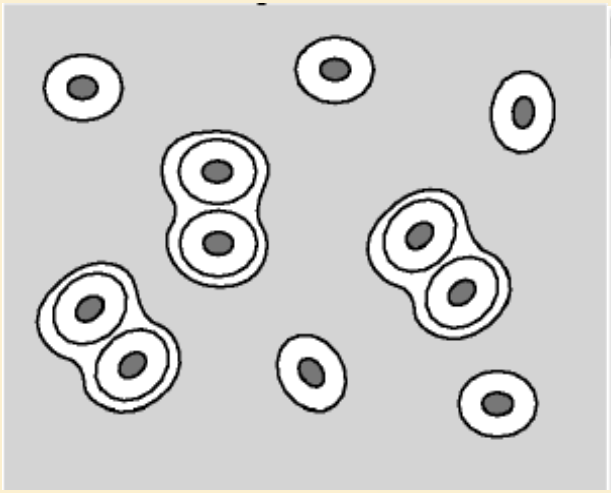




- **absence of blood and lymphatic vessels and nerves (**nutrients – diffusion from blood vessels of connective tissues).

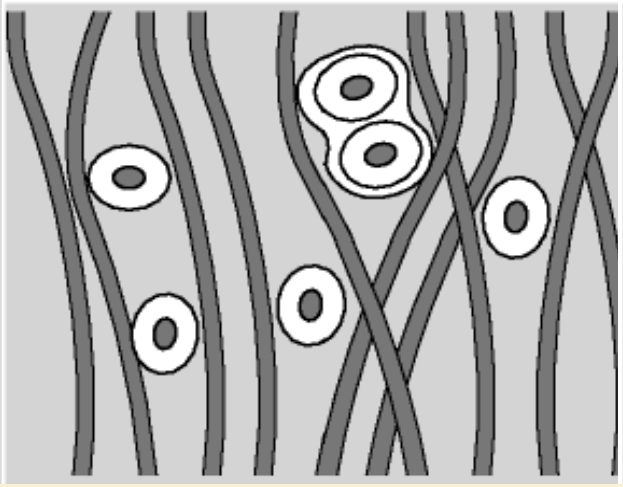
Types of cartilage

Hyaline cartilage



Type II collagen

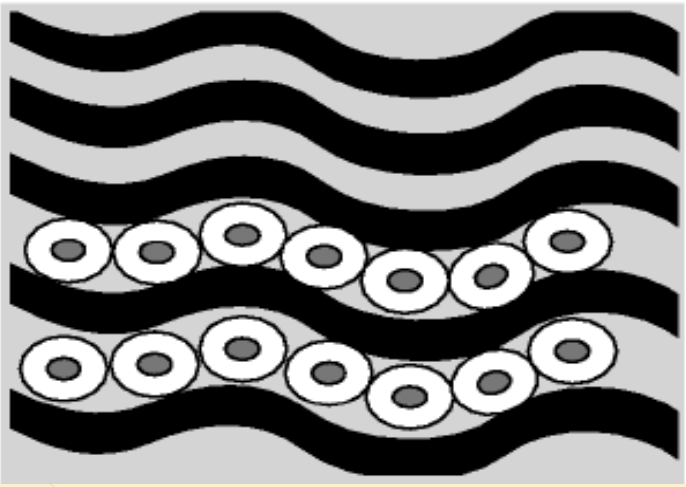
Elastic cartilage



Type II collagen and **elastic fibers**

-flexibility

Fibrocartilage

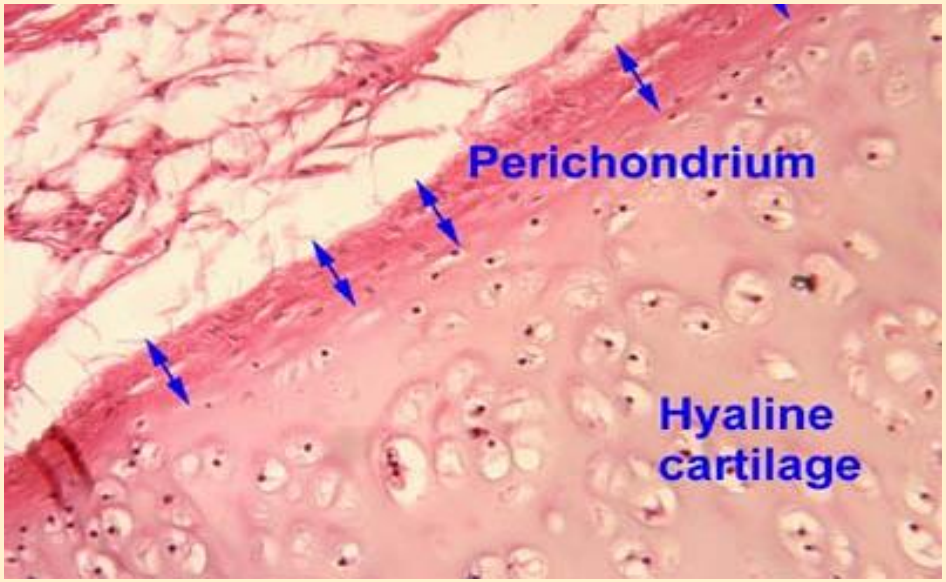


Type I collagen -

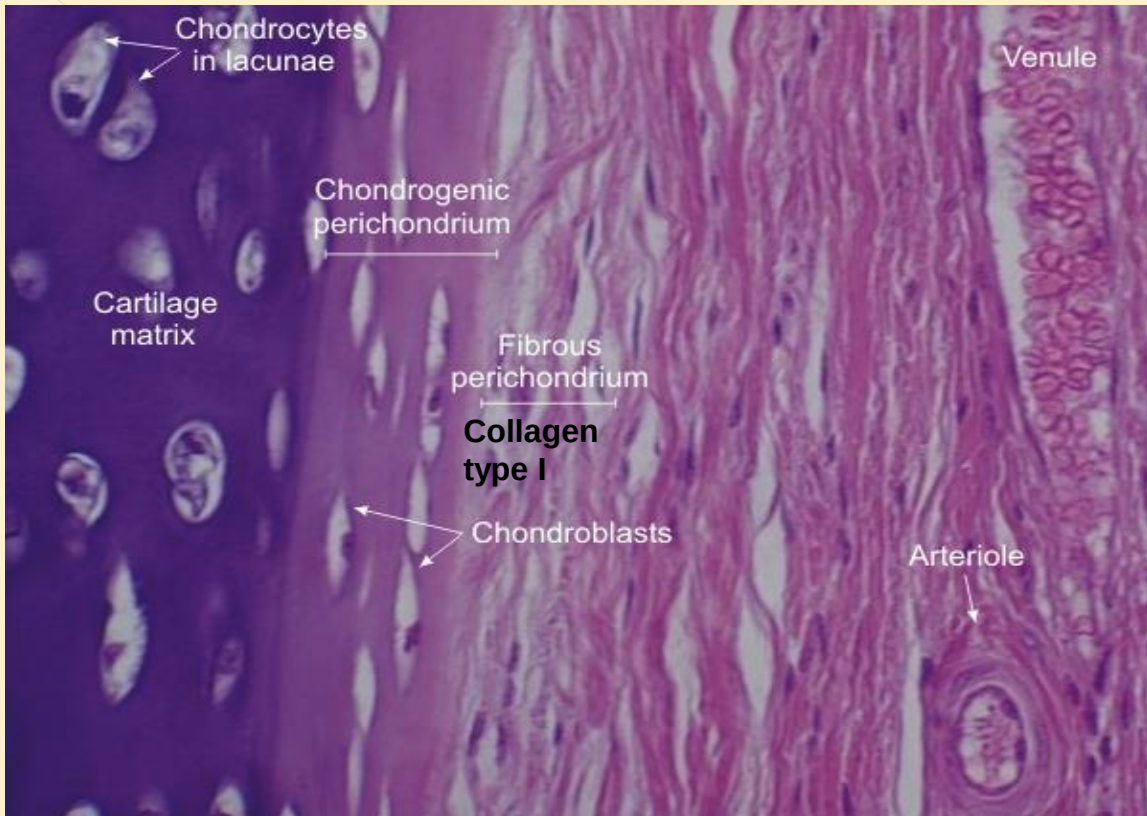
- resistant to stretching

Perichondrium

- connective tissue sheath covering cartilage
- present in most hyaline and elastic cartilages (**absent in fibrocartilage**)



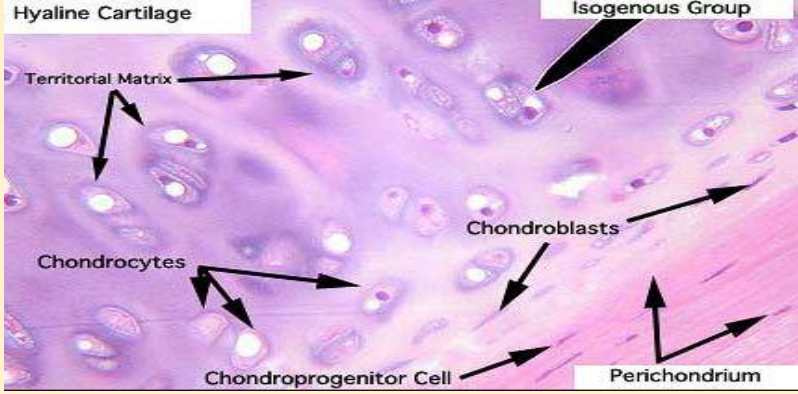
- an **outer** fibrous layer and **inner** cellular layer
- is vascular - vessels supply nutrients to the cell of cartilage



Cells of cartilage

1. **Chondrogenic cells**

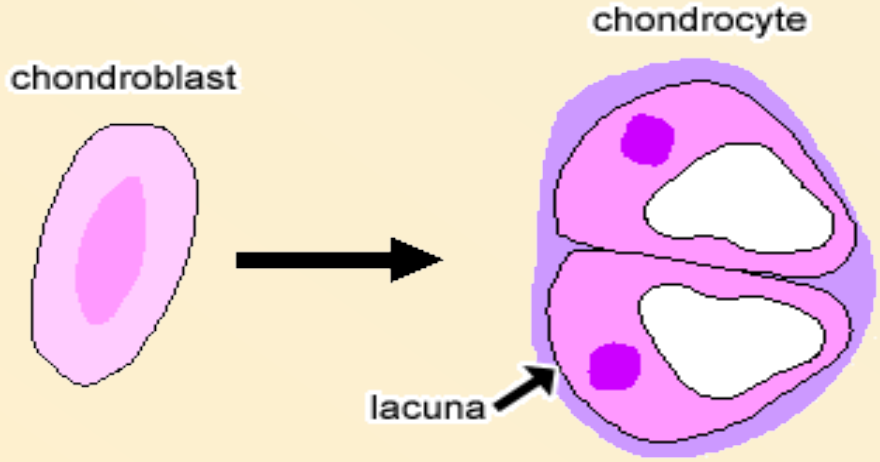
- in perichondrium
- differentiate into chondroblasts

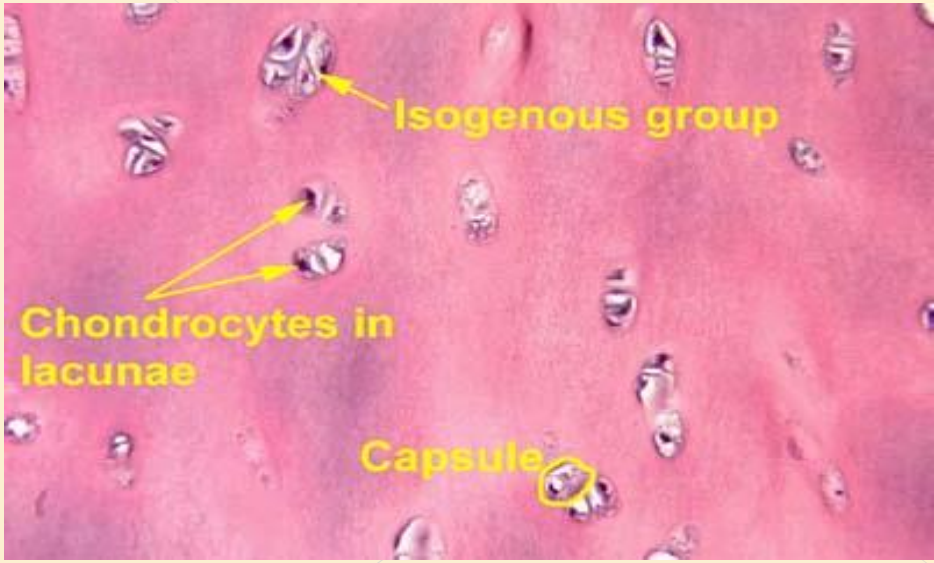


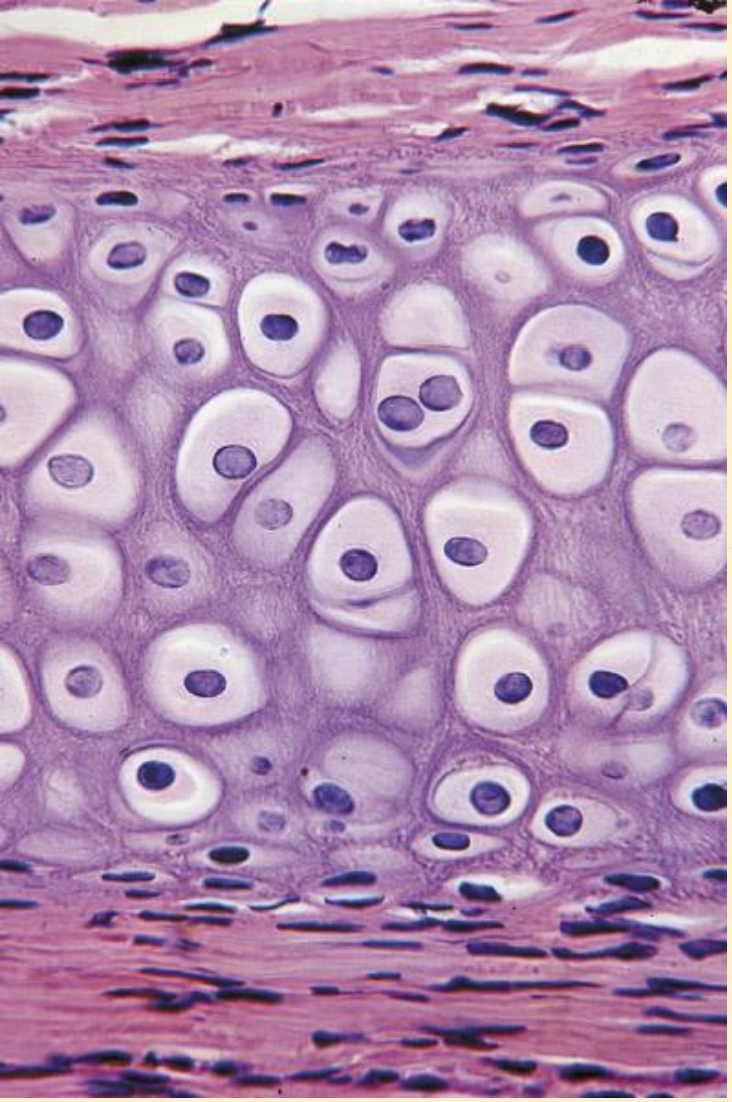
2. **Chondroblasts** – „young" chondrocytes - produce matrix components (growth of cartilage)

3. **Chondrocytes –** chondroblsts surrounded by matrix

- rest in cartilage lacunae (capable of cell division)
- form **isogenous groups** (cluster of 2 - 4 cells in lacunae)





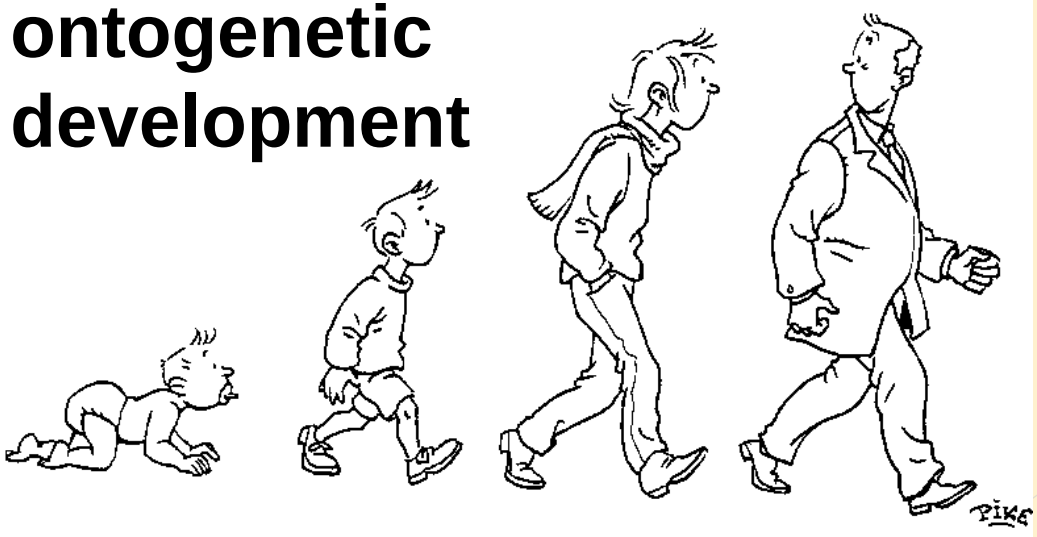


Perichondrium

Hyaline cartilage

the most abundant - chondrocytes (in lacunae) - isogenous groups

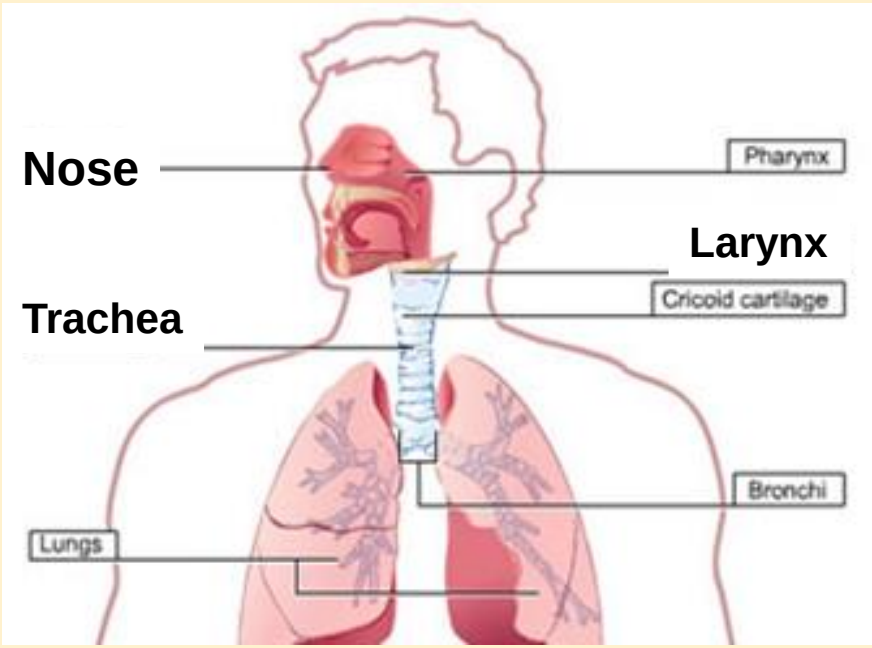
ontogenetic development

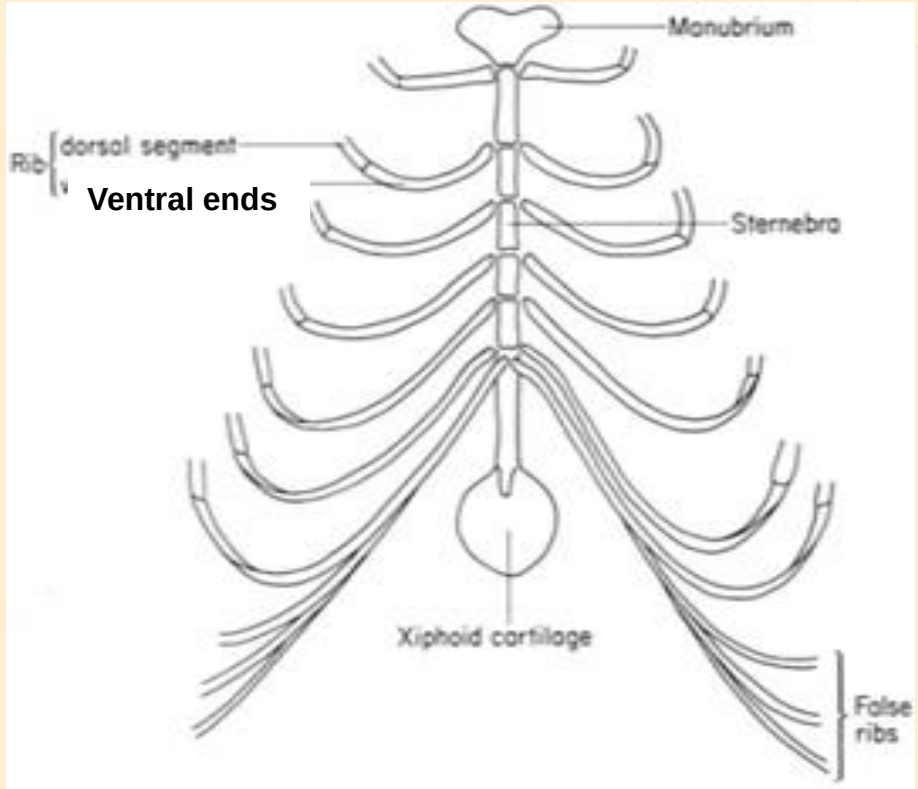


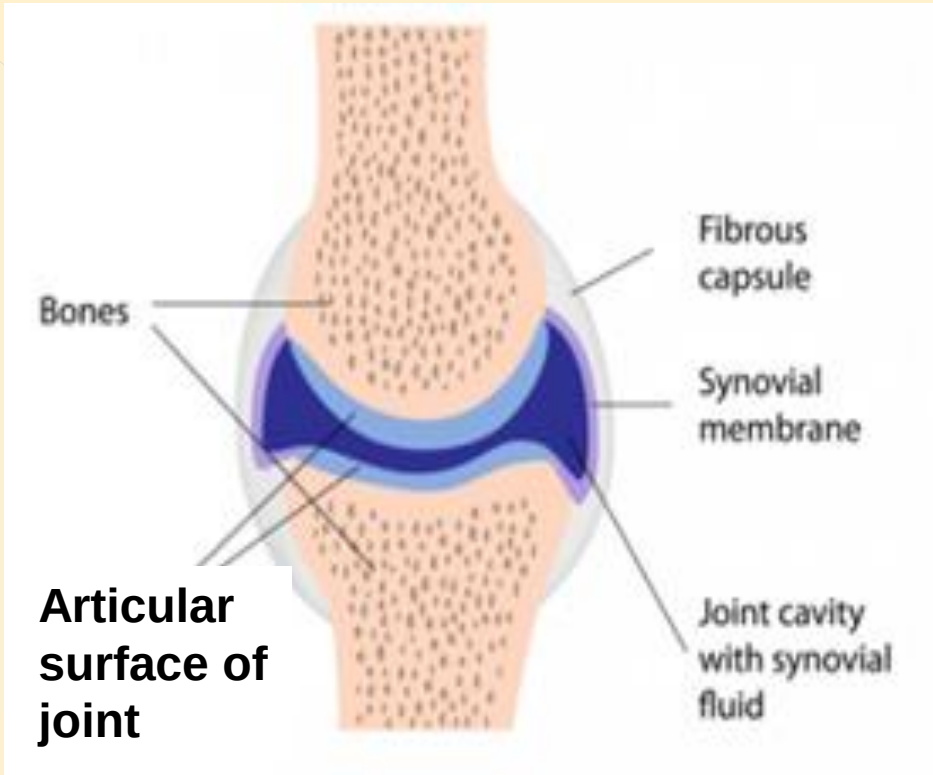
- cartilage templates (models) of many bones during embryonic development - cartilage is transformed into bone

Hyaline cartilage in adults:

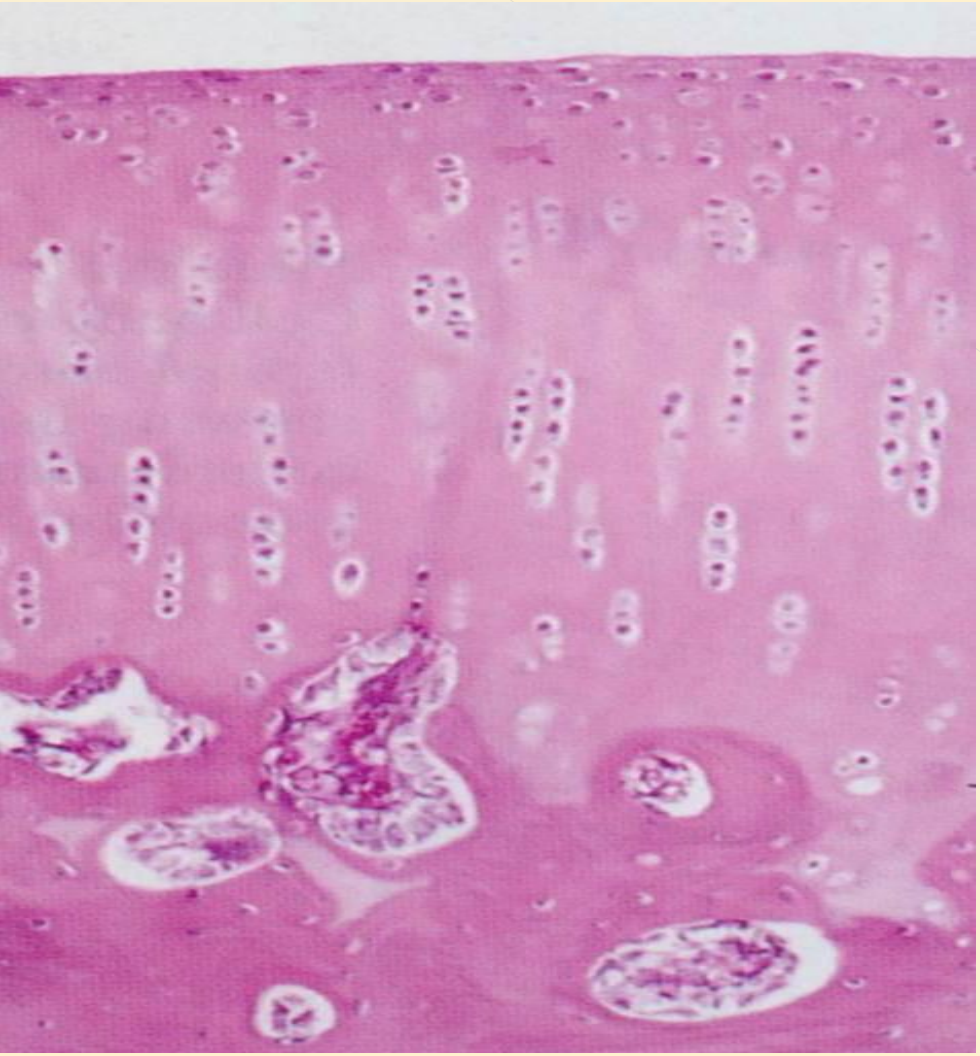
- Nose
- Larynx
- Trachea
- Ventral ends of ribs
- Articular surface of joints

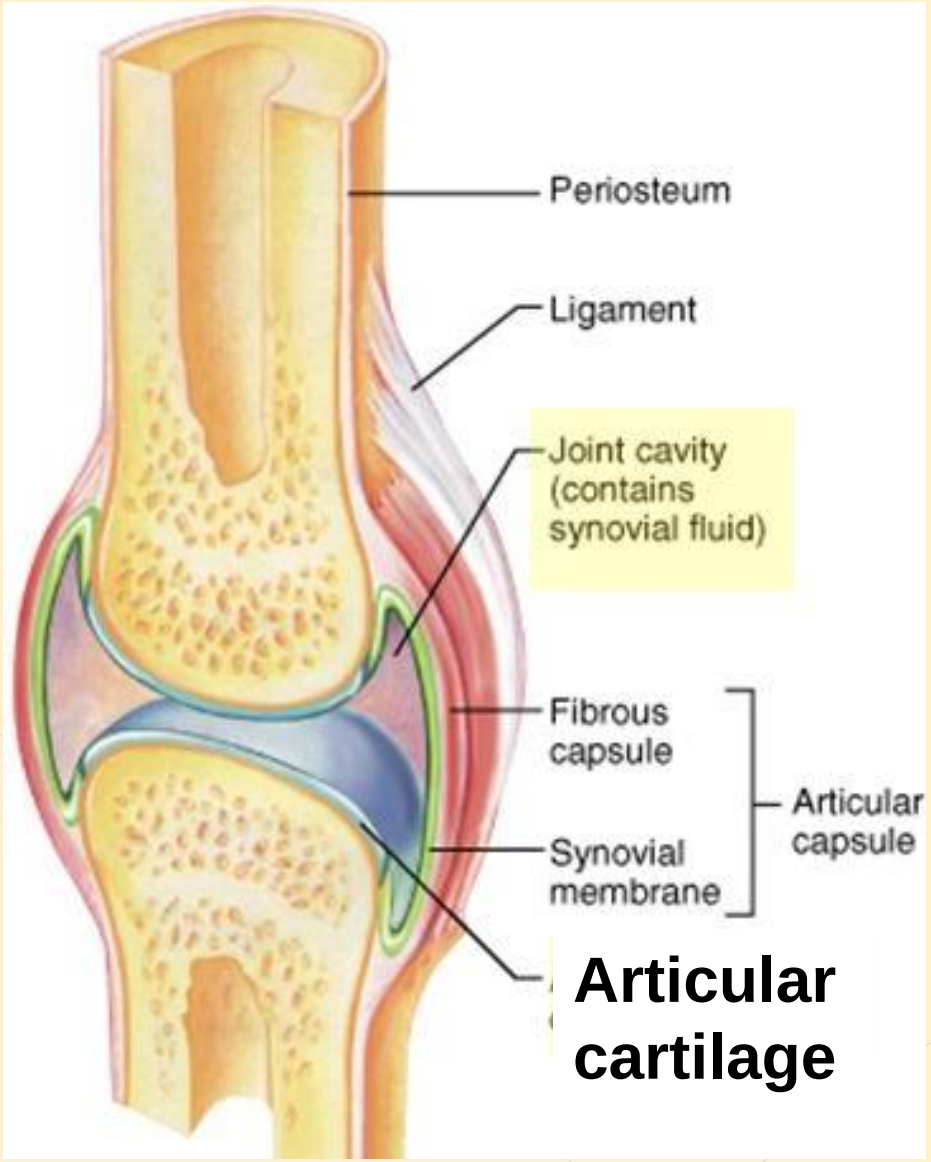




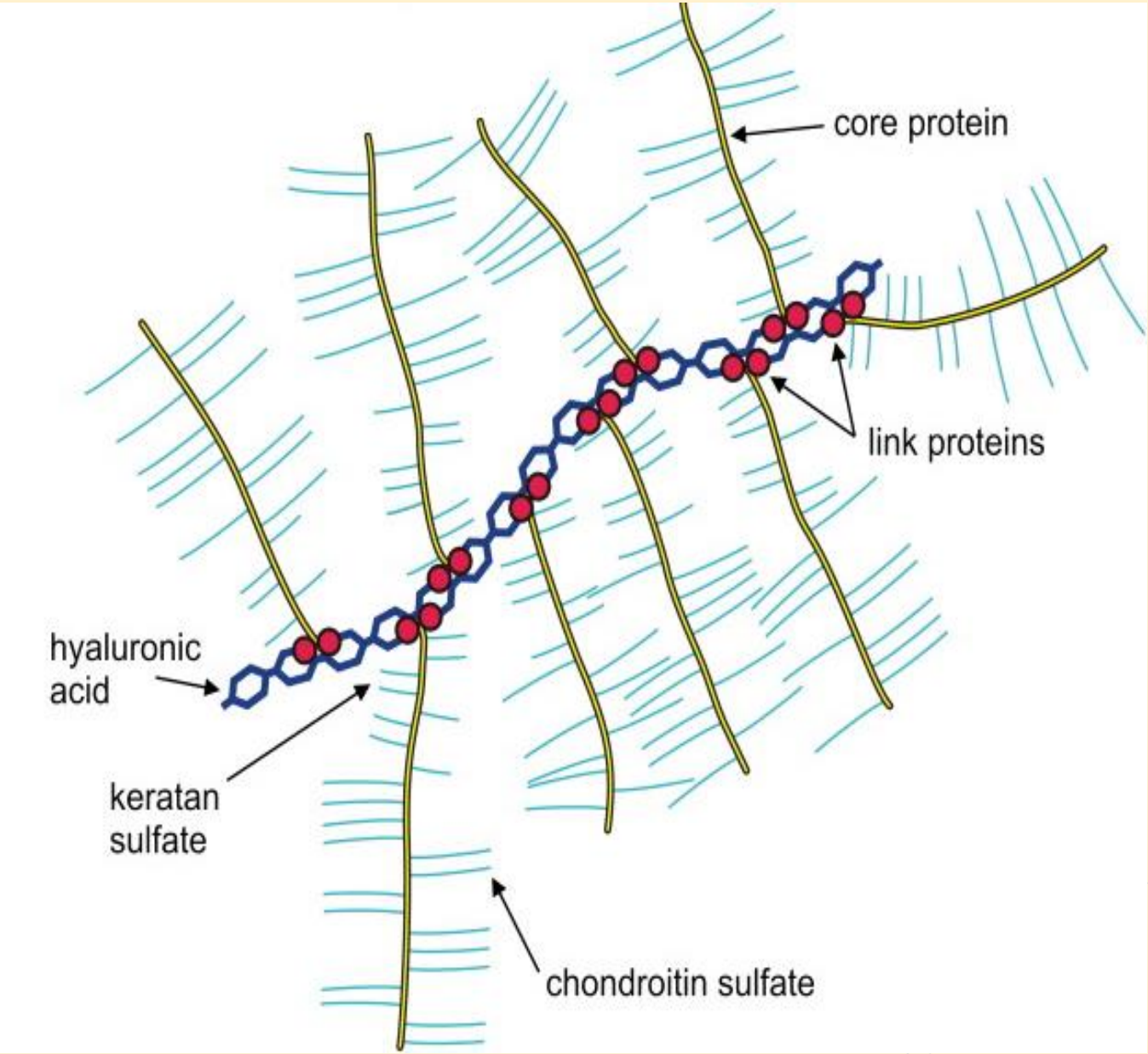


Hyaline cartilage of articular surface of joints - not surrounded by perichondrium

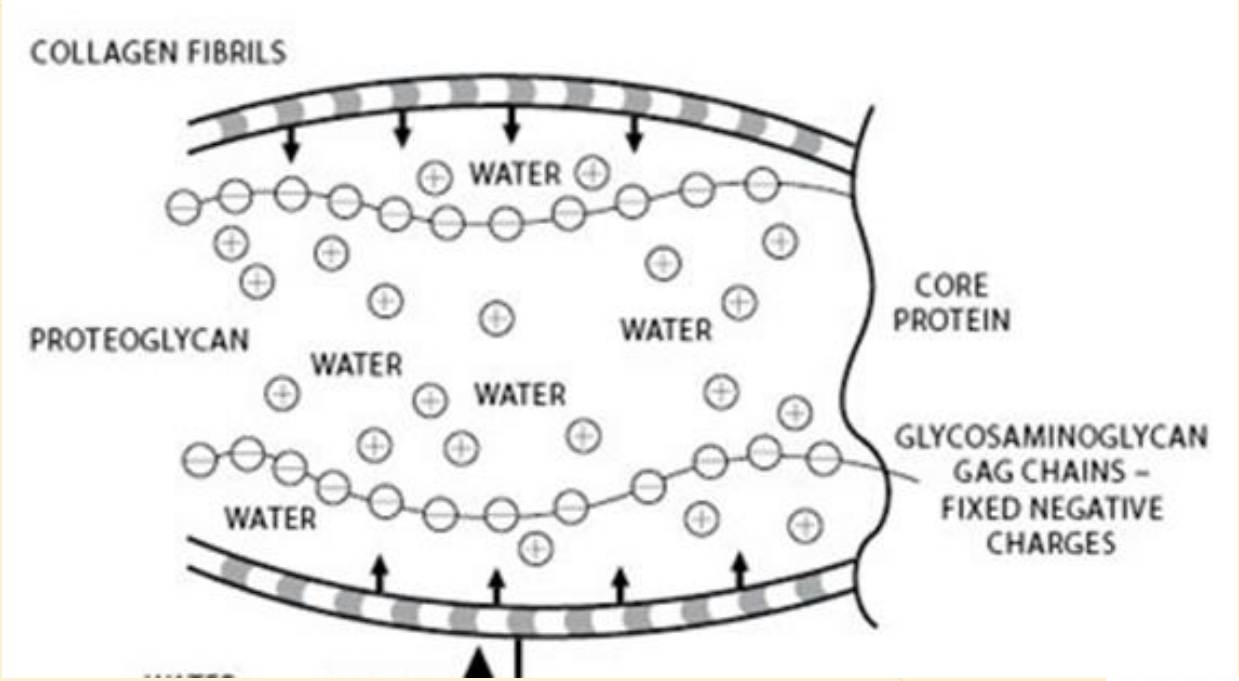




The main proteoglycan of cartilage – **aggrecan (**hydration)

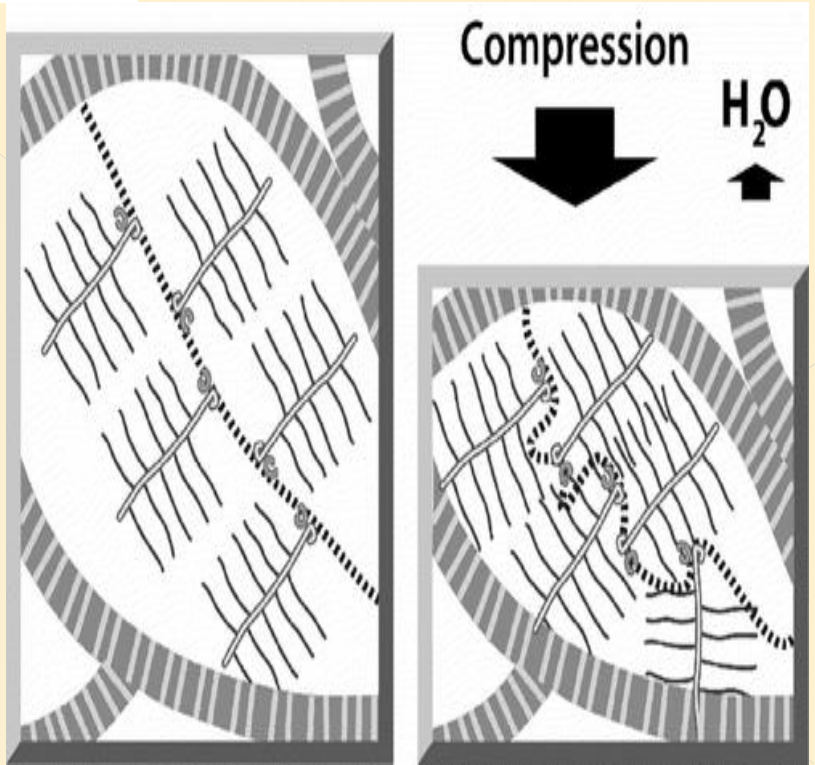


Proteoglycans - noncovalently linked to hyaluronic acid



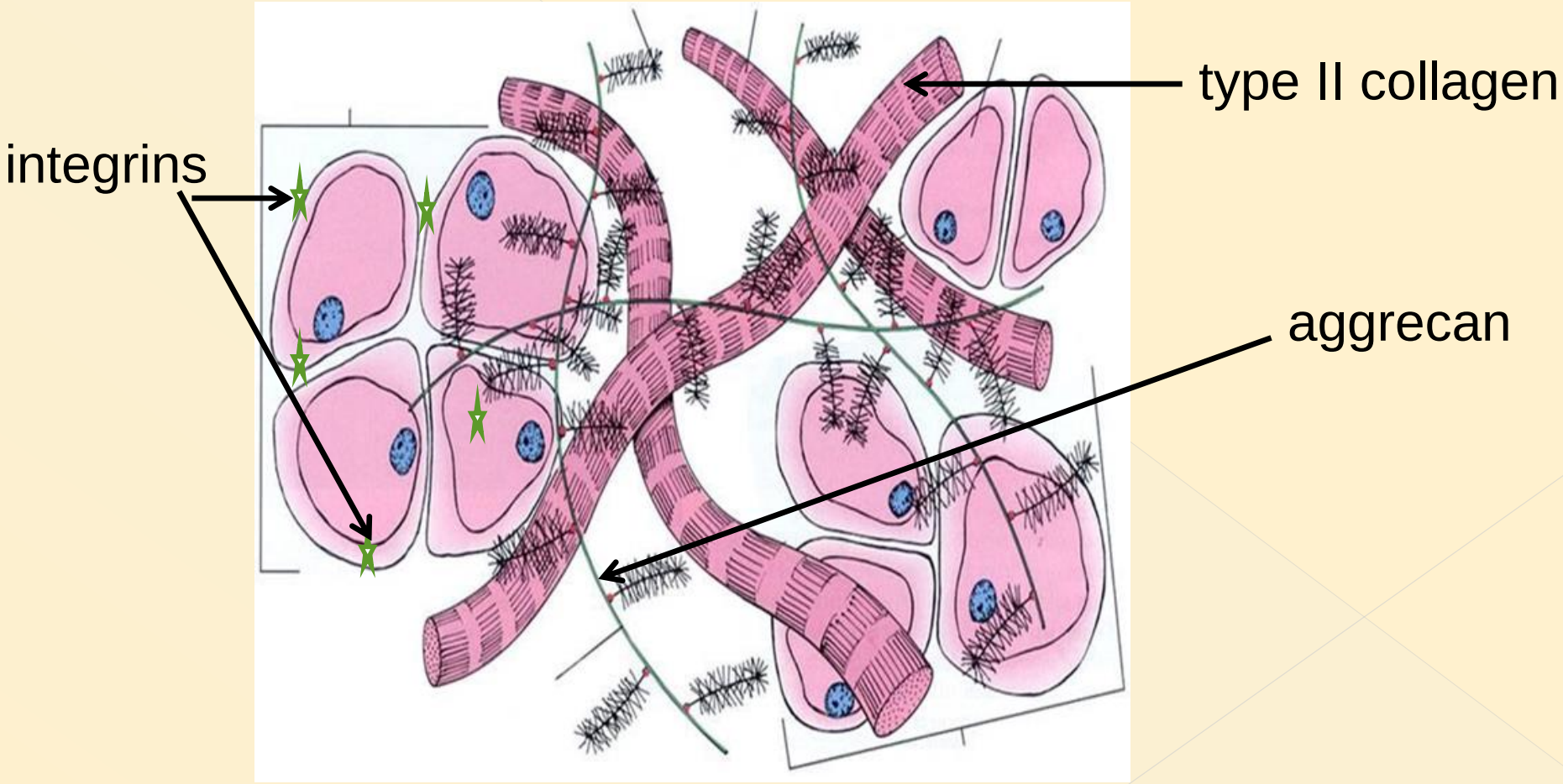
GAGs - bind water (hydration) and attract cations (sodium ions) (**are negatively charged)**

- up to 80% of the wet weight of cartilage is water - **the ability of cartilage to resist forces of compression** (the water is squeezed from spaces between GAGs, molecules repel each other).



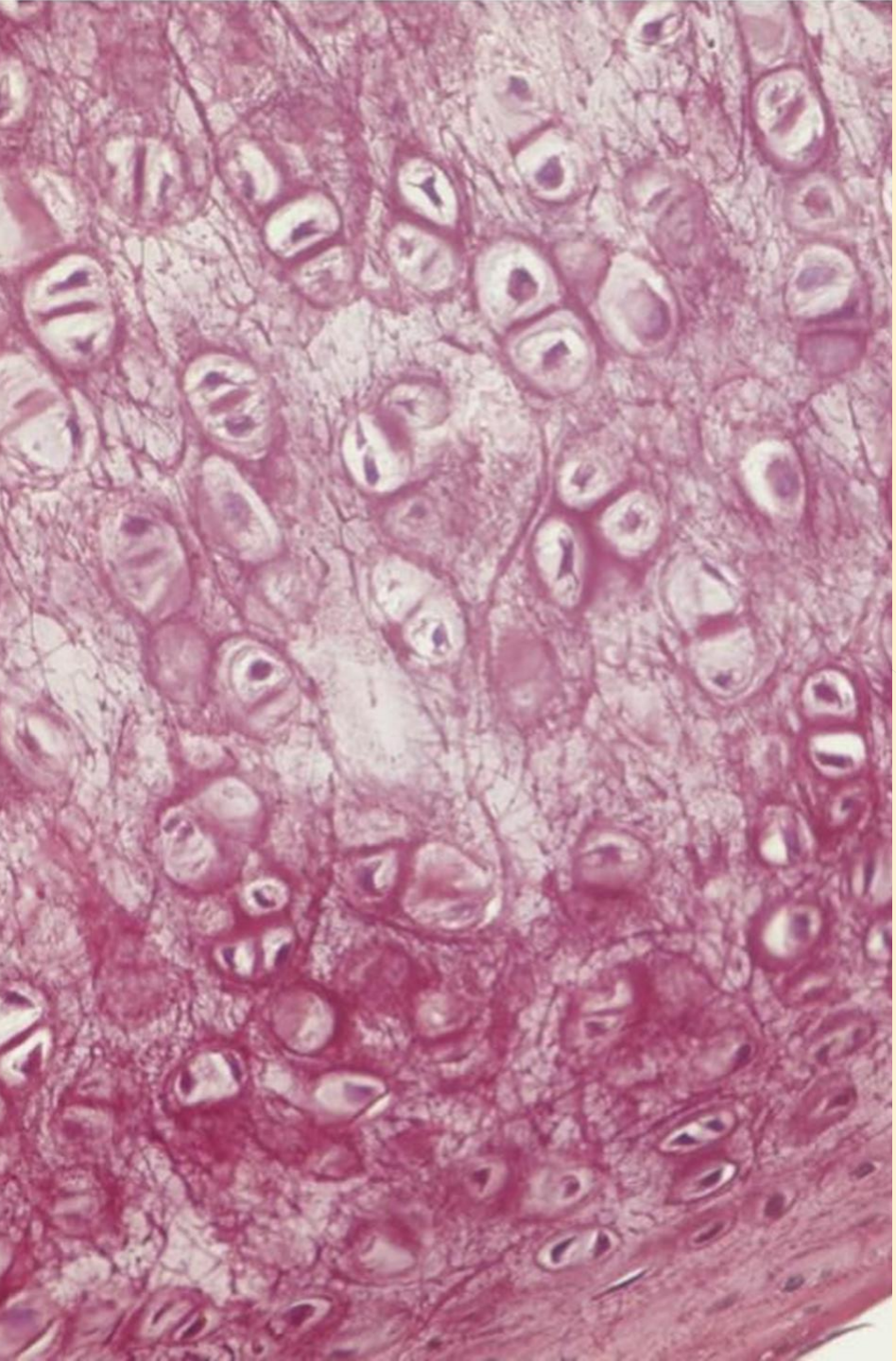
The main glycoprotein of cartilage:

Chondronectin - binding sites for type II collagen, aggrecan and integrins of chondrocytes - **attachment of chondrocytes to the extracellular matrix**



Types of growth of cartilage

- **Interstitial growth** – cells of isogenous groups produce matrix **enlarging the cartilage from within**
- **Appositional growth** – chondrogenic cells of inner layer of perichondrium undergo division and differentiate into chondroblasts which manufacture matrix. **Cartilage grows by adding to its periphery**

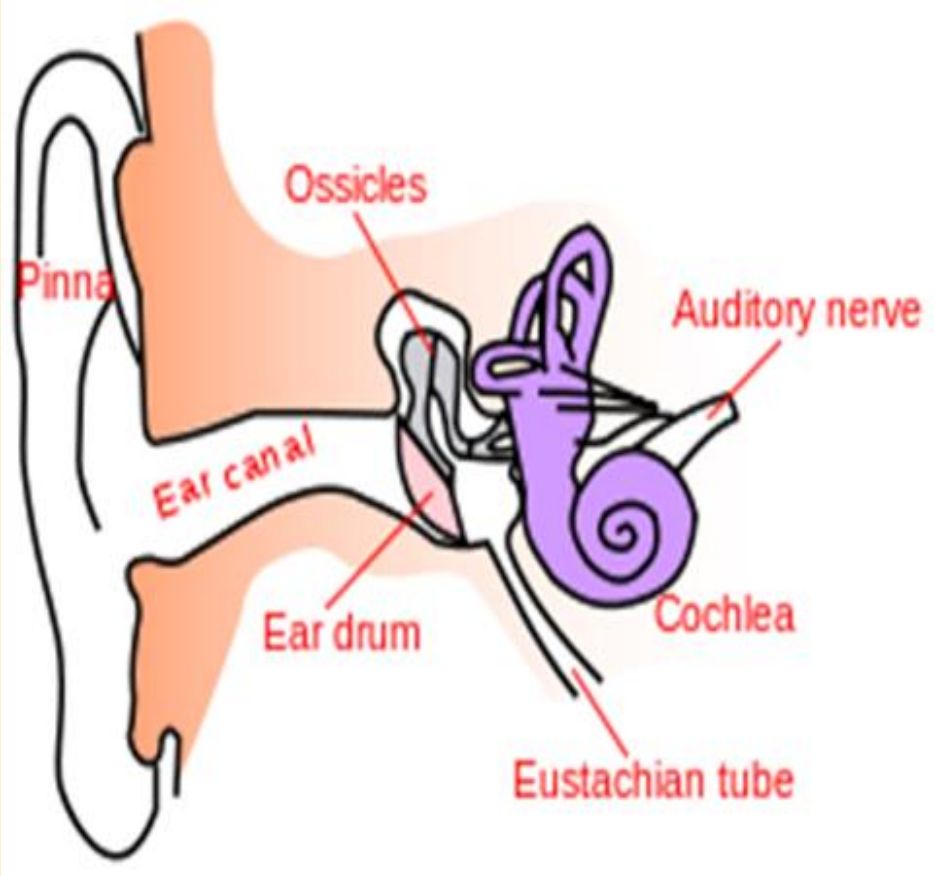


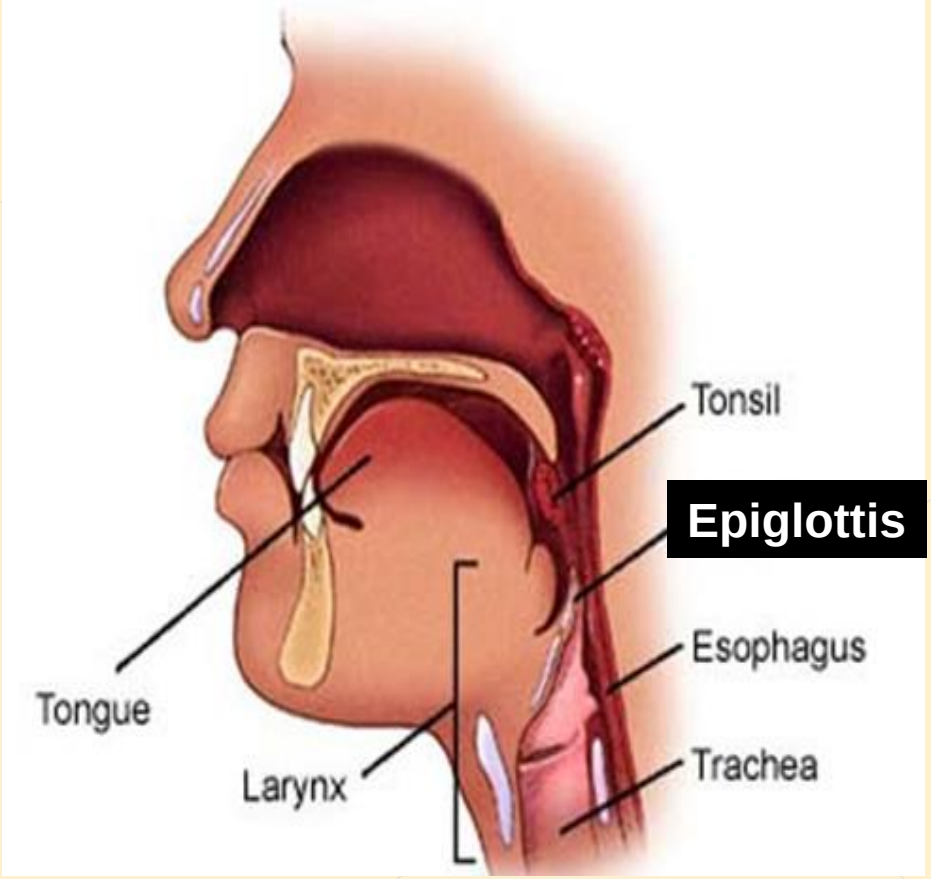
Elastic cartilage

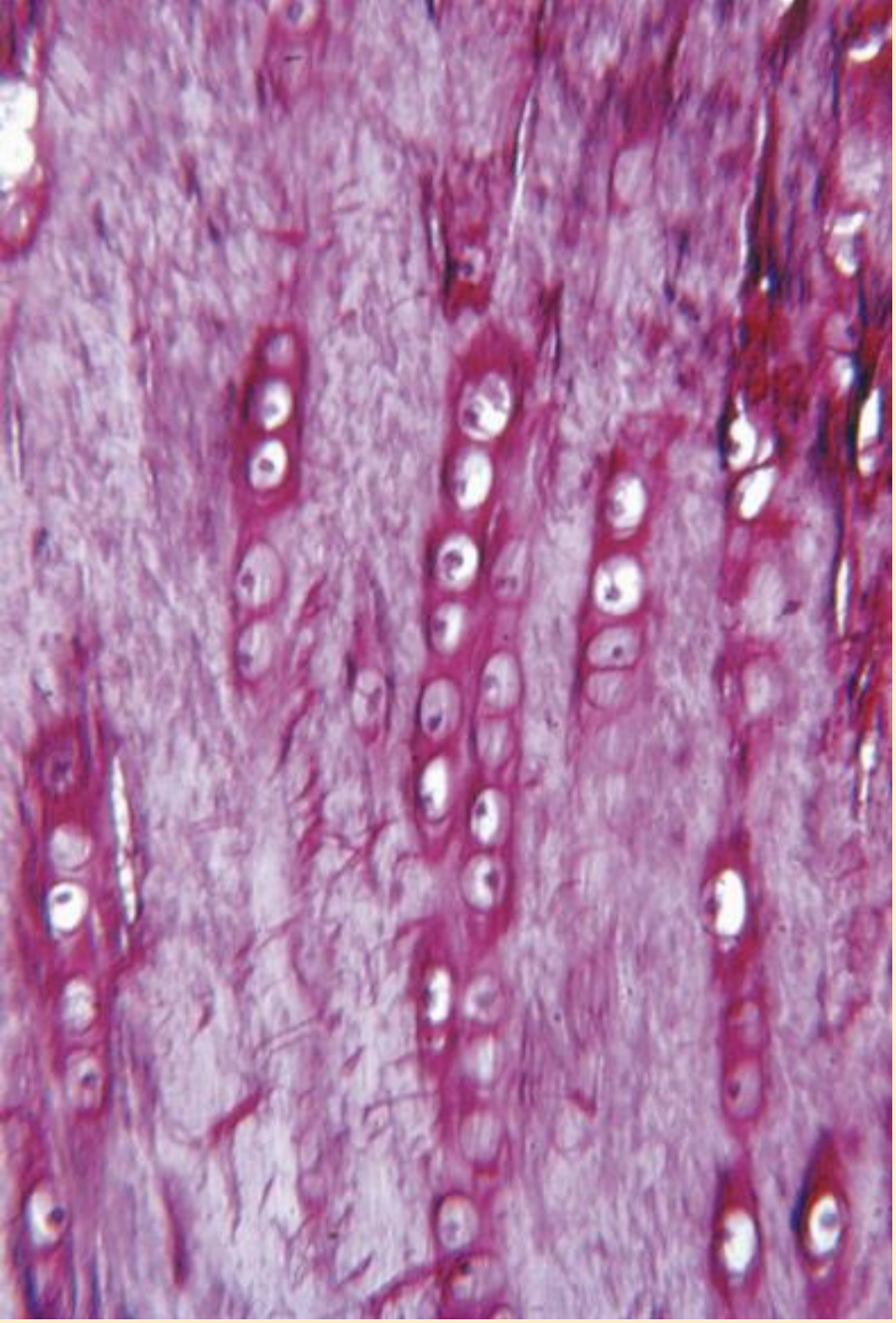
- branching **elastic fibers** (flexibility) and **collagen type II,** abundant chondrocytes

Elastic cartilage stained with orcein - elastic fibers

Elastic cartilage
- pinna of the ear,
- external and internal auditory tubes,
- epiglottis,
- larynx







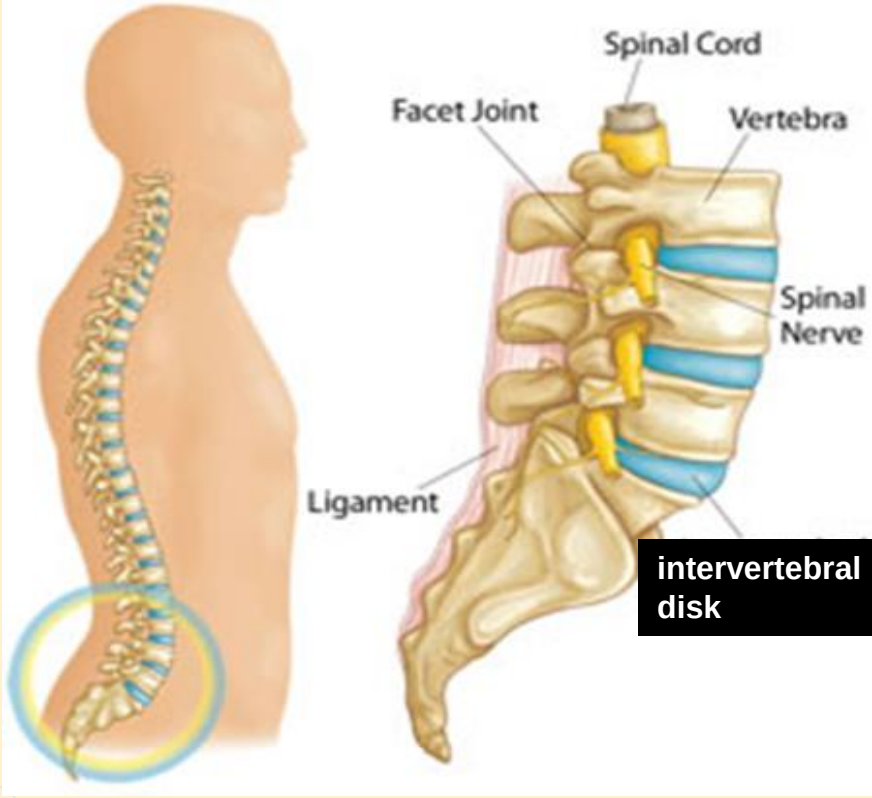
Fibrocartilage

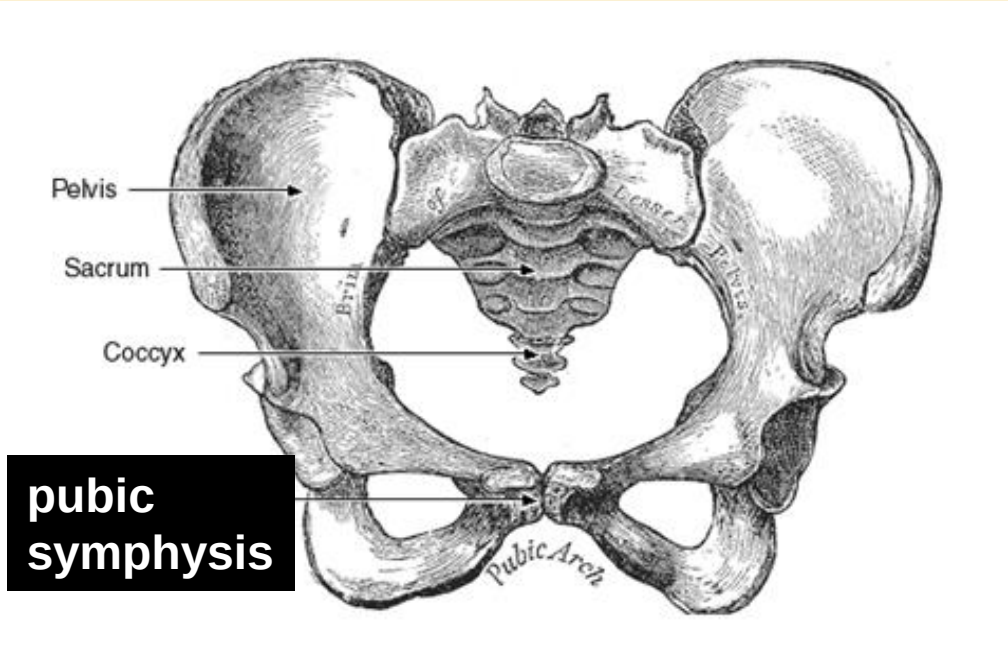
- **does not possess a perichondrium!**
- bundles of type **I collagen**
- chondrocytes aligned in parallel rows

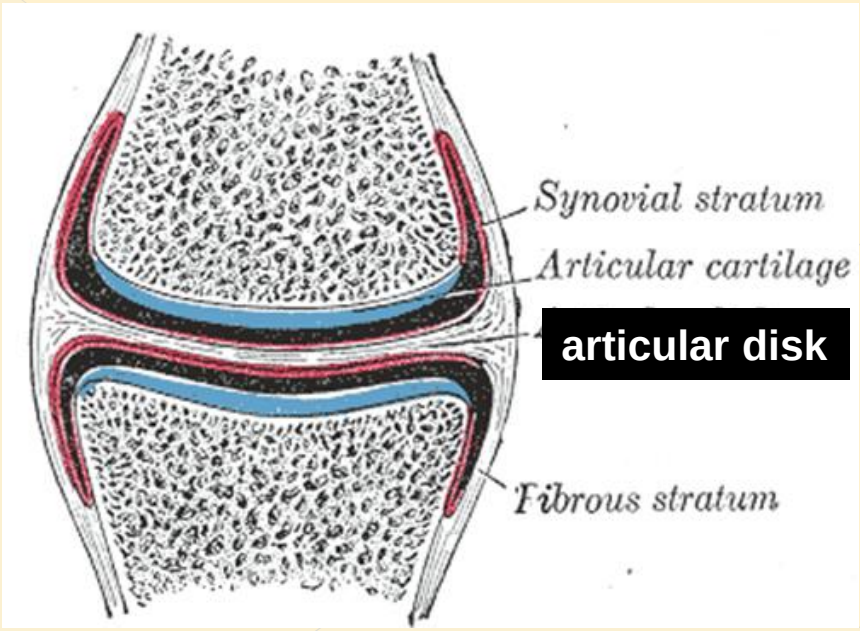
- similar to the dense connective tissue

Fibrocartilage

- intervertebral disks,
- pubic symphysis,
- articular disks





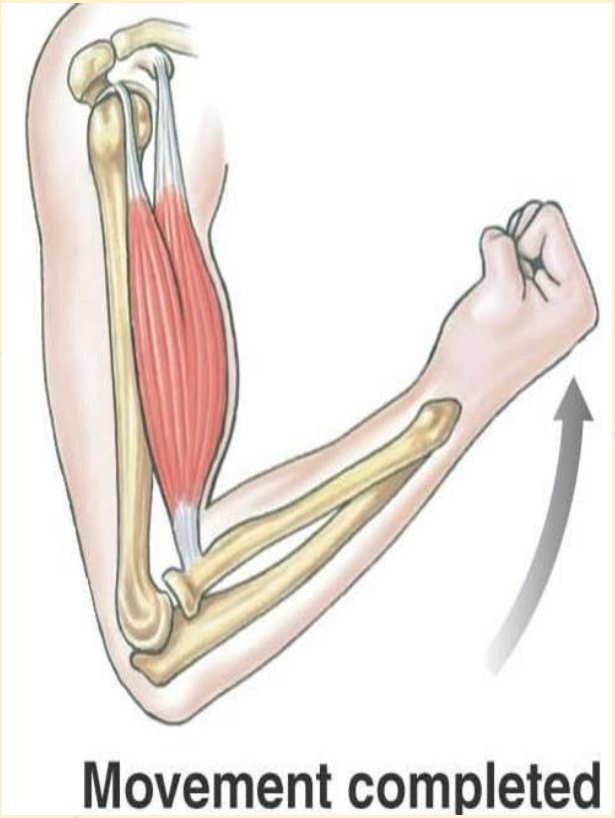


BONE

- **dynamic** tissue (changes shape in relation to the stresses placed on it)
- pressure – resorption
- tension – development of new bone

Bones:

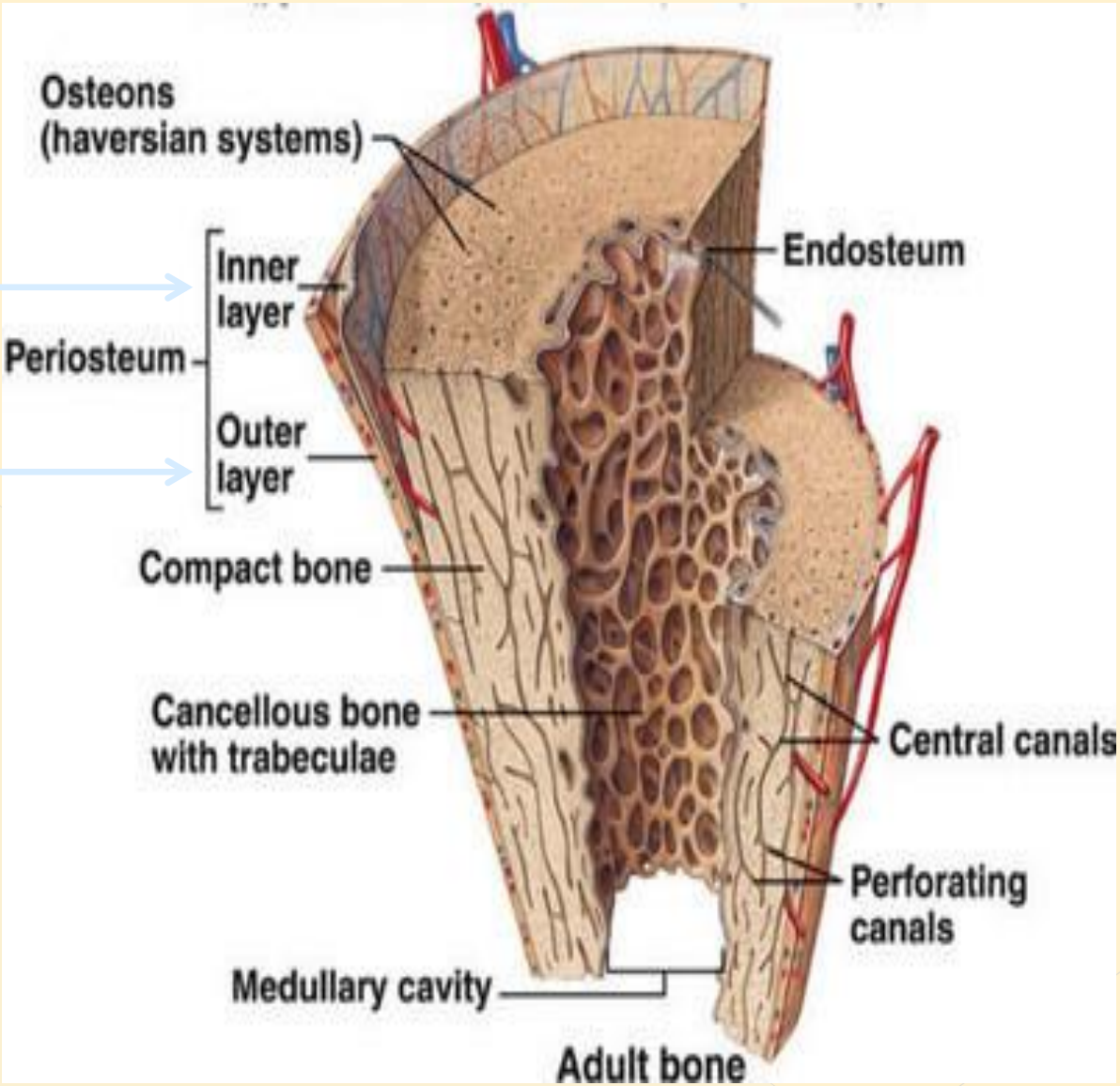
- support and protect organs
- are a reservoir for calcium
- serve as levers for muscles



Bone
- periosteum

Inner cellular layer (osteoprogenitor cells)

Outer layer of dense fibrous connective tissue



The central cavity - lined with **endosteum** – osteoprogenitor cells and osteoblasts

Bone Matrix

Inorganic component

- about 65% of dry weight
- calcium and phosphorus = **hydroxyapatite crystals**

Bone matrix is one of the hardest tissue

Organic component

- about 35% of dry weight of bone

Proteoglycans

Collagen type I
80%-90% of the organic components

Glycoproteins:
osteocalcin
osteopondin
sialoprotein

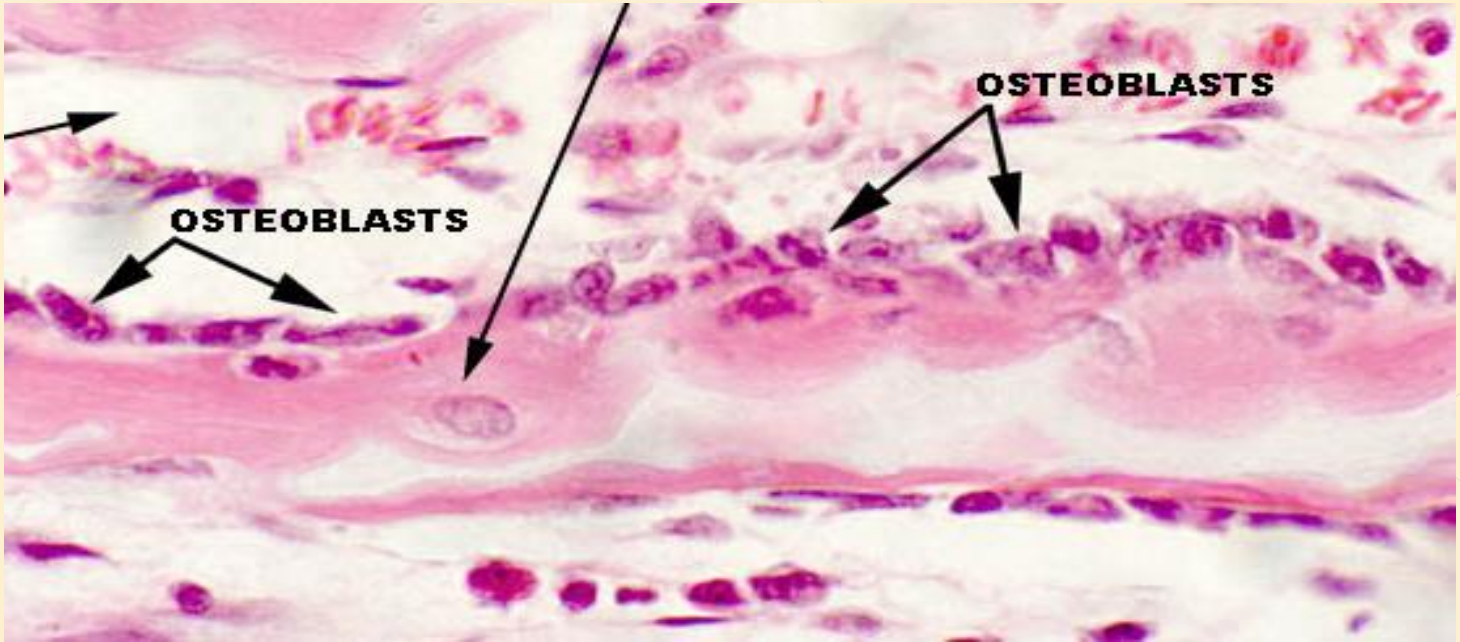
Binding of integrins and matrix components

Osteoprogenitor cells (stem cells of bone)

- in the periosteum and endosteum, under the influence of **BMPs (bone morphogenetic proteins),** differentiate into:

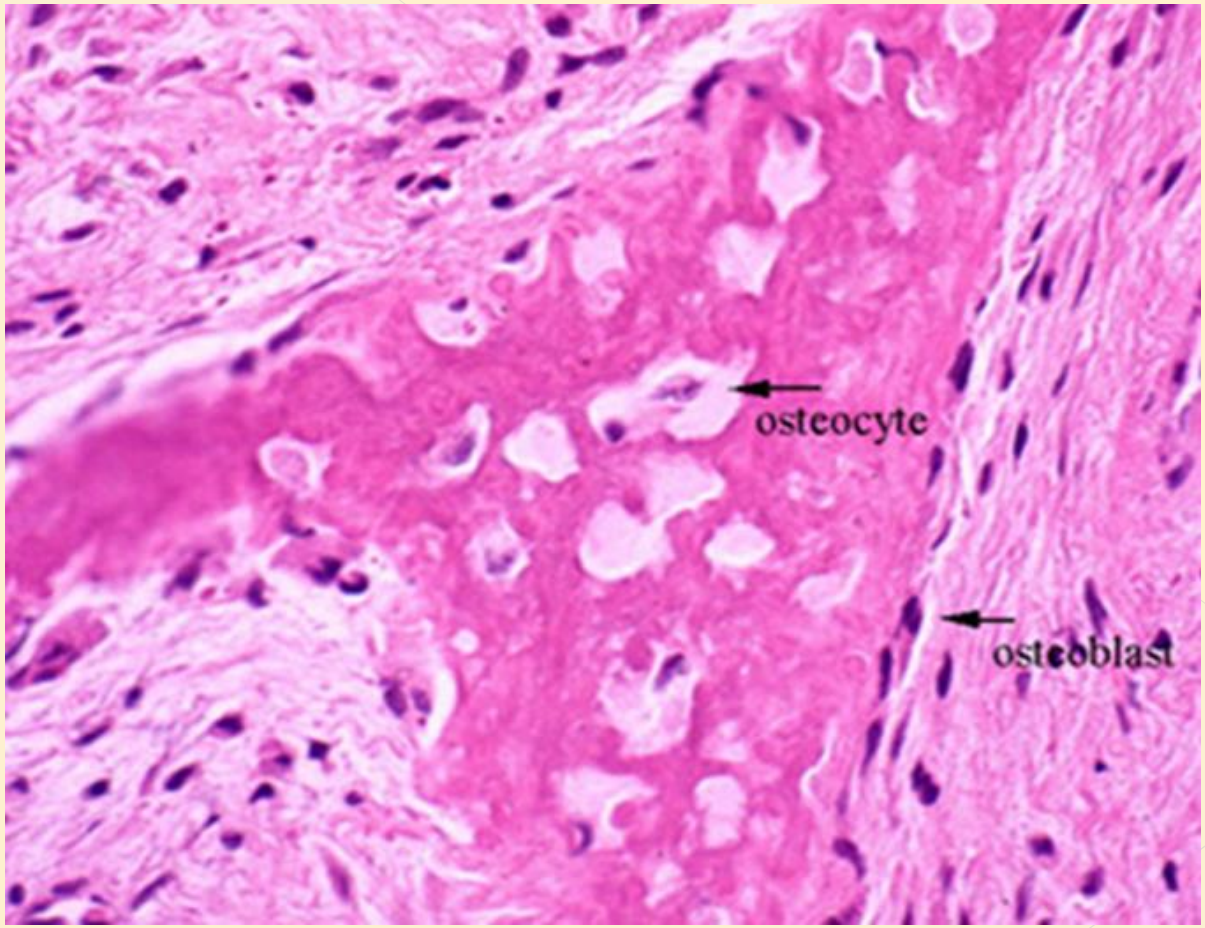
Osteoblasts (bone forming cells)

- synthesis of organic components of the bone matrix.
- on the surface of the bone
- trapped in the forming bone differentiate into:



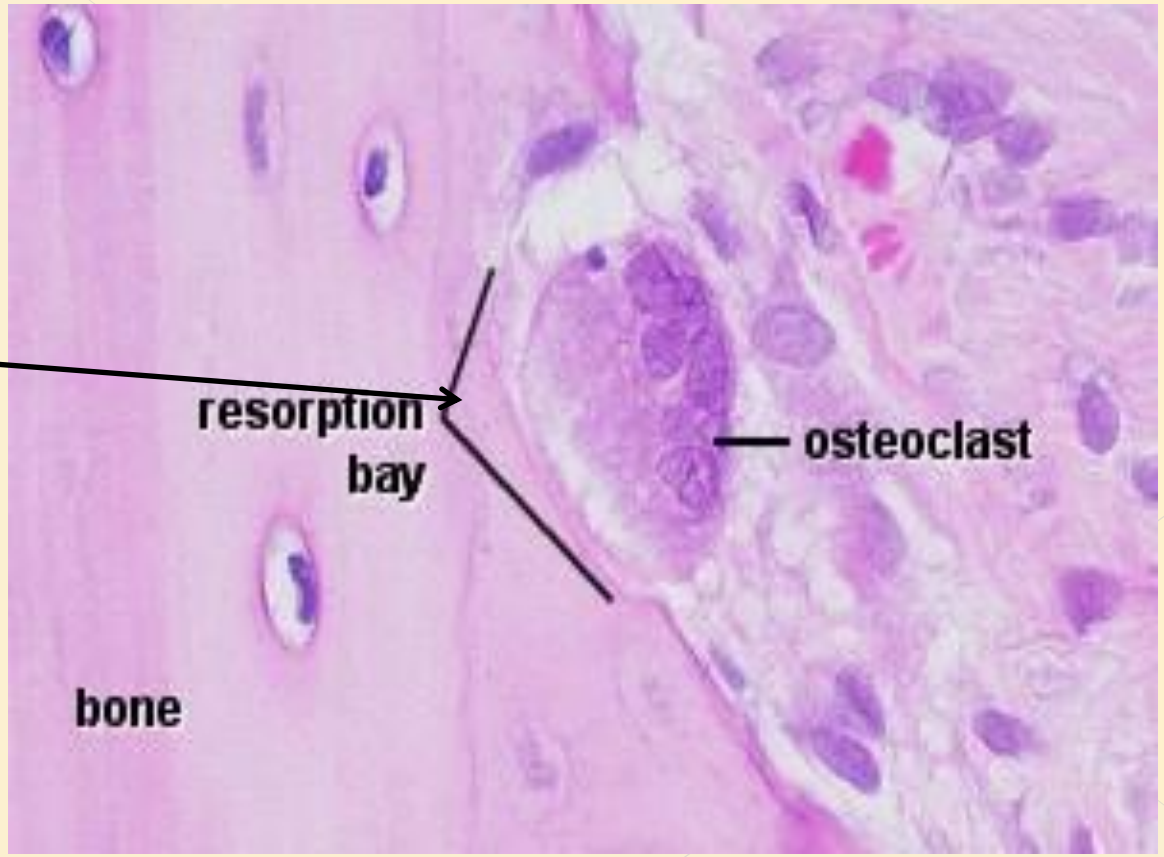
Osteocytes

- mature bone cells
- osteocytes and their processes reside inside spaces called lacunae and canaliculi, respectively

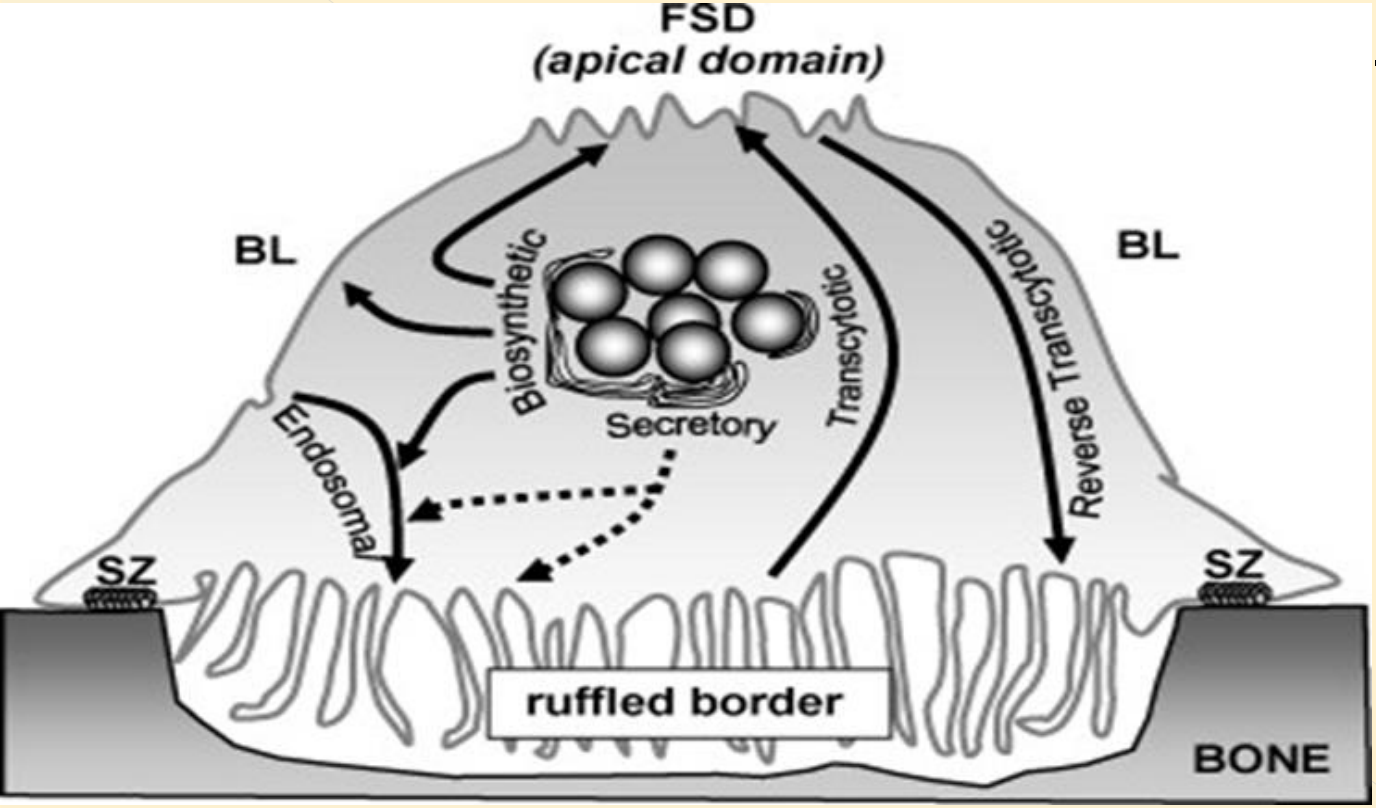


Osteoclasts - bone-resorbing cells

- large, multinucleated cells
- in Howship's lacunae – regions of bone resorption
- activity of osteoclasts - stimulated by **parathyroid hormone** (parathyroid gland) - inhibited by **calcitonin** (thyroid gland)

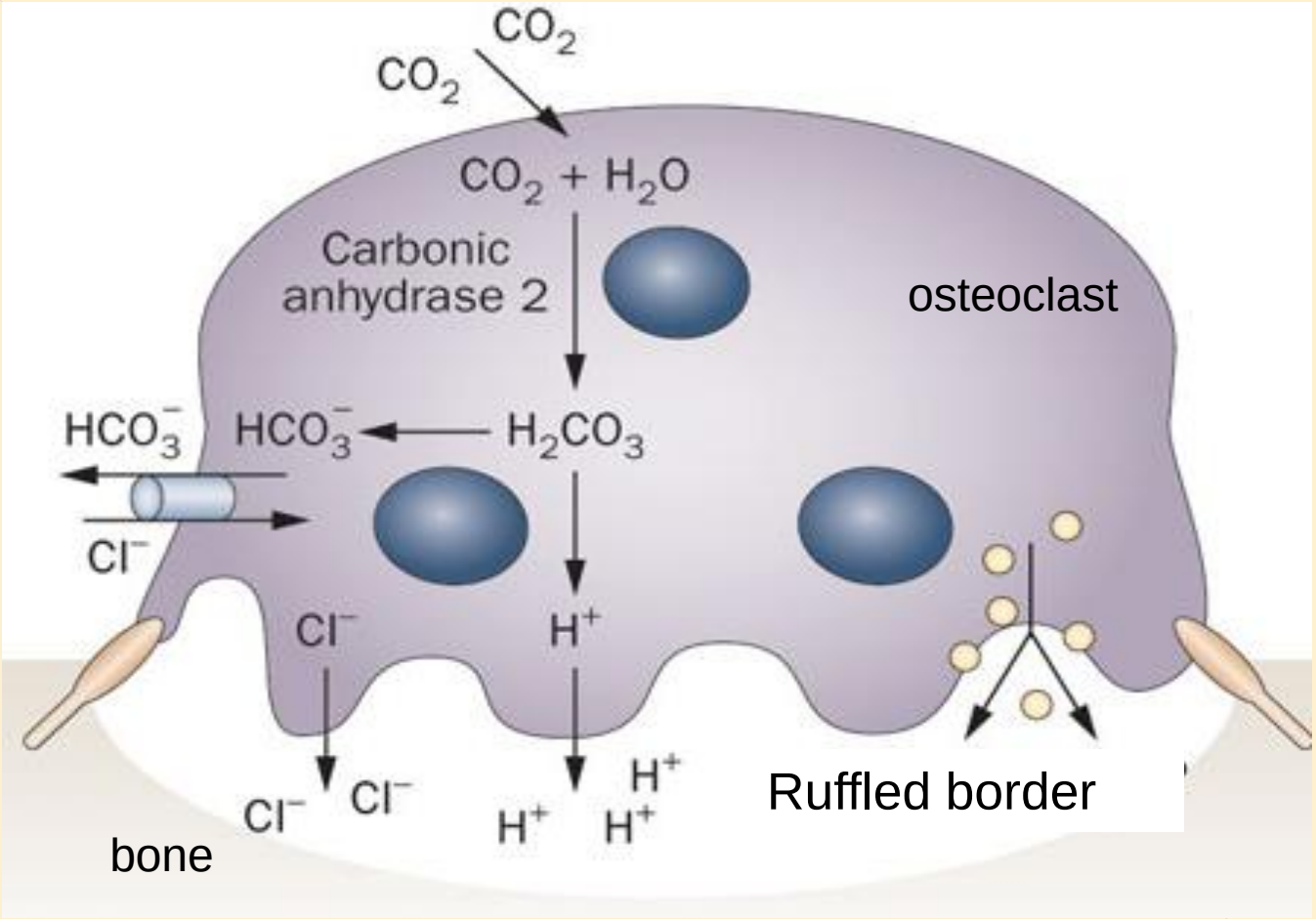


- ruffled border – a part of the cell directly involved in bone resorption - (finger-like processes)



- **organic components** of the matrix destroyed by lysosomal enzymes

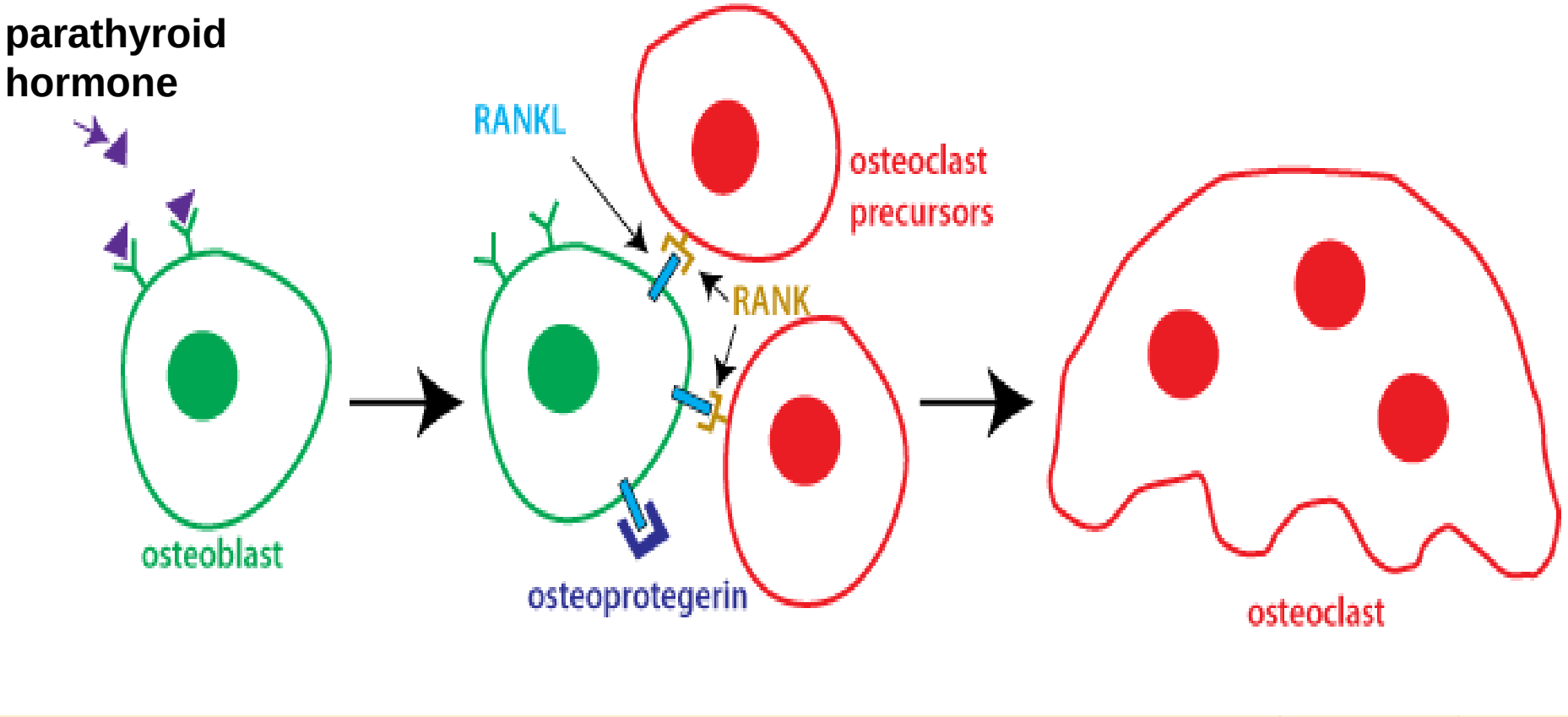
- lysosomal enzymes (**catepsin K, collagenase, gelatinase**) released into the space between the ruffled border and the bone matrix - break down the **organic components** of the matrix



- **inorganic components** of the bone matrix dissolved in acidic environment

- **carbonic anhydrase** - formation of carbonic acid - in the cytoplasm H_2CO_3 dissociates into H+ and bicarbonate ions
- proton pumps - transport H+ ions between ruffled border and bone, reducing the pH of environment

Resorption of the bone - stimulated by **parathyroid hormone (parathyroid glands)**



The relationship between osteoblasts and osteoclasts

M-CSF
Macrophage colony-stimulating factor

osteoblast

macrophage

macrophage

macrophage

RANK

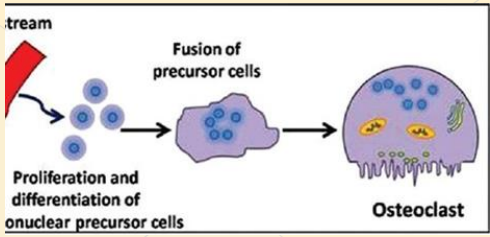
osteoclasts precursors

- proliferation
- induction of expression of RANK on the **osteoclasts precursors**

RANKL

RANK + RANKL

osteoclasts precursors



Osteoclast differentiation inhibited by **osteoprotegerin (OPG)**

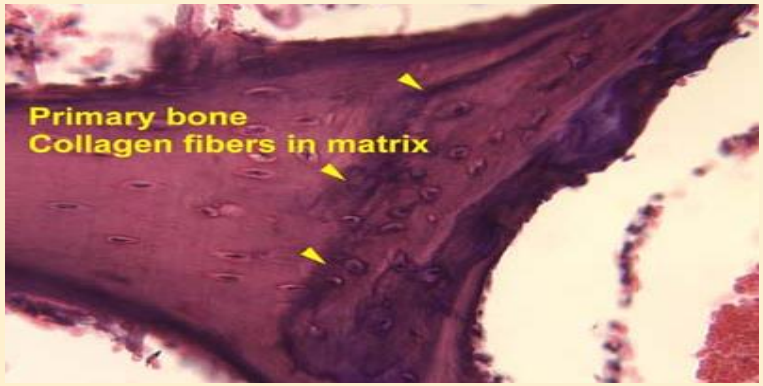
- produced by osteoblasts - binds RANKL - inhibition of osteoclasts formation

Osteoclasts precursors differentiate into the multinucleated osteoclasts

Bone Types

Primary bone
= immature bone
= woven bone

- first bone in fetal development and bone repair
- abundant osteocytes,
- irregular bundles of collagen, low mineral content



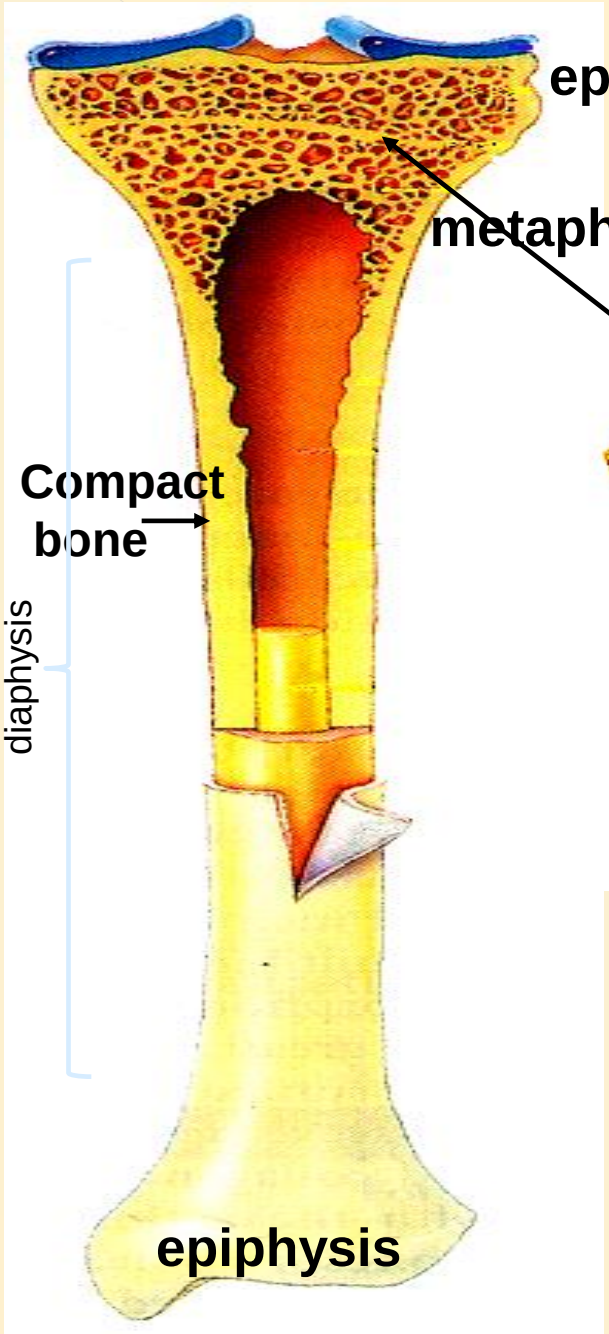
Secondary bone
= mature bone
= lamellar bone

- parallel or concentric bony lamellae, osteocytes between lamellae
- high mineral content

Compact bone

Spongy bone

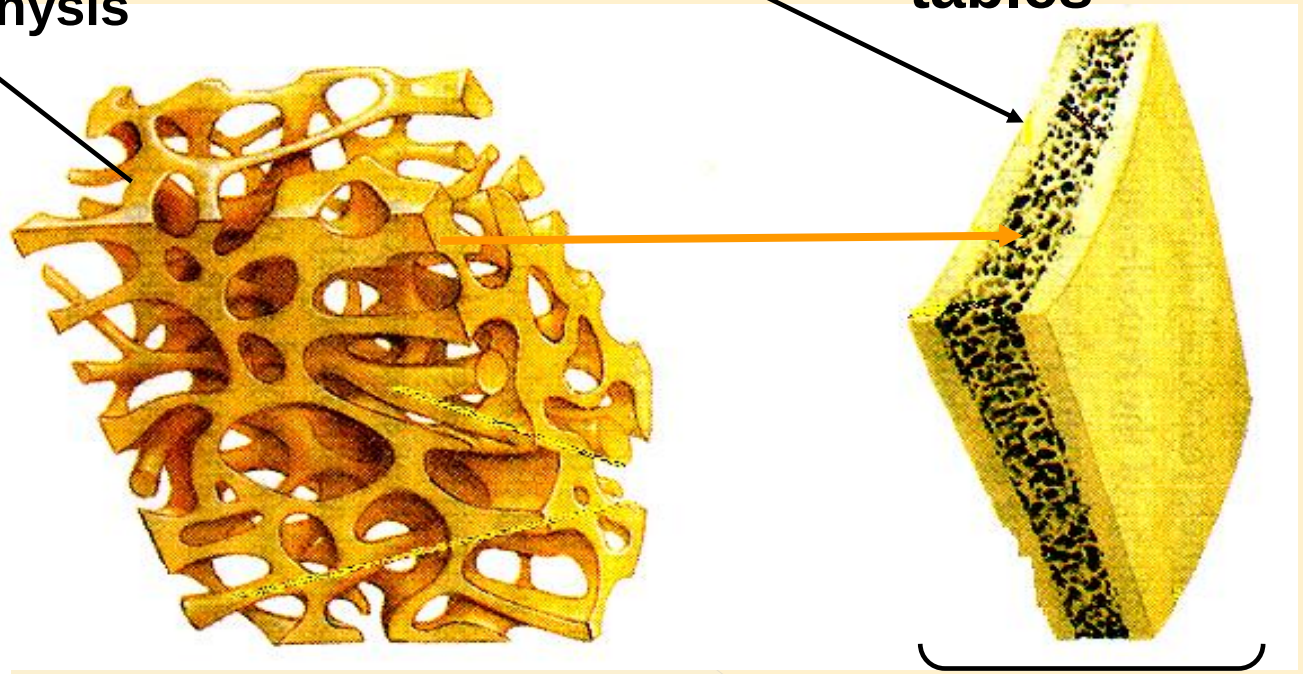
Long bone



Secondary bone

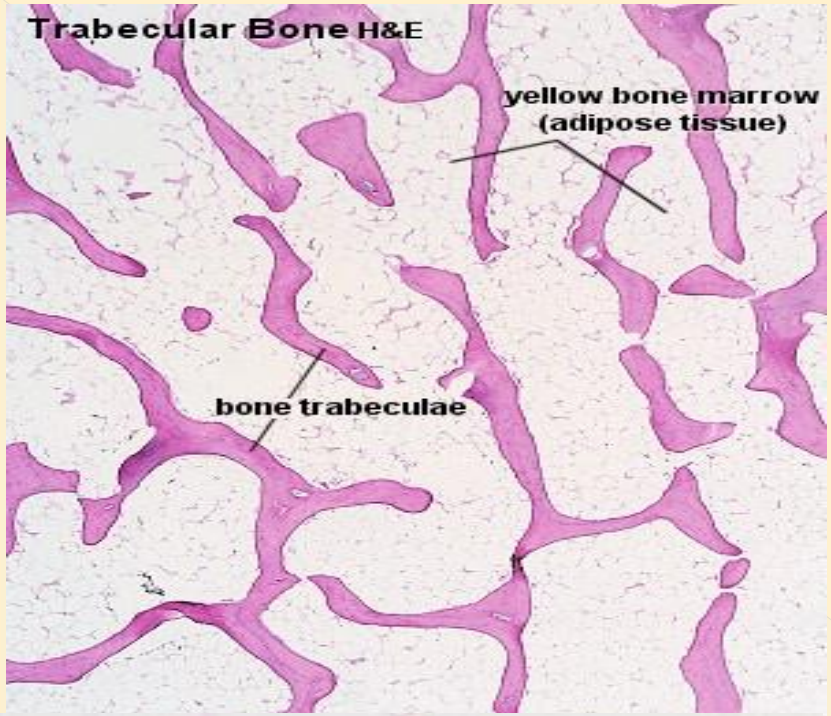
Compact bone

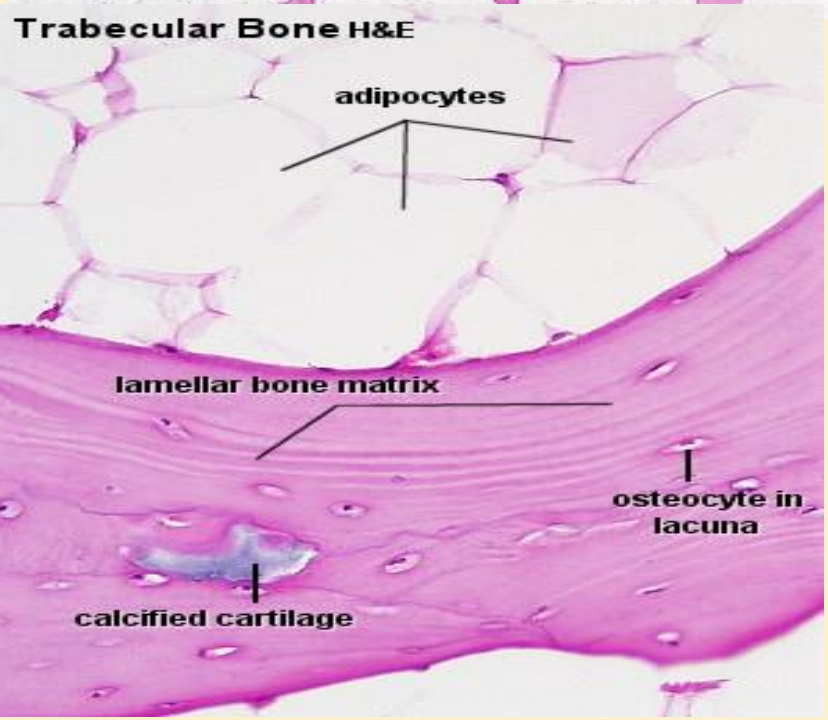
Inner and outer tables



Spongy or cancellous bone

Flat bone





spongy bone = cancellous bone = trabecular bone

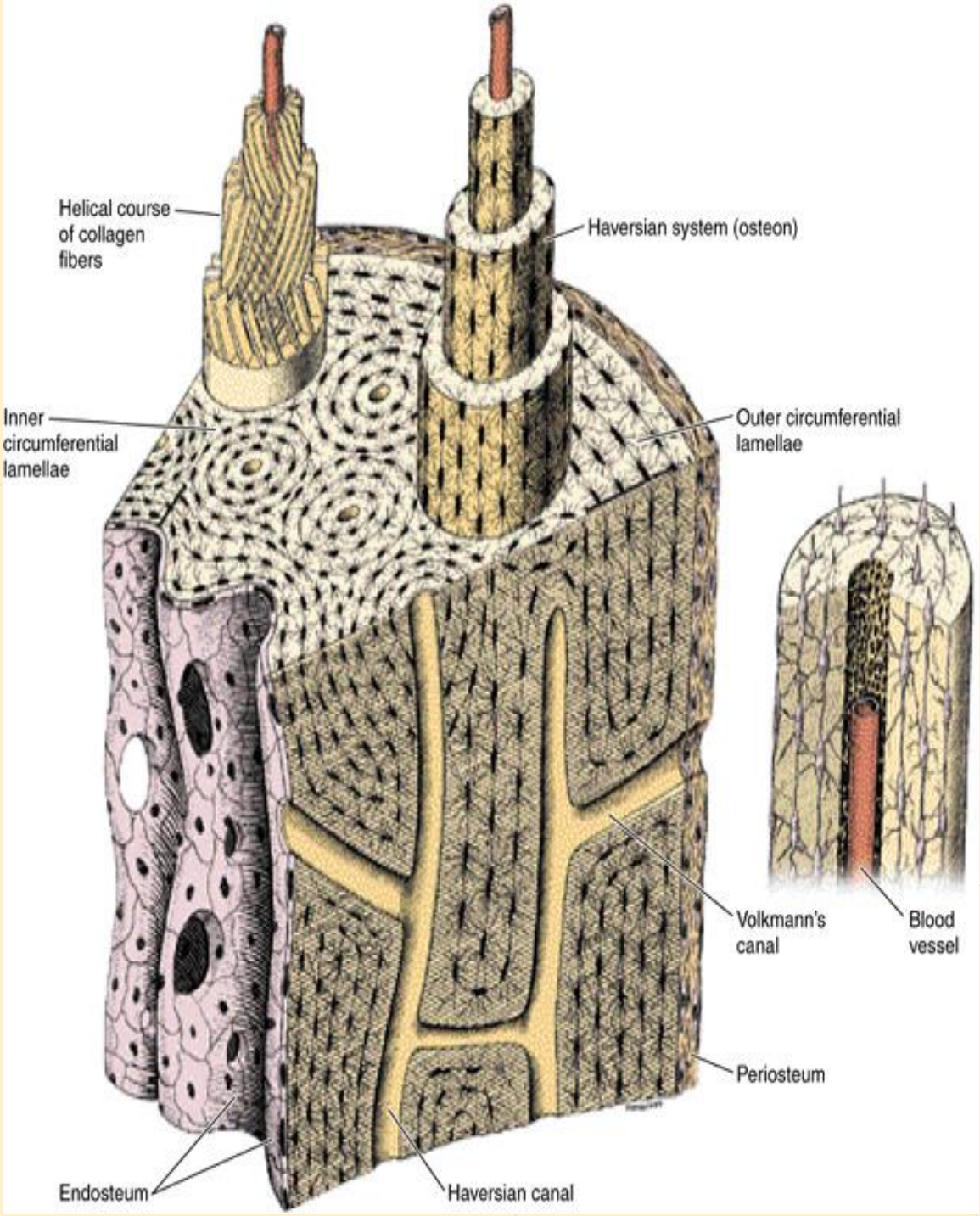
- interconnected meshwork of bony trabeculae
- spaces between trabeculae are filled by red or yellow bone marrow

- osteocytes in lacunae
- matrix of trabecular bone is formed by lamellae
- **Haversian systems are not present**

Compact bone

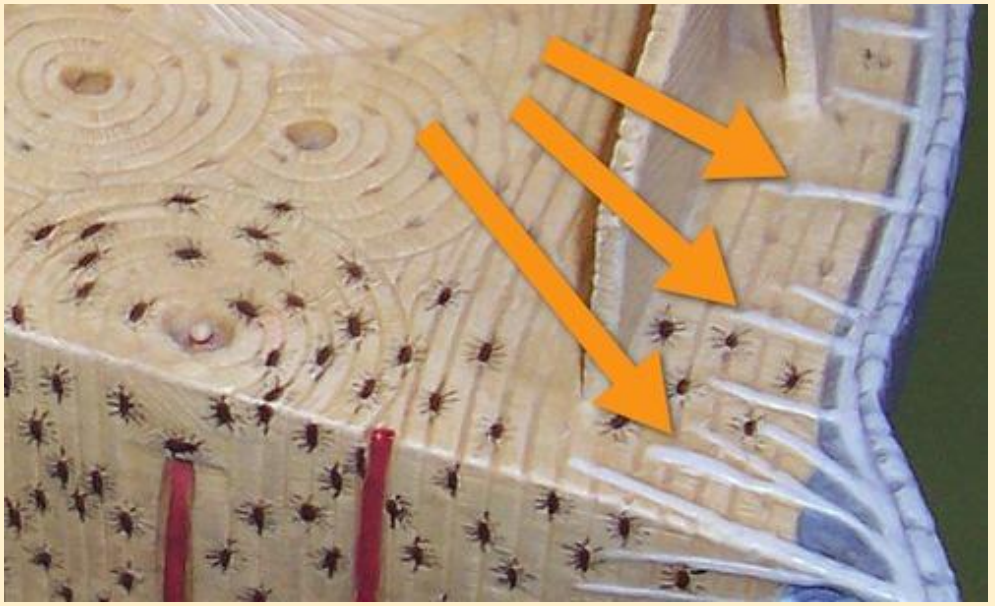
Four lamellar systems

1. Outer circumferential lamellae
2. Inner circumferential lamellae
3. Osteons (Haversian systems)
4. Interstitial lamellae



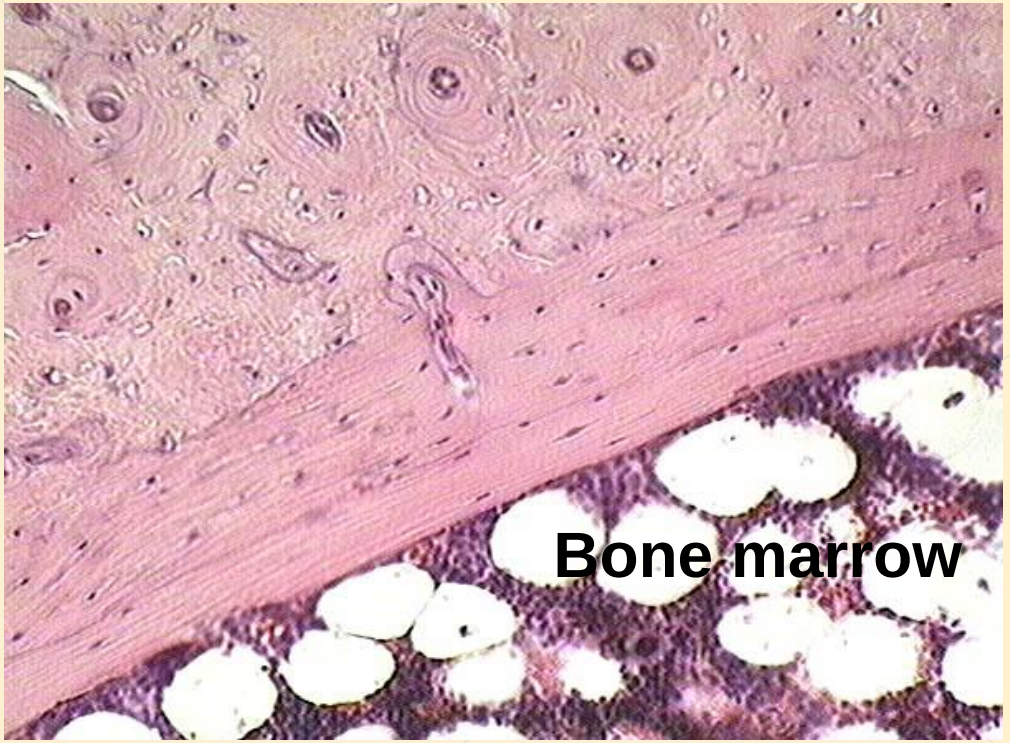
Outer circumferential lamellae

- just deep to the periosteum
- **Sharpey's fibers (type I collagen)** anchoring the periosteum to the bone



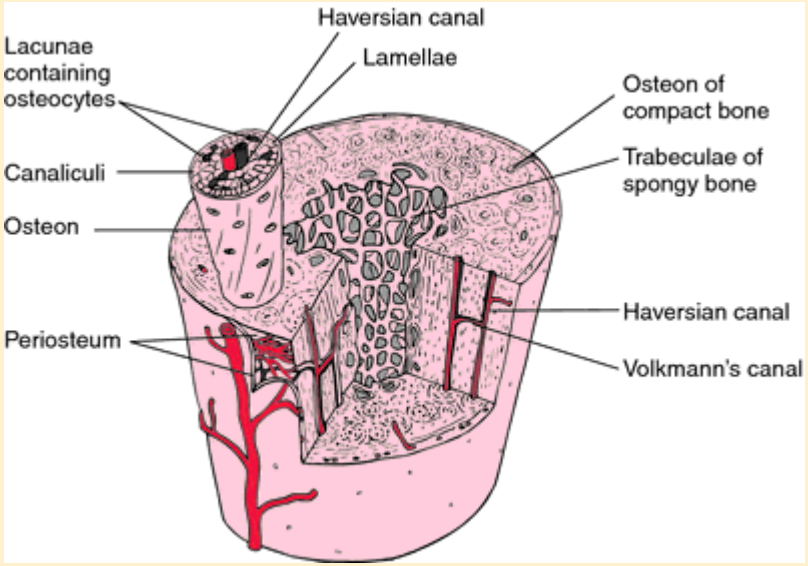
Inner circumferential lamellae

- completely encircle the marrow cavity

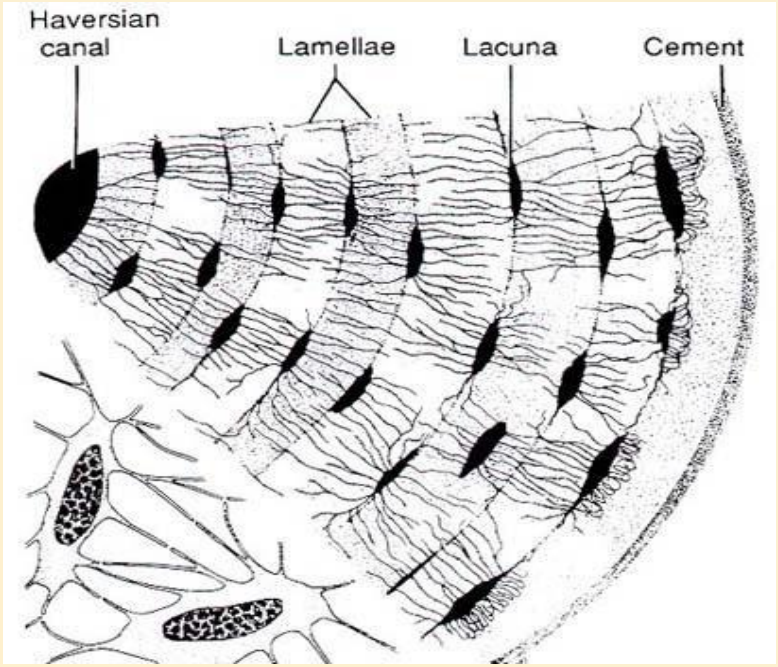


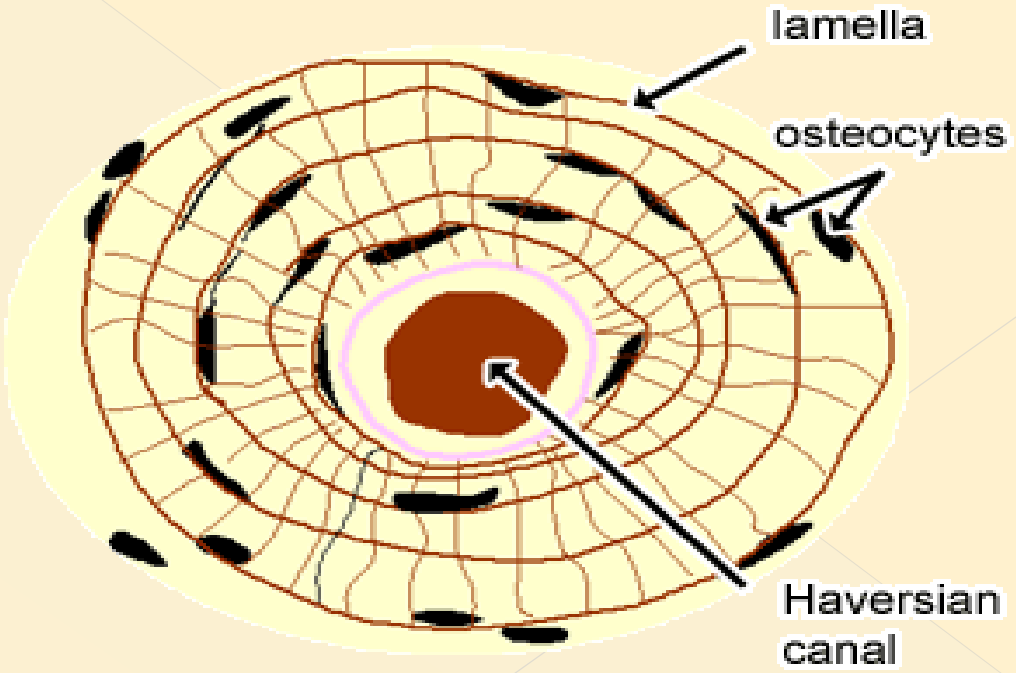
The bulk of compact bone is composed of:

HAVERSIAN SYSTEMS (OSTEONS)

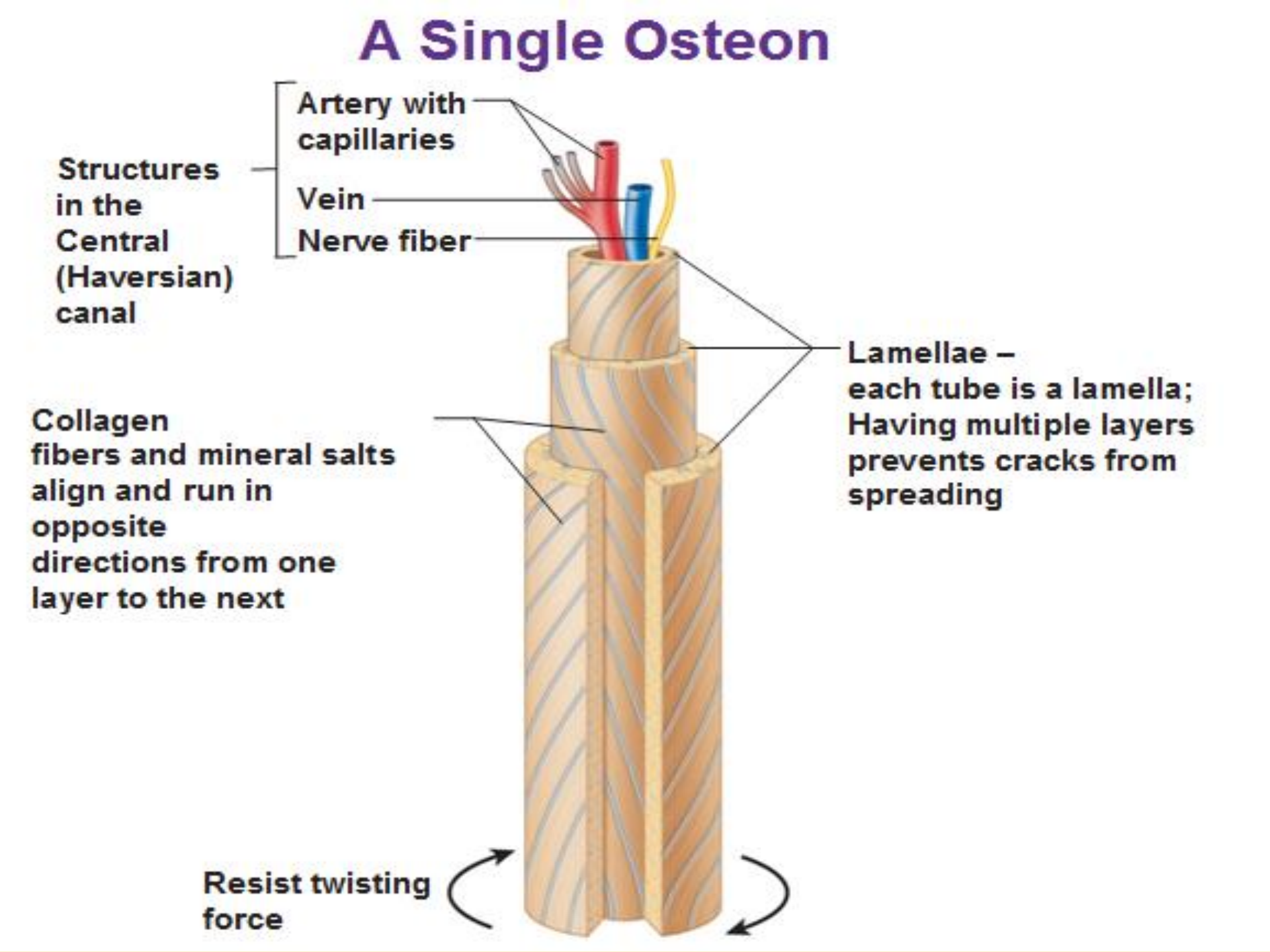


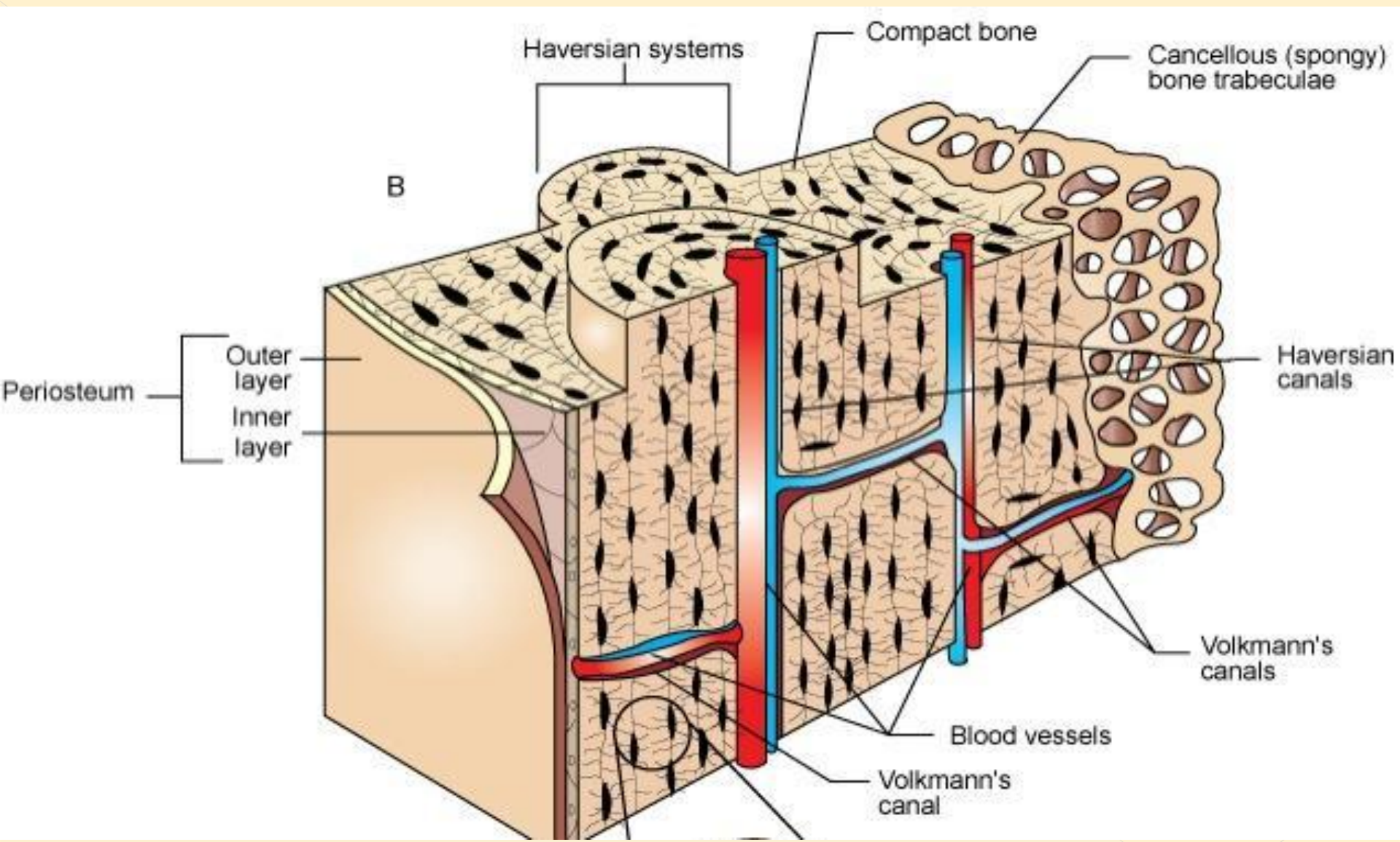
Haversian system - lamellae concentrically arranged around **haversian canal**





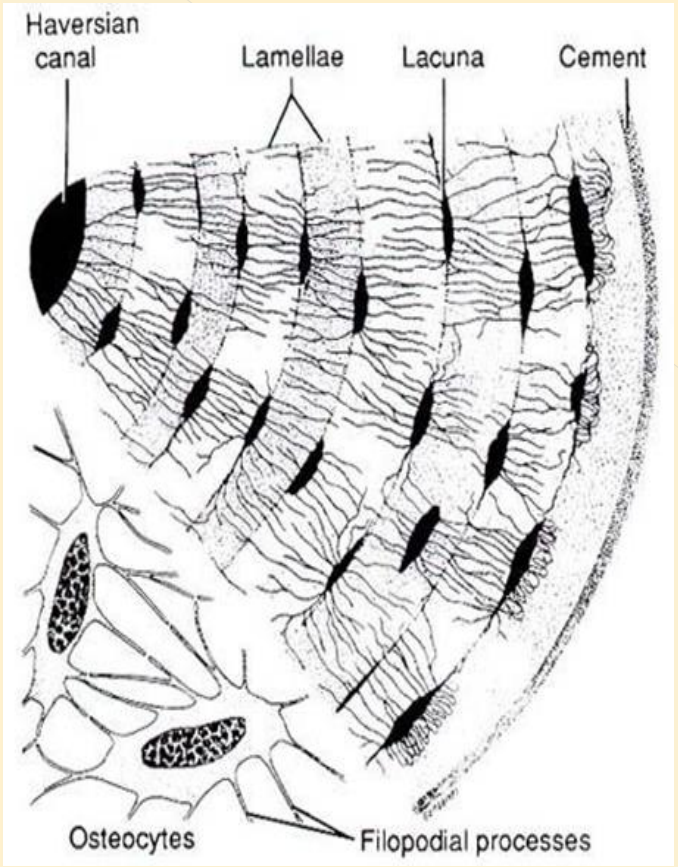
A Single Osteon

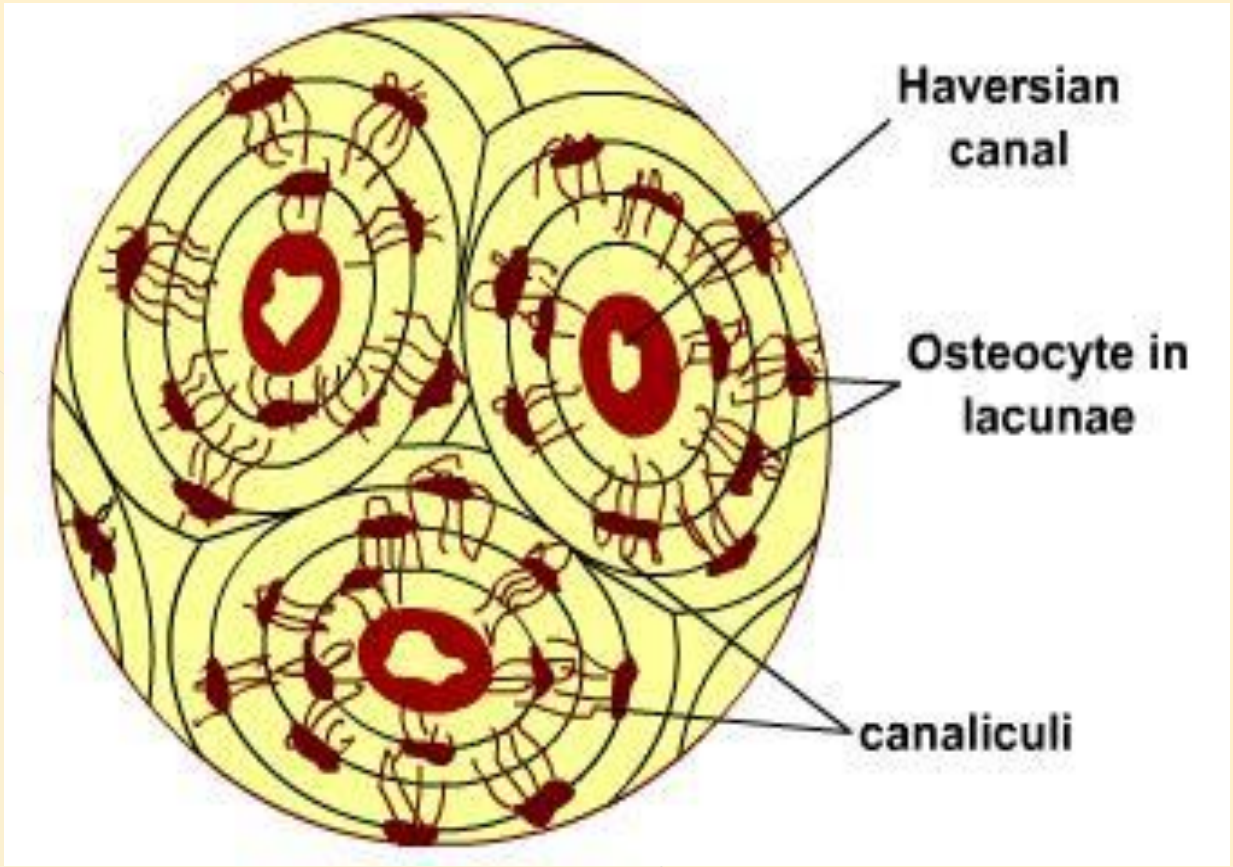




Volkmann's canals - establish connections of the Haversian canals with the inner and outer surfaces of the bone

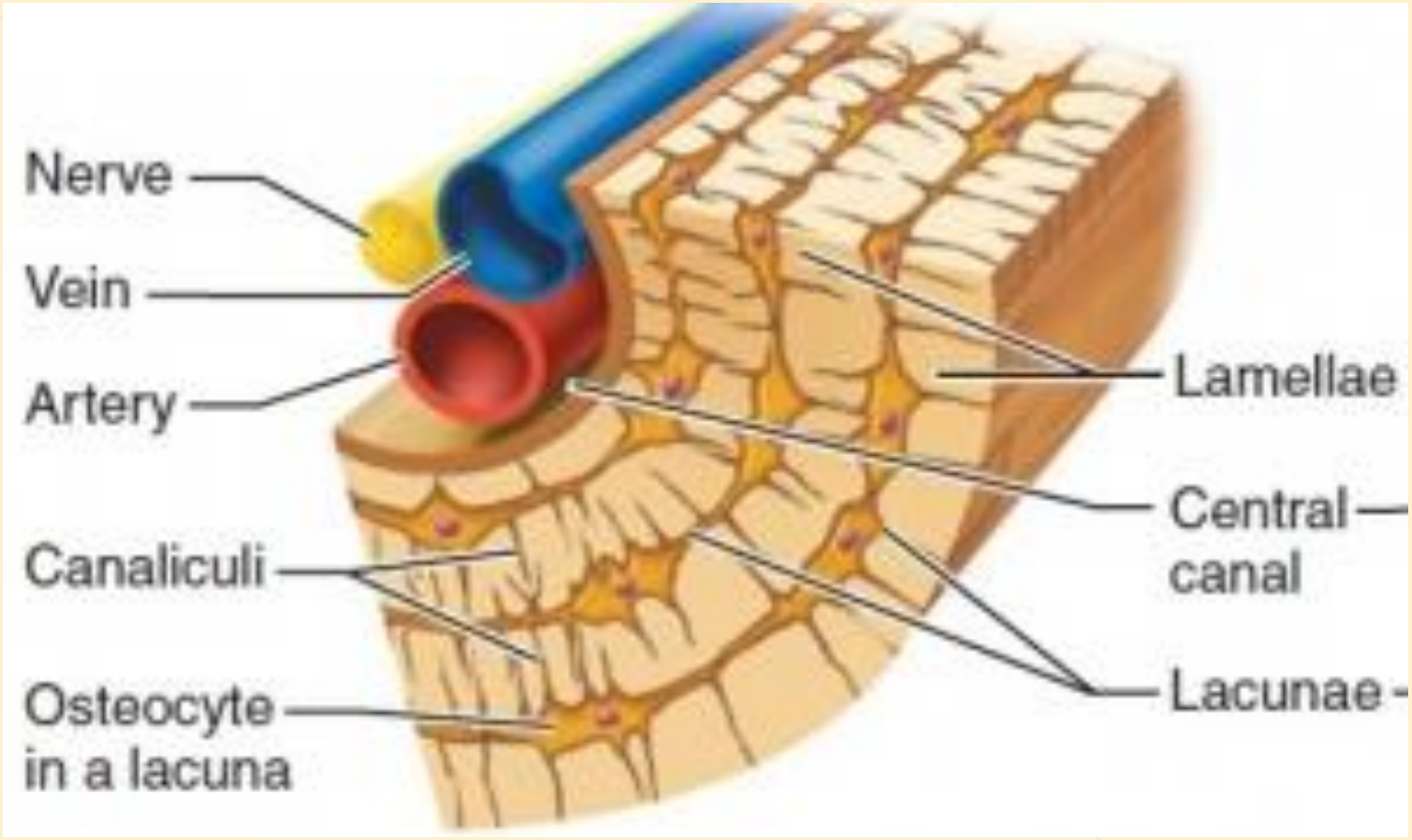
Osteocytes in bone lacunae



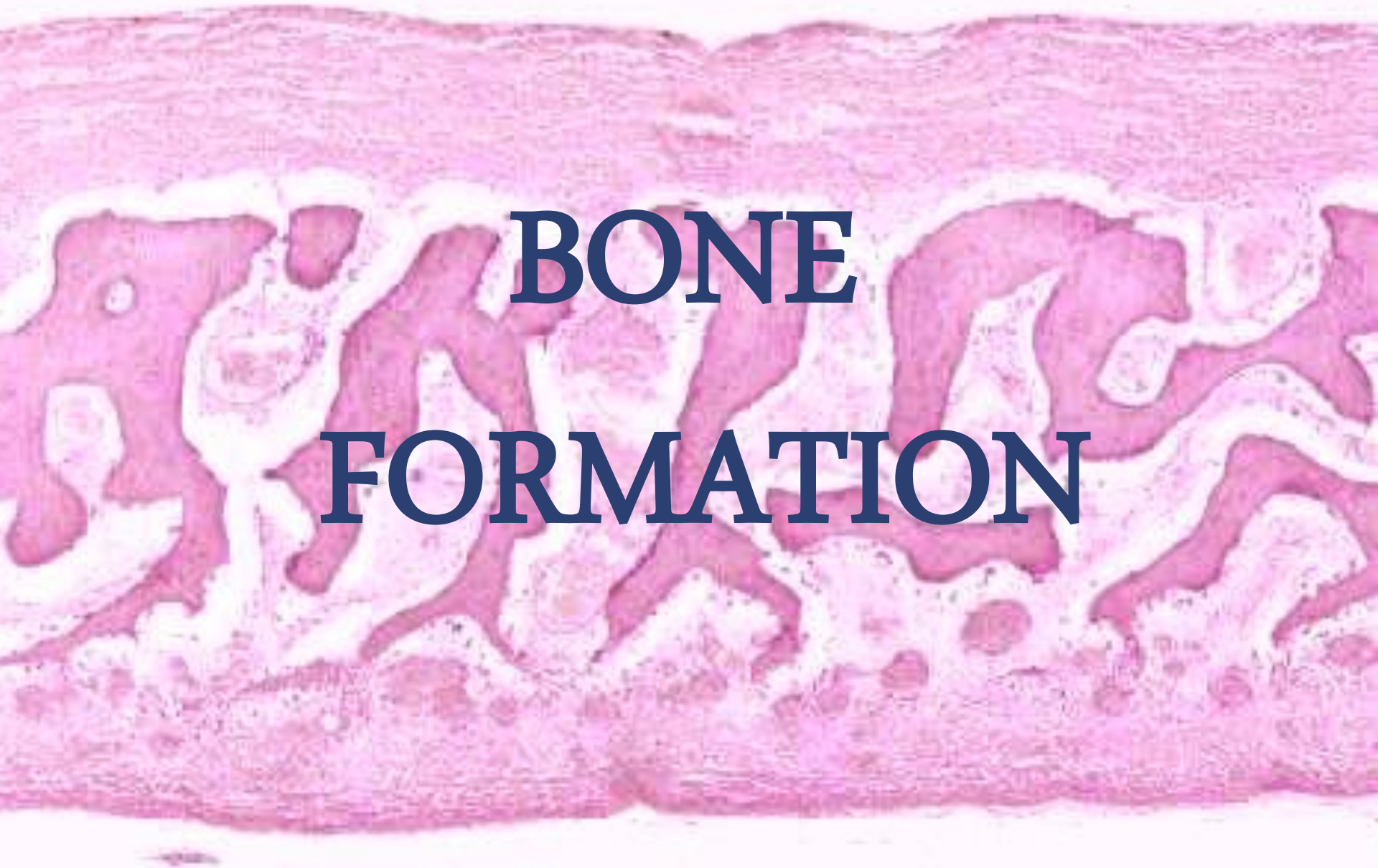


- cytoplasmic processes make contact with similar processes of neighboring osteocytes (**gap junctions** - ions and small molecules move between the cells
- cytoplasmic processes located in **canaliculi**

How do osteocytes get nutrients?



- canaliculi are places for exchange of nutrients and waste products
- nutrients from blood vessels of Haversian canal are transported from cell to cell through gap junctions



BONE FORMATION

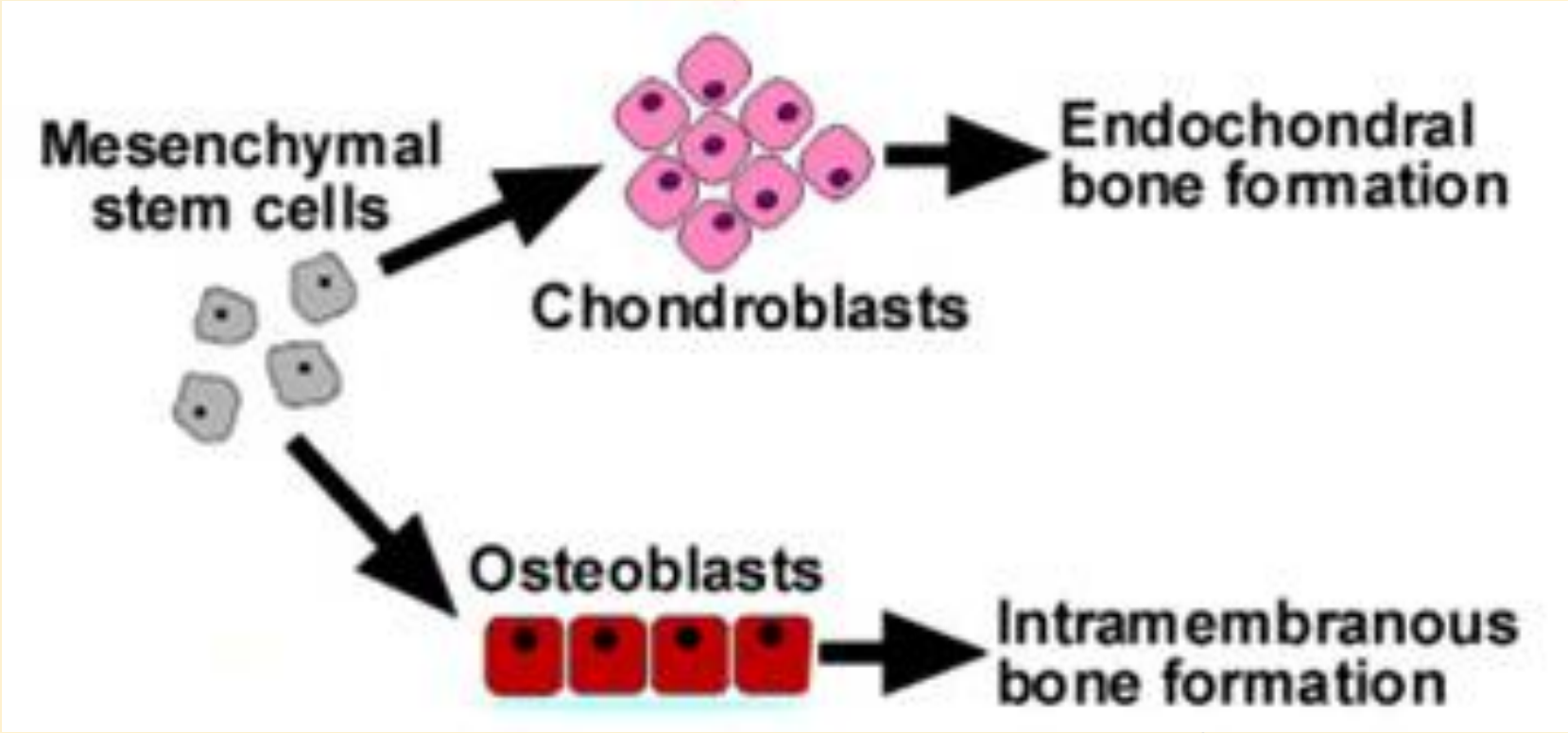
Bone formation

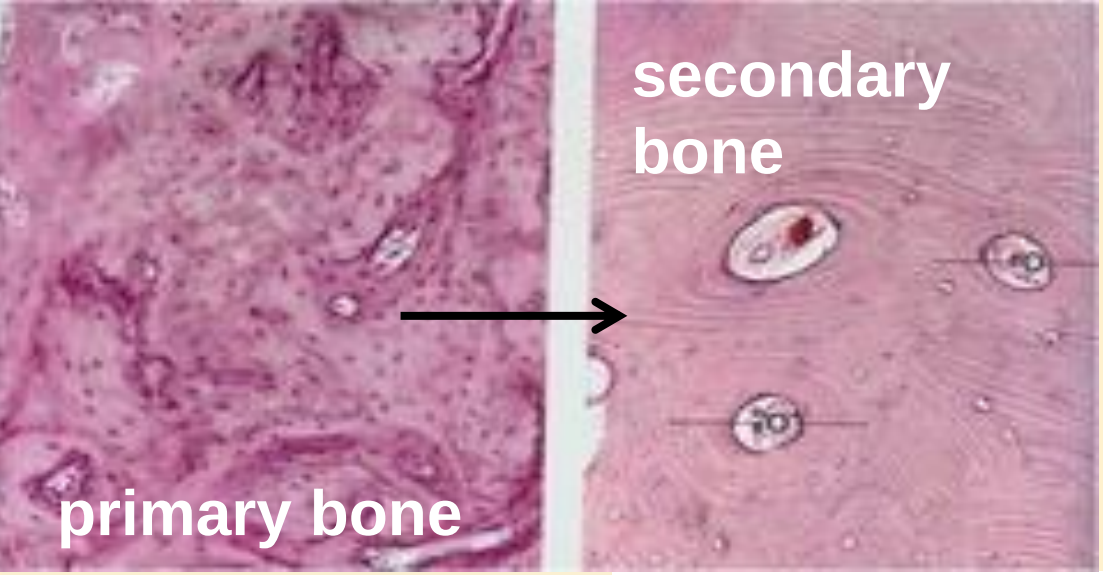
intramembranous

- **flat bones**
- in a richly vascularized mesenchymal tissue

endochondral

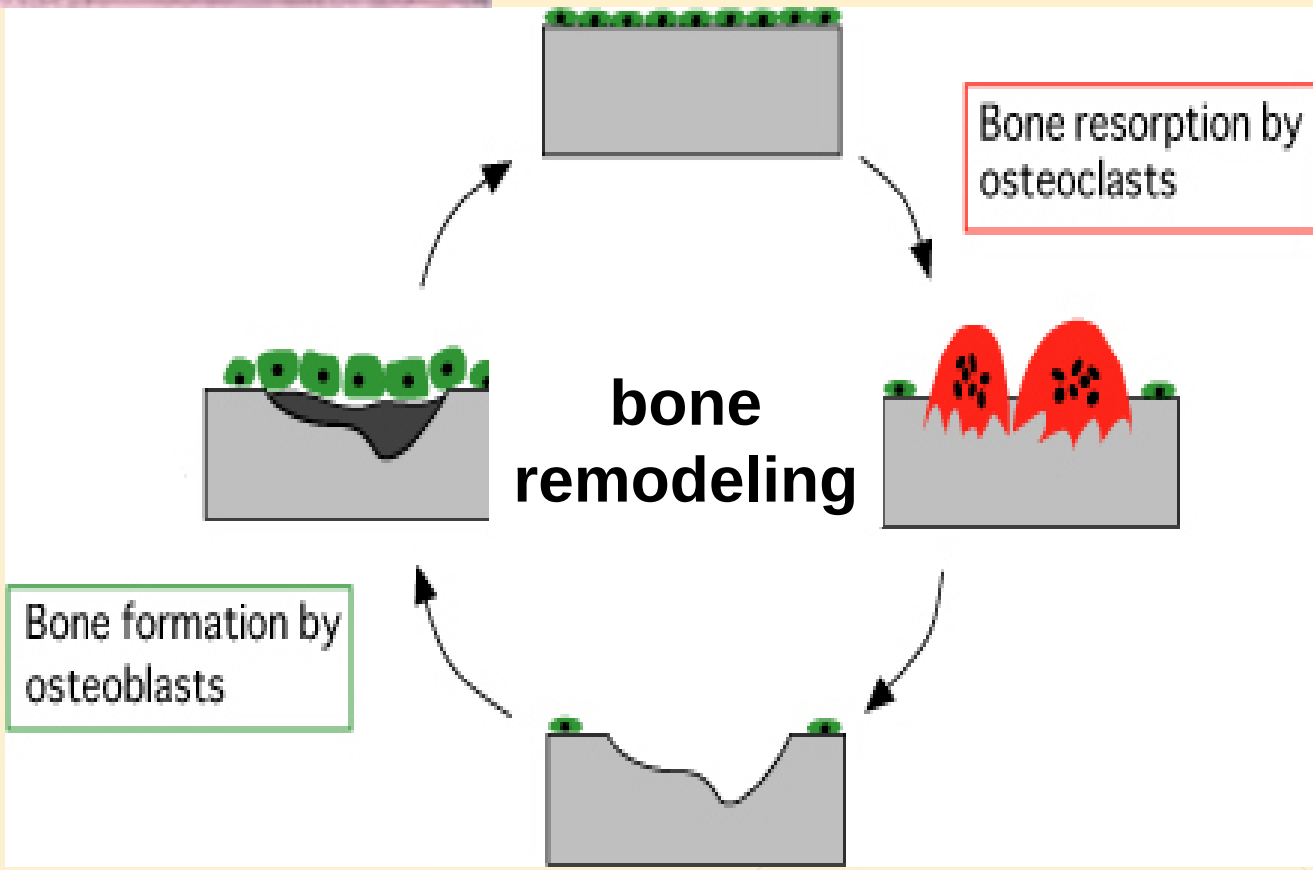
- **long and short bones**
- formation of a miniature **hyaline cartilage model**





The first bone – primary bone - replaced by **secondary bone** (continuous activity of osteoclasts and osteoblasts).

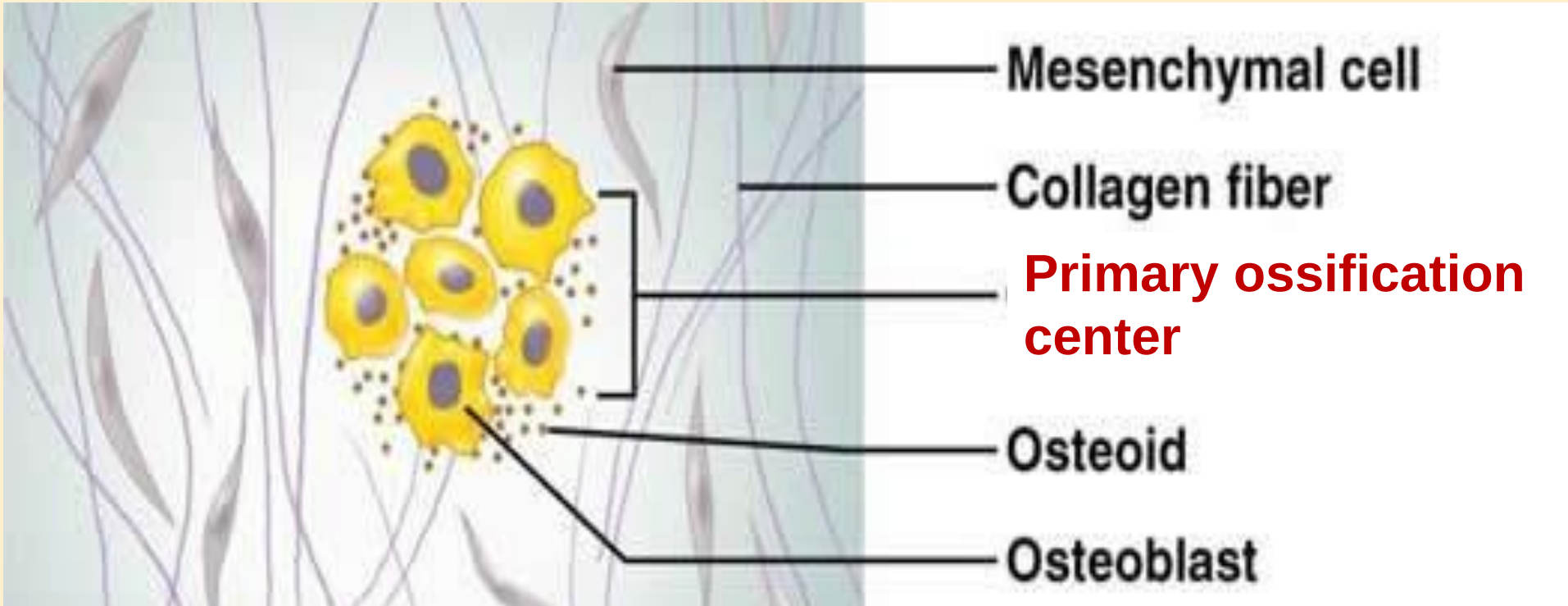
Secondary bone - resorbed and replaced throughout the life – **bone remodeling**.



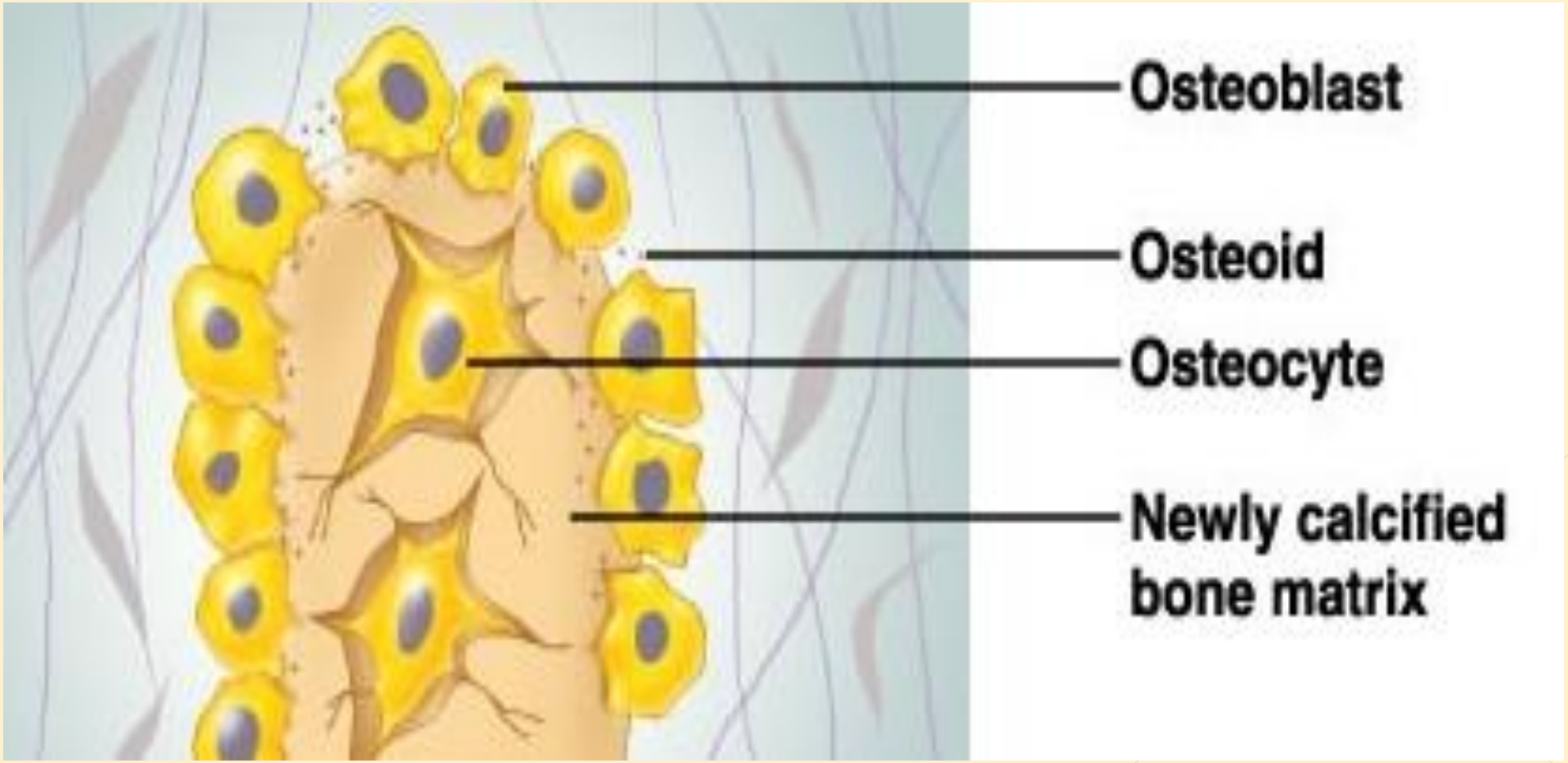
Intramembranous bone formation -

mesenchymal cells - form clusters

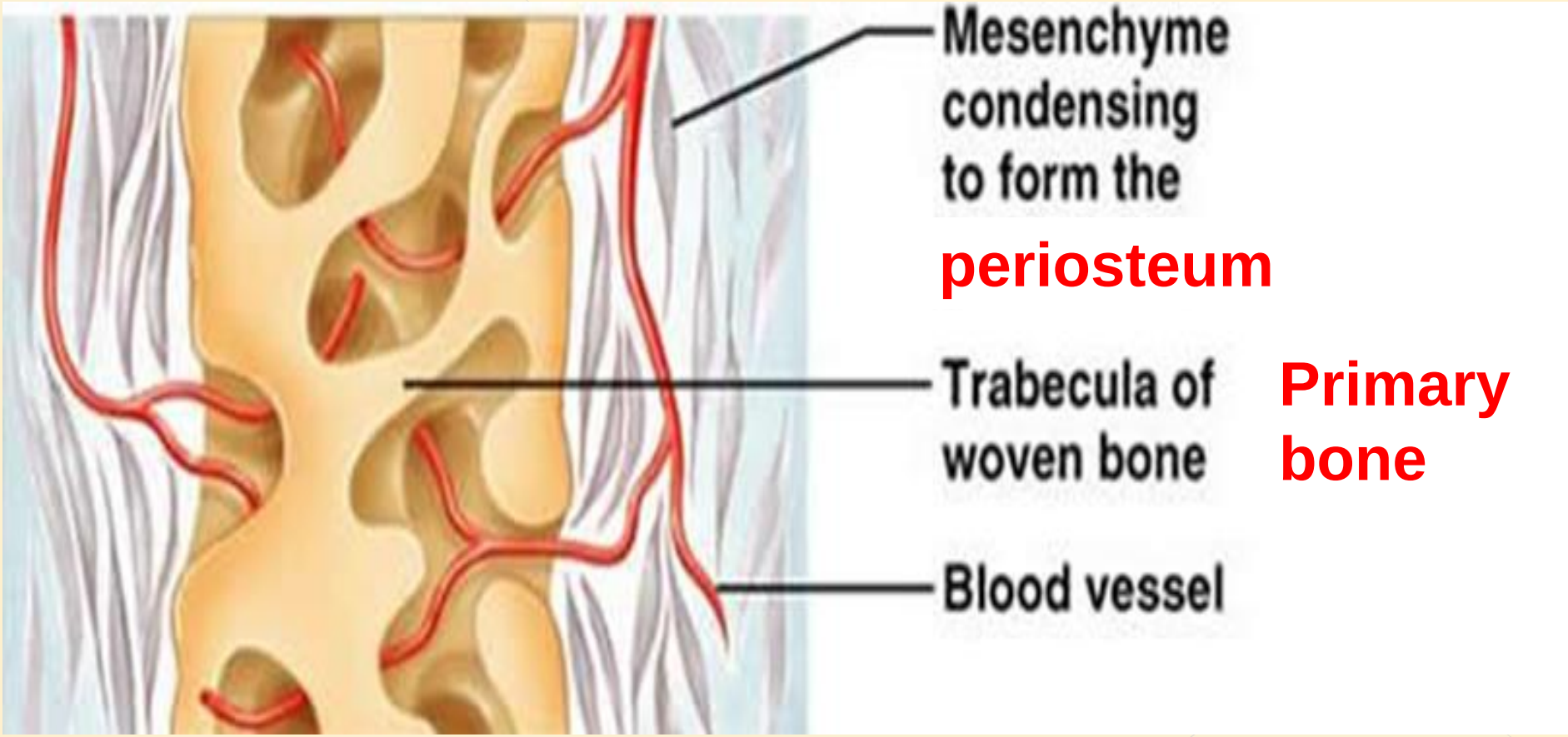
- differentiate into **osteoblasts** (**bone morphogenetic protein** (**BMP 2,4,6**) and **TGFβ** - Transforming growth factor beta
- secrete bone matrix (osteoid) - **primary ossification center.**



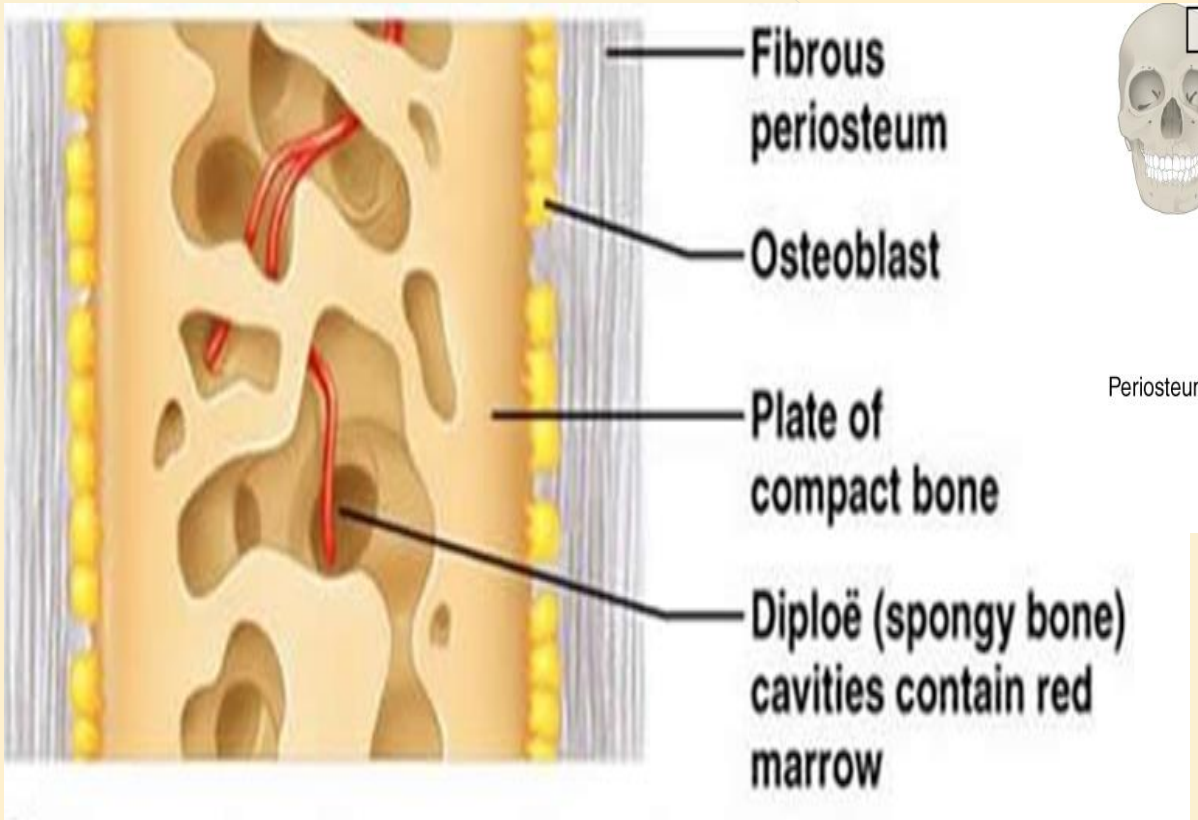
- **calcification of osteoid** - osteoblasts are trapped in matrix – **osteoblasts** become **osteocytes**

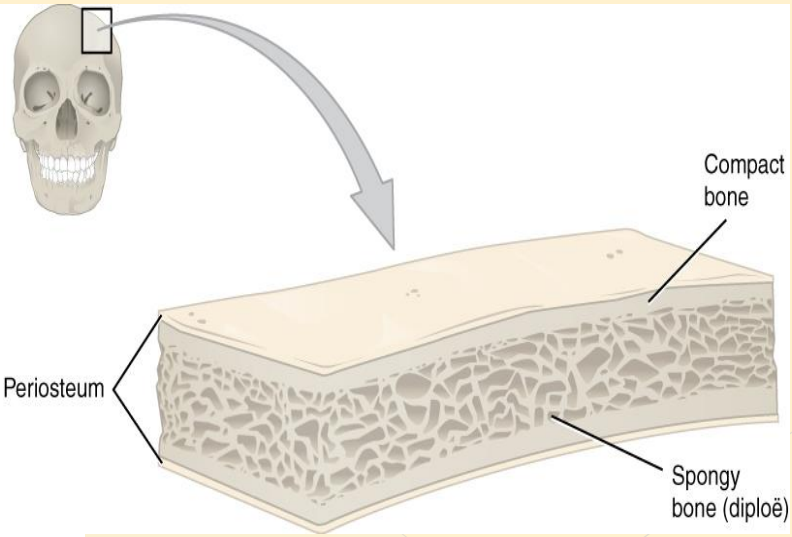


- **network of trabeculae -** calcified osteoid with osteocytes – **primary bone**
- blood vessels from mesenchyme enter the bone
- mesenchymal cells form **periosteum**

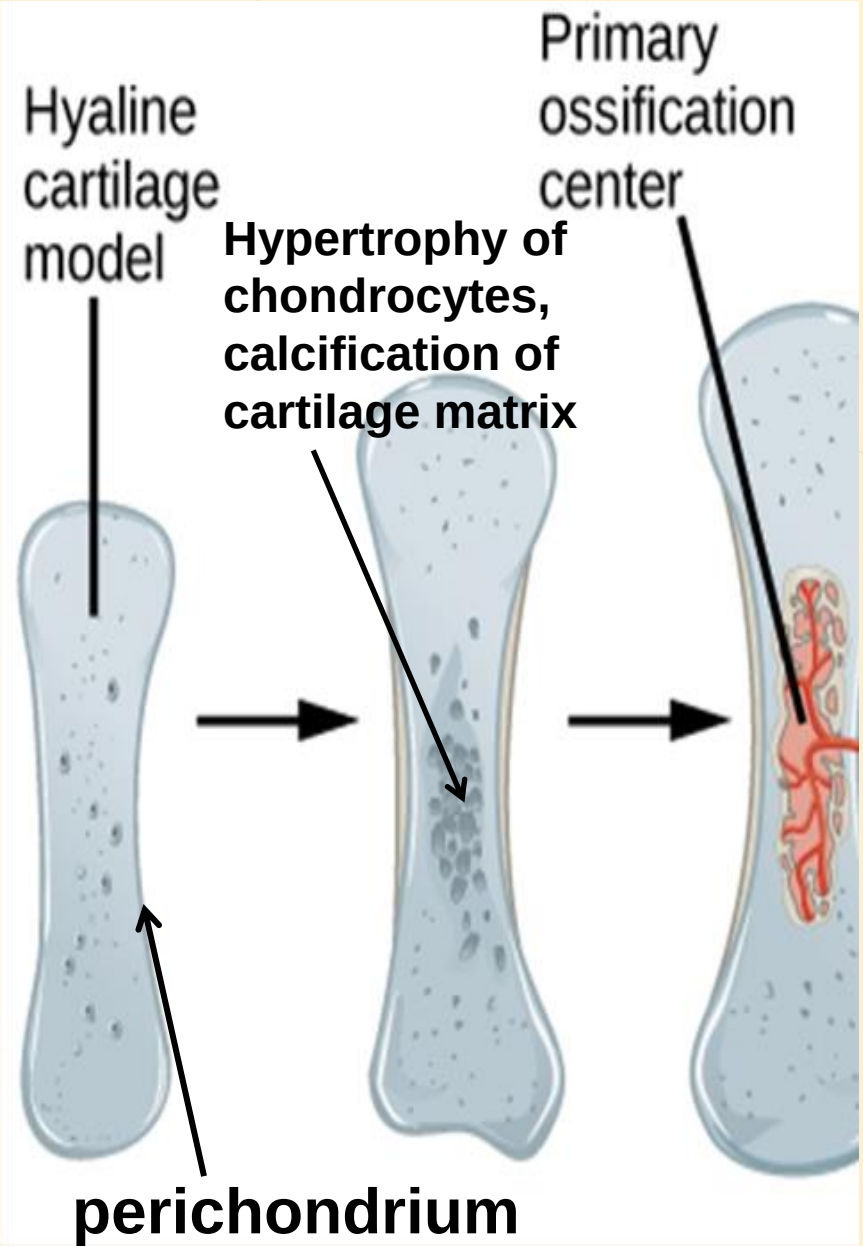


- primary bone - replaced by **secondary (lamellar) bone**
- vascular mesenchymal tissue (in **spongy bone**) transformed into **bone marrow**
- trabeculae on the periphery replaced with lamellar (**compact**) bone – **inner and outer tables**



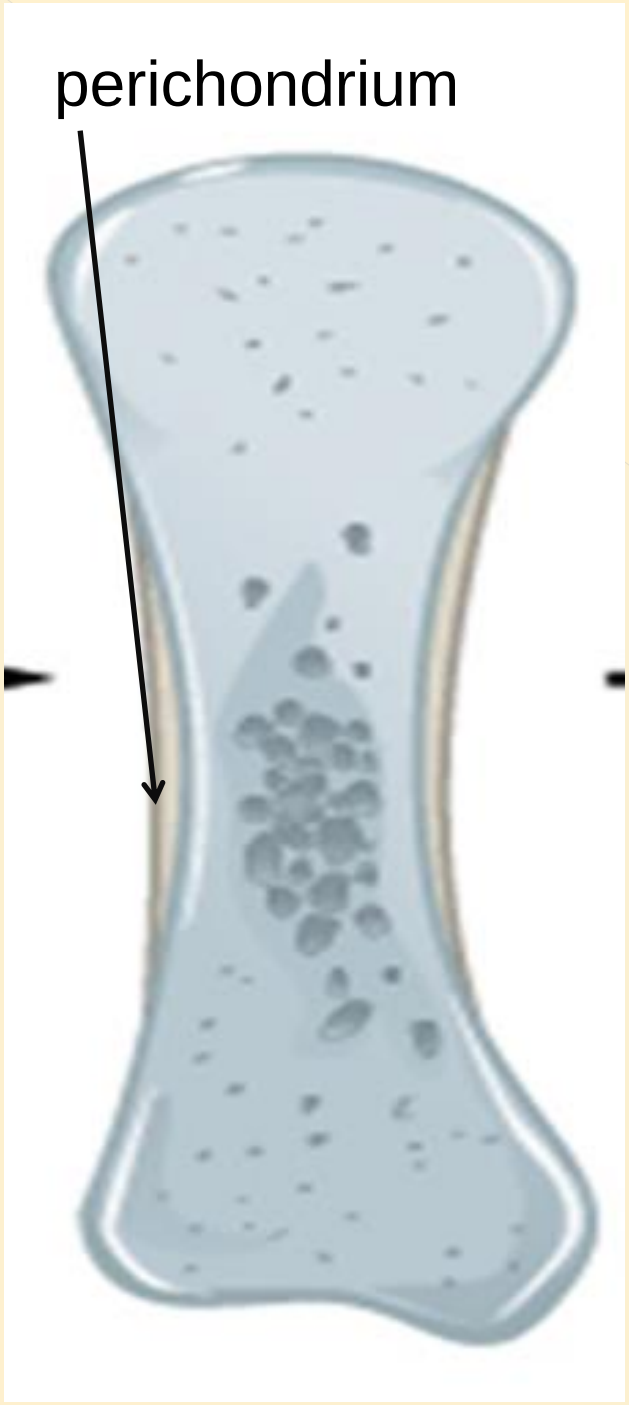


Formation of the primary ossification center - formation of miniature hyaline cartilage model



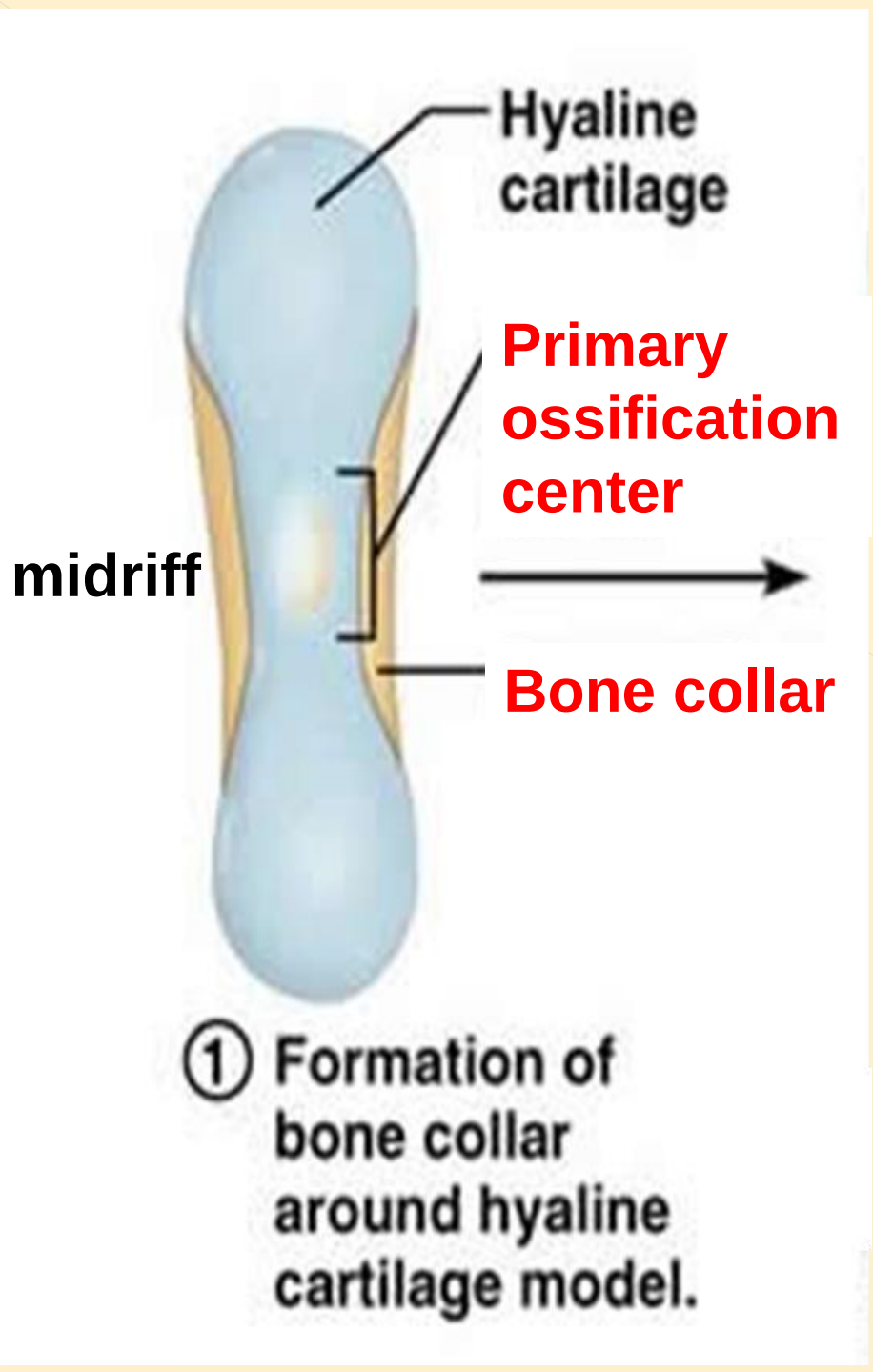
Primary ossification center:

- **hypertrophy** of chondrocytes in the center, production of **type X collagen** and accumulation of **glycogen** by chondrocytes (energy source).
- **calcification** of cartilage matrix between hypertrophied chondrocytes.



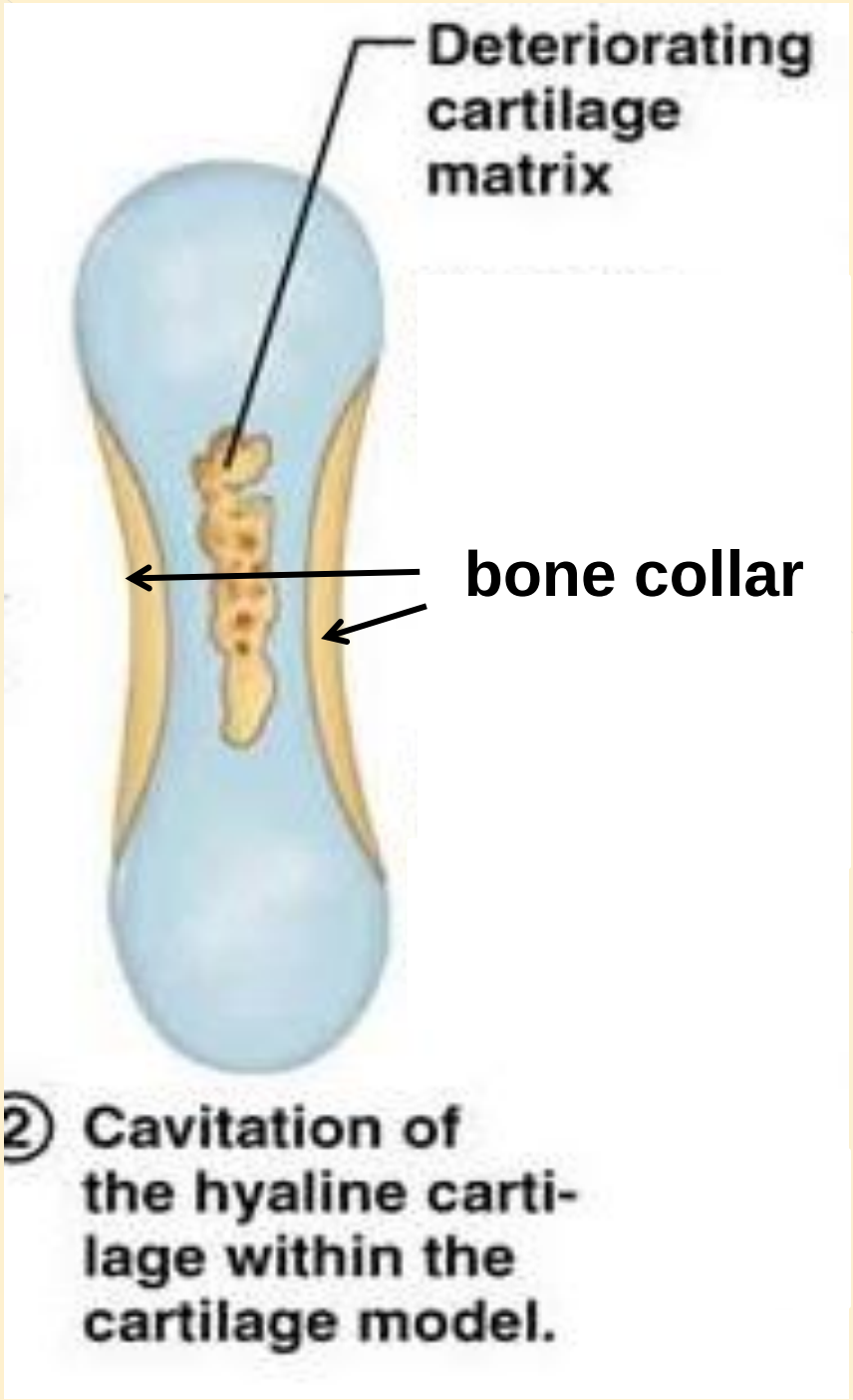
Endochondral bone formation

- transformation of chondrogenic cells of perichondrium into **osteoprogenitor cells**, later **osteoblasts** (**VEGF** - Vascular endothelial growth factor) - vascularization of perichondrium at the midriff of diaphysis



- Osteoblasts (from perichondrium) secrete bone matrix **– bone collar** on the surface of the cartilage model (**perichondrium becomes periosteum**)

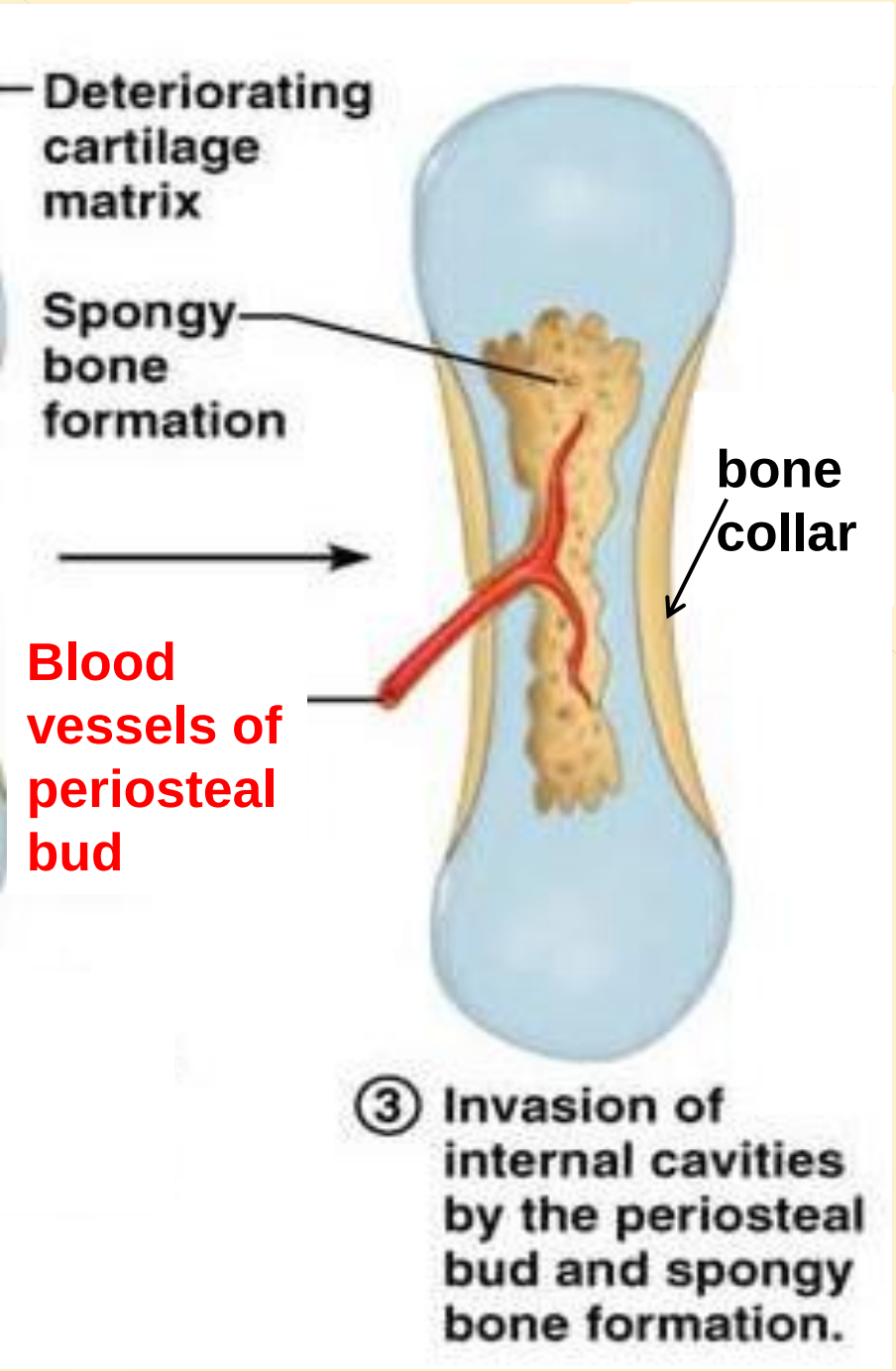
- **bone collar is formed by intramembranous bone formation (**hyaline cartilage model doesn't participate in this process) **– bone growth in width.**



Endochondral bone formation

- the diffusion of nutrients to hypertrophied chondrocytes is inhibited by the bone collar - **chondrocytes die**.

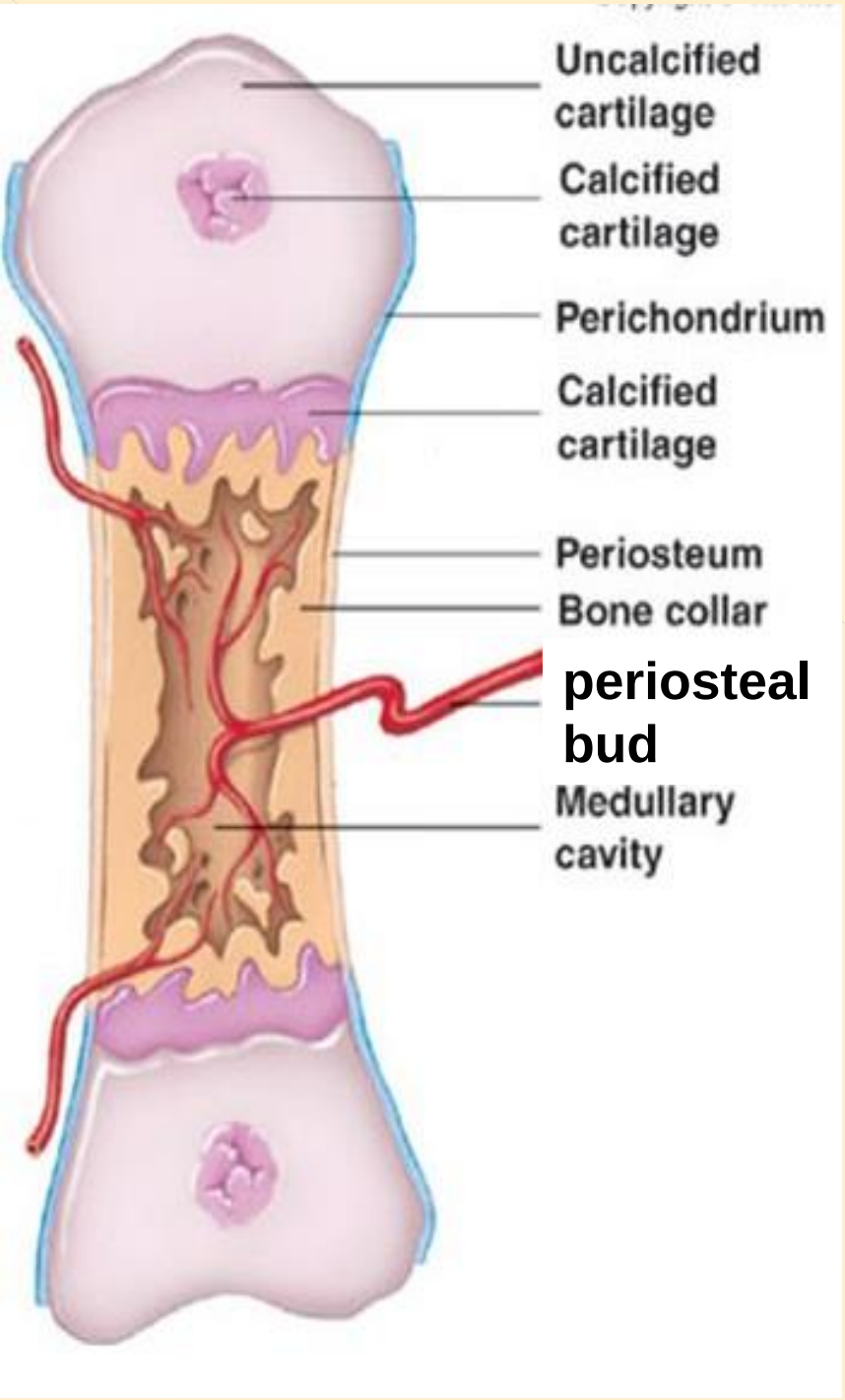
- empty lacunae connected to each other (cavitations) form future **marrow cavity**.



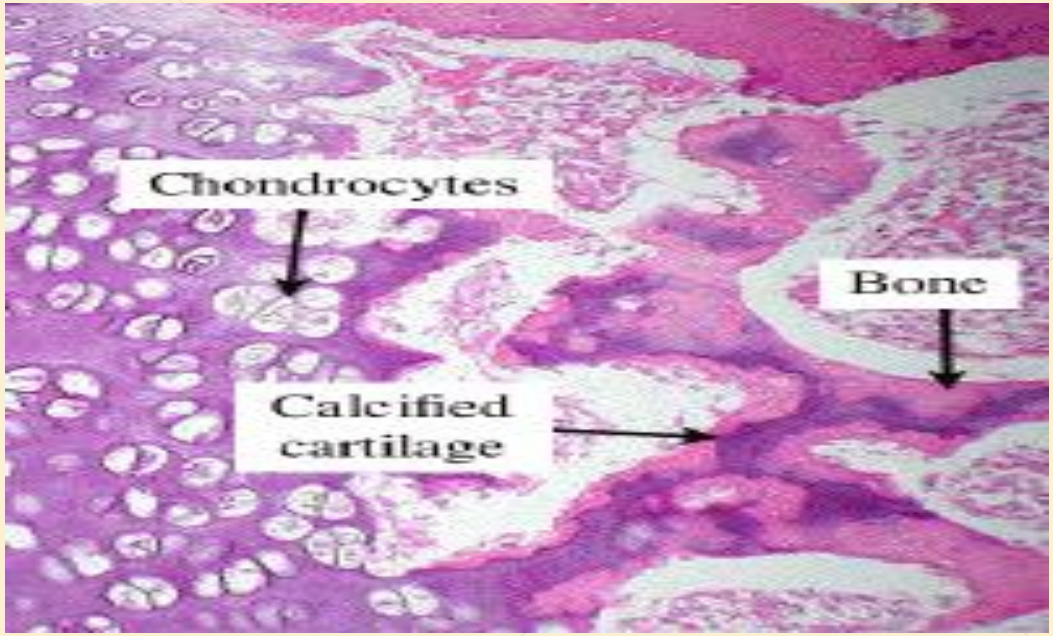
Endochondral bone formation

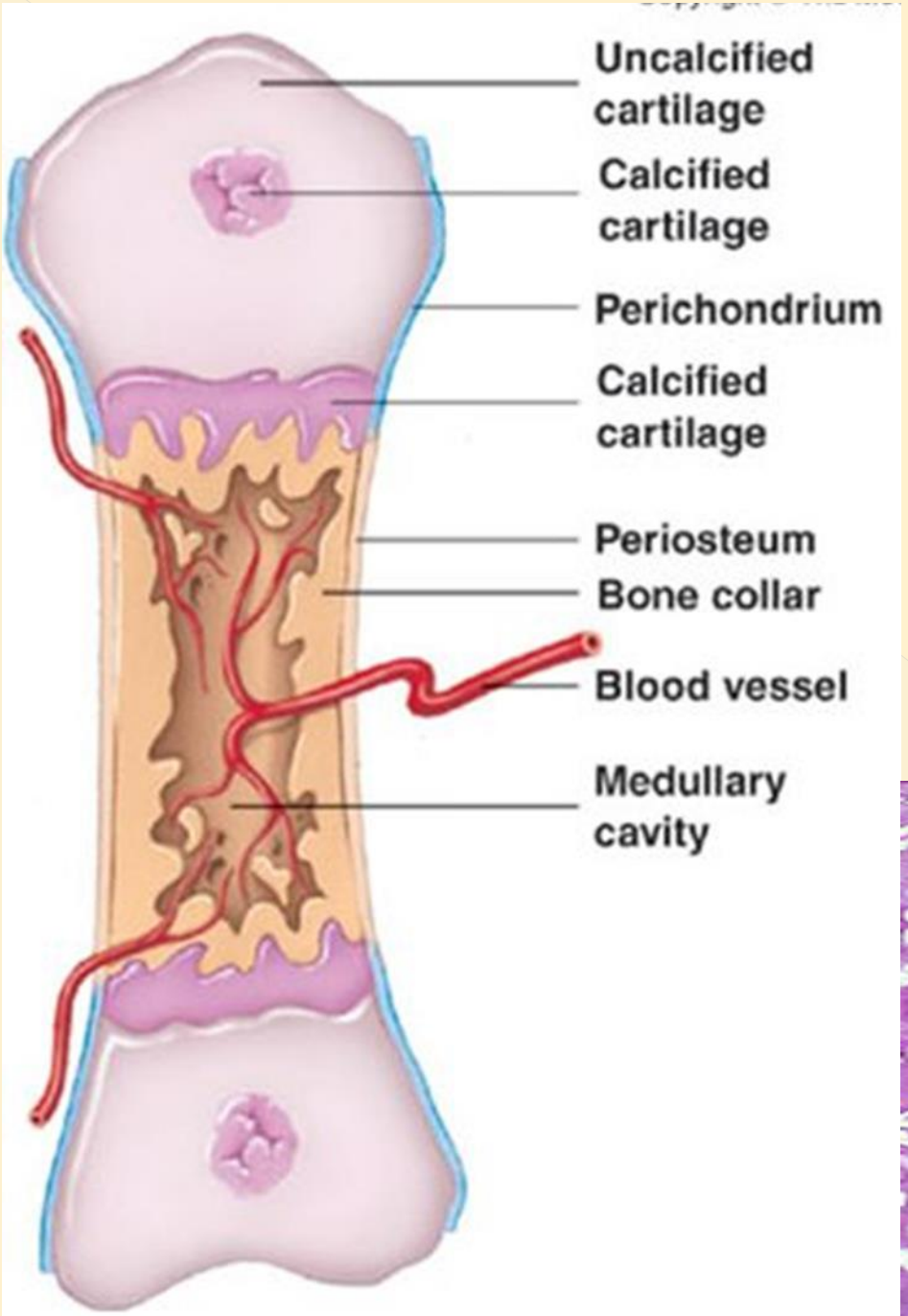
- **Osteoclasts** (fusion of macrophages of mesenchymal cells) digest holes in the bone collar.

- **periosteal bud - osteogenic bud** (**osteoprogenitor** and **hematopoietic** cells and blood vessels) enters the concavities.



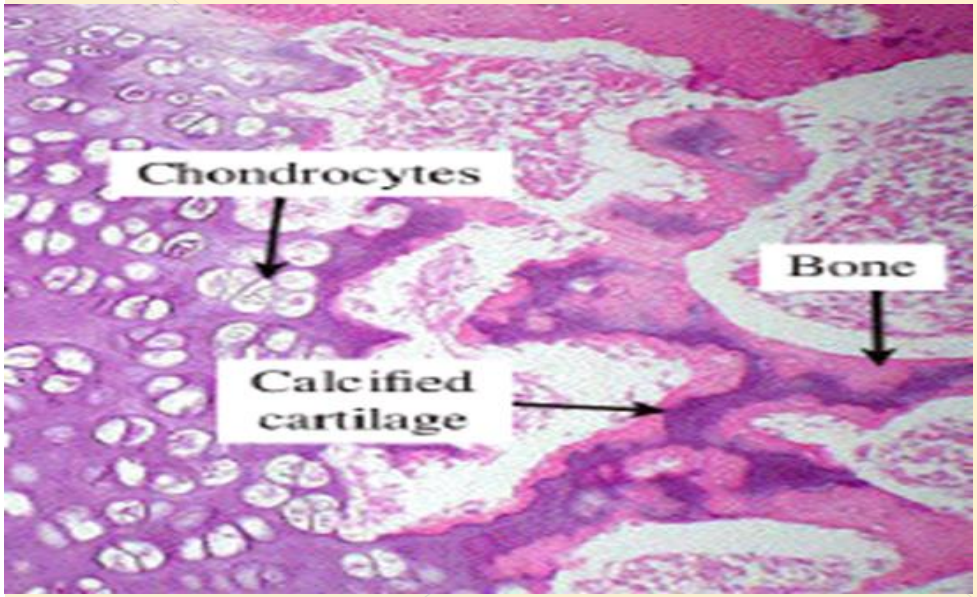
- osteoprogenitor cells divide, form **osteoblasts** - elaborate the bone matrix on the surface of the calcified cartilage matrix - formation of a **calcified cartilage/calcified bone complexes**.



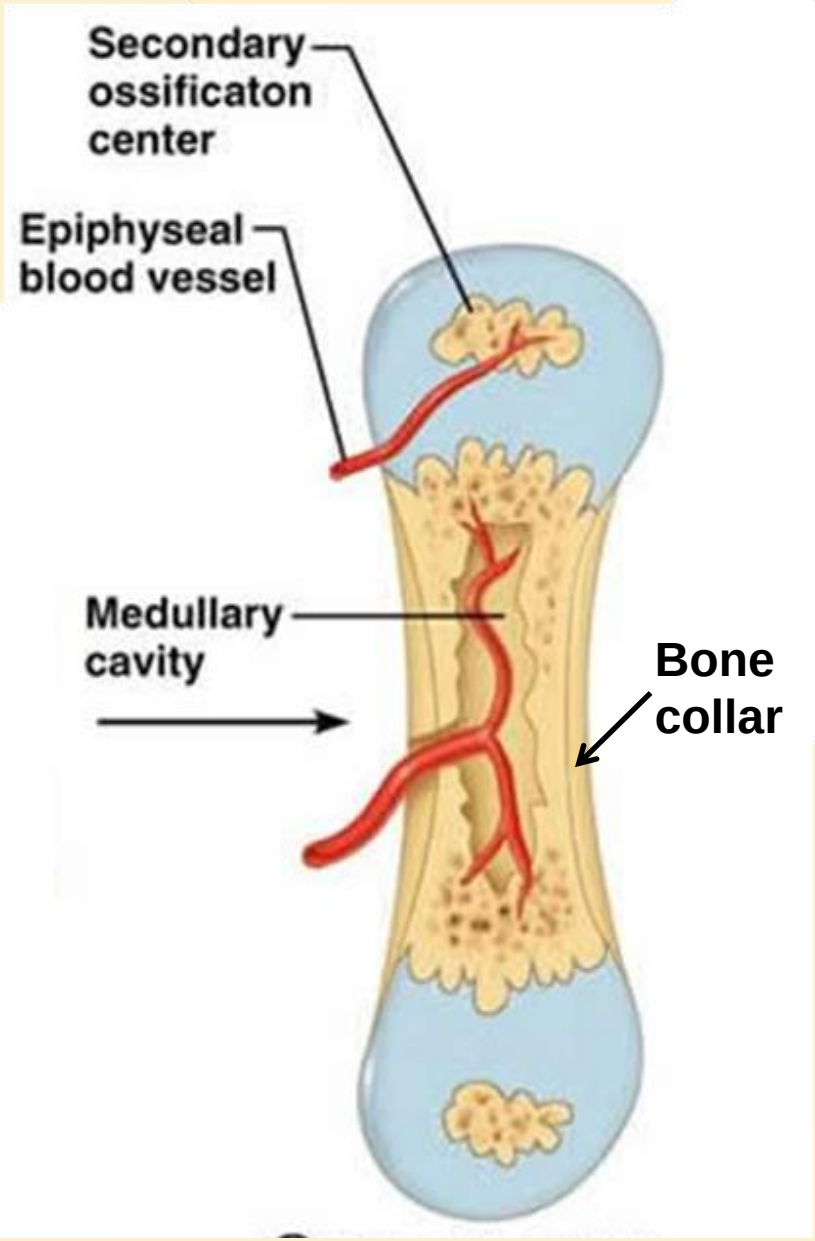


- osteoclasts resorb the calcified cartilage/calcified bone complexes, bone marrow cavity enlarges.

- the **cartilage of diaphysis is replaced by the bone and bone marrow**



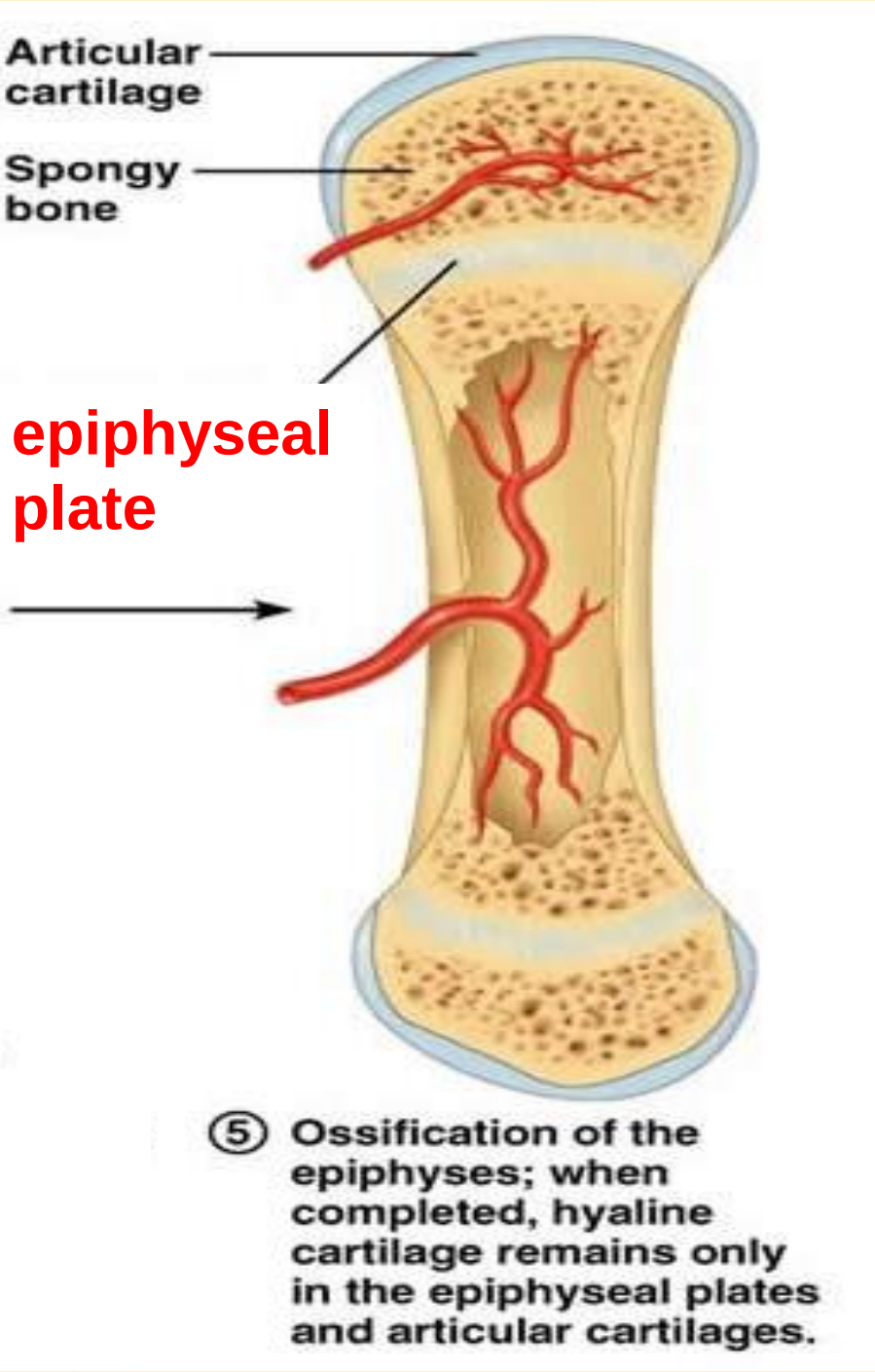
Endochondral bone formation - ossification of epiphysis



Formation of **secondary ossification centers** at both epiphyses:

- **osteoprogenitor** cells invade the epiphysis, differentiate into **osteoblasts**
- osteoblasts secrete bone matrix on the cartilage scaffold

Bone collar is not formed !

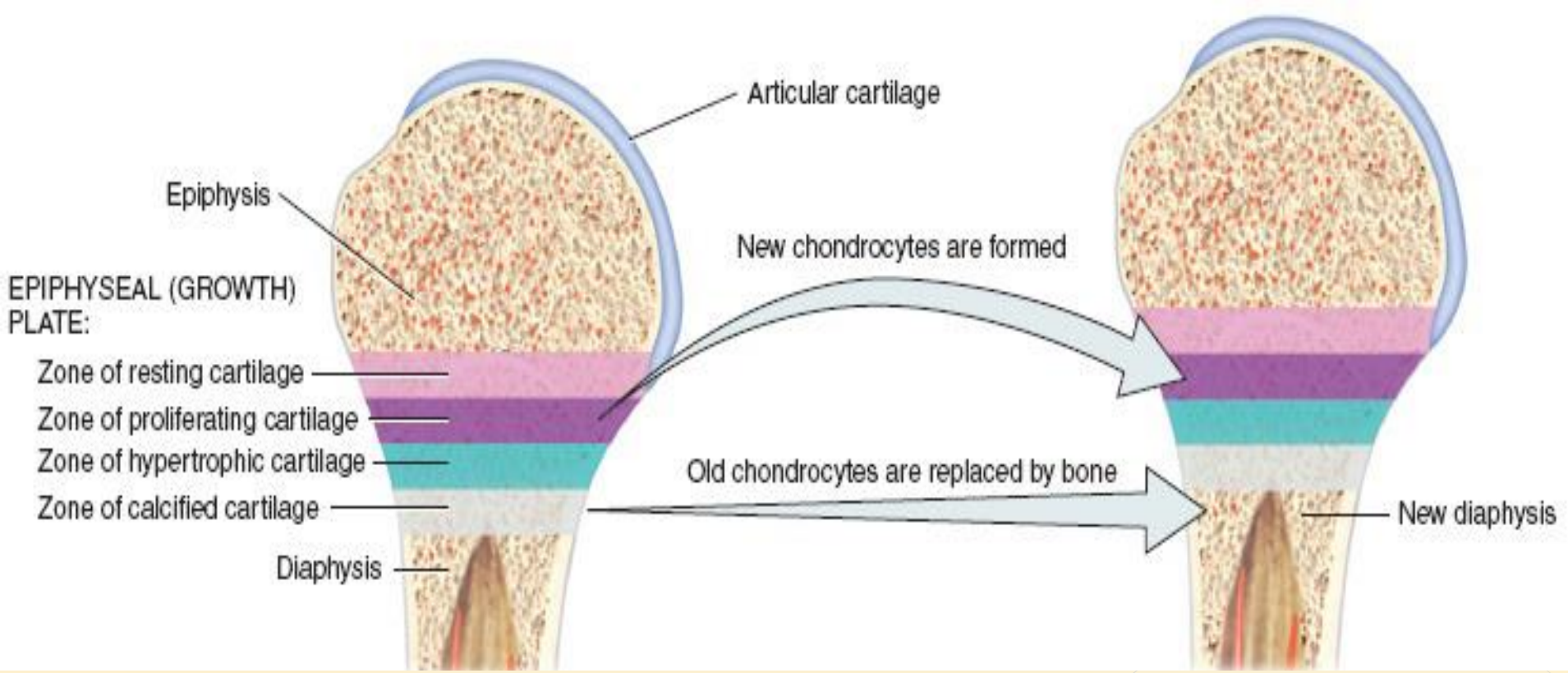


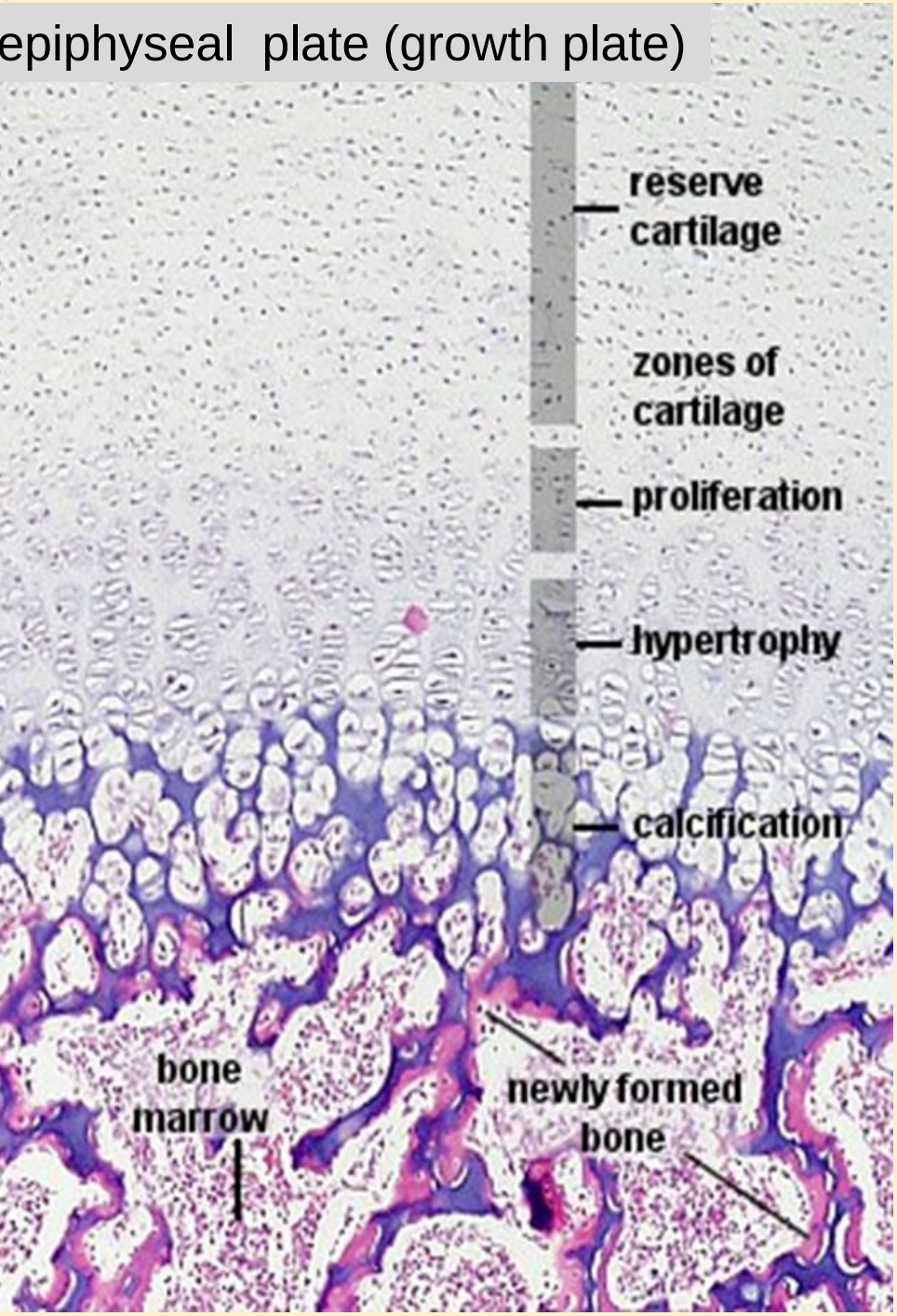
Endochondral bone formation

- the cartilage of epiphyses and cartilage of diaphysis are replaced by bone except at the articular surface and the **epiphyseal plate.**

bone growth in length - epiphyseal plate (growth plate)

- **Reserve cartilage**
- **Proliferation**
- **Maturation and hypertrophy**
- **Calcification**
- **Ossification**

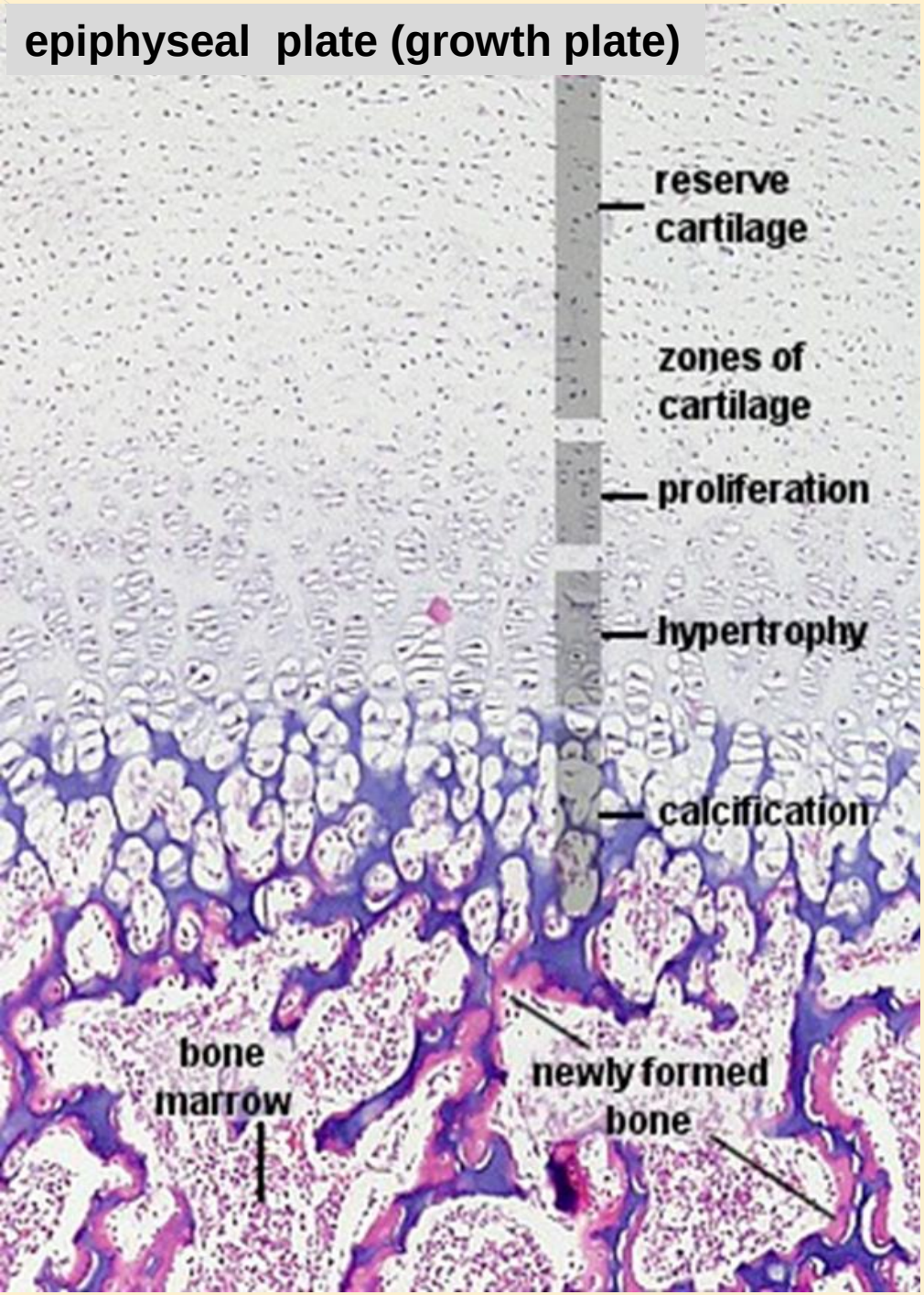




Reserve cartilage – chondrocytes randomly distributed in the matrix

Proliferation – cell proliferation - rows of cells (**IGF1** - **Insulin-like growth factor 1** (somatomedin C) – **liver** - (**growth hormone - hypophysis**)

Maturation and hypertrophy - chondrocytes hypertrophy. The matrix between lacunae – narrow, **lacunae - large**

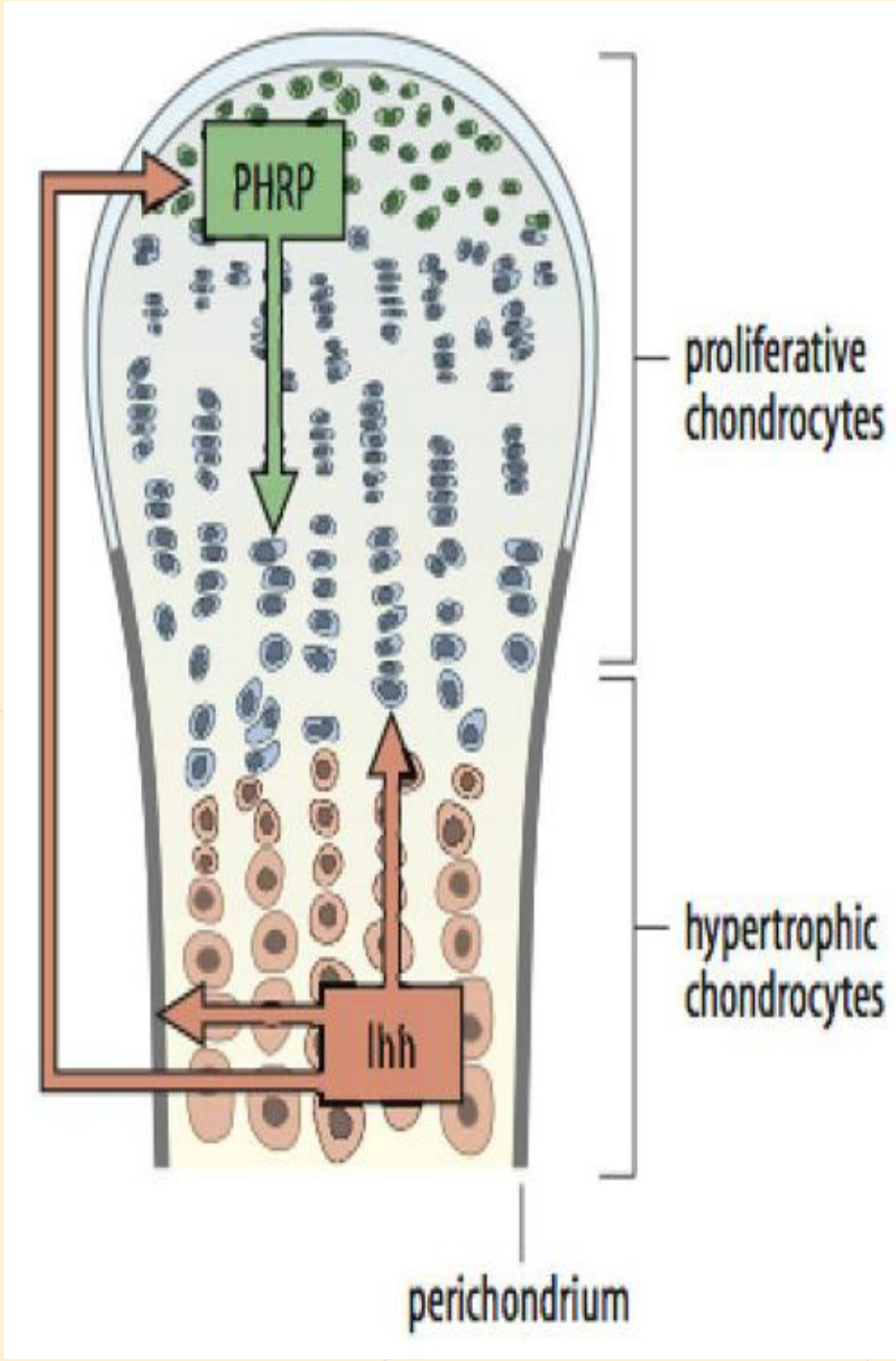


Calcification – lacunae confluent, hypertrophied **chondrocytes die** and **cartilage matrix becomes calcified**

Ossification – osteoprogenitors cells differentiate into **osteoblasts**, elaborate osteoid that becomes calcified on the surface of calcified cartilage.

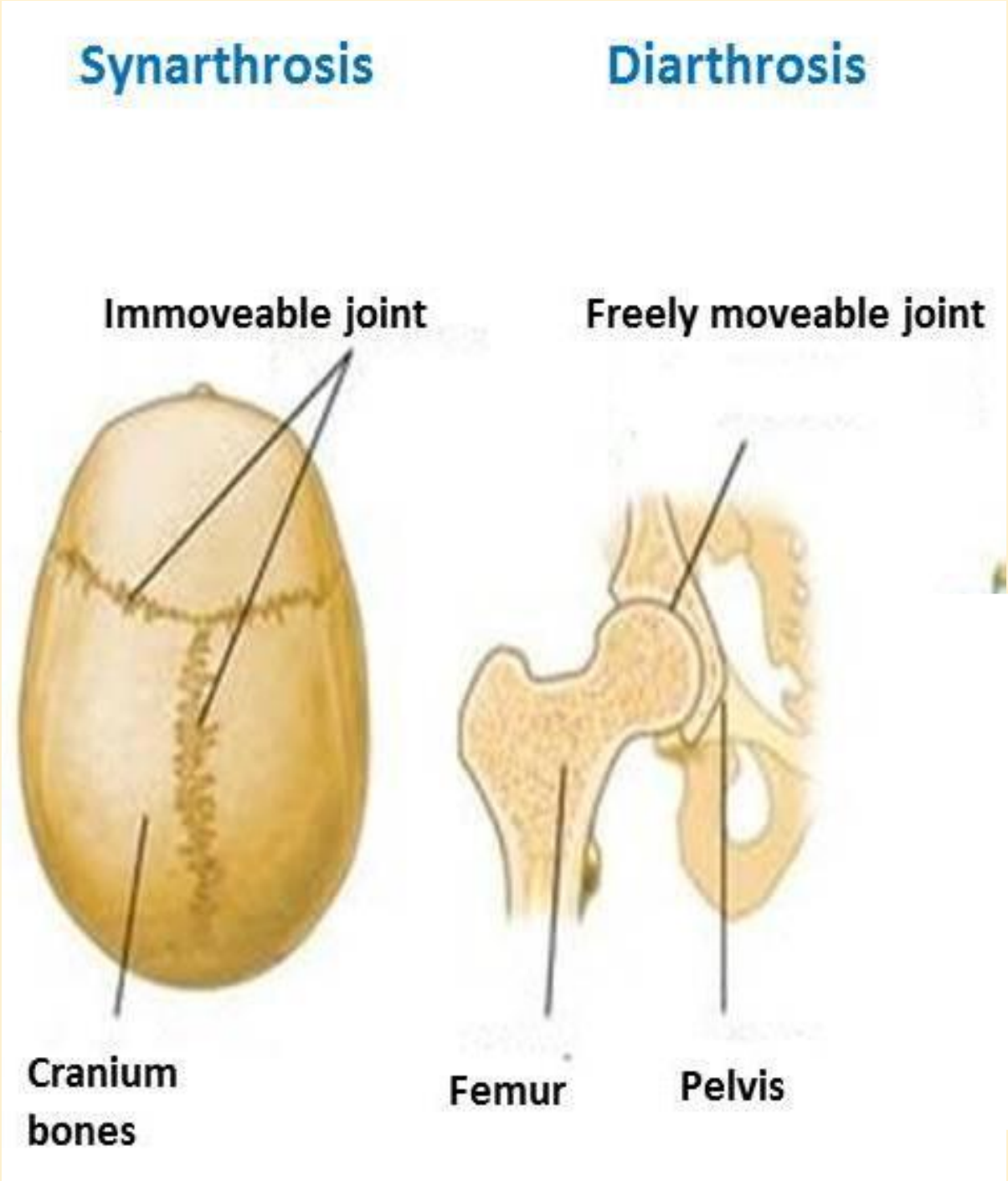
proliferation of chondrocytes

- **parathyroid-hormone-related protein (PHRP)** (resting chondrocytes) - maintains proliferation of chondrocytes
- **Indian hedgehog (ihh) -** hypertrophic chondrocytes – inhibits chondrocyte differentiation
- **indian hedgehog (ihh) -** stimulates production of **parathyroid hormone-related protein (phrp)**

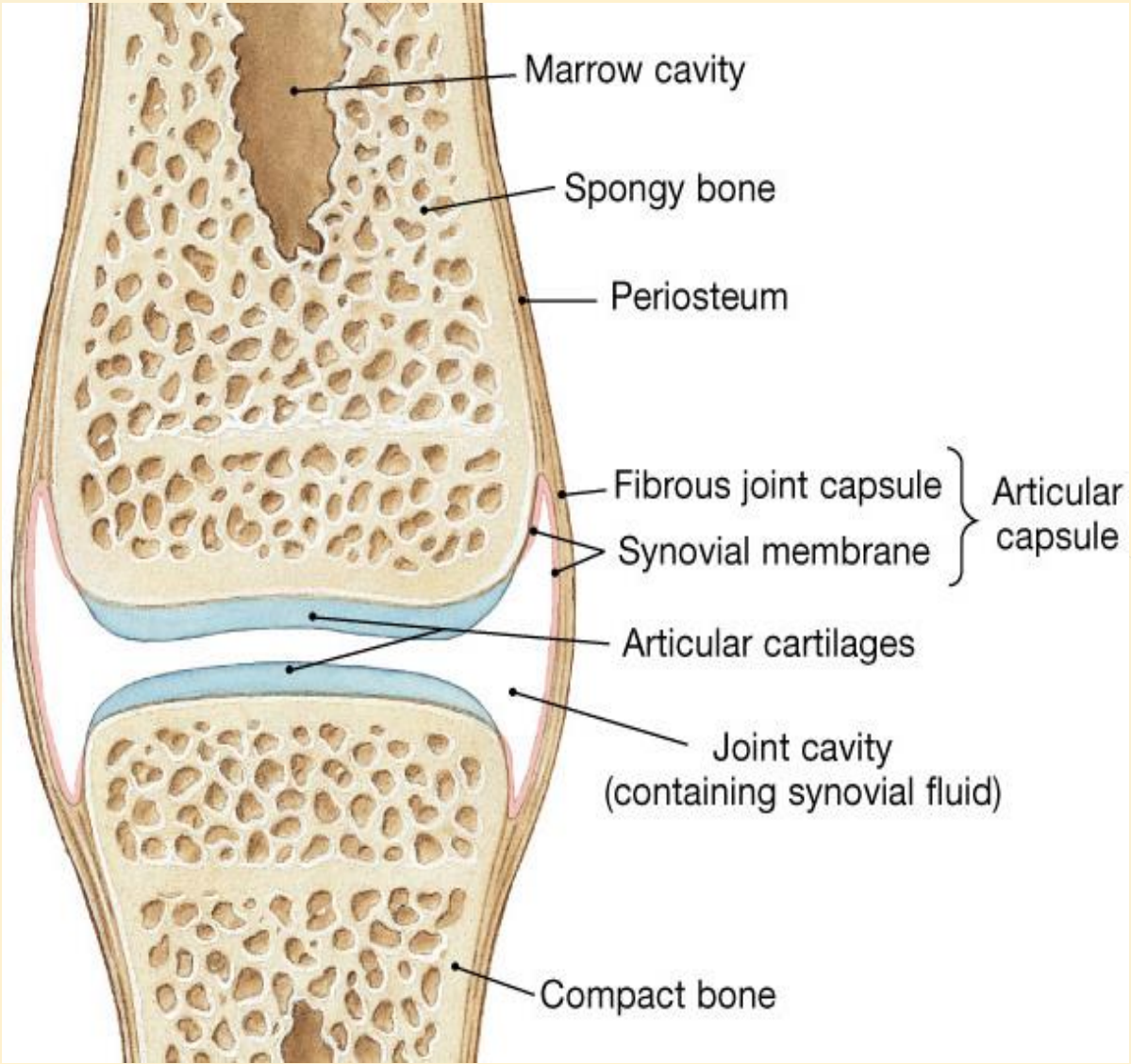


Joints - connections between two bones

- **Synarthroses** - limited movement
- **Diarthroses** - synovial joints, free movement



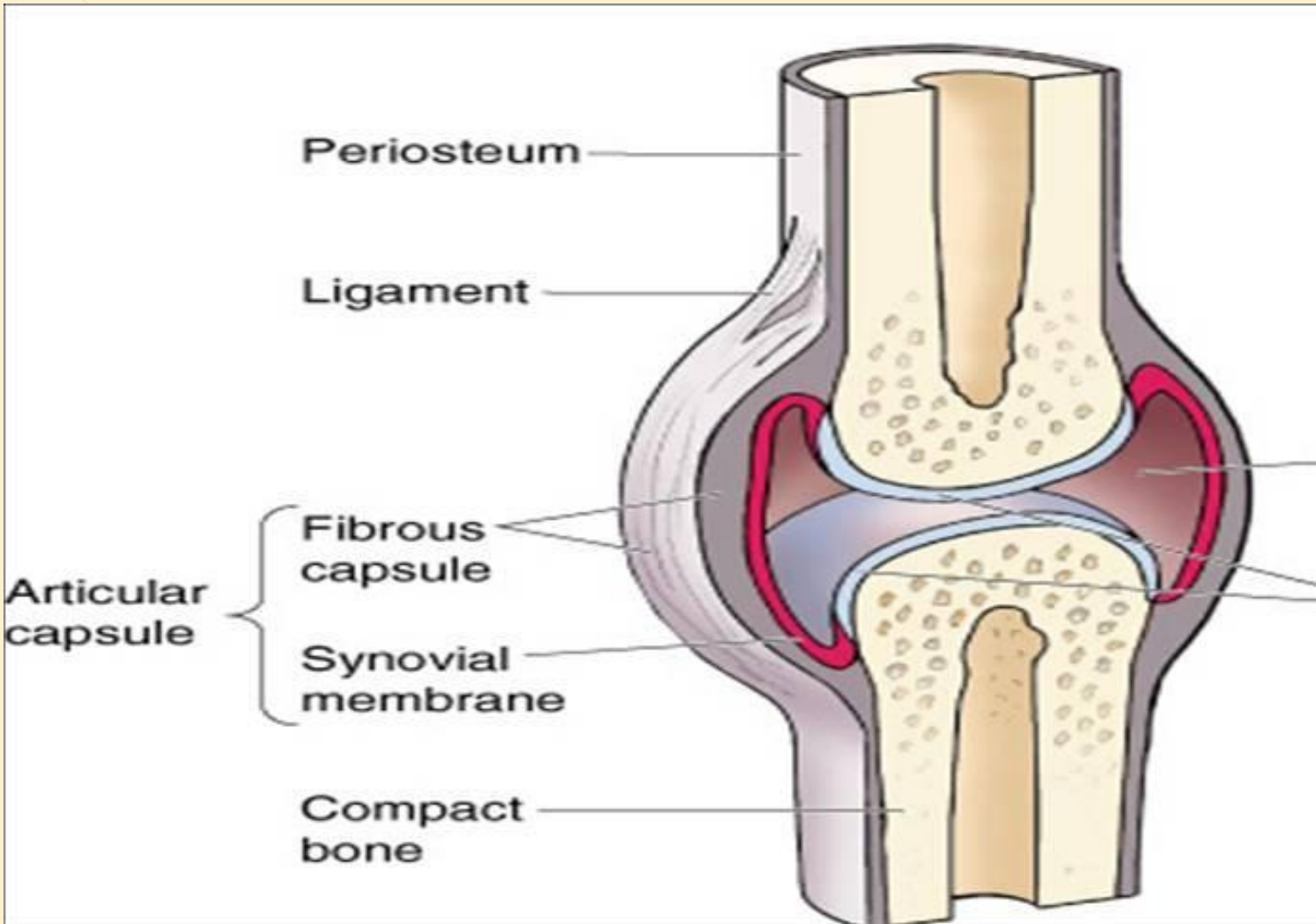
Articular capsule
- **fibrous capsule**
- **synovial membrane**



- **Diarthroses** (synovial joints, free movement)

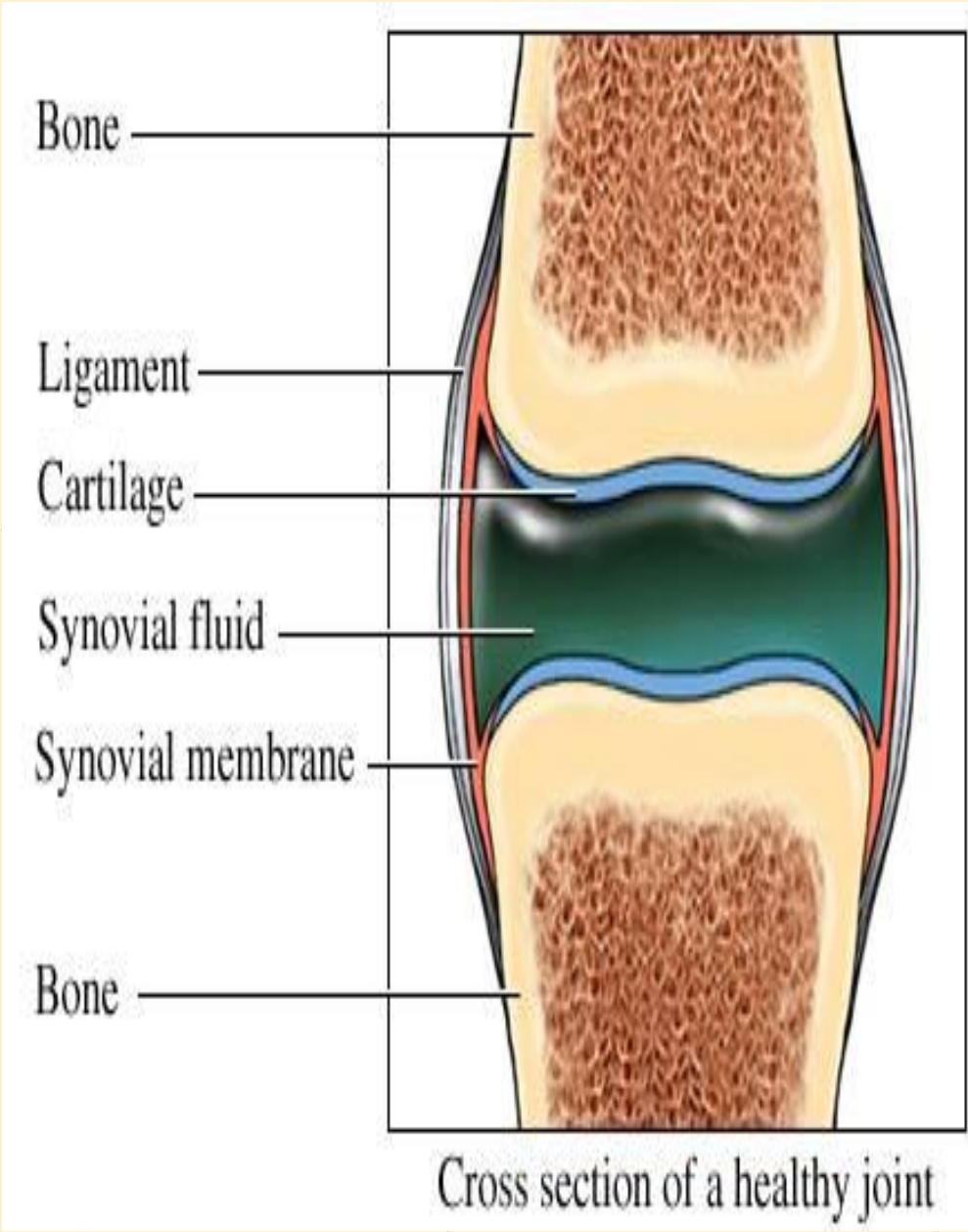
Cells of synovial membrane

- **Type A** cells- **macrophages** (remove microbes and the debris)
- **Type B** cells- **fibroblasts** (production of **synovial fluid**)



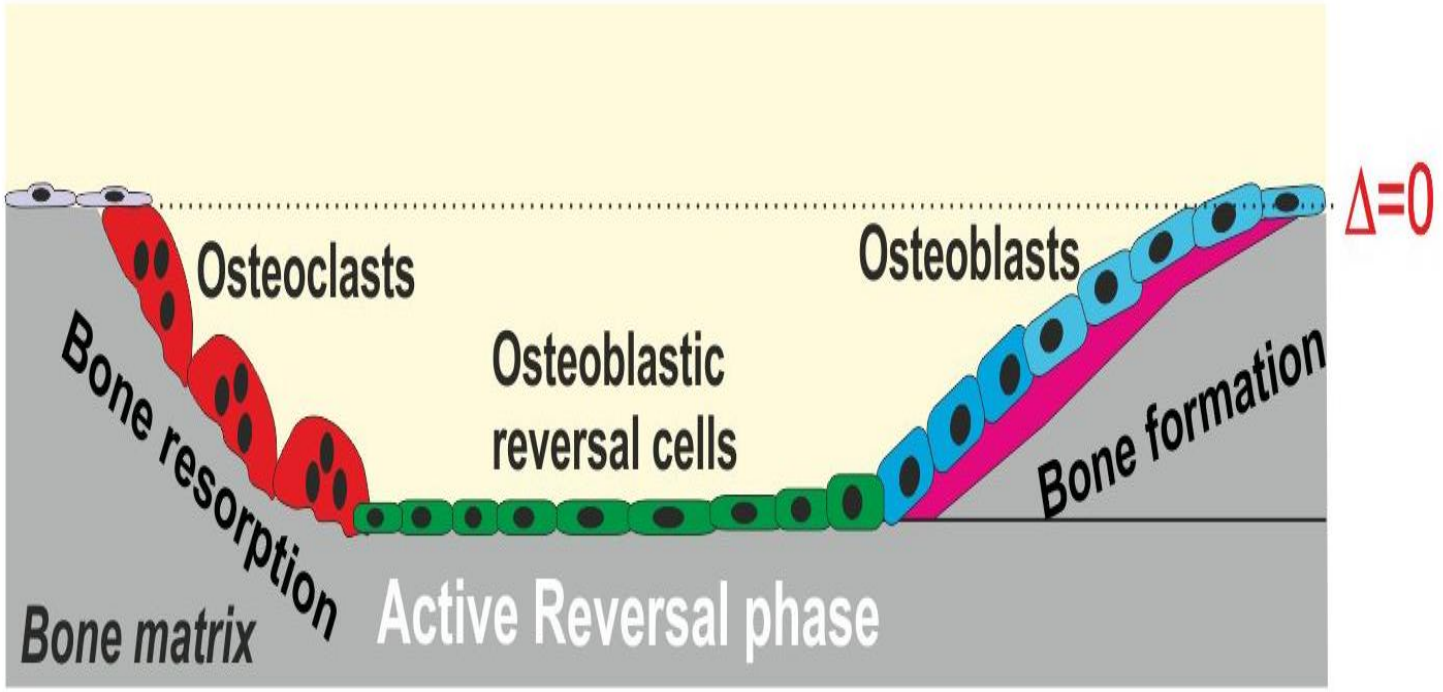
synovial fluid

- reduces friction between the articular cartilage during movement
- **hyaluronic acid** and **lubricin** secreted by fibroblast-like cells
- supplies oxygen and nutrients and removes carbon dioxide and metabolic wastes from the chondrocytes of cartilage



Coupling between bone resorption and bone formation

Coupled and balanced bone remodeling

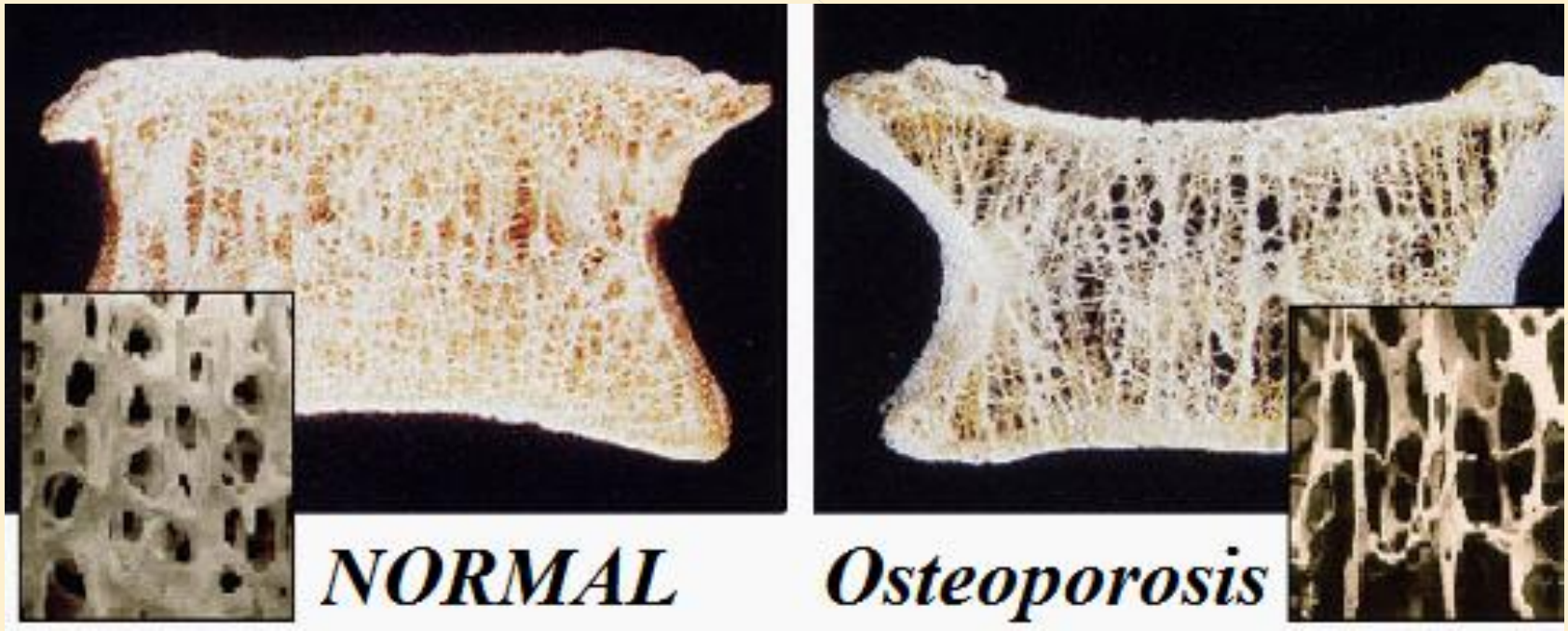


- in adult new **bone development is balanced with bone resorption** - **coupling** (bone resorption, bone replacement) continues throughout life.

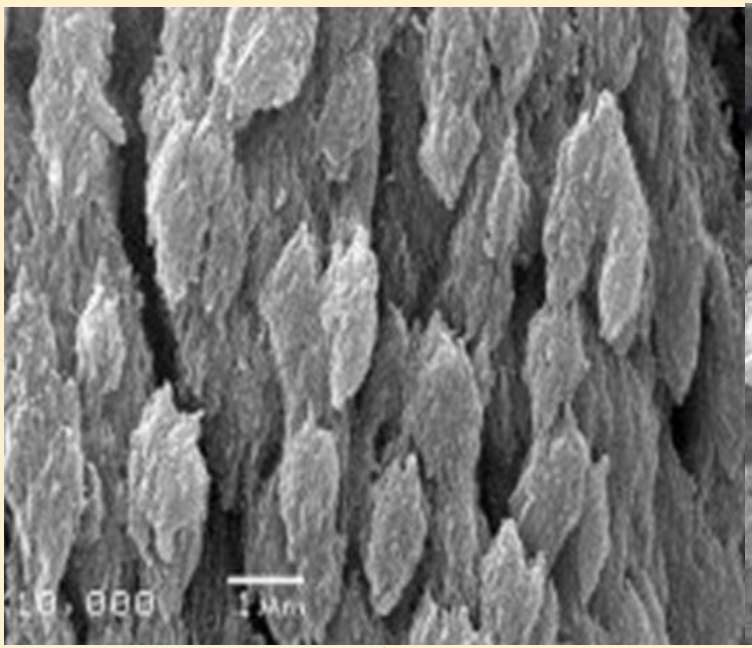
OSTEOPOROSIS (porous bones) **-** defect in bone remodeling

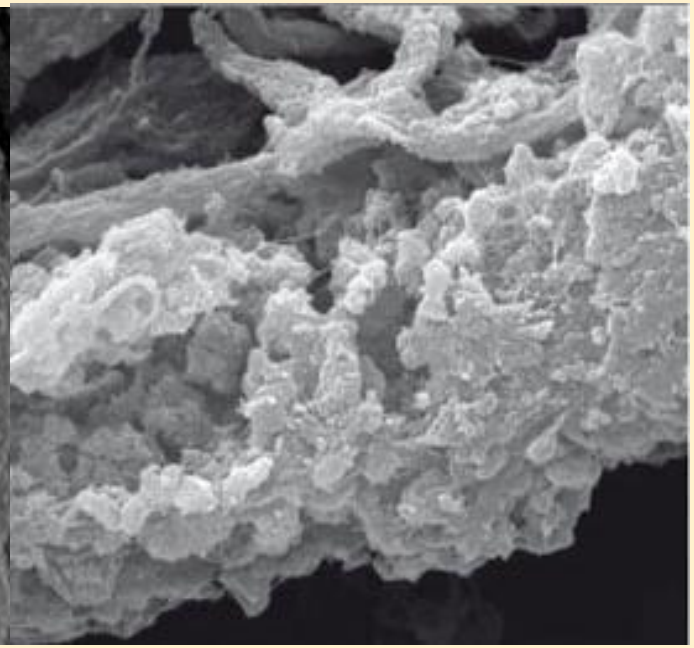
Osteoporosis - **decrease in bone mass and density** - increased risk of fracture.

- osteoporosis - in women **estrogen deficiency (menopause)**, in men a decrease in testosterone level (testosterone is converted to estrogen) - binding of estrogens to osteoblasts - production of osteoid



Calcification



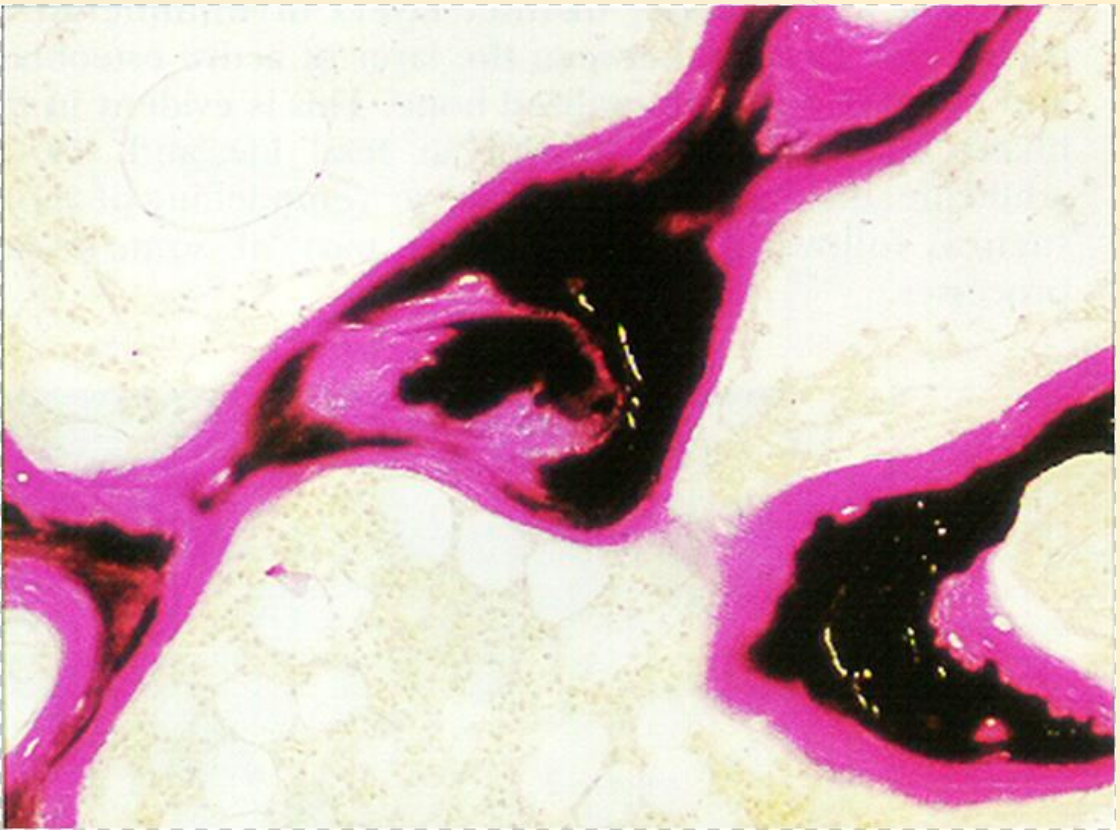


- **osteoblasts** release membrane-bounded vesicles (**calcium and phosphate ions**, and calcium binding proteins - **osteonectin**).
- vesicles – crystallization, new-formed **hydroxyapatite crystals** pierce the membrane and are released.

Crystals - deposited on the surface of collagen molecules (**nidi of crystalization**).

Osteomalacia (incorrect mineralization)

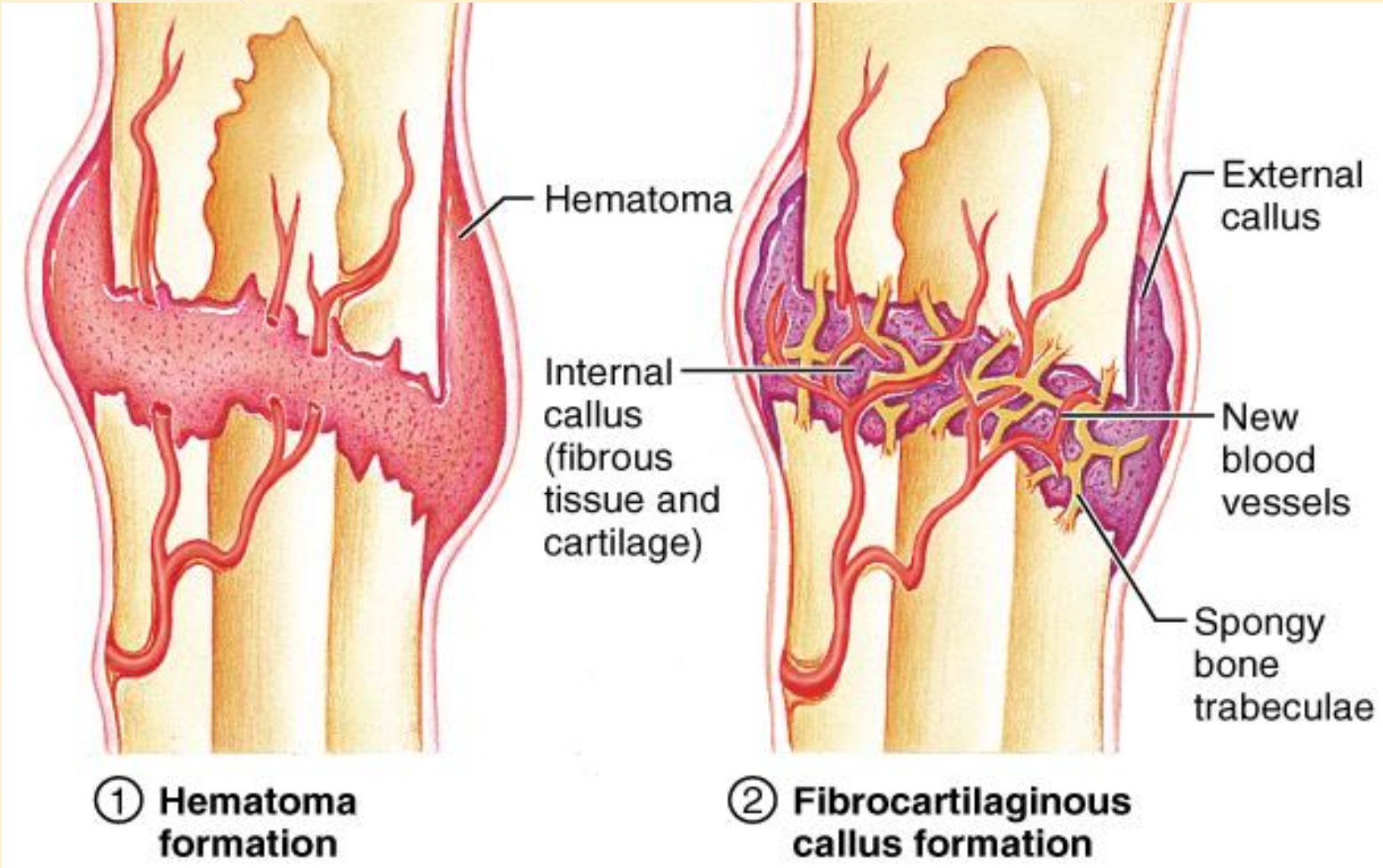
- insufficient Ca^{2+} and PO_4^{3-} ions concentration. If the level of Ca^{2+} ions is low (inadequate dietary or malabsorption- incorrect absorption in the small intestine) or if PO_4^{3-} level is low (excessive loss in the urine) - mineralization is impaired.



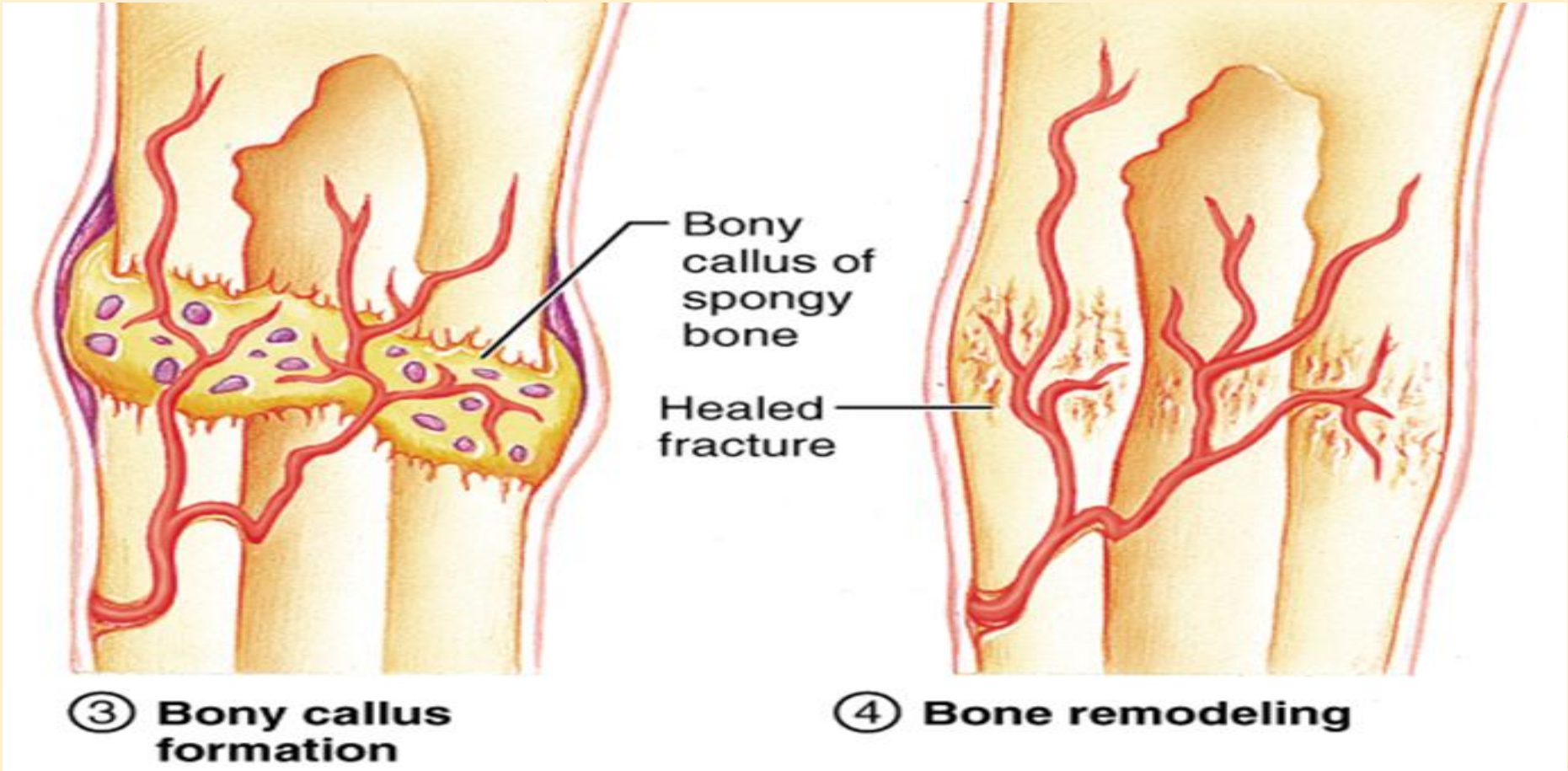
- unmineralized osteoid (magenta) and the central zone of mineralized bone (black).

- bone fracture - blood from vessel torn by the fracture - blood begins to clot - a **fracture hematoma –** the clot is invaded by capilaries, **progenitor cells** from endosteum and multipotential cells of bone marrow – **internal and external callus**

Bone repair

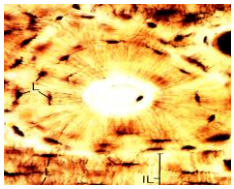


- clot - replaced by **fibrocartilage**
- osteoblasts - trabeculae of spongy bone - convert fibrocartilage to **bony callus**
- osteoblasts - compact bone, osteoclasts reabsorb spongy bone to make medullary cavity if needed



Seminar: Development of various types of bone tissue; rebuilding of bones.
Practical class: Bone formation.

- **capsule of synovial joint (slide # 15),**
- **intramembranous ossification (slide # 17),**
- **endochondral ossification – late stage (slide # 18),**
- **synovial membrane of joint capsule (slide # 59),** **(Fragment of synovial membrane from human knee joint. A layer of synoviocytes rests on the cushion of fat cells. Numerous blood vessels are present. The layer of synoviocytes contains both fibroblasts (F cells) and macrophages (M cells), but they are difficult to distinguish without special staining. F cells usually have elongated nuclei with the long axis parallel to the surface of the synovial membrane. Nuclei of M cells are usually larger and more rounded. L. general structure of synovial membrane; H. a layer of synoviocytes.)**


osteon

- **vascular system of bone and bone marrow cavity (fig. # 63),**
- **osteoporosis (text # 38),**
- **the role of cell-to-cell interactions in osteoclast formation (text & fig. # 56),**
- **changes occurring in bones in osteoporosis (fig. # 86),**
- **osteogenic groove and perichondral ring (text & photo # 28).**

Fig. # 63

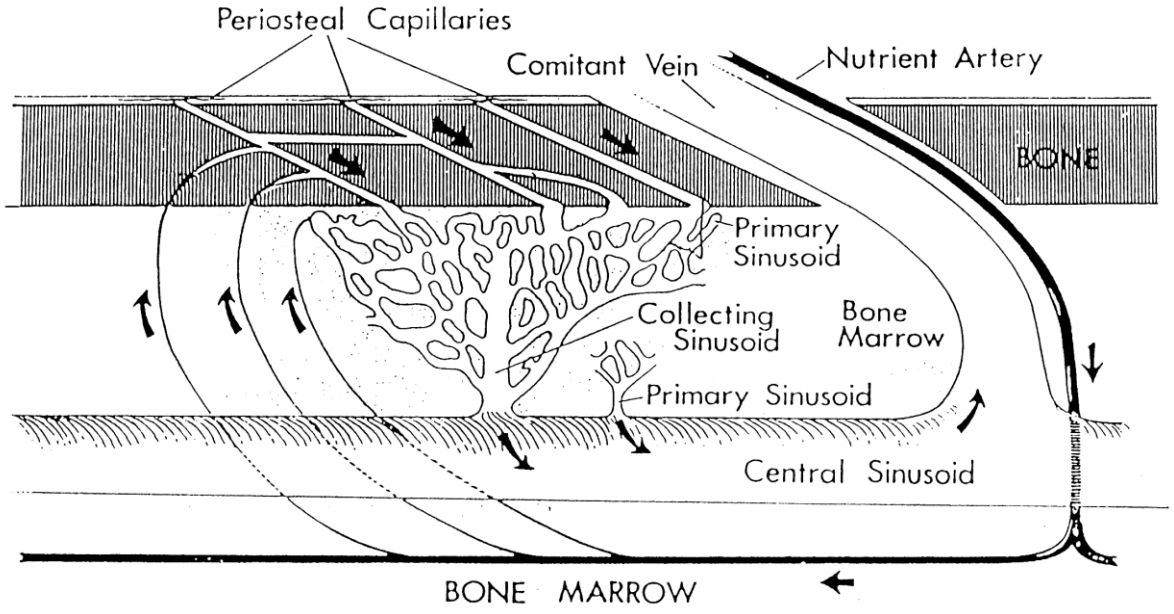


Diagram of the circulation in the bone marrow.

The blood supply to the sinusoids is primarily through the osteal vessels. Direct connections of the terminal branches of the nutrient artery are very rare. Most of the arborizations of the nutrient artery enter the bone, where they anastomose with the intraosteal vessels which connect with the sinusoidal network at the osteomyeloid juction. The venous drainage is through the large central sinusoid, sometimes via collecting sinusoids, sometimes directly via primary sinusoids.

TEXT # 38

OSTEOPOROSIS- DIAGNOSTIC & DENSITOMETRIC EVALUATION

A. Sawicki, K. Włodarski

Osteoporosis is a metabolic bone disorder characterized by reduction of bone mass and in consequence of increased fragility.

Tab. I

Classification of osteoporosis

Local	**Generalized**	
	primary	secondary
idiopathic	involutive	
	Typ I (post menopausal)	Type II (aging)

Diagnosis: Based on interview, presence of some bone involution risk factors and on densitometric evaluation of skeleton.

Tab. II

Risk factors for osteoporosis development

A. Genetic

advanced age, female sex, tiny body

B. Hormonal

postmenopausal state, premature cessation of ovary function, lack of child birth

C. Life habit

reduced Ca and vit. D intake, low protein diet, exess of Na, coffee, immobilization, smoking, alkohol abuse

D. Drugs

anti-epileptic drugs, anti-thrombin drugs, aluminium salts, glucocorticoides

E. Diseeses facilitating secondary osteoporosis development

- of alimentary tract,
- of kidney,
- endocrinopathies,
- collagenosis,
- systemic.

Osteoporosis is a painless disorder. When no evident fractures are claimed, the osteoporosis can be diagnosed by densitometric evaluation of skeleton, providing that osteomalatia (demineralisation of bone), hyperfunction of parathyroid glands and neoplastic diseases are excluded. For evaluation of the advancement and dynamic of osteoporosis several laboratory tests should be taken: the level of Ca and P, of alkaline phosphatase in serum, rtg of vertebral column and pelvis. The involution of bone mass exceeding the 30% of normal value can be recognized on regular radiographs, but this range is near to the spontaneous bone fractures. For evaluation of osteoporosis status before fractures appear the radiographs of vertebrae in lateral projection are usually considered.

Two densitometric methods for quantitative evaluation of bone mass are used:

1) dual roentgen photon absorption (DXA),
2) ultrasounds.

Both methods are sensitive, give quite reproducible results and enable diagnosis of early stage of bone loss as well as the monitoring of the bone mass changes. The range of densitometric examination depends on the patient age and goal of examination. For prophylactic screening of population the cancanelous bone and forearm bones are of choice. In advanced osteoporosis and for monitoring the therapeutic effects in adults 40 – 50 years

old, the lumbar spine (anterior – posterior projection) is adviced. In a more advanced age (after 65 years) when the increase of hip fractures risk appears, then the evaluation of distal femur plus bone mineral density of femur neck and of Ward's triangle are recommended. The classification of bone loss based on bone mineral density (BMD) evaluation, according to WHO is based on the difference between the mean value of BMD for population of young people and the value determined for a given patient (so called T-score)

(T-score = ± standard deviation for the mean value)

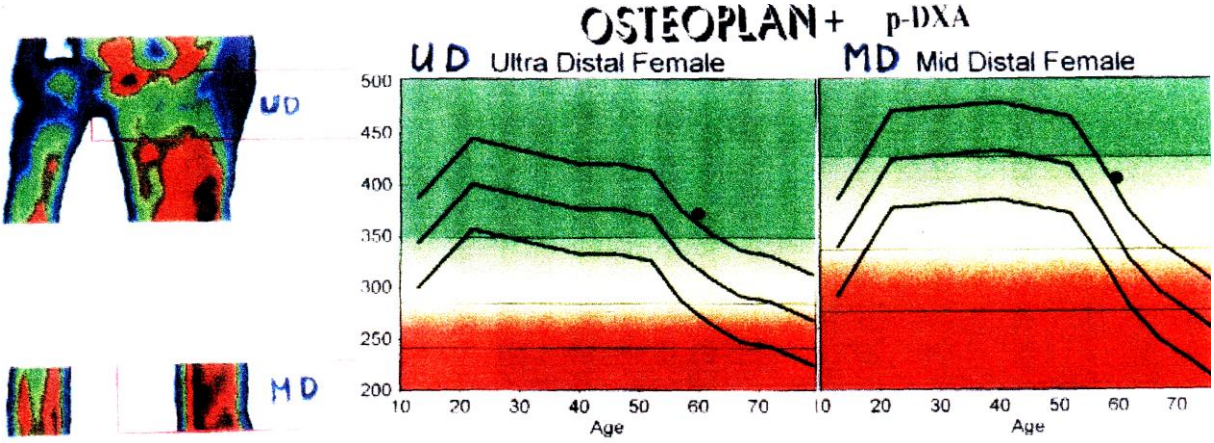
1. Normal value: T-score between +1 and –1,
2. Osteopenia (decrease of bone mass in the bone): T-score below –1,
3. Osteoporosis: T-score below –2,5,
4. Advanced osteoporosis: T-score below –2,5 plus bone fractures.

Legend to the densitograms:

BMD – bone mineral density
Z – age
Neck – femoral neck
Troch – trochanter
Inter – intertrochanteral
Ward's – Ward's trangle
UD – Ultra Distal
MD -- Mid Distal

Fig. 38.
Examples of bone densitograms:

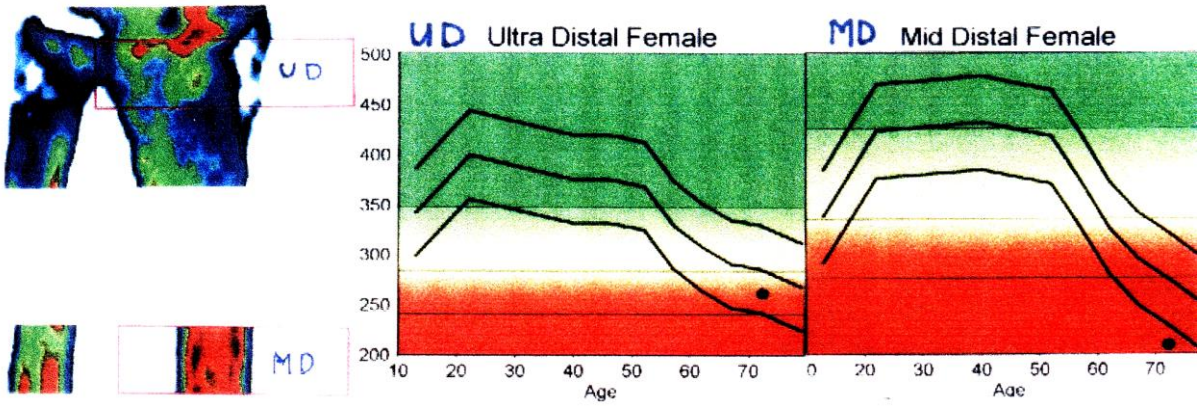
38 A. High BMD of forearm in 60♀



	Ultra Distal			Mid Distal		
	Value	Z-Score	T-Score	Value	Z-Score	T-Score
sBMD(mg/sqcm)	373 □	1.22	-0.64	671 □	1.23	-0.58
BMC(mg)	1.080			869		

T-Score classification: ↔-1 Osteopenia, ↔-2,5 Oseeoporisis, ↔-3,5 Severe Osreoporosis.
For definition and explanation do consult the user manual.

38 B. Reduced BMD in forearm of 73♀ patient.

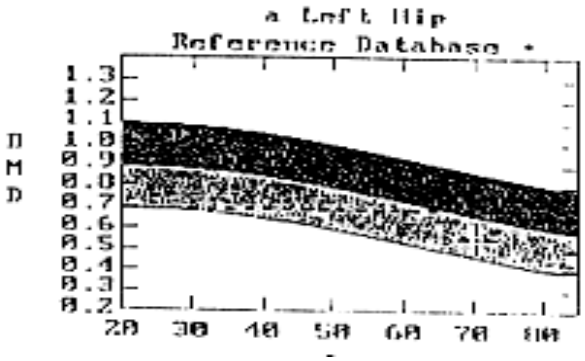


	Ultra Distal			Mid Distal		
	Value	Z-Score	T-Score	Value	Z-Score	T-Score
sBMD(mg/sqcm)	262 □	-0.54	-3.16	508 □	-1.40	-4.78
BMC(mg)	718			710		

T-Score classification: ↔-1 Osteopenia, ↔-2,5 Oseeoporisis, ↔-3,5 Severe Osreoporosis.
For definition and explanation do consult the user manual.

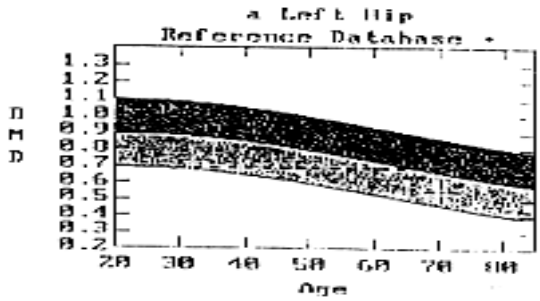
green – normal value
yellow – limits of mineral density for osteopenia
red – reduced mineral density for osteoporosis

38C. Femoral bone mineral density in osteoporotic patient (♀73 year)



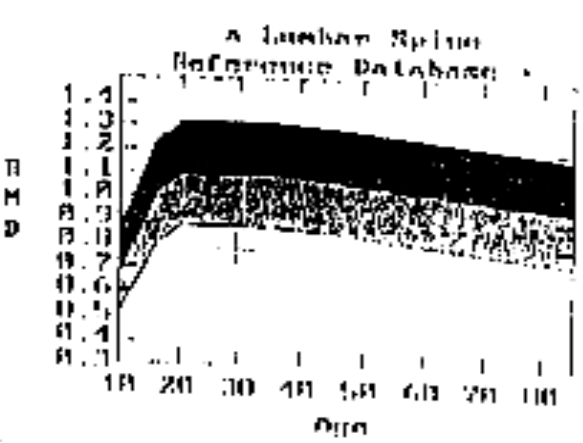
Region	*BDM*	*T*	*Z*
Neck	0.546	-3.49 61% (22.0)	-1.17 82%
Troch	0.554	-1.86 77% (30.0)	-0.23 96%
Inter	0.818	-2.35 71% (29.0)	-0.70 89%
Total	0.672	-2.53 69% (28.0)	-083 87%
Ward's	0.288	-4.62 36% (20.0)	-1.45 64%

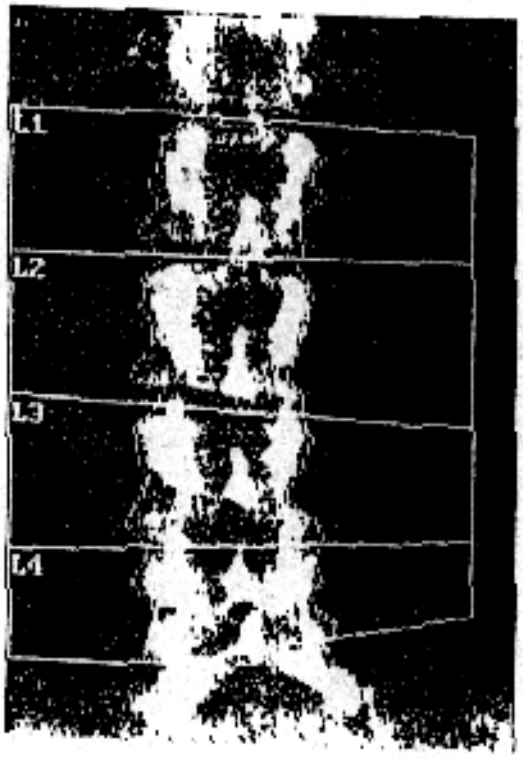
38D. Femoral bone mineral density slightly reduced, according to the age – osteopenia.



Region	BDM	T	Z
Neck	0.706	-1.88 79% (22.0)	-0.86 89%
Troch	0.554	-1.75 78% (30.0)	-1.19 84%
Inter	0.941	-1.47 82% (29.0)	-0.95 88%
Total	0.777	-1.65 80% (28.0)	-1.07 86%
Ward's	0.584	-1.93 73% (20.0)	-2.22 96%

38E. Lumbar spine mineral density profoundly reduced (♂29 % year)





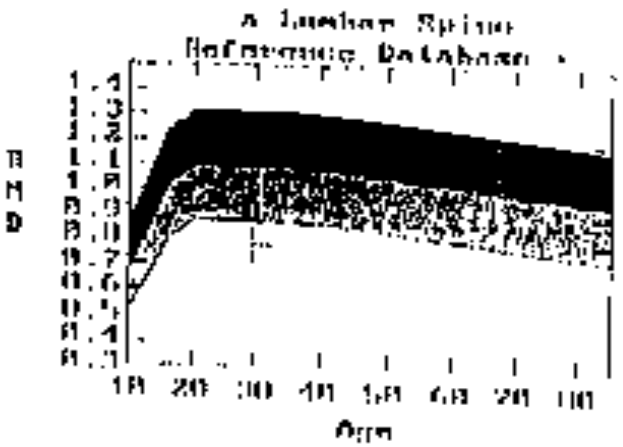
Region	BDM	T(30.0)	Z
L1	0.746	2.38 74%	2.38 74%
L2	0.760	3.04 69%	3.04 69%
L3	0.761	3.11 69%	3.11 69%
L4	0.856	2.63 75%	2.63 75%
L1 L4	0.778	2.85 71%	2.85 71%

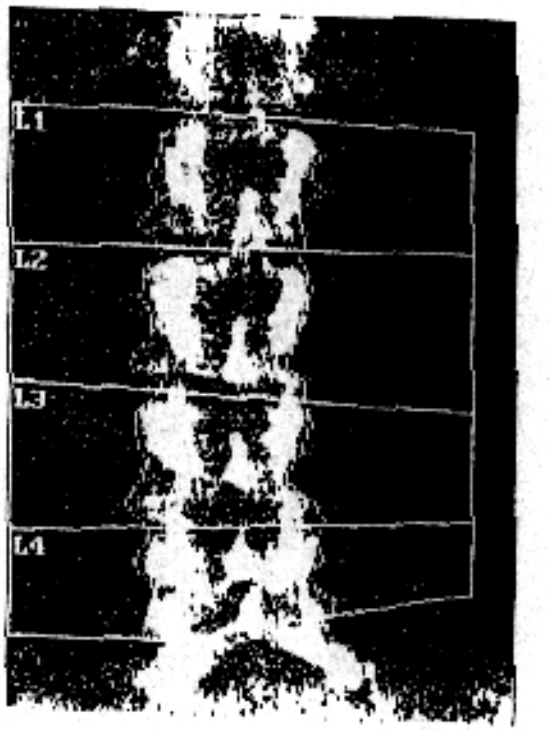
- Age and sex matched

T – peak bone mass

Z - age matched

38F. Lumbar spine mineral density slightly reduced (♀52 year)





Region	*BDM*	*T(30.0)*		*Z*	
L1	**0.914**	**-0.10**	**99%**	**+0.71**	**109%**
L2	**1.039**	**+0.10**	**101%**	**+1.00**	**112%**
L3	**0.934**	**-1.36**	**86%**	**-0.41**	**95%**
L4	**0.861**	**-2.32**	**77%**	**-1.35**	**85%**
L1 L4	**0.932**	**-1.05**	**89%**	**-0.12**	**99%**

- **Age and sex matched**

T – peak bone mass

Z - age matched

Text & fig. 56
The role of cell to cell interactions in osteoclast formation

Osteoclasts are hematopoietic cell derived, coming into being by fusion of precursor mononuclear cells. This precursor cells are derived from CFU-GM (Colony Forming Units - Granulocyte Macrophage) and branches from the monocyte-macrophage lineage early during the differentiation process.

Osteoclastogenesis - process of osteoclast formation, and bone resorption are modulated by (1) systemic hormones, (2) locally acting cytokines and (3) cell to cell interactions (osteoblast/stromal cell-osteoclast/precursor).

Cells forming colonies of monocyte-macrophage lineage in vitro, in presence of GM-CSF (Granulocyte Macrophage - Colony Stimulating Factor) and vitamin D_3 - $1,25(OH)_2D_3$ forms four groups of cells in the culture: granulocytic, macrophage, mixed hematopoietic lineages and polygonal. This polygonal cells express calcitonin receptor, and fuse to form osteoclast-like multinucleated cells that resorb calcified bone matrix. In response to calcitonin they react strongly with 23c6 monoclonal antibody that identifies the osteoclast vitronectin receptor, and express high levels of TRAP (Tartaric Resistant Acid Phosphatase) - a marker enzyme for osteoclasts.

It has been found that osteoblasts and bone marrow stromal cells, but not osteoclasts, express receptors for hormones responsible for bone resorption like: vitamin D_3, and PTH (parathormon). In response to this hormones osteoblosts and stromal cells release several locally acting factors: MCSF (Macrophage Colony Stimulating Factor), interleukins (IL-1, IL-6, IL-11), PGE_2 (prostaglandin E_2). MCSF appears to affect mature osteoclasts as well as its precursors (MCSF receptors (c-fms) are expressed in mature osteoclasts and precursor cells).

In mature osteoclasts MCSF enhanced motility and prevented apoptosis of osteoclasts.

The effect of microenvironment, which involves osteoblasts and/or stromal cells, seems to play a very important role in the osteoclast formation. This cell to cell system is composed of three elements: (1) **OPGL** – Osteoprotegerin Ligand also called ODF (Osteoclast Differentiation Factor)

produced by bone marrow stromal cells or osteoblasts, (2) **RANK receptor** (Receptor Activator of NFκB) for OPGL/ODF present on cell membrane of osteoclasts and its precursors, (3) **OPG** (Osteoprotegerin) also called OCIF (Osteoclastogenesis Inhibitory Factor) – decoy receptor for OPGL synthesized by osteoblasts and marrow stromal cells, released to microenvironment.

OPGL/ODF is synthetised by osteoblasts and marrow stromal cells in response to $1,25(OH)_2D_3$, PTH, PGE_2 or IL-11 with presence of MCSF.OPGL/ODF is a 40 kD polypeptide, anchored to cell membrane of osteoblasts and stromal cells. Gene expression of this peptide was also found in cells of several tissues, but firs of all in lymphocyte T and dendritic cells.

OPGL/ODF act on osteoclasts and its precursors via RANK receptor present on its cell membrane. For that reason OPGL is also called RANK ligand (RANK-L).

Receptor RANK is a member of superfamily of TNF receptors – **TNFRSF-11A** (Tumor Necrosis Factor Receptor Superfamily Member 11A) (fig. 2). Activation of RANK receptor leads to differentiation of osteoclasts precursors to mononuclear osteoclasts. There are also present markers of differentiation of this cells: MMP-9 (metaloproteinase – 9), calcitonin receptor, and vitronectin receptor. In the next stage the fusion of mononuclear osteoclasts to multinuclear cells and its activation is observed.

Activated T lymphocyte can stimulate osteoclastogenesis, and leads to bone and cartilage destruction. Expression of OPGL/ODF was found in activated T lymphocyte present in synovial fluid in patient with rheumatoid arthritis. This can explain the destruction of and cartilage and local osteopenia during rheumatoid arthritis.

Osteoprotegerin (OPG) was for the first time described in 1997 as a molecule which inhibit differentiation process and activated osteoclasts. In the same year has been isolated from fibroblast culture, as a factor that inhibit osteoclastogenesis in vitro, mediated by addition of $1,25(OH)_2D_3$, PTH, PGE_2 or IL-11 and was named as OCIF. OPGL/OCIF as well as RANK belongs to superfamily of TNF receptors (**TNFRSF 11B** – Tumor Necrosis Factor Receptor Superfamily Member 11B) (fig.2).

In 1998 gene for human osteoprotegerin has been localized on 8 chromosome. OPG/OCIF mRNA transcripts were found in osteoblasts and stromal cells as well as in kidneys, liver, thyroid and several fetal tissues.

Osteoprotegerin (OPG/OCIF) is synthetised as a 40 kD polypeptide, composed of 380 amino acids with 21 aa signaling peptide. In Golgi apparatus after glicolyzation a 110-120 kD homodimer is formed and then secreted out of the cell. In OPG/OCIF molecule in N-terminal region, four rich in cysteine domain has been localized. This domains are responsible for inhibition of osteolastogenesis. In the C-terminal region a heparin binding domain has been found.

OPG/OCIF molecule inhibits osteoclastogenesis by binding to OPGL and block contact of osteoblasts and stromal cells with RANK receptor present on cell membrane of osteoclast and its precursors. For that reason OPG is called DR-3 (Decoy Receptor-3).

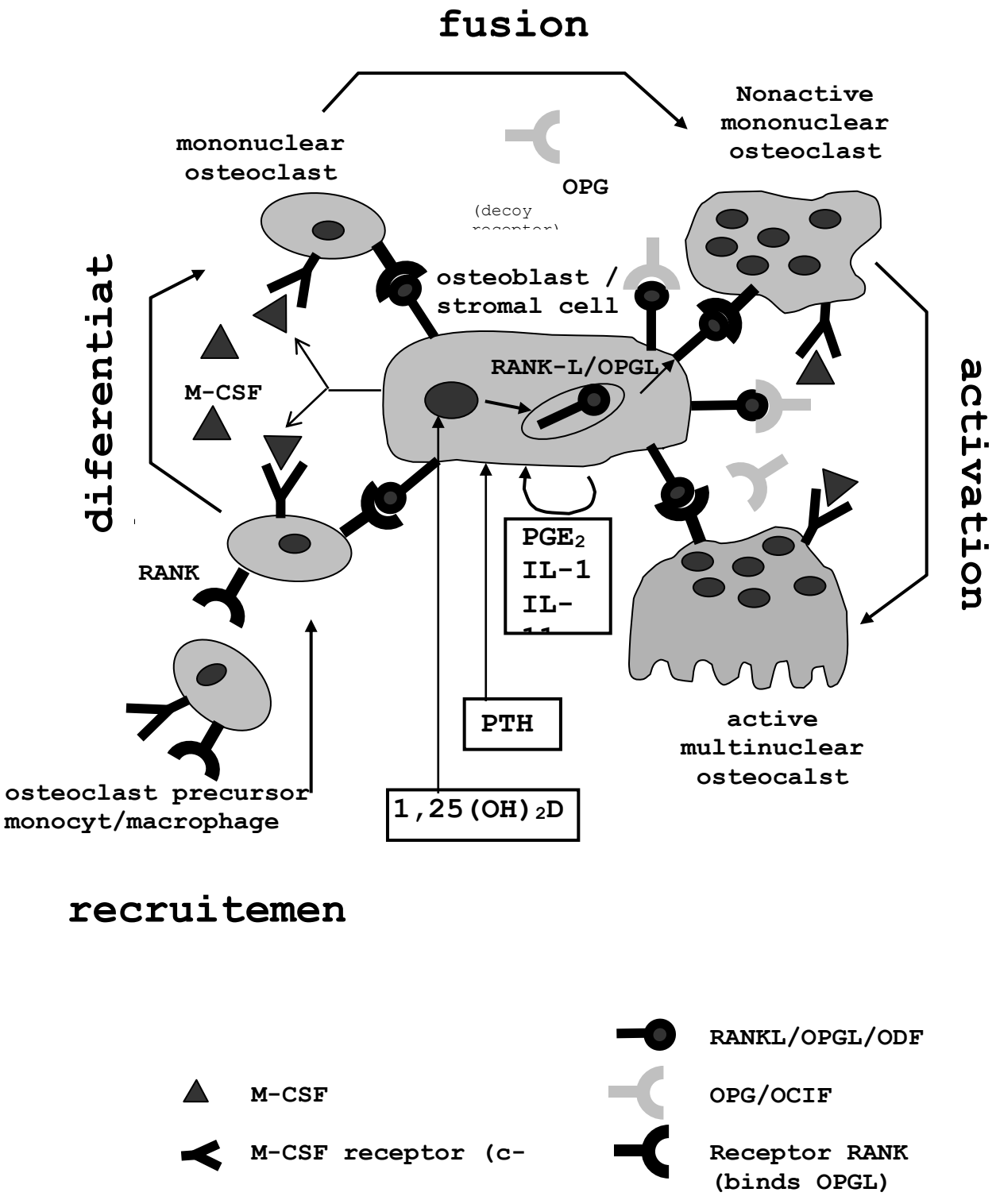
In transgenic mice with over expression of osteoprotegerin gene and in mice treated with egzogenic recombinant osteoprotegerin an osteopetrosis was observed. In ovarectomized mice treated with osteoprotegerin the bone resorption process has been decreased and increase in bone mass was observed. In osteoprotegerin knock' out mice the osteoporosis was observed due to increased osteoclastogenesis.

The expression of osteoprotegerin in osteoblasts and stromal cells increases during hypercalcemia. There was found also, that TGF-β (Transforming Growth Factor-β) inhibits osteoclastogenesis by stimulation of expression of osteoprotegerin gene in that cells.

Estrogen also increases the expression of osteoprotegerin gene in osteoblasts. In the opposite $1,25(OH)_2D_3$, PTH and glicocorticoids suppress osteoprotegerin gene.

It seems that inhibition of expression of osteoprotegerin gene by glicocorticoids and increased expression of osteoprotegerin ligand may be one of the main mechanism of glicocorticosteroid induced osteopenia. There was also found that glicocorticosteroids inhibit osteogenesis by induction of apoptosis of osteoblasts.

Based on: *Dziedzic-Gocławska A., Tyszkiewicz J., Uhrynowska-Tyszkiewicz I.: "Wybrane mechanizmy sterujące procesem przebudowy tkanki kostnej pływające na przebieg osteoporozy" Nowa Klinika, 7: 666-674, 2000*

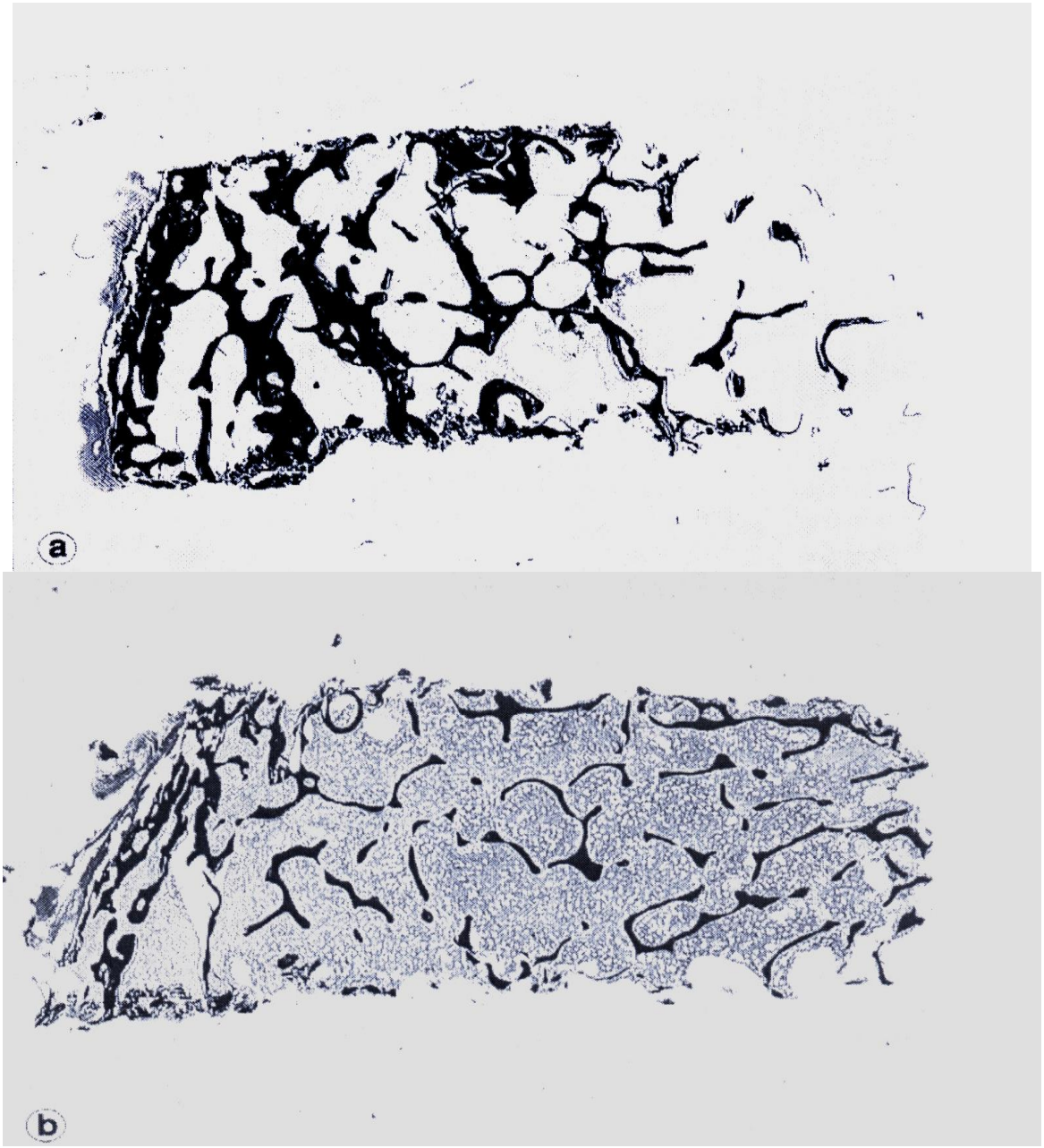


Ryc. 1. Scheme of osteoclastogenesis. This cell to cell system is composed of three elements:
(1) **OPGL** – Osteoprotegerin Ligand also called ODF (Osteoclast Differentiation Factor), produced by bone marrow stromal cells or by osteoblasts,
(2) **RANK receptor** (Receptor Activator of NFκB) for OPGL/ODF, present on the cell membrane of osteoclasts and its precursors,
(3) **OPG** (Osteoprotegerin), also called OCIF (Osteoclastogenesis Inhibitory Factor) – decoy receptor for OPGL, synthesized by osteoblasts and marrow stromal cells, released to microenvironment.

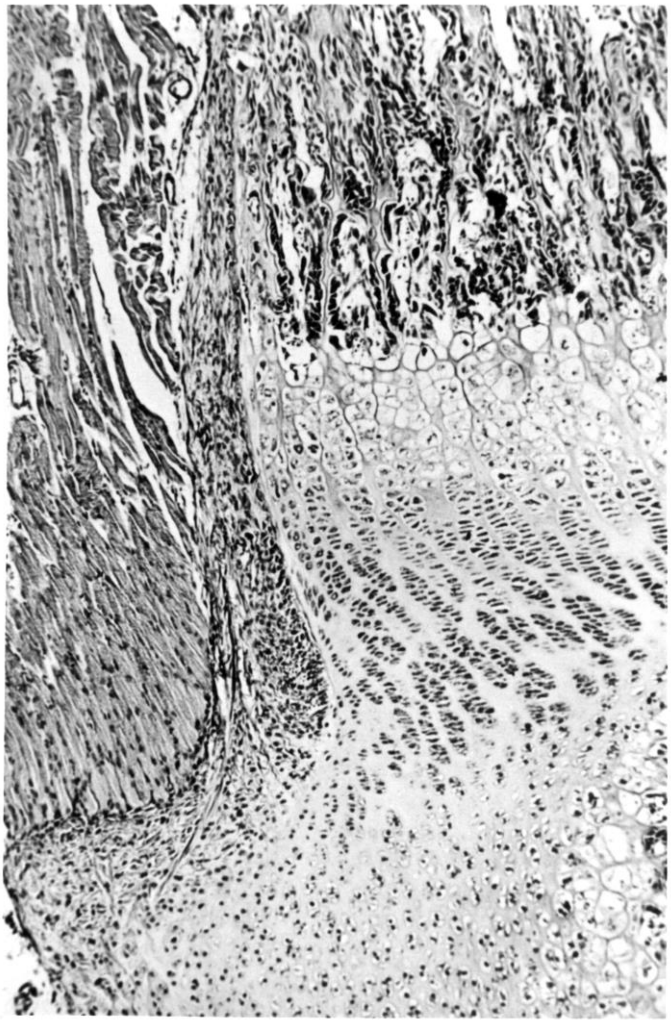
OPGL/ODF	**OPGL** osteoprotegerin ligand **ODF** osteoclast differentiation factor **SOFA** stromal cell-derived osteoclast formation activity **TRANCE** tumor necrosis factor related activation induced cytokine **TNFSF 11** tumor necrosis factor superfamily member 11
OPG/OCIF	**OPG** osteoprotegerin **OCIF** osteoclastogenesis inhibitory factor **DcR3** decoy receptor 3 **TNFRSF 11B** tumor necrosis factor receptor superfamily 11B **TRIAL-R5** T-cell receptor apoptosis inducing ligand-receptor 5 **FDCR-1** follicular dendritic cell receptor-1 **TR1** TNF-receptor-like molecule 1
RANK RECEPTOR	**RANK** receptor activator of nuclear factor κB (NFκB) **TNFSRF 11A** tumor necrosis factor receptor superfamily member 11A **ODAR** osteoclast differentiation and activation receptor

Fig. 2. Synonyms of molecules involved in cell to cell interaction in osteoclastogenesis

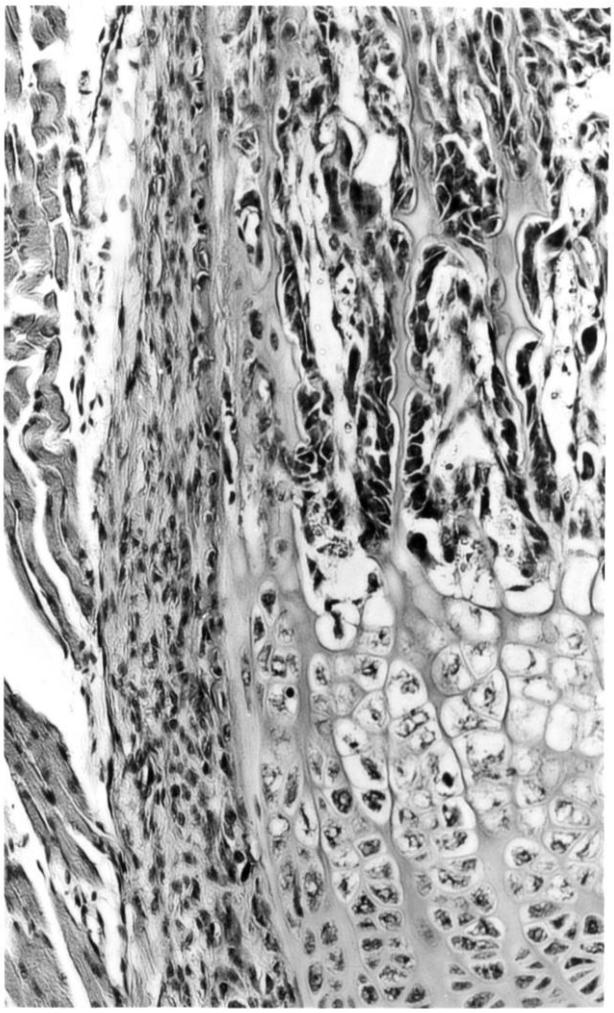
Fig. # 86



a. Epon–embedded normal bone from transiliac bone biopsy. Cortical and spongy bone is stained in dark.
b. Similar bone taken from osteoporotic patient. Cortical bone is thinner, bone trabeculae are less numerous and thinner than in ,,a".



fot. 28a



fot. 28b

OSTEOGENIC GROOVE AND PERICHONDREAL RING

On the periphery of epiphyseal plate osteogenic groove (Ranvier's) ***fot. 28a*** and Lacroix' perichondreal ring ***fot. 28b*** are present. Both elements are different parts of the same structure, but because of different function they play, they are described separately.

Fot. 28a. Osteogenic groove containing round and oval cells, arranged in cuneus form, penetrate the epiphyseal cartilage at the level of their resting zone. These cells intensively proliferate and are involved in appositional growth of cartilage. Apart from cells involved in appositional growth of cartilage, the osteogenic groove contains fibroblasts which produce bundles of collagen fibres covering osteogenic groove and anchoraging cells to the cartilage, as well as cells differentiating into osteoblasts, which produce bone in the perichondreal ring.

Fot. 28b. Perichondreal ring is made of densely packed collagen fibres, surrounding epiphyseal cartilage at the level of junction with bone. Fibres run horizontally, vertically and obliquely. This ring connects fibroblasts and fibres of osteogenic groove with the periosteal membrane and with subperiosteal bone of metaphysis. The inner layer of perichondreal ring is made of bone, but sometime this bone is absent.

Prof. S. Moskalewski – author

Prof. K. Włodarski – translator