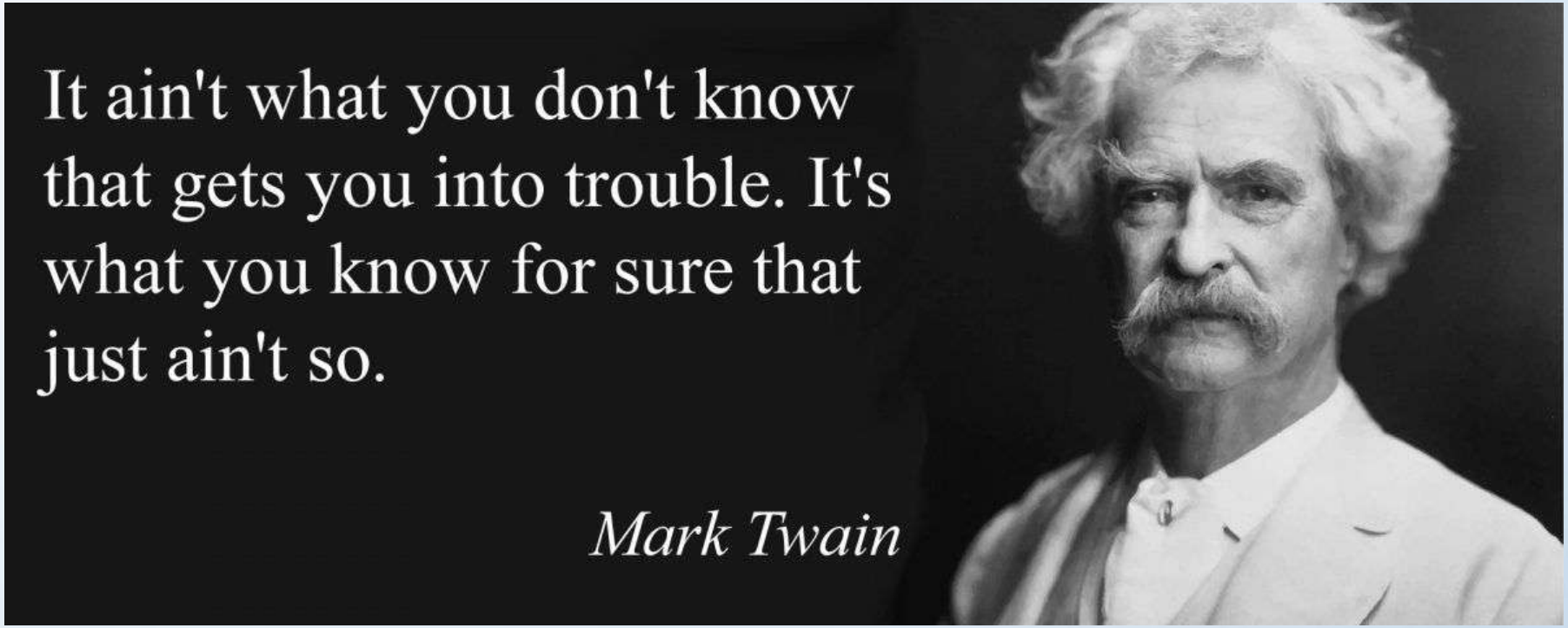
Basic Sciences

By Dr Daivik T Shetty

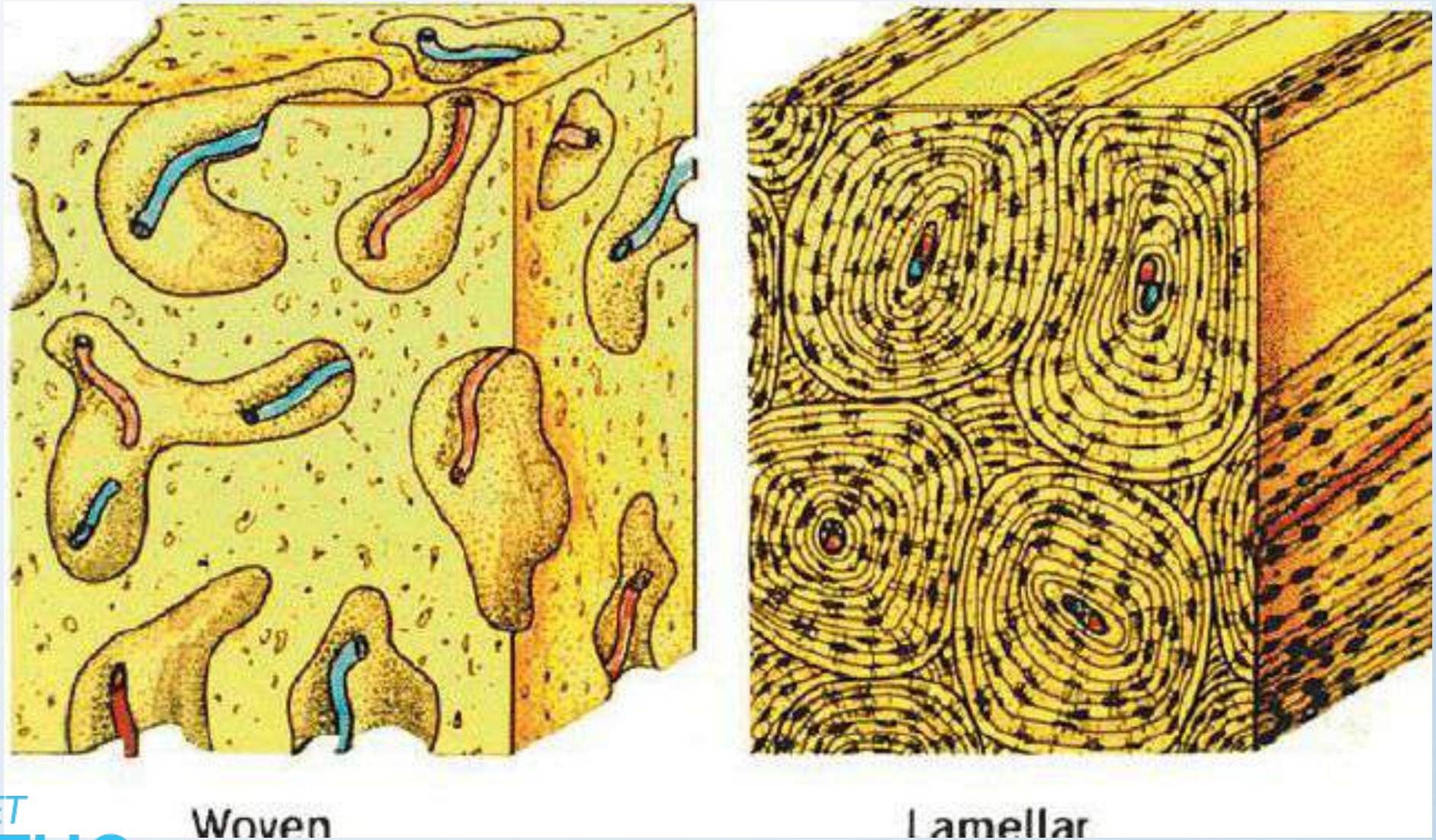


Bone

HISTOLOGY OF BONE

- Lamellar
  - Normal Cortical
  - Normal cancellous bone

- Woven
  - Immature
  - Pathological

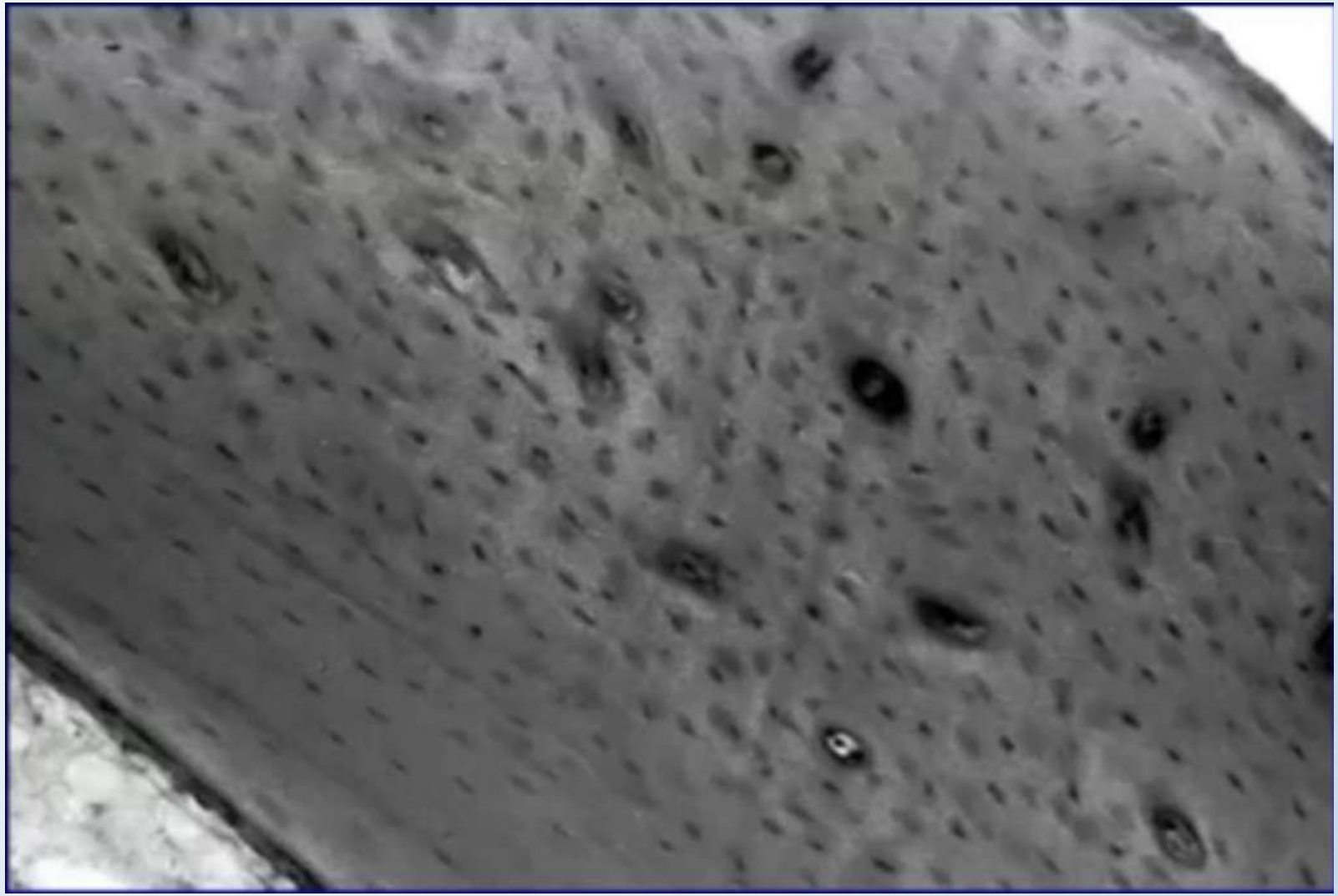


LAMELLAR BONE

- Organized
- Less cellularity
- Stress oriented (**WOLFF'S LAW**)

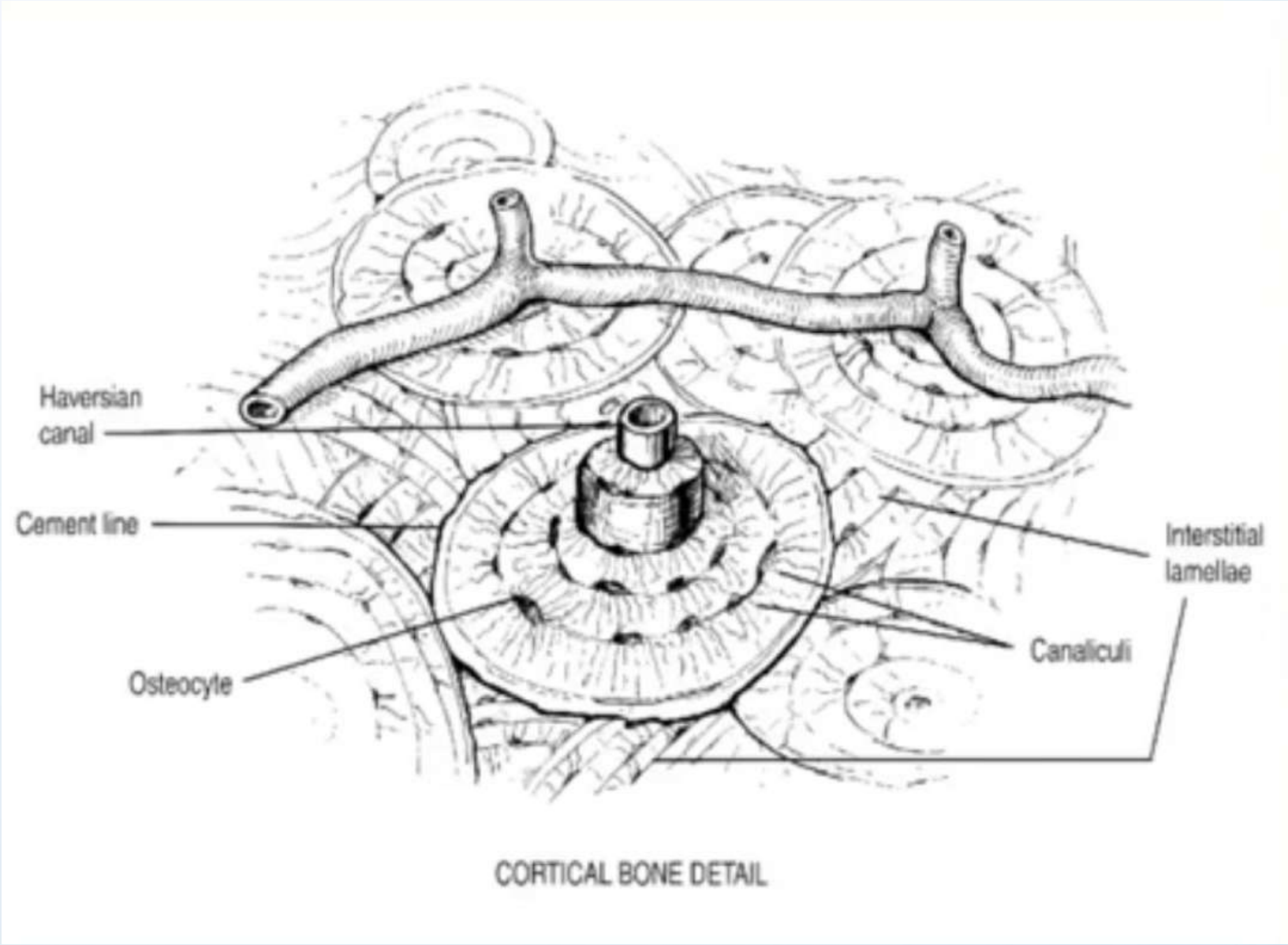
CORTICAL BONE (COMPACT BONE)

- 80% of skeleton
- Slow turnover rate
- Composed of **Osteons**
- High Young's modulus (E)
- High resistance to torsion and bending



OSTEONS OR HAVERSIAN SYSTEM

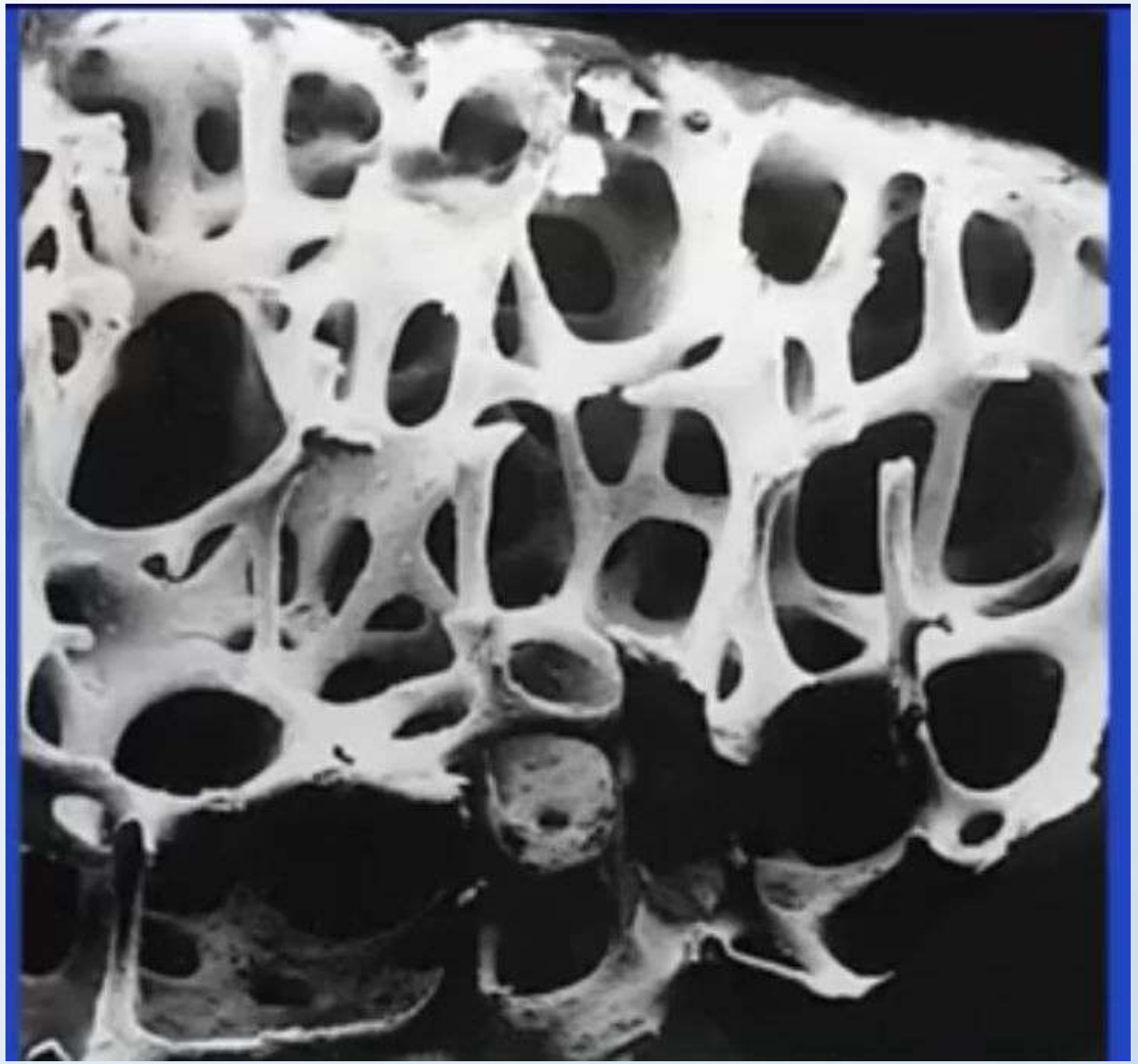
- Functional unit of bone
  - VOLKMANN'S CANAL (vessels)
  - INTERSTITIAL LAMELLAE
  - CEMENT LINES
  - CANALICULI



CORTICAL BONE DETAIL

Cancellous (Trabecular bone)

- Less dense than cortical bone
- High turnover rate
- More elastic than cortical bone
- Smaller Young's modulus (E)



WOVEN BONE

- Immature – fracture callus or pathological (infection , malignancy , stress reaction)
- Weak
- Random organization
- Increased turnover
- Not stress oriented
- More cellularity

Cellular biology of bone

- Osteoblasts
- Osteocytes
- Osteoclasts
- Osteo progenitor cells

Which of the following is considered a key characteristic of osteoblasts?

a) Produce acid phosphatase

b) Responsive to parathyroid hormone

c) Produce Osteocalcin when stimulated by 24 25 dihydroxy vitamin D

d) Resorb mineralized bone

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OSTEOBLASTS

- Bone forming cells

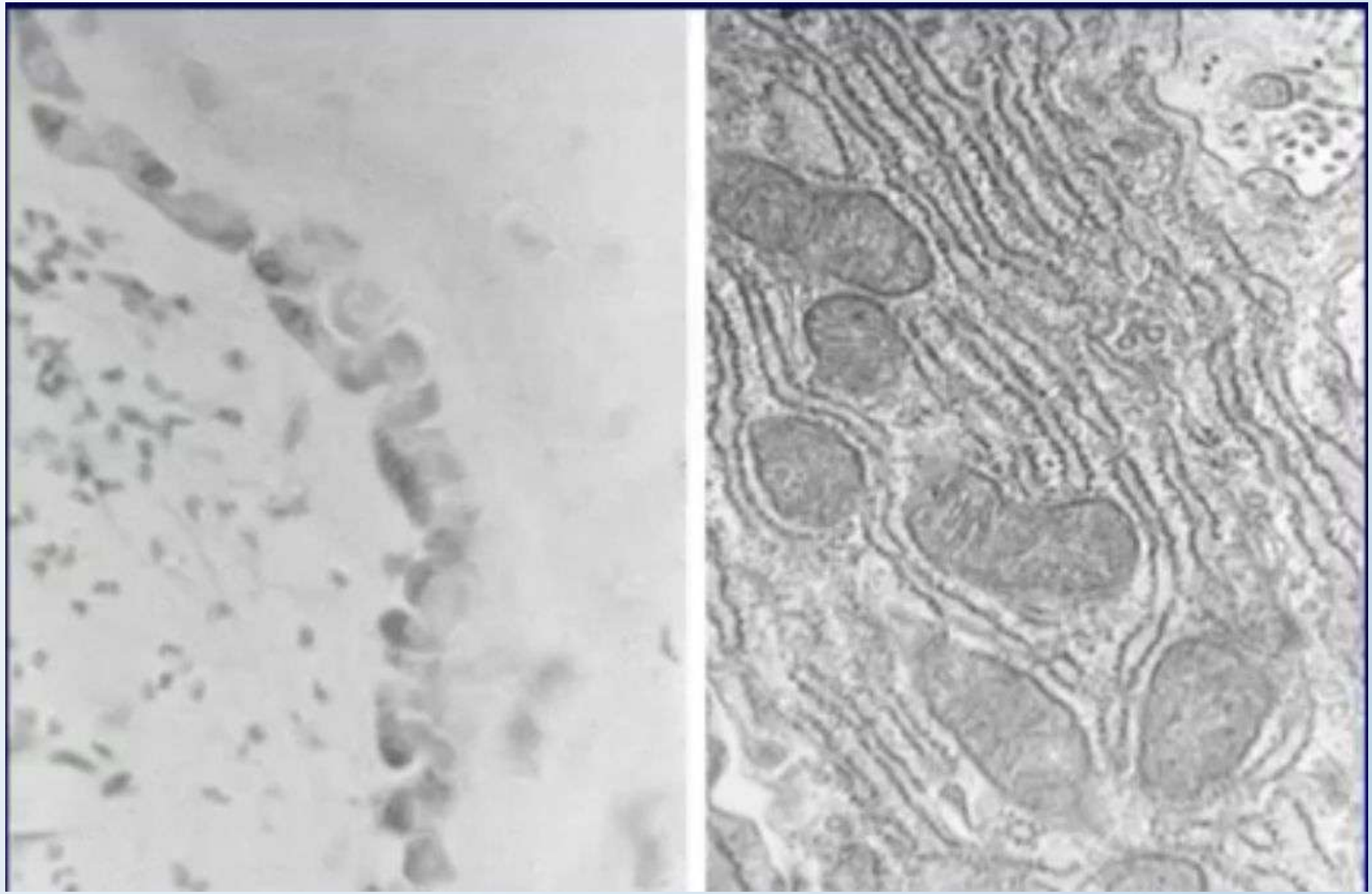
✔ Derived from **undifferentiated mesenchymal cells**

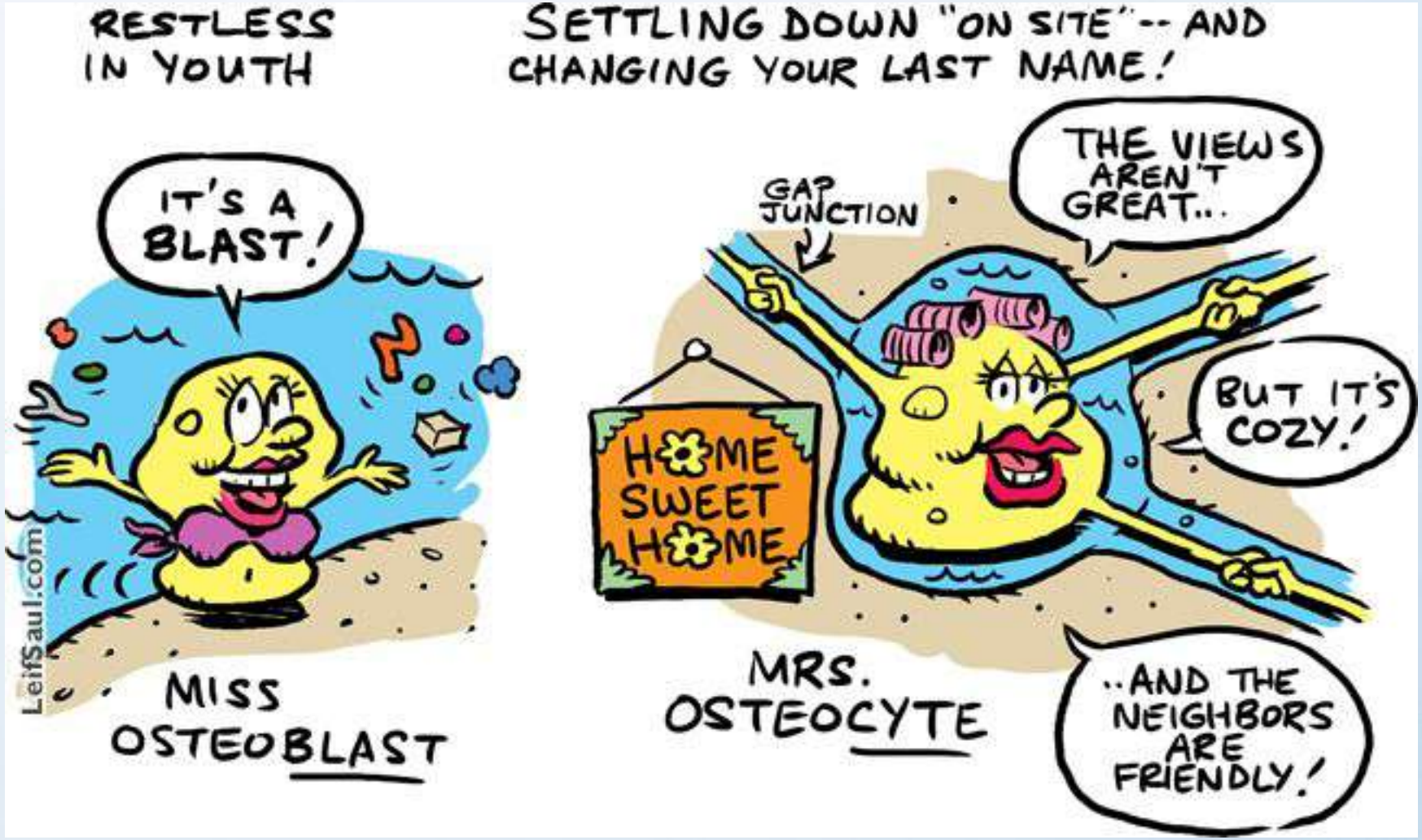
✔ **Synthesize type 1 collagen**

✔ High alkaline phosphatase activity

Osteoblast receptors

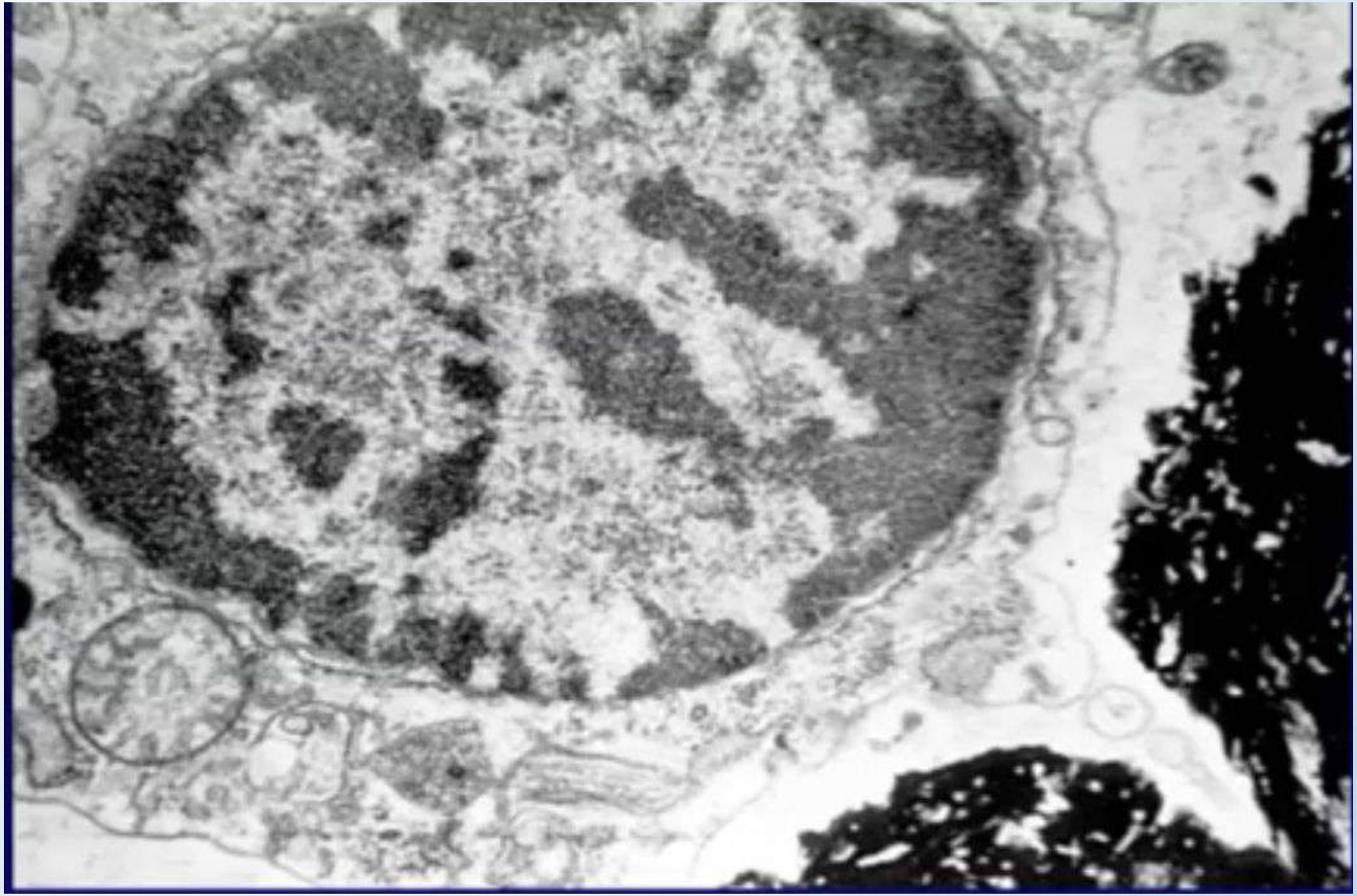
- **PARATHYROID HORMONE (PTH)**
  - Releases secondary messenger to stimulate osteoclastic activity
- **1,25 dihydroxyvitamin D**
  - Stimulates matrix , alk phos synthesis and production of bone specific proteins (Osteocalcin)
- **Glucocorticoids**
  - Inhibits synthesis of DNA , production of collagen and synthesis of osteoblastic proteins
- **Prostaglandins**
  - Activates adenylate cyclase mediated bone resorption
- **Estrogen**
  - Anabolic and anticatabolic





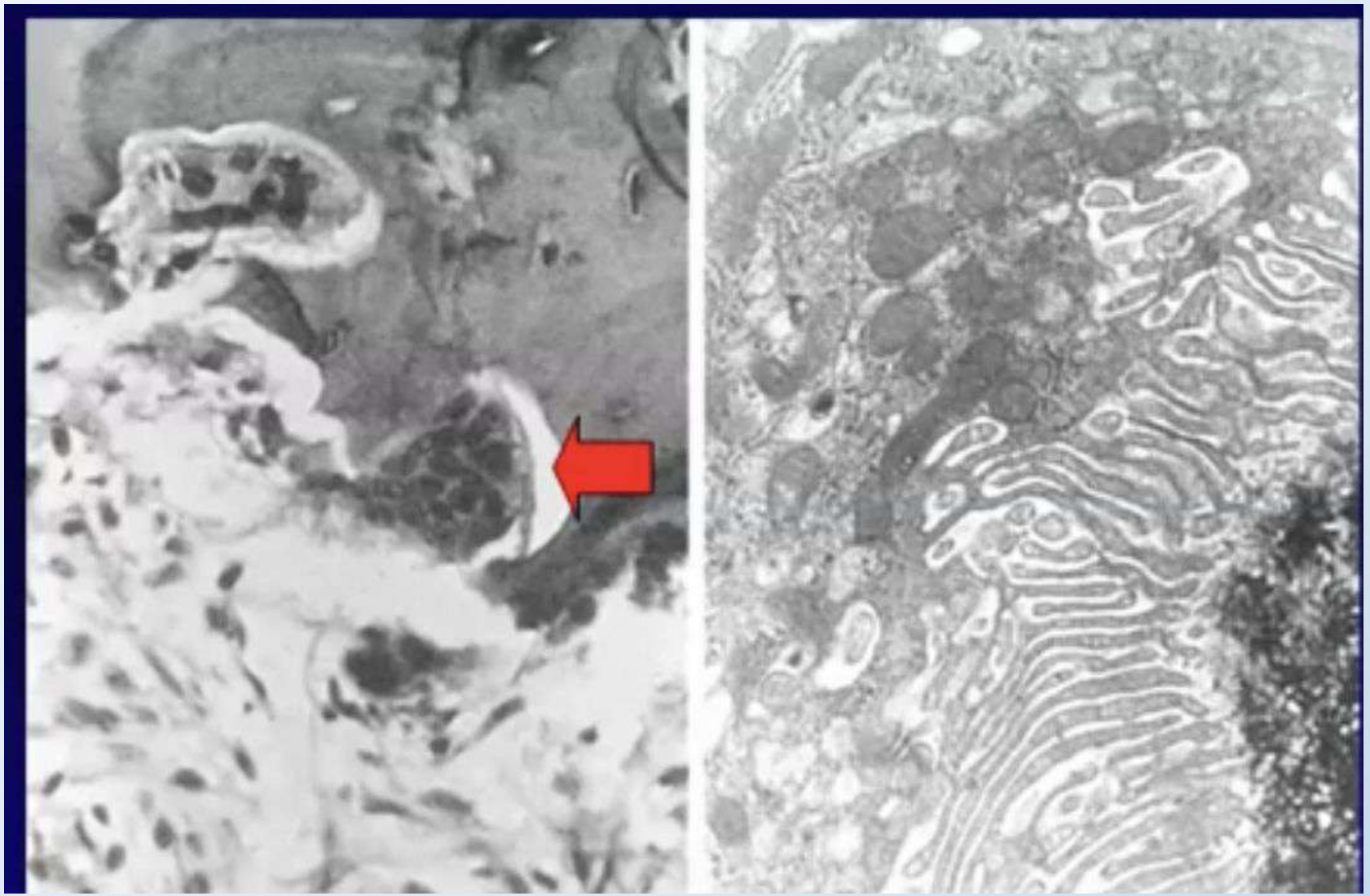
OSTEOCYTES

- 90% of cells in mature skeleton
- Former osteoblasts trapped in new matrix
- Maintain bone
- Control extra cellular concentration of calcium and phosphorus
- Directly stimulated by calcitonin and inhibited by PTH



OSTEOCLASTS

- Resort bone
- Multinucleated irregularly shaped giant cells
- **Originate from monocytes**
- Ruffled border – increase surface area for resorption
- Bone resorption at **Howship's lacunae**



OSTEOCLASTS

- Bone formation and resorption coupled
- Osteoclasts synthesize **tartarate resistant acid phosphatase**
- Bind to bone surface via **bone anchoring protein (integrins)**
- **Possess specific receptors for calcitonin which inhibit bone resorption**

Which of the following has been implicated in the Patho genesis of OSTEOARTHRITIS ?

a) Interleukin 1

b) Cyclooxygenase 1

c) BMP 2

d) T cells

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a) **Interleukin 1**

b) Cyclooxygenase 1

c) BMP 2

d) T cells

OSTEOCLASTS

- **IL-1-** Potent stimulator for osteoclastic bone resorption and has been found in membranes surrounding loose total joint arthroplasties

- May have role in osteoarthritis

A fully differentiated osteoclasts has receptors for which of the following protein?

a) Parathyroid hormone

b) Calcitonin

c) Interleukin 2

d) Cholecalciferol

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OSTEOPROGENITOR CELLS

- Precursor of osteoblasts
- Mesenchymal cells lining Haversian canals, endosteum
- Differentiate after receiving appropriate stimulus

BONE MATRIX

- Organic components (40%)

- Inorganic components (60%)

You start losing bone density due to osteoclast overactivity	
more osteoblasts are recruited	
The bone tissue formed is unorganised	

ORGANIC COMPONENTS

- Collagen
- Proteoglycans
- Non collagenous matrix proteins

✔ Glycoproteins

✔ Phospholipids

✔ Phosphoprotiens

- Growth factors
- Cytokines

COLLAGEN

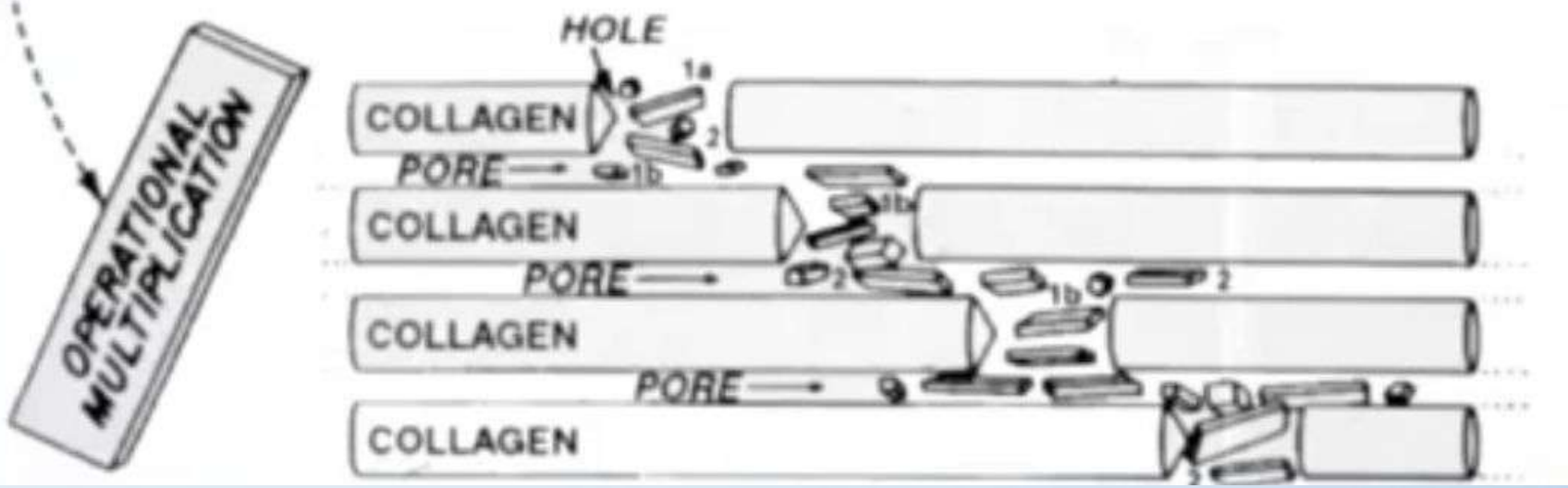
- Tensile strength of bone
- Type 1 collagen (90% of organic matrix)
- Triple helix (2 ã1 and 2 ā2 chains) - FIBRIL
- Mineral deposition in pores and hole zones
- Cross linking decreases solubility and increases tensile strength

MINERAL ACCRETION: *BIOLOGICAL CONSIDERATIONS*

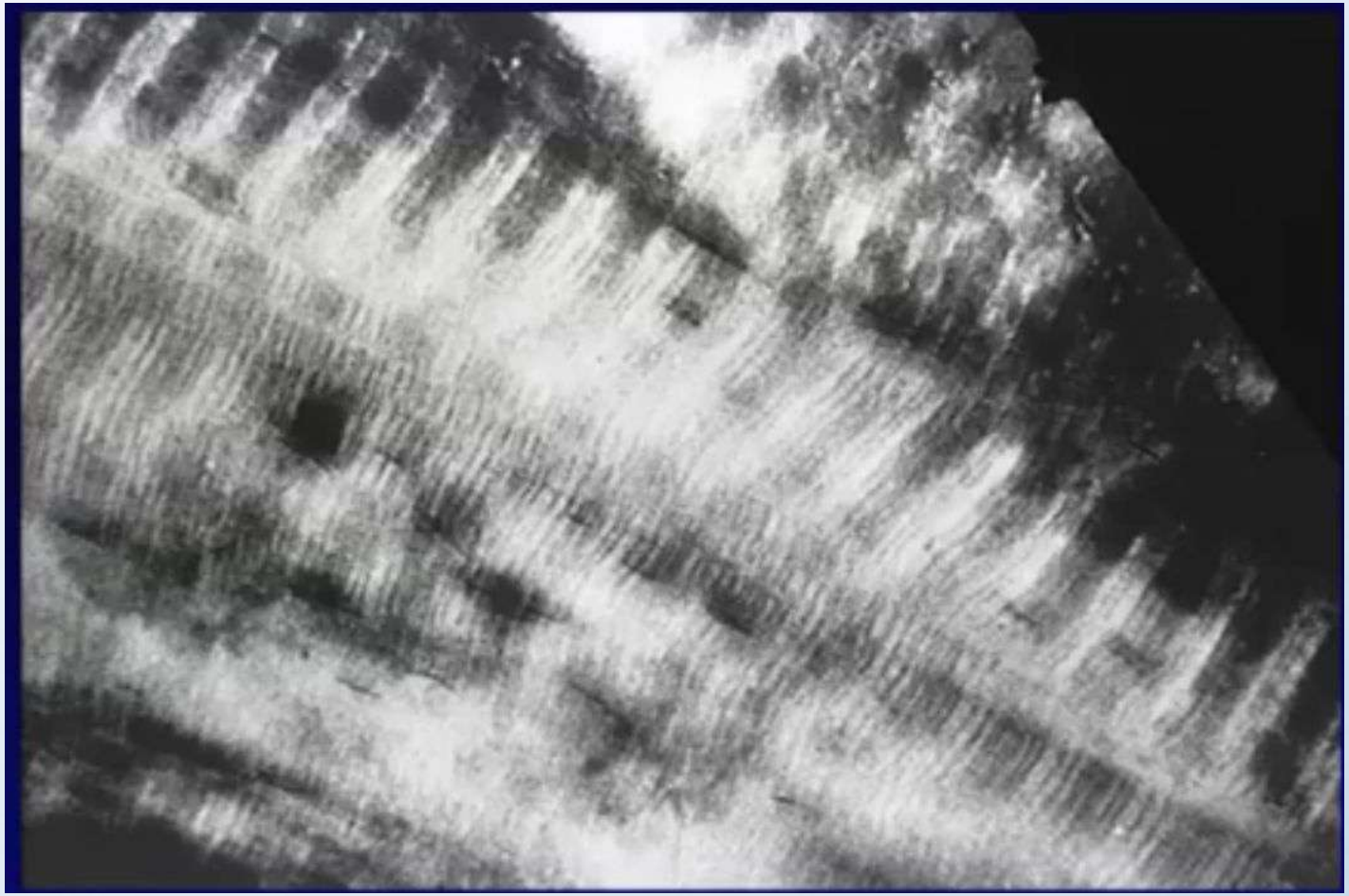
HETEROGENEITY WITHIN A COLLAGEN FIBRIL

PROGRESSIVELY INCREASING MINERAL MASS DUE TO:

1. INCREASED NUMBER OF NEW MINERAL PHASE PARTICLES (NUCLEATION)
   a. HETEROGENEOUS NUCLEATION BY MATRIX IN COLLAGEN HOLES (? PORES
   b. 2° CRYSTAL INDUCED NUCLEATION IN HOLES AND PORES
2. INITIAL GROWTH OF PARTICLES TO ~ 400Å x 15–30Å x 50–75Å



EM OF BONE COLLAGEN



Over time, you lose collagen, neurons, hair, memory ...
Ahhh, but fat, the fat is faithful, that stays until the end!

PROTEOGLYCANS

- **Compressive strength of bone**

- Composed of GAG protein complexes

Matrix proteins

- Promotes mineralization and bone formation
  - Osteocalcin (bone GLA protein)
  - Osteonectin
  - Osteopontin

Osteocalcin

- Produced by osteoblasts
- **Most abundant noncollagenous matrix protein**
- Attracts osteoclasts
- Regulates bone density
- Inhibited by PTH and stimulated by 1,25 dihydrovitamin D
- Can be measured in serum or urine as a marker of bone turnover (Paget's,hyperparathyroidism)

Matrix proteins

- **OSTEONECTIN**

✔ Secreted by platelets, osteoblasts

✔ Matrix mineralization

- **OSTEOPONTIN**

✔ Cell binding protein

CYTOKINES AND GROWTH FACTORS

- Present in small amounts
- Involved in cell differentiation,activation , growth and turnover
- TGF-beta, IGF, IL-1, IL-6, BMP

INORGANIC COMPONENT (MINERAL)

- 60% of dry weight of bone

- **Calcium hydroxyapatite**
  - Compressive strength of bone

HYDROXYAPATITE

Ca 10 (PO4)6 (OH)2

BONE REMODELING

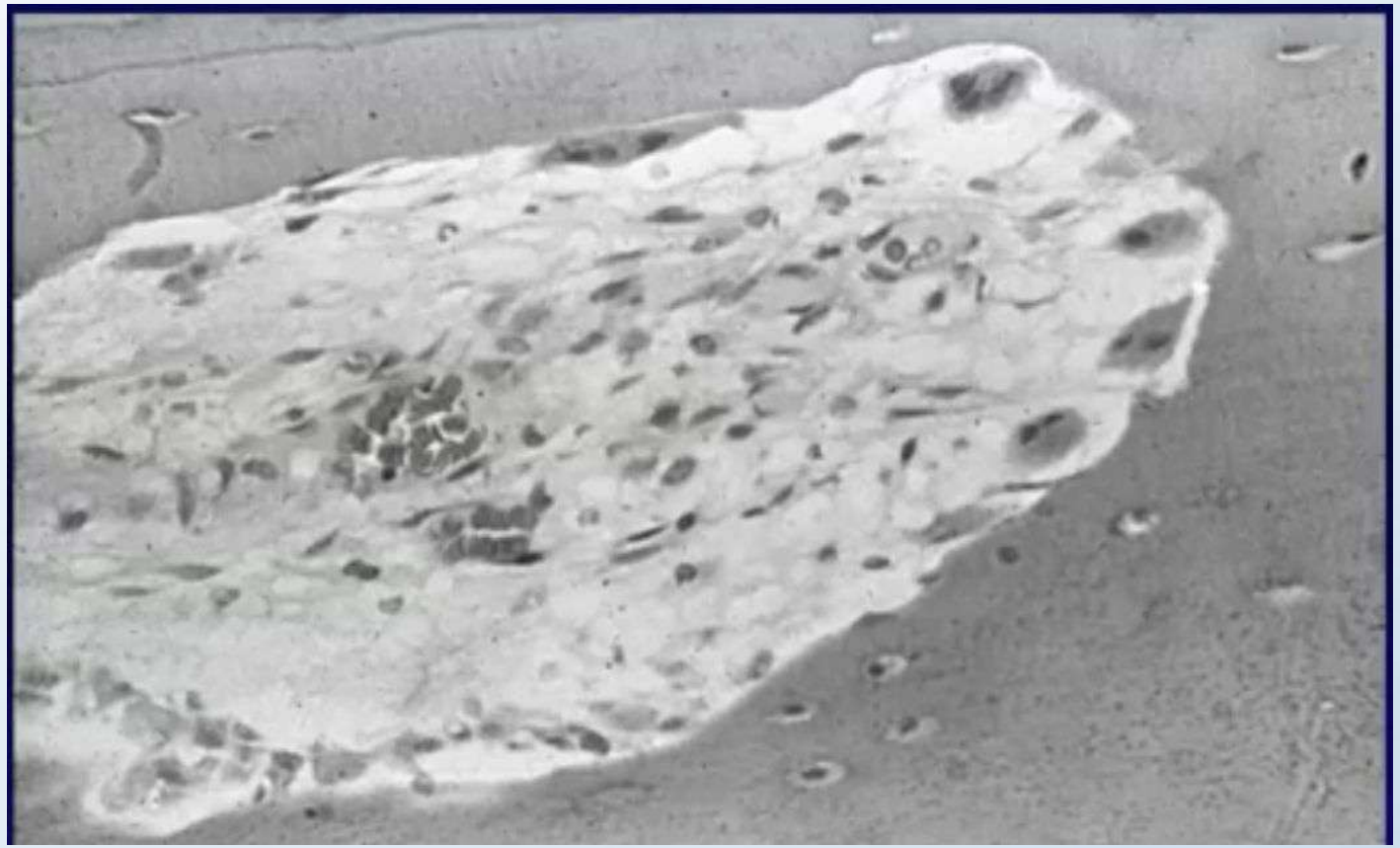
- Modulated by systemic hormones and local hormones

- Affected by stress – **WOLFF's LAW**

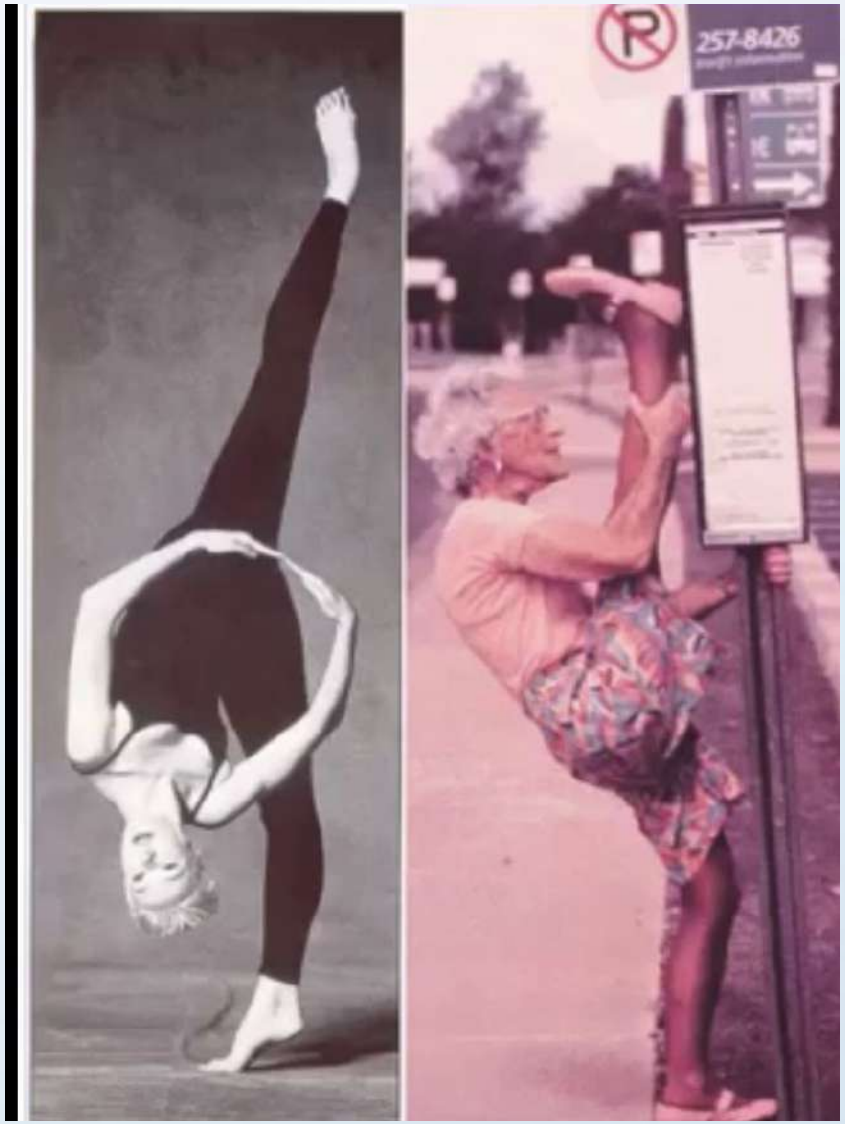
CORTICAL BONE REMODELLING

- Osteoclastic tunneling (cutting cones)
- Capillaries
- Osteoblasts

EM OF A CUTTING CONE



Basic science of Cartilage



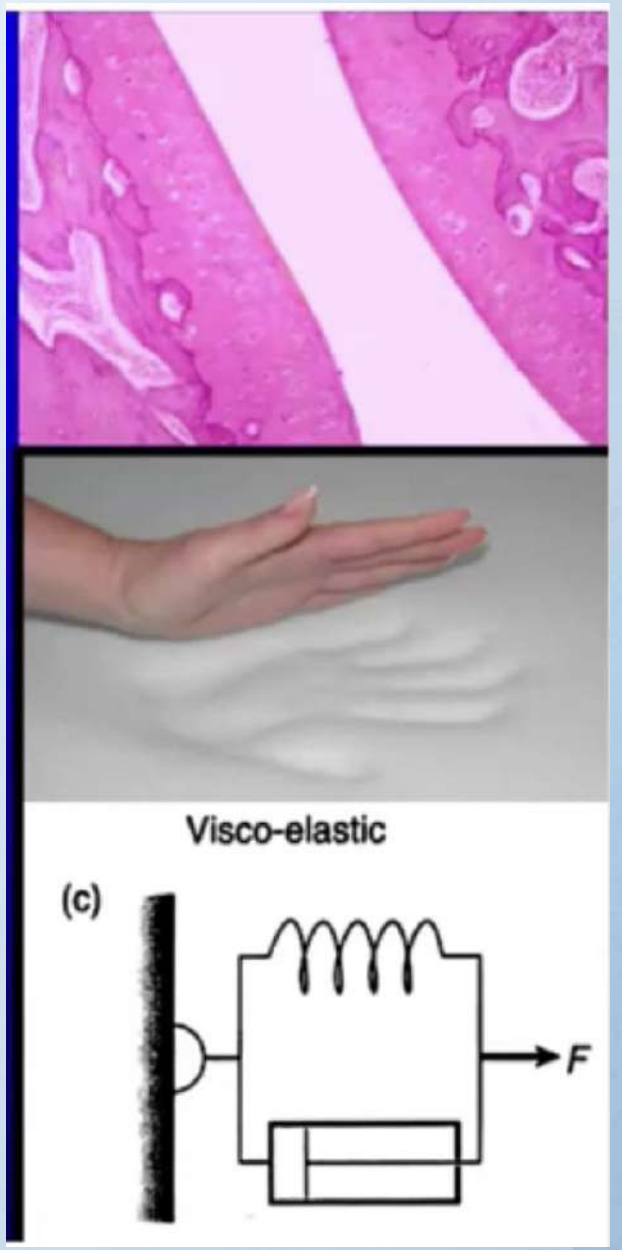
ARTICULAR CARTILAGE – HYALINE CARTILAGE

- 2mm thin slick smooth cushion
- Avascular, aneural and alymphatic
- **Nutrition – diffusion from synovial fluid**

- **Anisotropic** – properties vary with direction
- **Biphasic** – properties of both liquid and solid
- **Viscoelastic**

Viscous – thick liquid resists flow

Elastic – springs back to same shape



Function

- Distribute load : Proteoglycans resist compression
- **Impact loads up to 25 N/mm2**

- Decreased friction

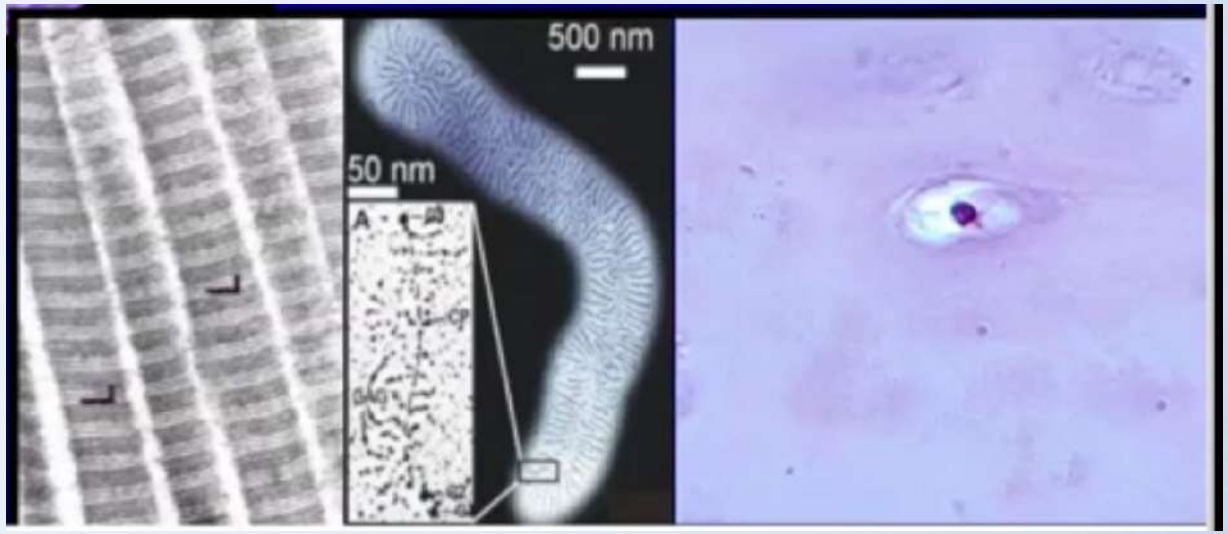
- HEALS POORLY

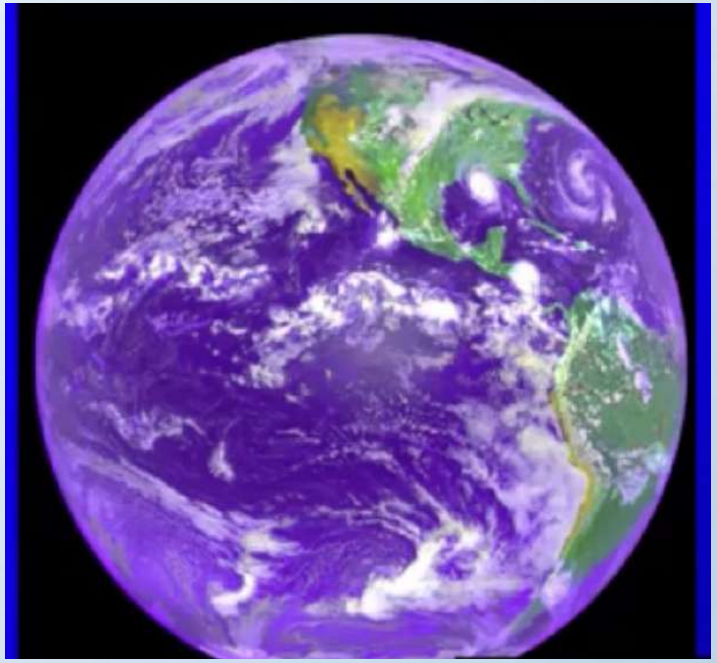
Chondral injury – not at all

Osteochondral injury – poorly

COMPOSITION

- Water – 74%
- Collagen – 15%
- Proteoglycans- 10%
- Chondrocytes – 1%
- Adhesive and lipids - <1%





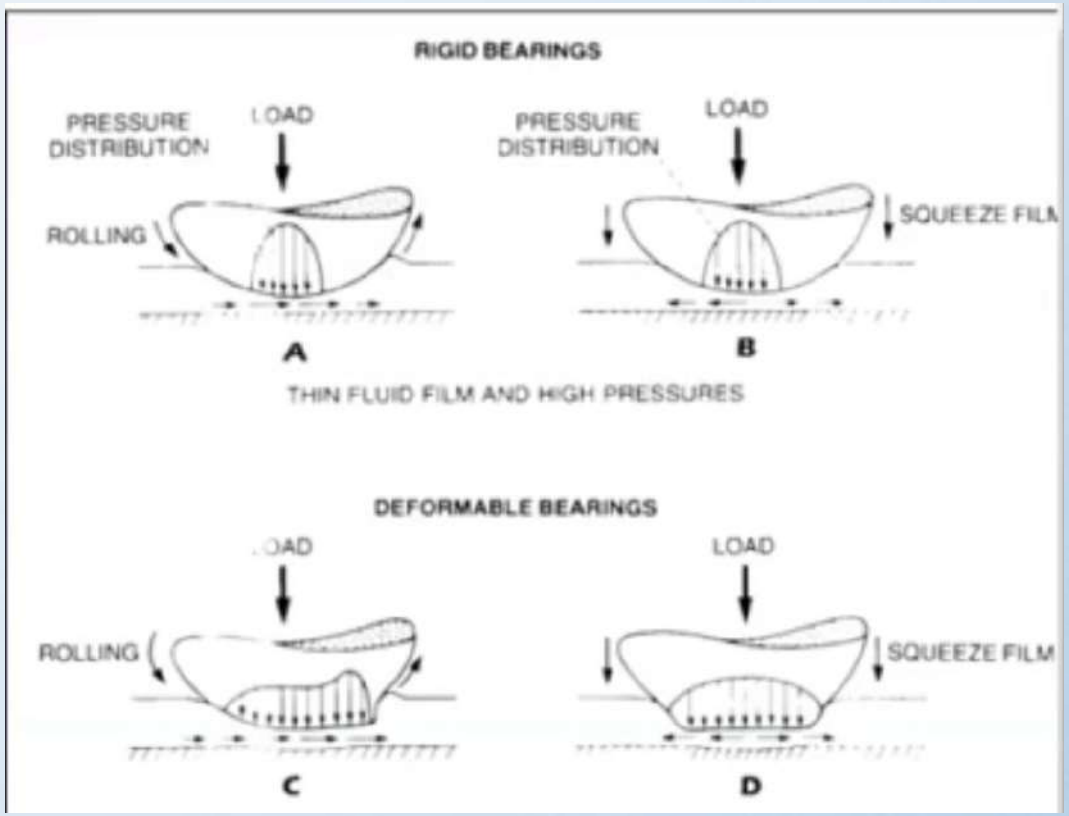
Water

- Fluid leaves hydrophilic Proteoglycans with deformation of load returns without load
- Carries nutrient
- Elastohydrodynamic lubrication

METAL ON METAL = .57 CoF

OILED MOM = .06

HUMAN JOINT = .003



Mention the type of cartilage present near CALCIFIED CARTILAGE

a) Type IX

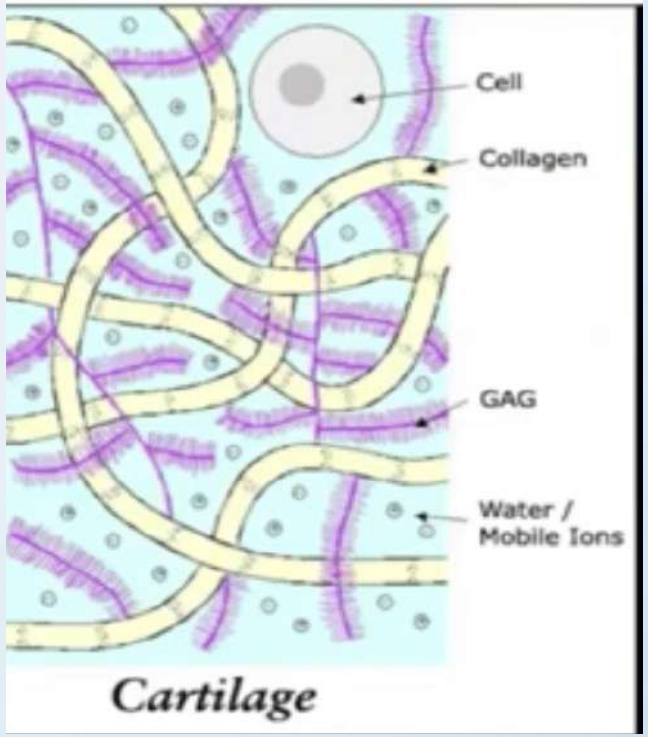
b) Type I

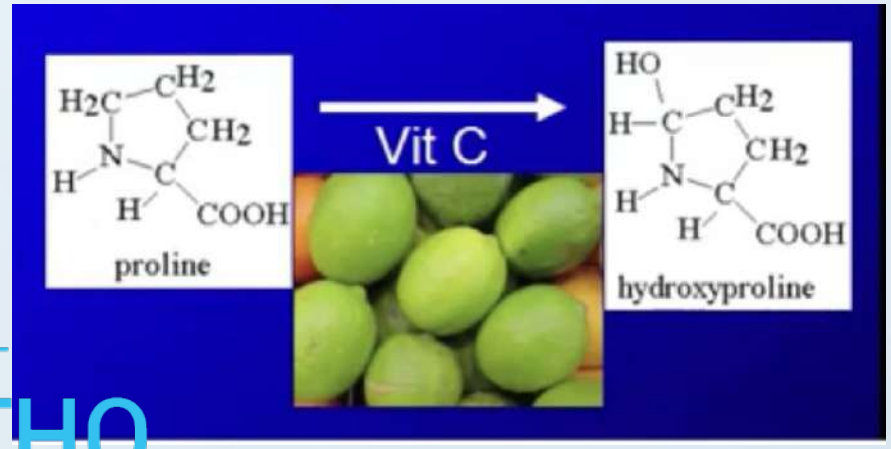
c) Type XI

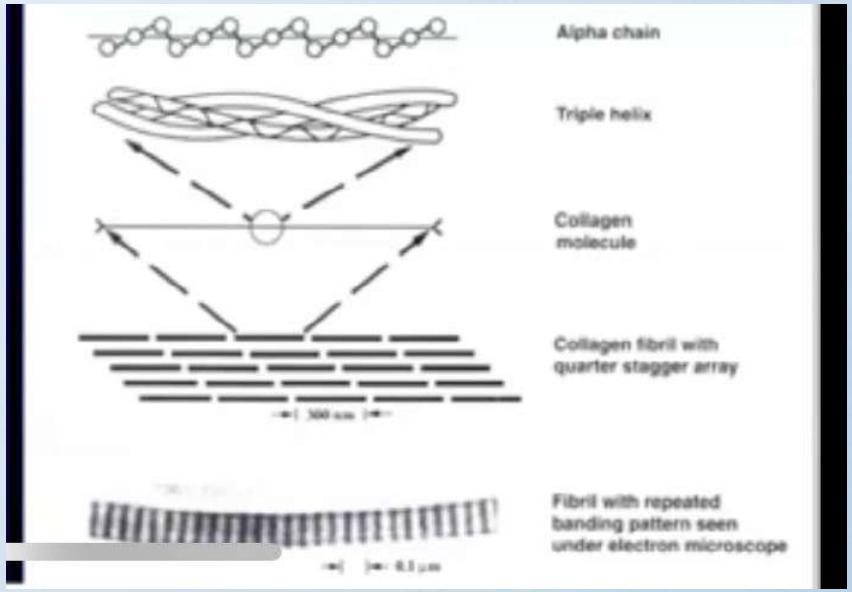
d) Type X

Collagen

- Tensile stiffness and strength
- Forms tight fibril mesh work to mechanically entrap Proteoglycans
- 3 alpha pro collagen chains wound together in triple helix

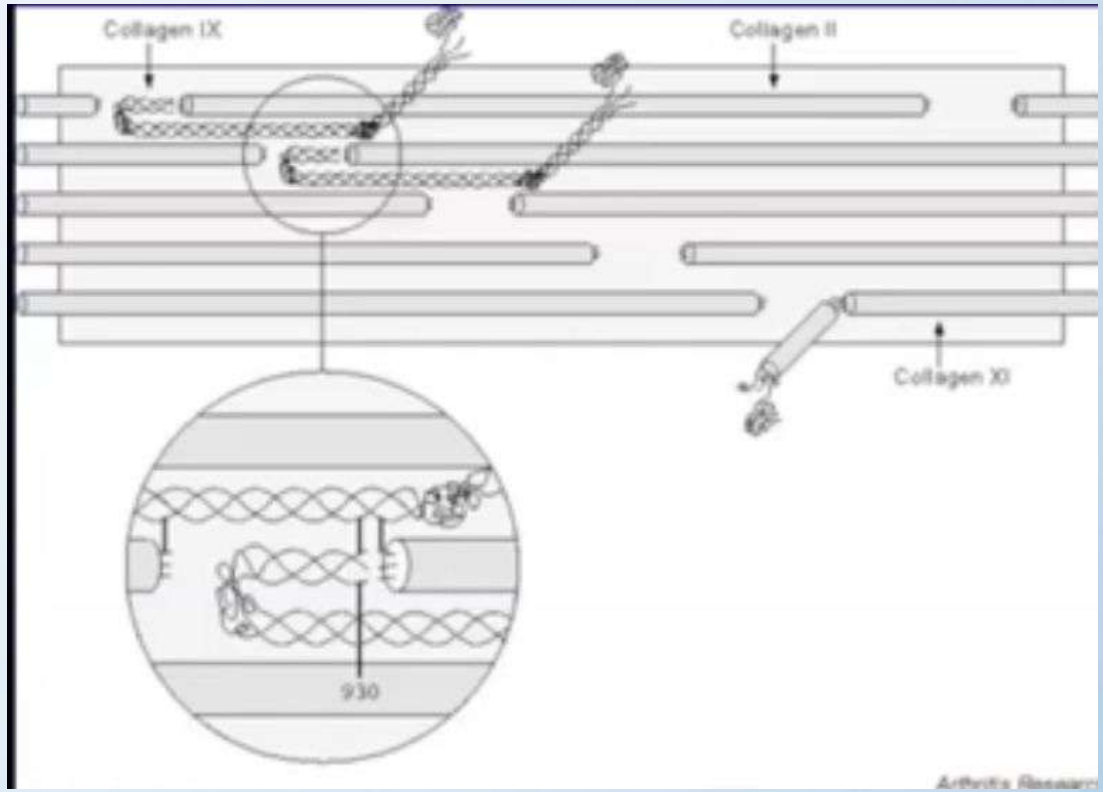






COLLAGEN

- Types in cartilage



Type 2 – 90-95% of cartilage

Type IX - links between II and II , IX

Type XI - links II and II

Type X – calcified zone of articulation cartilage

Hypertrophic zone growth plate – **ONLY NEAR CALCIFIED CARTILAGE**

Mention the type of cartilage present near CALCIFIED CARTILAGE

a) Type IX

b) Type I

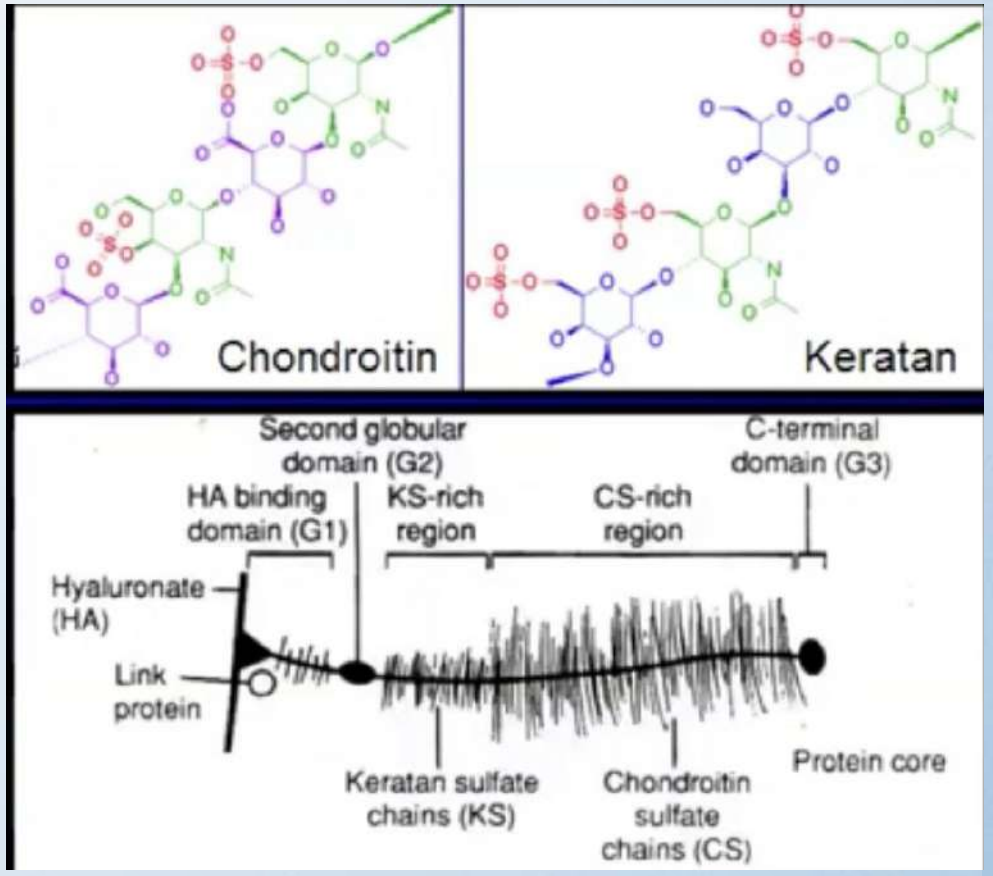
c) Type XI

d) Type X

PROTEOGLYCAN

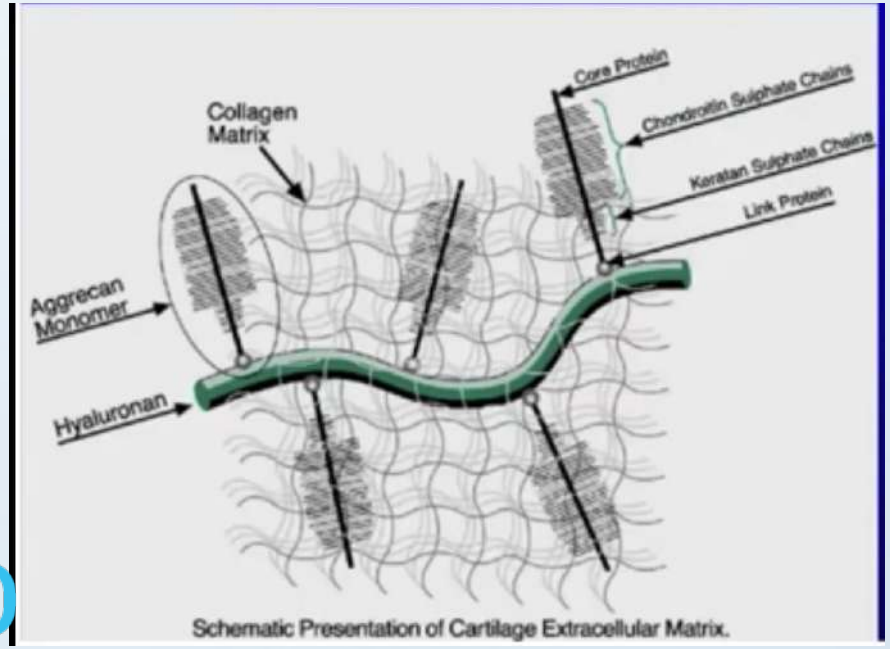
- 95% CHONDROTIN SO4
- 5% KERATAN SO4

- Long chain of negative charge – like charges repel
- Negative charge attract cations
- Increase osmotic pressure
- Inflates elastic mesh work
- Strength in compression

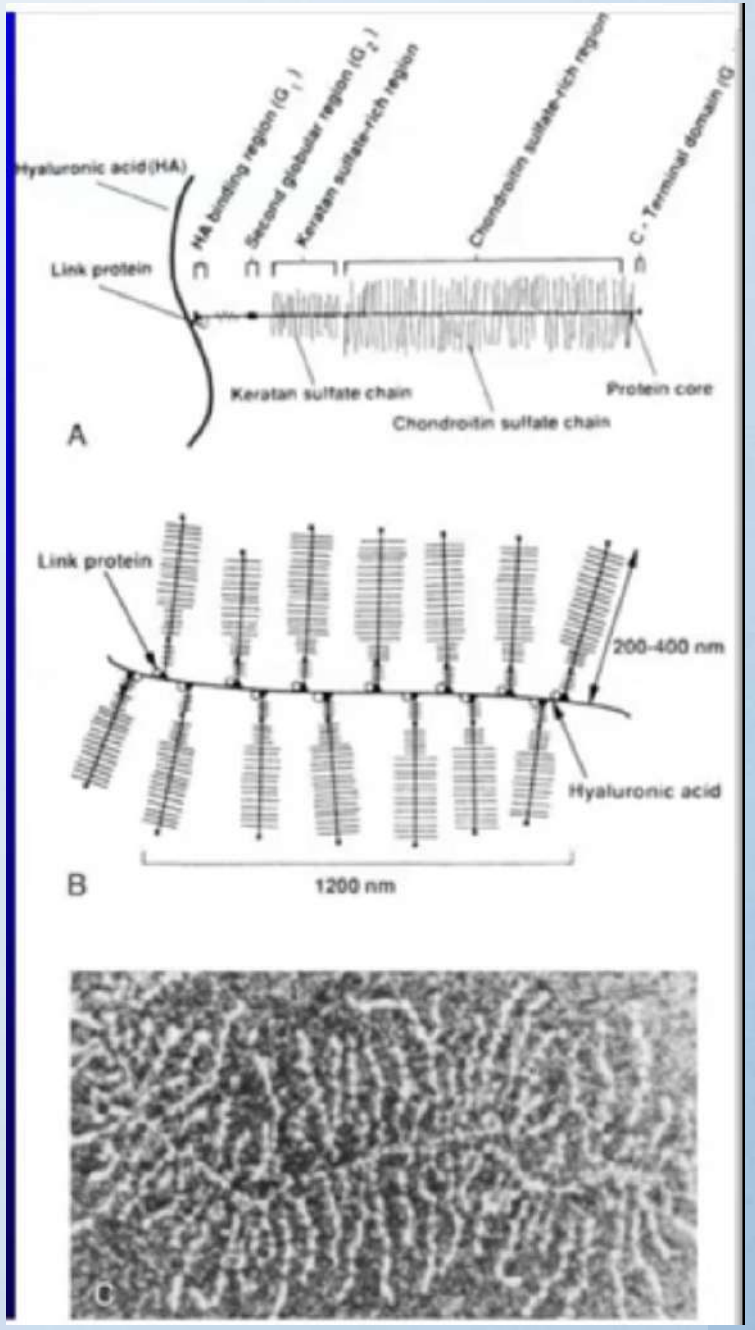


Proteoglycans – Aggrecan

- Aggrecan – mega molecules of matrix
- Monomers bound to hyaluronan backbone
- Fills interfibillar space of matrix
- Large aggregates provide structure

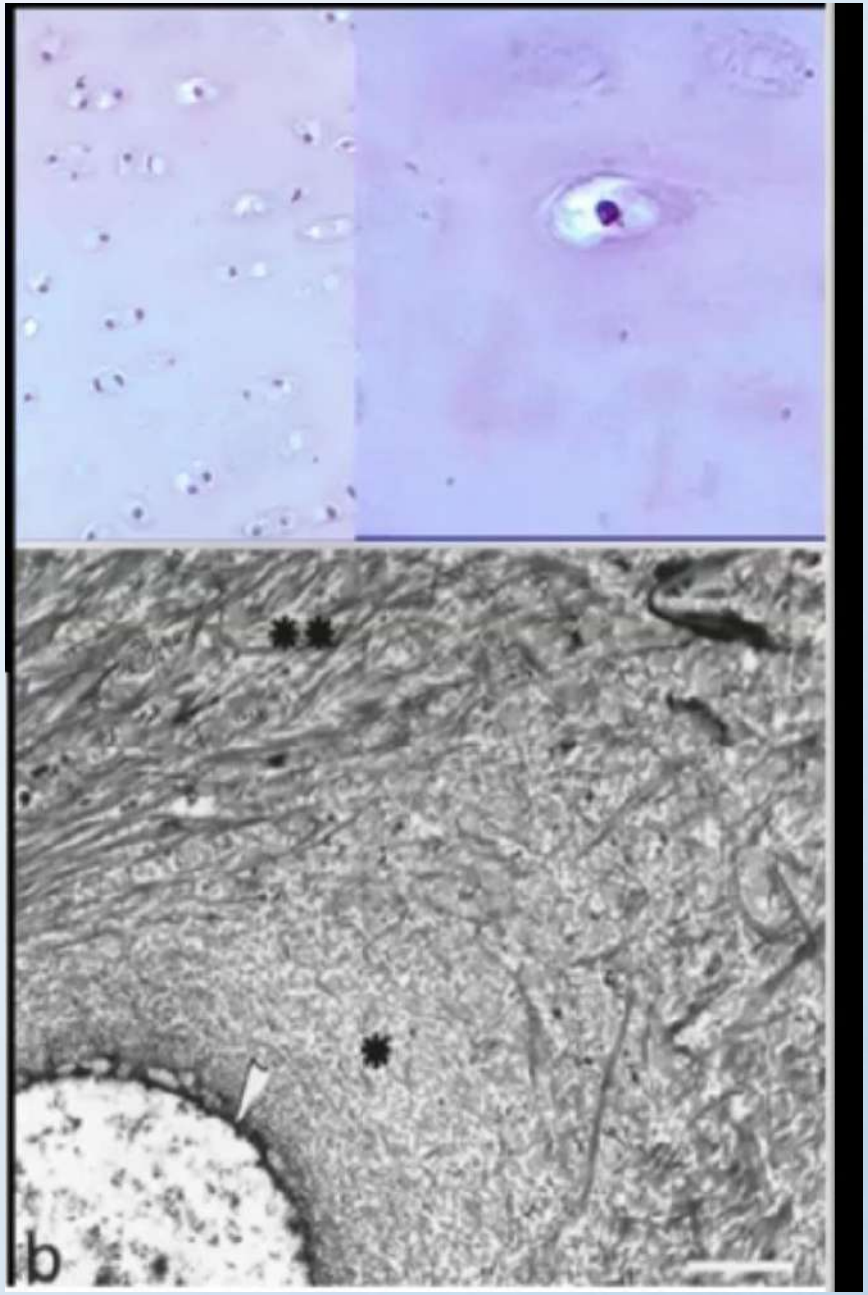


Schematic Presentation of Cartilage Extracellular Matrix.

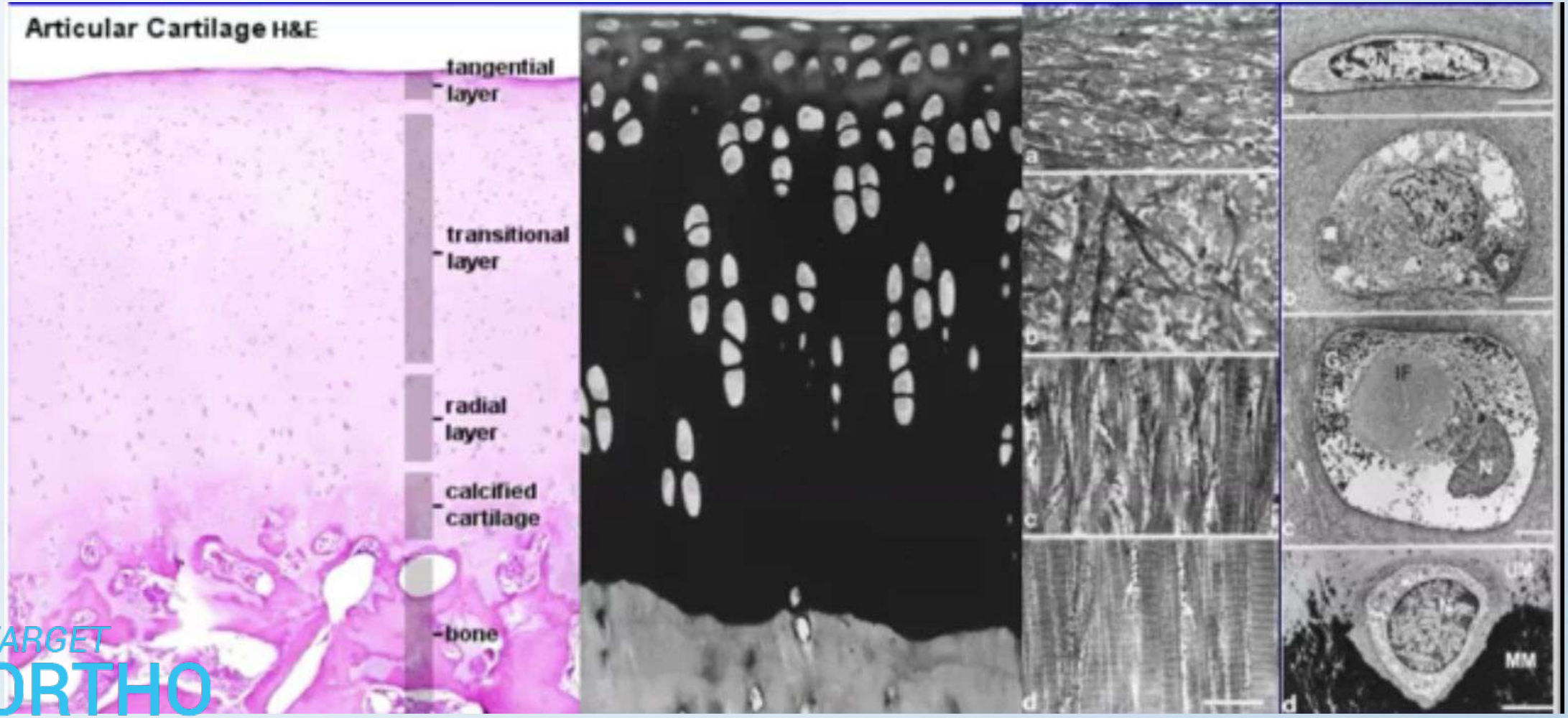


Chondrocytes

- Only cell of cartilage
- Anaerobic metabolism
- Metabolically active
- Synthesize matrix
- Maintain matrix structure
- Sense mechanical changes

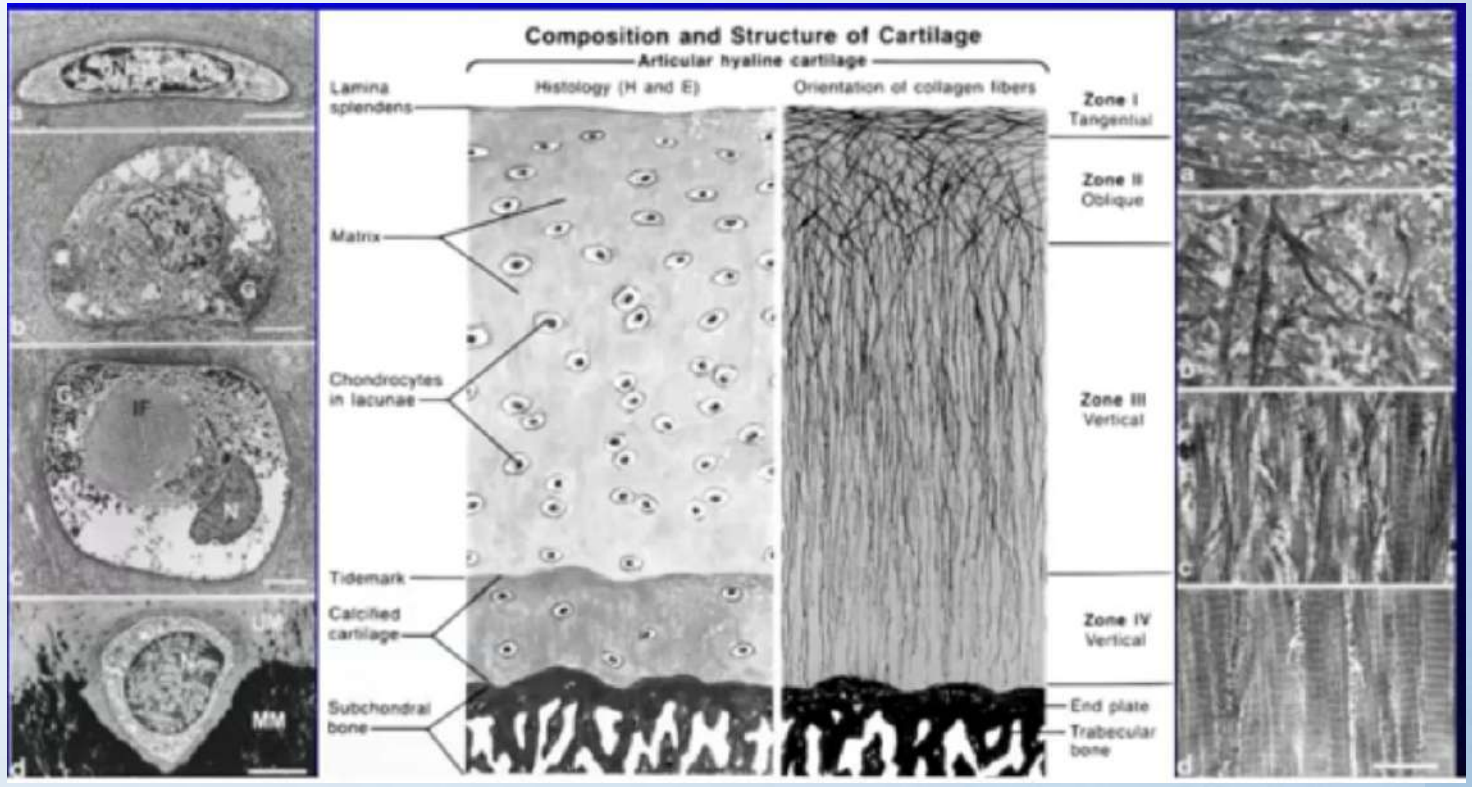


Layers



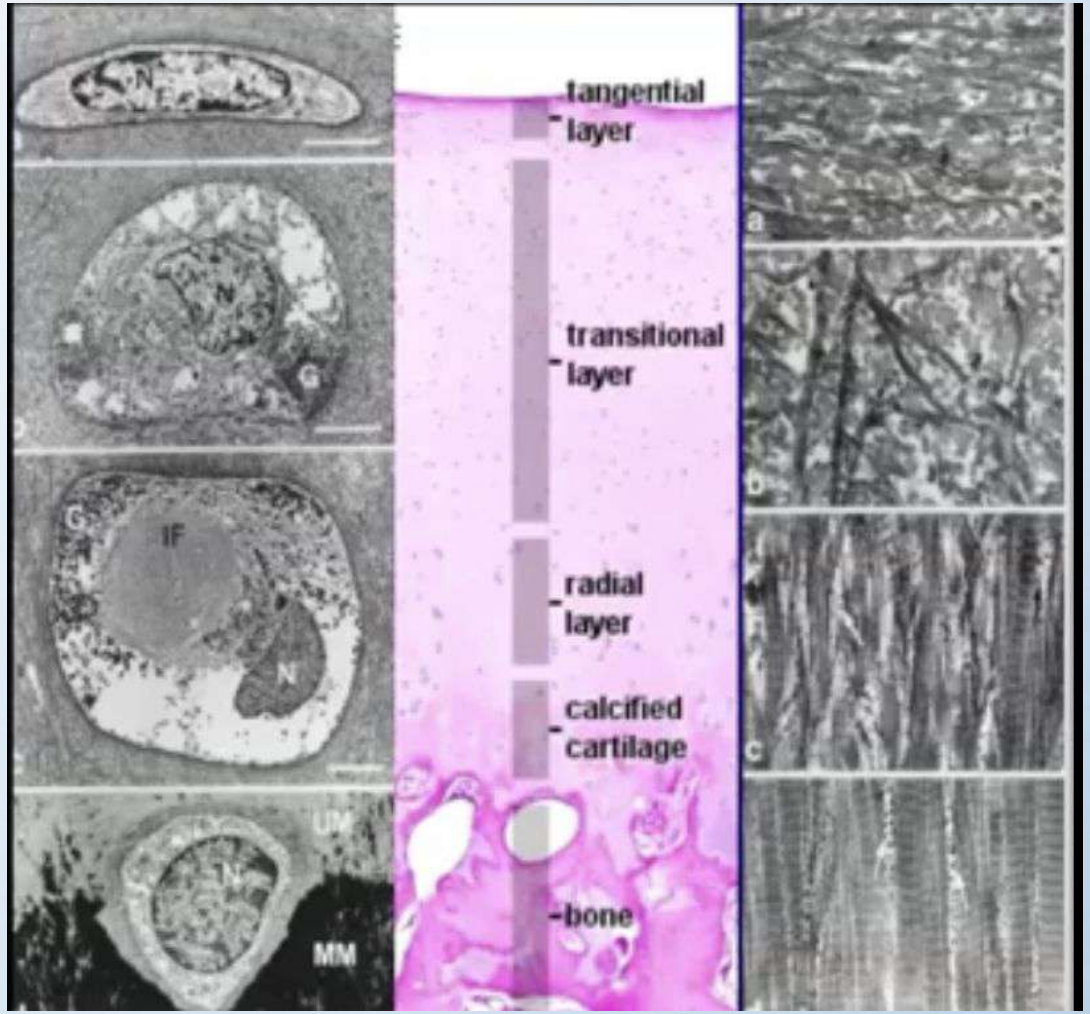
Superficial cartilage layer TANGENTIAL ZONE

- Highest collagen
- Fibers oriented tangentially
- Strength against sheer
- Highest water
- Least PG synthesis
- Flat chondrocytes



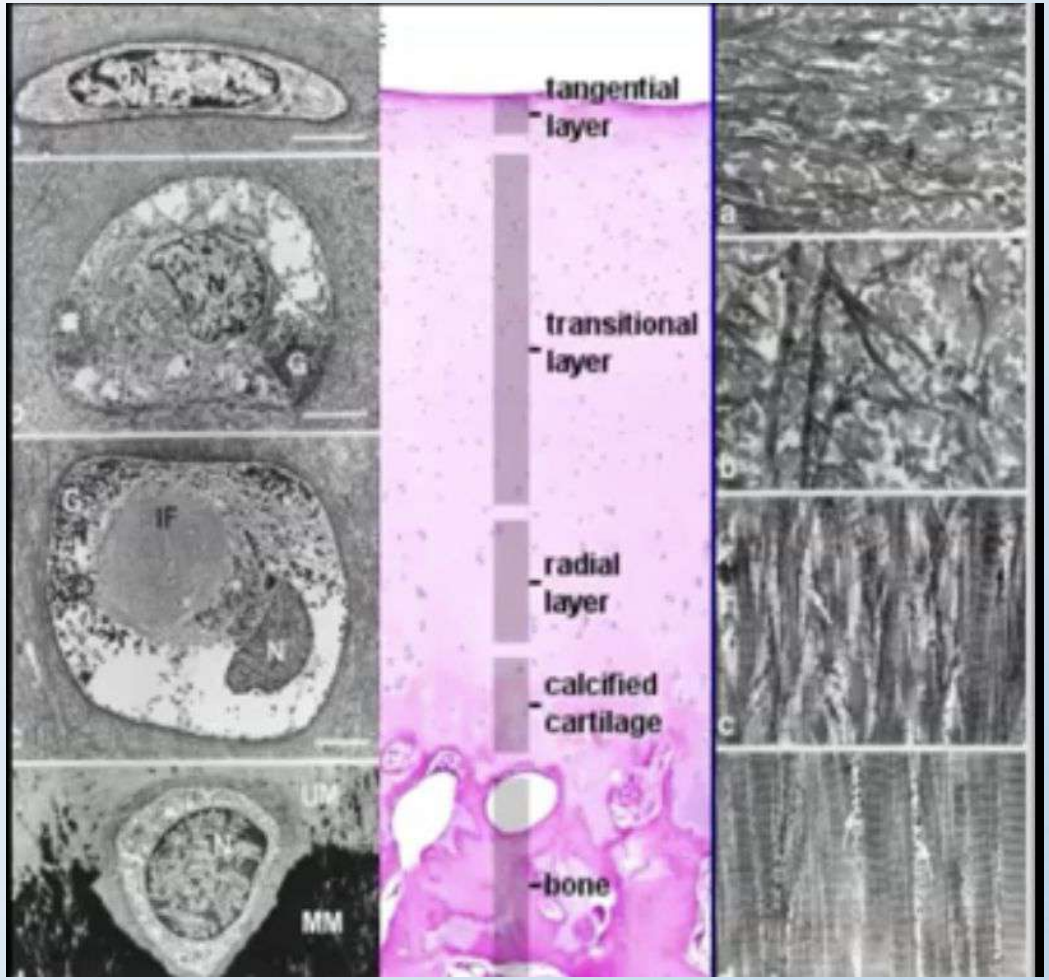
Middle layer – Transitional zone

- Oblique collagen fibers
- Largest collagen diameter
- Highest PG
- Lowest H20



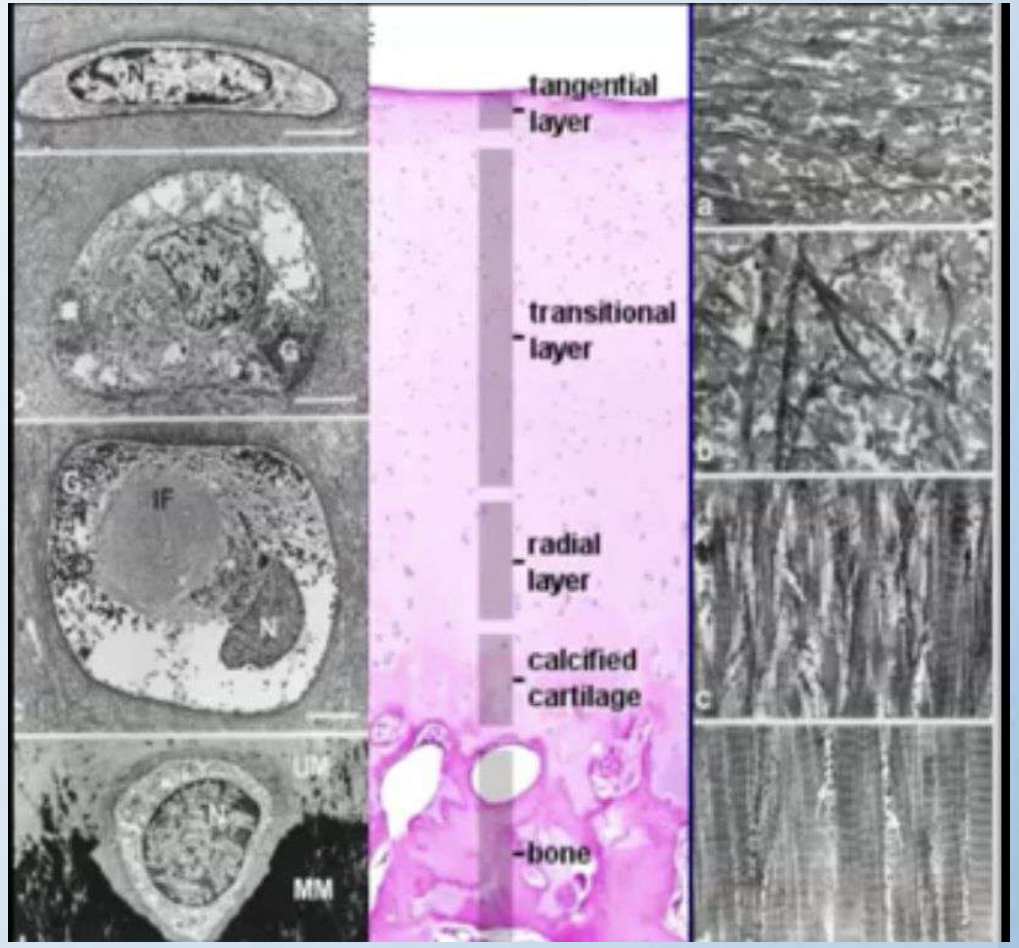
Deep cartilage layer – Radial zone

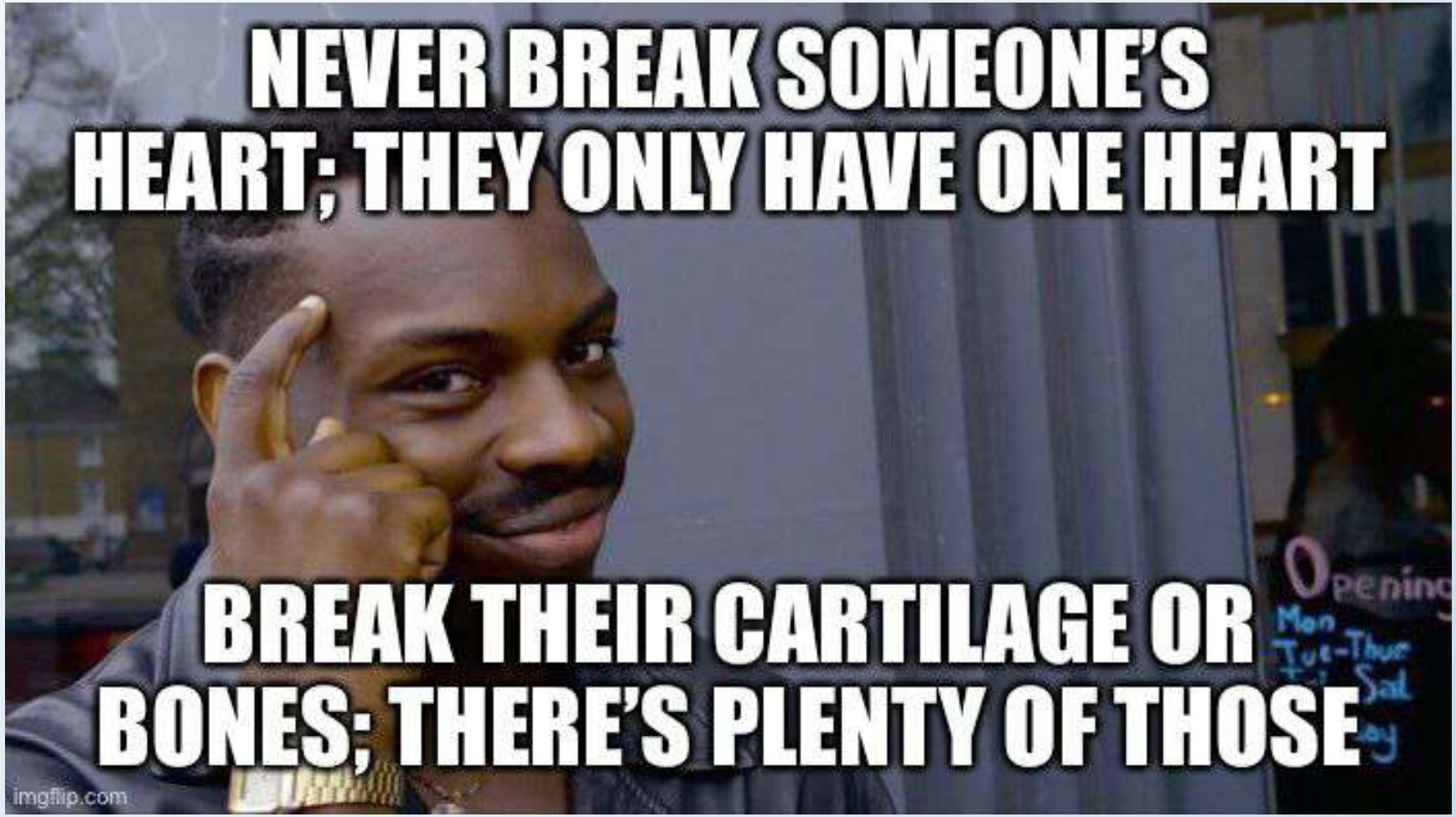
- Vertical collagen fibers
- Plump chondrocytes
- Highest level of PG
- Lowest H20



Calcified cartilage zone

- Starts at tidemark
- Transition to bone
- Injuries below heal with fibrocartilage
- Type X cartilage is found here





BONE CIRCULATION

- 5-10 % of cardiac output

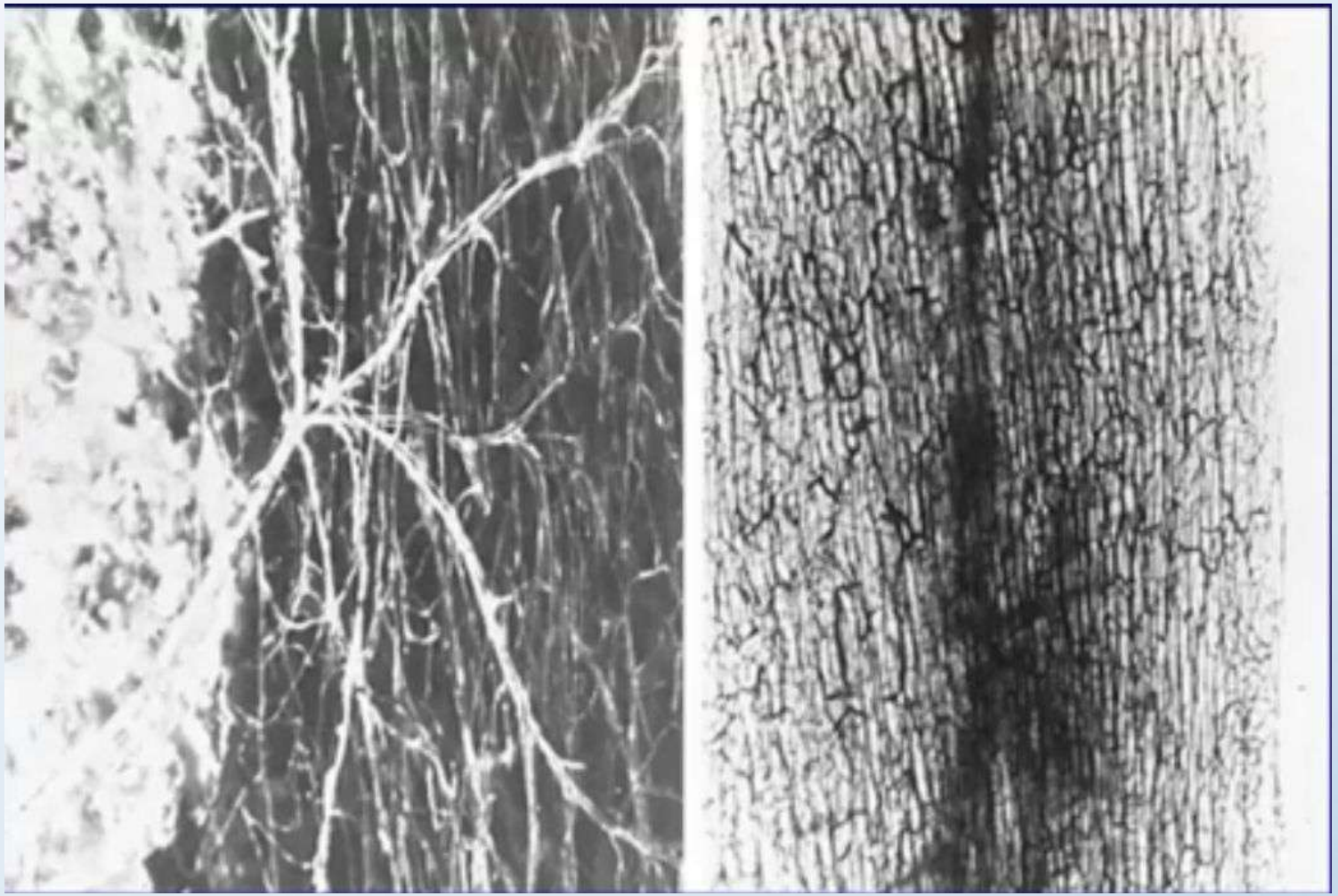
- 3 sources to long bones

✔ Nutrient artery system

✔ Metaphysical-epiphyseal system

✔ Periosteal system

BONE CIRCULATION



Nutrient artery system

- Originate from major arteries
- Enter diaphysis through nutrient foremen and enters medullary cavity

- Supplies at least 2/3rd of cortex through vessels that transverse the Haversian system
- High pressure

BONE CIRCULATION

- Metaphyseal-Epiphyseal system

✔ Arises from periarticular vascular plexus

- Periosteal system

✔ Primarily capillaries

✔ Low pressure

✔ Supply outer 1/3rd of diaphyseal cortex

At what time after fracture is vascular response (blood flow rate)at the fracture site maximized ?

a) Immediately after injury

b) 2 weeks

c) 4 weeks

d) 6 weeks

BLOOD FLOW IN FRACTURE HEALING

- Blood flow delivers nutrients to site of fracture
- Initial response – decreased flow
- Blood flow increases within hours and **peaks at two weeks**
- **Flow returns to normal 3-5 months**
- Major determinant of fracture healing

At what time after fracture is vascular response (blood flow rate)at the fracture site maximized ?

a) Immediately after injury

b) 2 weeks

c) 4 weeks

d) 6 weeks

TISSUE SURROUNDING BONE

- **PERIOSTEUM**

Tough connective tissue membrane surrounding bone

Highly developed in children

Responsible for growth in bone diameter

Inner cambium layer

- ✔ Vascular , contains osteoblastic progenitor cells
- ✔ Responsible for bone diameter and periosteal callus in fracture healing

BONE MARROW

- SOURCE OF OSTEOPROGENITOR CELLS

- **RED MARROW** – hematopoietic

40% water 40% fat 20% protein

- **YELLOW MARROW** – inactive, aged

15% water 80% fat 5% protein

Enchondral ossification is seen in all except

a) Physics longitudinal growth

b) Distraction osteogenesis

c) Fracture callus

d) Embryonic long bone formation

Types of bone formation

- ENCHONDRAL
- INTRAMEMBRANOUS
- APPOSITIONAL

ENCHONDRAL BONE FORMATION

- ***Bone replaces a cartilage model***

Cartilage is not converted to bone!

Examples

Embryonic long bone formation

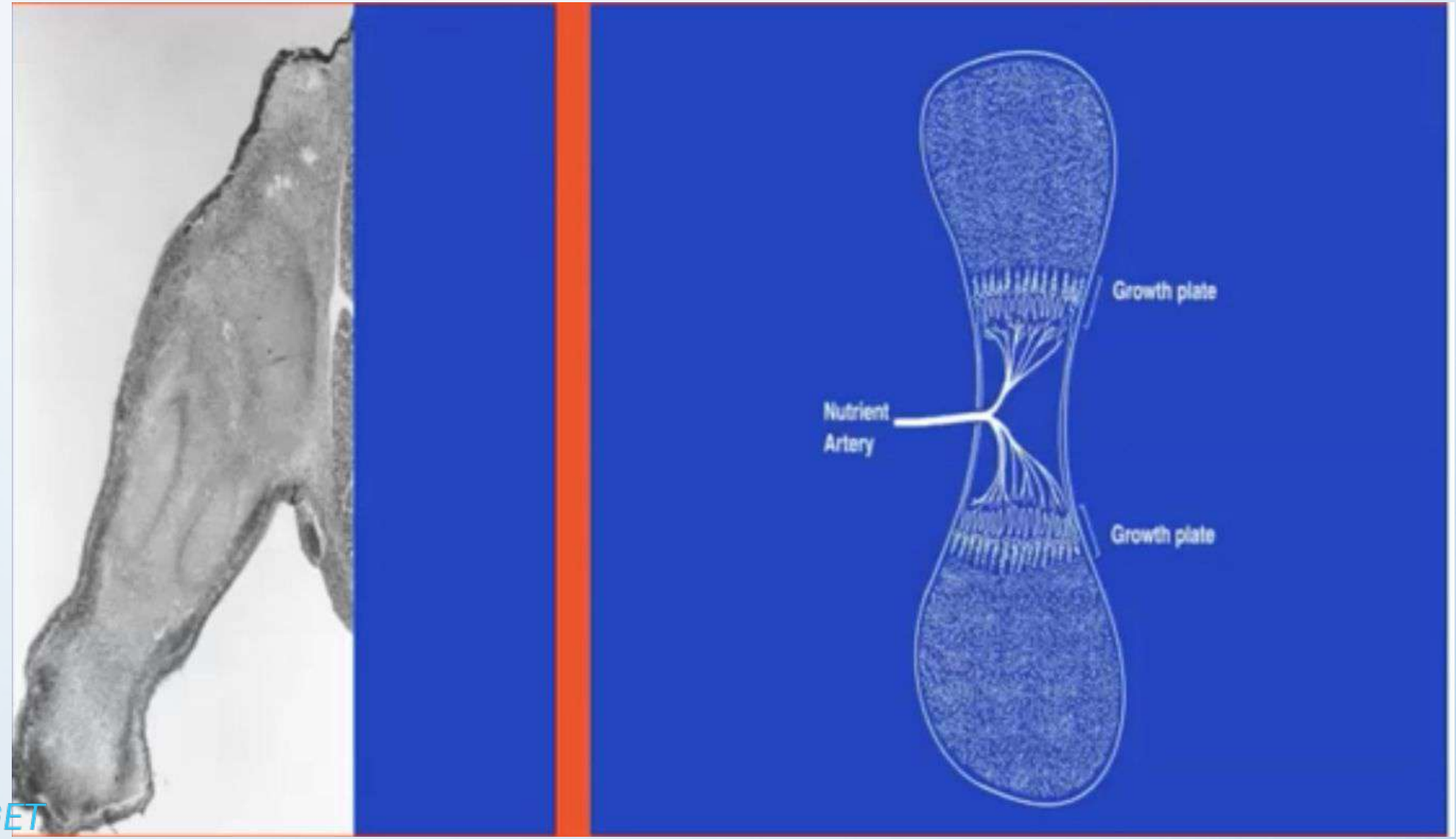
Longitudinal growth – physis

Fracture callus

DMB matrix enhanced bone formation

EMBRYONIC LONG BONE

- Mesenchymal anlage (6 weeks in utero)
- Vascular buds invade mesenchymal model and bring osteoprogenitor cells
- Cells differentiate into osteoblasts and form Primary centers of ossification (8 weeks)
- Secondary centers of ossification develop at bone ends
- Cartilage model grows by apposition and interstitional growth, bone replaces cartilage model



Enchondral ossification is seen in all except

a) Physics longitudinal growth

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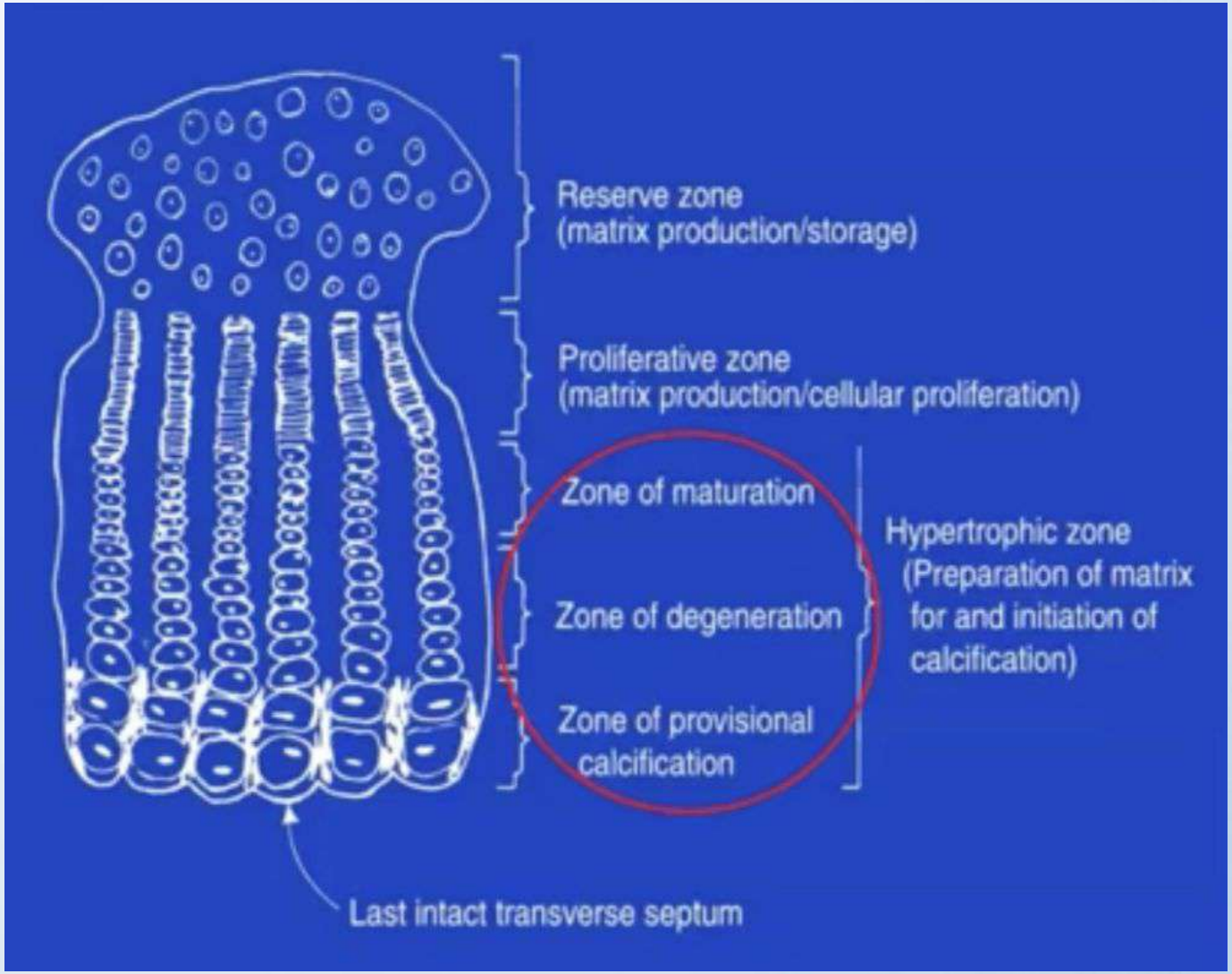
PHYSIS

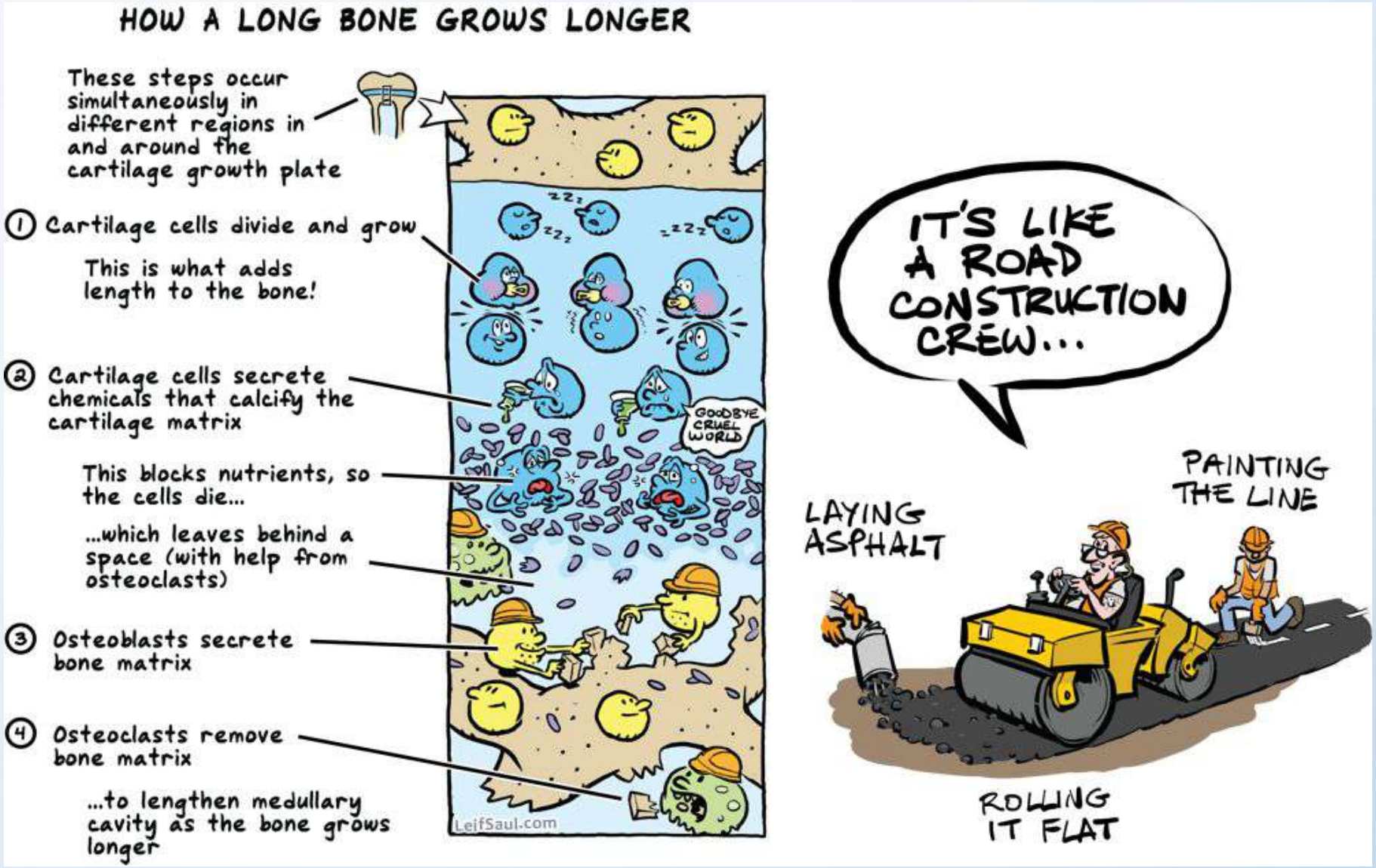
- Horizontal growth plate (physis)
- Spherical growth plate (epiphysis)
- Both have same arrangement, but spherical less organized
- Divided into zones

PHYSIS ZONES

- RESERVE
- PROLIFERATIVE
- HYPERTROPHIC
- METAPHYSIS



HOW A LONG BONE GROWS LONGER



RESERVE ZONE

- Matrix production
- Cells store lipids, glycogen, proteoglycan for later growth
- Low oxygen tension

PROLIFERATIVE ZONE

- Longitudinal growth
- Chondrocytes arranged in columns
- Top cell is dividing cell
- Increased oxygen tension inhibits calcification
- Cellular proliferation and matrix production

HYPERTROPHIC ZONE

- Divided into 3 zones
  - MATURATION
  - DEGENERATION
  - PROVISIONAL CALCIFICATION

- Cells increase in size, accumulate calcium and then die
- Osteoblasts migrate from vessels located in metaphysis and use cartilage as a scaffold

METAPHYSIS

- Adjacent to physis
- Osteoblasts use cartilage scaffold

- PRIMARY SPONGIOSA – calcified cartilage bars – mineralized to form woven and remodeled to form secondary spongiosa
- Cortical bone remodels in response to stress

The common pathologic process that occurs in patients with rickets regardless of cause is failure to

a) Adequately synthesize Chondroitin sulfate

b) Resorb the primary spongiosum

c) Adequately synthesize type 2 collagen

d) Mineralize the matrix in the zone of provisional calcification

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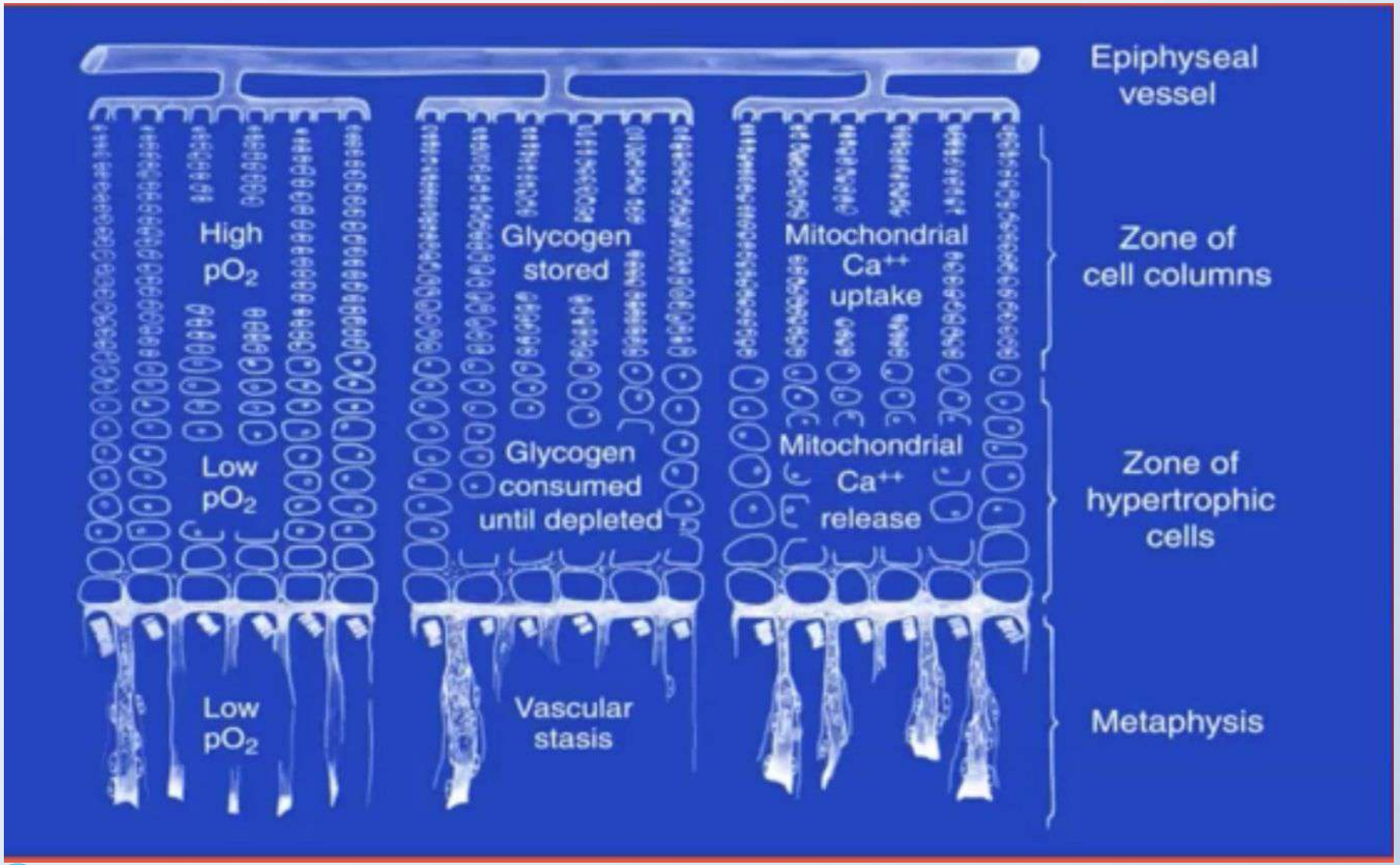


PERIPHERY OF PHYSIS

- **GROOVE OF RANVIER**

Supplies chondrocytes to the periphery of the growth plate for lateral growth (width)

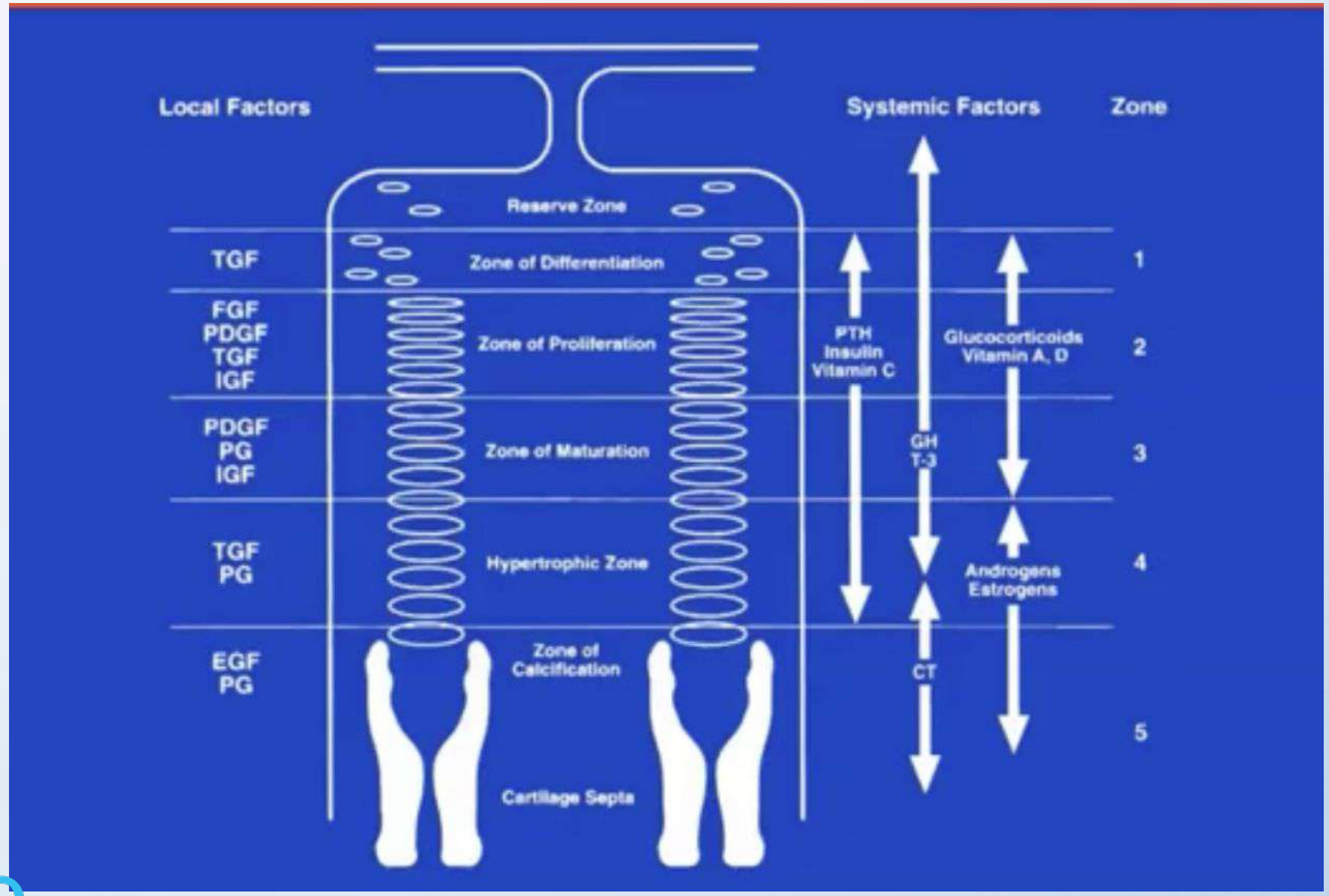
- **PERICHONDRIAL RING OF LACROIX**

Dense fibrous tissue anchors and supports the physis

HORMONES AND GROWTH FACTORS

- Paracrine and autocrine influence on growth plate

- Endocrine influence on growth plate



RESERVE ZONE	GAUCHER DISEASE, DIASTROPHIC DWARFISM, KNEIST SYNDROME AND PSEUDOACHONDROPLASIA
PROLIFERATIVE ZONE	ACHONDROPLASIA GIGANTISM
HYPERTROPHIC ZONE	RICKETS, OSTEOMALACIA ENCHONDROMA MUCOPOLYSACCHARIDOSES (MORQUIO, HURLER) SCFE PHYSEAL FRACTURES – ZONE OF PROVISIONAL CALCIFICATION

INTRAMEMBRANOUS OSSIFICATION

- Occurs without a cartilage model

- Undifferentiated mesenchymal cells aggregate into layers and differentiate into osteoblasts which deposit organic matrix that mineralizes to bone

1. **Embryonic flat bone formation (pelvis, clavicle)**
2. **Distraction osteogenesis**
3. **Blastema bone (young children with amputations)**

APPOSITIONAL OSSIFICATION

- Osteoblasts align on existing bone surfaces and lay down new bone

1. **Periosteal bone enlargement (WIDTH)**
2. Bone formation phase of bone remodelling

During repair process of an unstable fracture the expression of major collagen types during callus formation can be best characterized by

a) Type 1 collagen only

b) Type 2 collagen only

c) Type 1 collagen early followed by type 2 collagen

d) Type 2 collagen early followed by type 1 collagen

BONE INJURY AND REPAIR

- Fracture repair – Continuum of events

- INFLAMMATION
- SOFT CALLUS FORMATION
- HARD CALLUS FORMATION
- REMODELING

Fracture Repair – Inflammation

- Bleeding at fracture site creates a fibrin clot (hematoma) which is a source of hematopoietic cells and growth factors

- Fibroblasts, mesenchymal cells and osteoprogenitor cells migrate to site forming granulation tissue

REPAIR

- Primary callus repair occurs within 2 weeks
- Bridging soft callus forms between bone ends and is converted to hard callus by **enchondral ossification**
- Medullary callus supplements bridging callus at a slower rate

REPAIR

- Amount of callus formation is indirectly proportional to the amount of immobilization of the fracture

- Primary cortical healing occurs with rigid immobilization (plate fixation) and resembles normal remodeling – **no visible callus**

Types of fracture healing based on stabilization

- CAST – Periosteal bridging callus – enchondral ossification
- COMPRESSION PLATE – Primary bone healing – Cutting cone remodelling (Haversian remodeling)
- IM NAIL – early – periosteal callus, late – medullary callus – enchondral ossification
- EX FIX – less rigid – periosteal, more rigid – primary bone healing
- INADEQUATE – hypertrophic nonunion – failed endochondral ossification- **TYPE 2 COLLAGEN**

Remodeling

- Starts at 2 weeks and continues well after fracture has healed (7 years)

- Bone assumes normal shape based on stress it experiences (WOLFF'S LAW)
- Woven bone replaced with lamellar bone

- Fracture healing complete with repopulation of marrow space

BIOCHEMISTRY OF FRACTURE HEALING

- **Mesenchymal === Collagen I , II , (III , V)**
- **Chondroid === Collagen II , IX**
- **Chondroid**  **Osteoid === I , II , X**
- **Osteogenic === Collagen I**

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During fracture healing which of the following cartilage types is expressed by hypertrophic chondrocytes as the ECM undergoes calcification ?

a) Type V

b) Type X

c) Type VI

d) Type III

During fracture healing which of the following cartilage types is expressed by hypertrophic chondrocytes as the ECM undergoes calcification ?

a) Type V

b) Type X

c) Type VI

d) Type III

Growth factor involved in fracture healing

- **BMP (bone morphogenetic protein)**

Osteoinductive === causes mesenchymal cells differentiation to osteoblasts

TARGET CELL FOR BMP

Undifferentiated perivascular mesenchymal cell

Transforming growth factor – Beta

- Induces mesenchymal cells to produce type 2 collagen and proteoglycan
- Present in fracture callus
- Regulates cartilage and bone formation in fracture callus

INSULIN LIKE GROWTH FACTOR II

- Stimulates type 1 collagen, cellular proliferation, cartilage matrix synthesis

PLATELET DERIVED GROWTH FACTOR

- Released from platelets

- Chemotactic = attracts inflammatory cells to fracture site

All of the following substances are osteoconductive EXCEPT

a) Autogenous bone graft

b) Calcium sulfate

c) Tricalcium phosphate

d) BMP 2

BONE GRAFTS

- Cancellous bone
- Cortical bone
- Osteochondral grafts
- Vascularized bone grafts

CORTICAL BONE GRAFTS

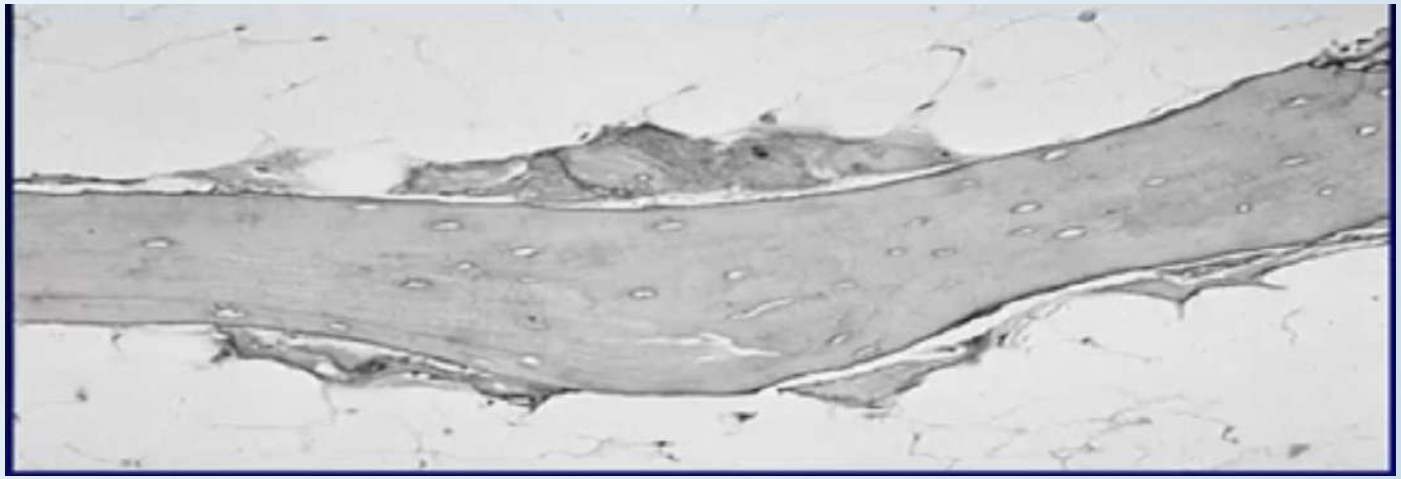
- Slow incorporation
- Remodeling of existing Haversian systems via resorption followed by deposition of new bone

- Weak during resorption phase (fatigue fractures)

Cancellous bone graft

- Revascularized quickly

- Osteoblasts lay down new bone on old trabeculae **(CREEPING SUBSTITUTION)**



SYNTHETIC BONE GRAFTS

- Calcium phosphate grafts – OSTEOCONDUCTIVE
- Calcium sulfate – OSTEOCONDUCTIVE
- Calcium carbonate – OSTEOCONDUCTIVE
- Coralline hydroxyapatite- OSTEOCONDUCTIVE

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The process of host repair following osteonecrosis is referred to as

a) Haversian remodeling

b) Osteoinduction

c) Osteogenesis

d) Creeping substitution

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Normal bone metabolism

1. Calcium
2. Phosphate
3. PTH
4. Vitamin D
5. Calcitonin
6. Estrogen
7. Corticosteroids
8. Thyroid hormone
9. Growth hormone

What is the primary effect of vitamin d ?

a) Strongly stimulates intestinal absorption of calcium and phosphate

b) Stimulates parathyroid hormone release

c) Stimulates calcium release from bone

d) Promotes urinary excretion of phosphate

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CALCIUM

- Bone is a reserve for 99% of body calcium
- Plasma calcium 1% - 50% free , 50% bound to albumin
- Calcium absorbed in duodenum by active transport (regulated by 1,25 (OH)2 Vit D and by passive transport (jejunum)
- Resorted in proximal tubules kidney

Calcium – Dietary requirements

- 600 mg/day – children
- 1300 mg/day – adolescents
- 750 mg/day – adult men / women
- 1500 mg/day - pregnant woman
- 2000 mg/day – lactating women
- 1500 mg/day – post menopausal women, fracture healing

Primary regulators of calcium

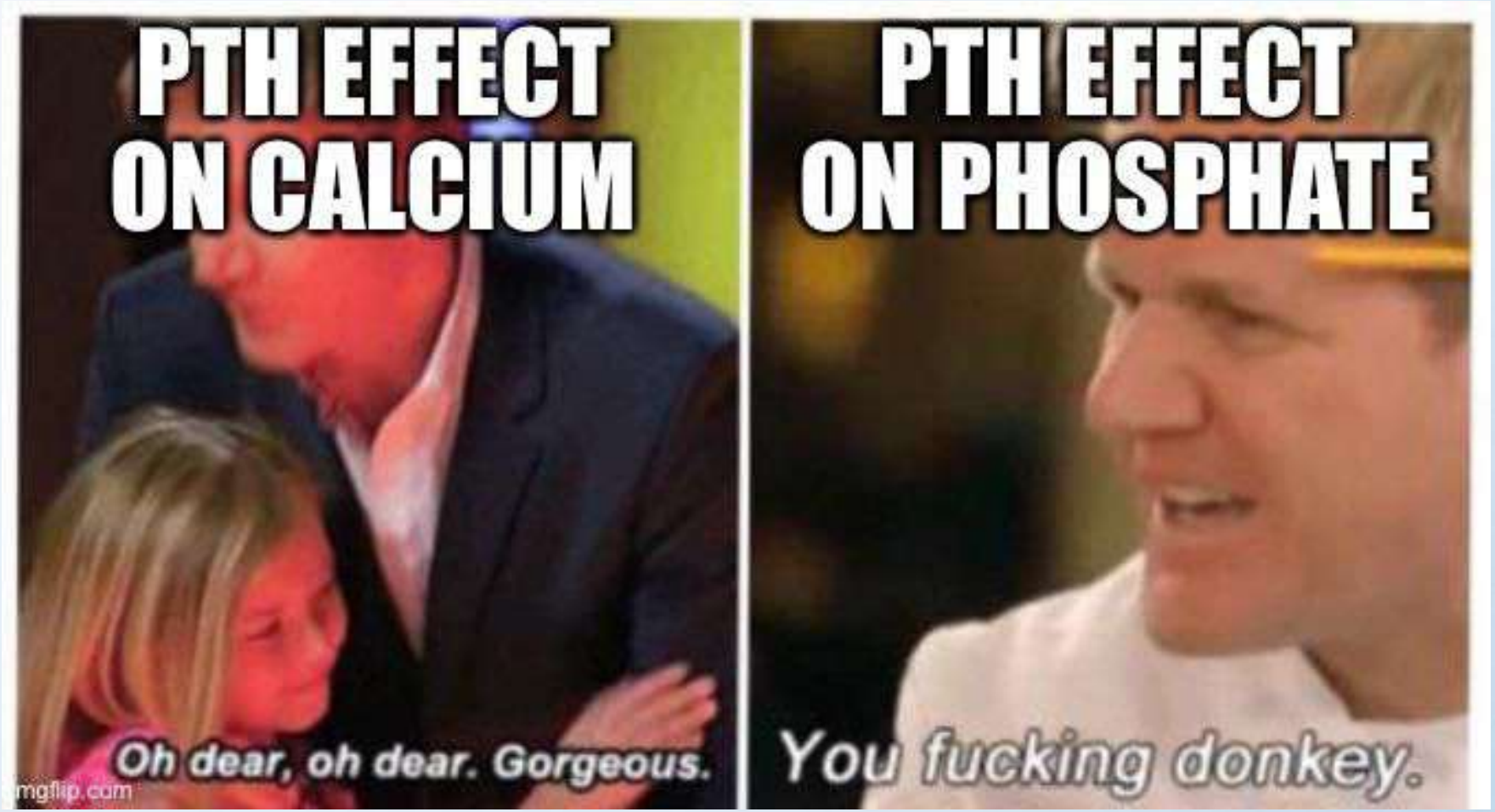
- PTH
- 1,25 (OH)2 Vit D

PHOSPHATE

- 85% of body stores in bone
- Plasma phosphate unbound and reabsorbed in proximal tubules of kidneys
- Dietary requirements- 1000 – 1500 mg/day

PARATHYROID HORMONE

- Synthesized and secreted from chief cells of 4 parathyroid glands
- **Directly activates osteoblasts which stimulate osteoclasts through 2nd messenger**
- Decreased calcium levels stimulate PTH release which act at intestine, kidneys and bone



What organ secretes calcitonin ?

a) Parathyroid

b) Thyroid

c) Kidney

d) Bone

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The major physiologic effect of calcitonin is :

a) Inhibition of bone resorption

b) Enhancement of bone deposition

c) To increase parathyroid hormone release

d) To produce osteoclastic cell multiplication

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Vitamin D is converted to 25 hydroxycholecalciferol in which organ ?

a) Skin

b) Kidney

c) Liver

d) Intestine

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