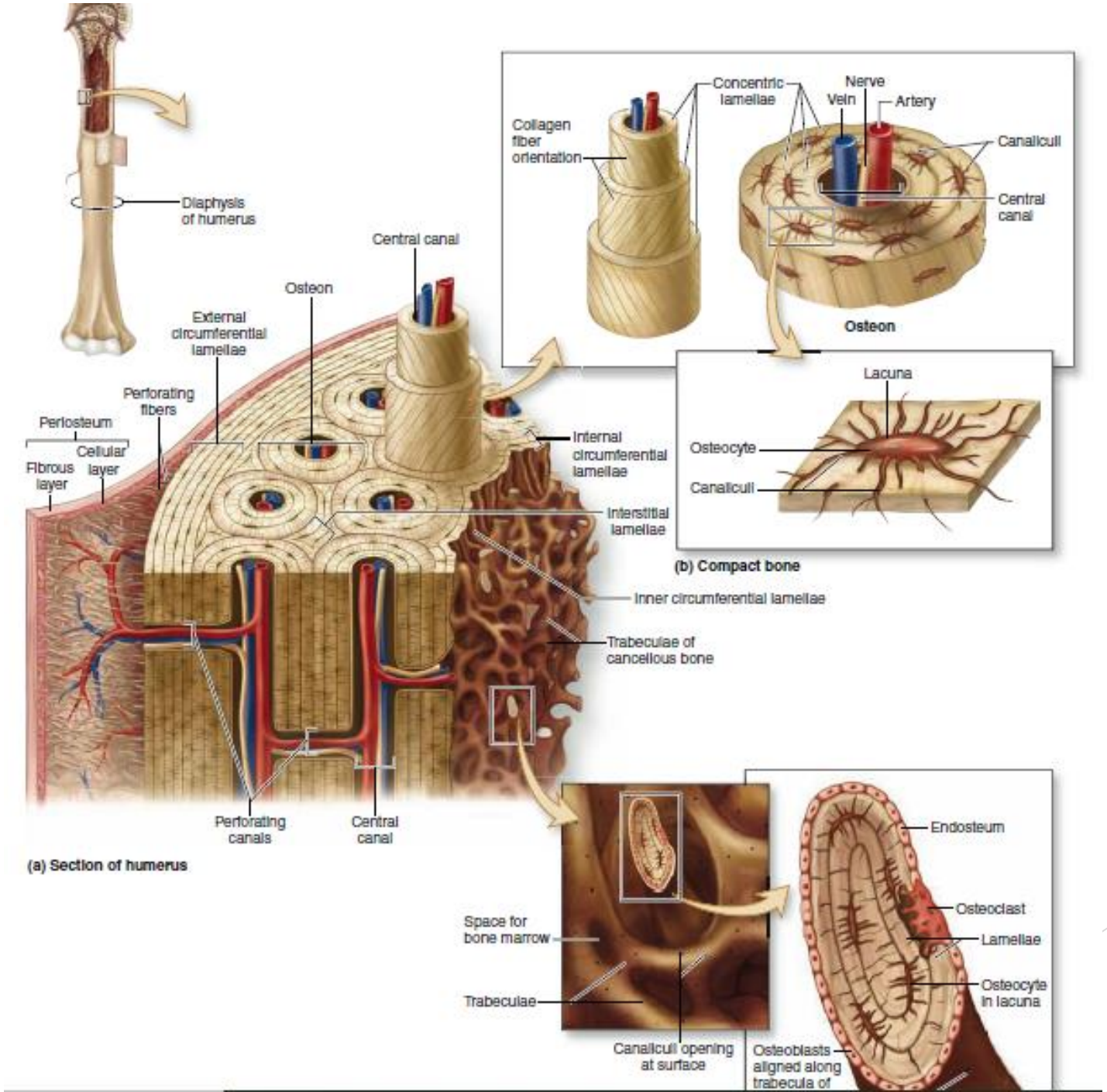
Bone

Hanan Jafar. BDS.MSc.PhD

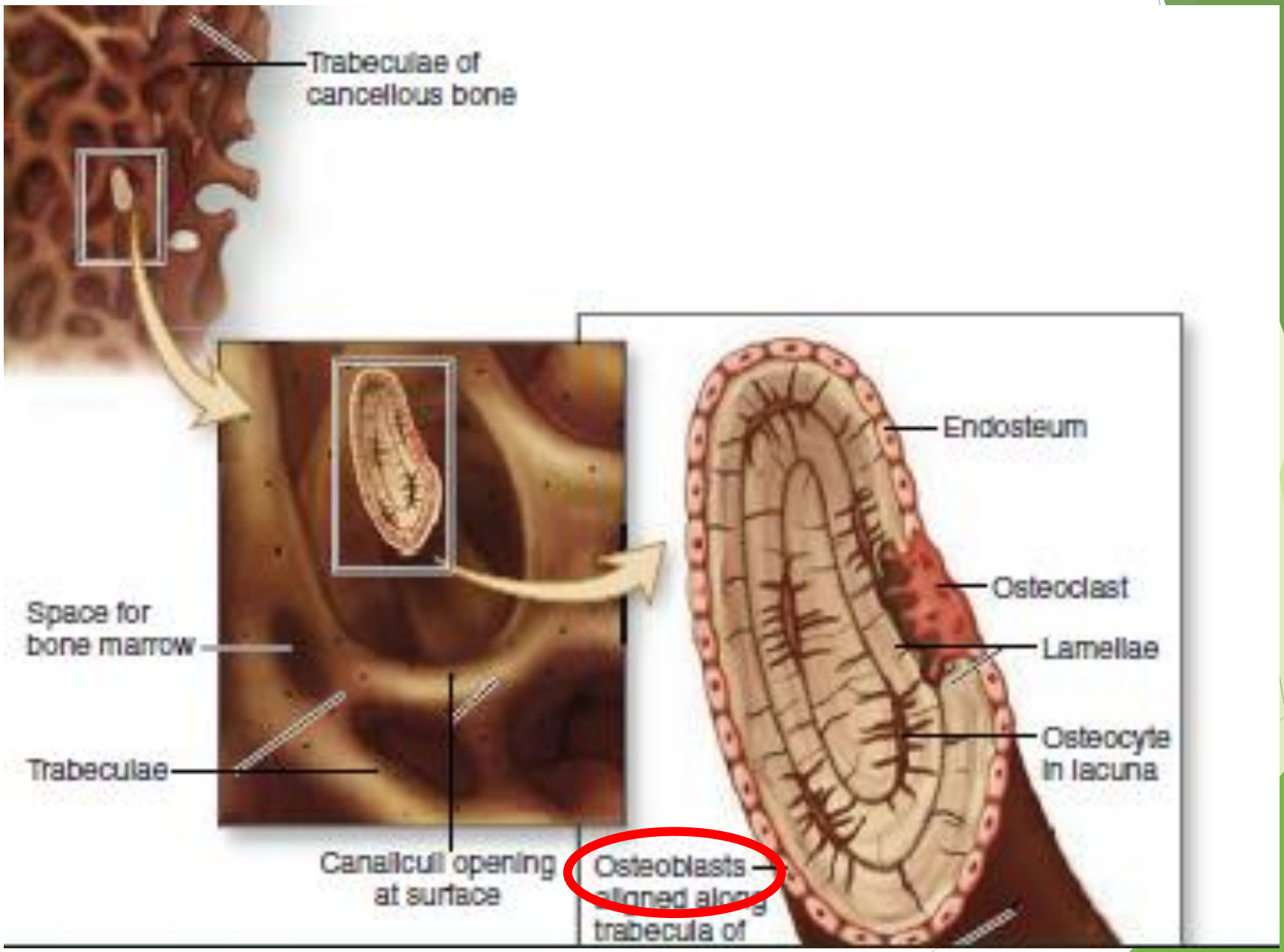
Bone tissue

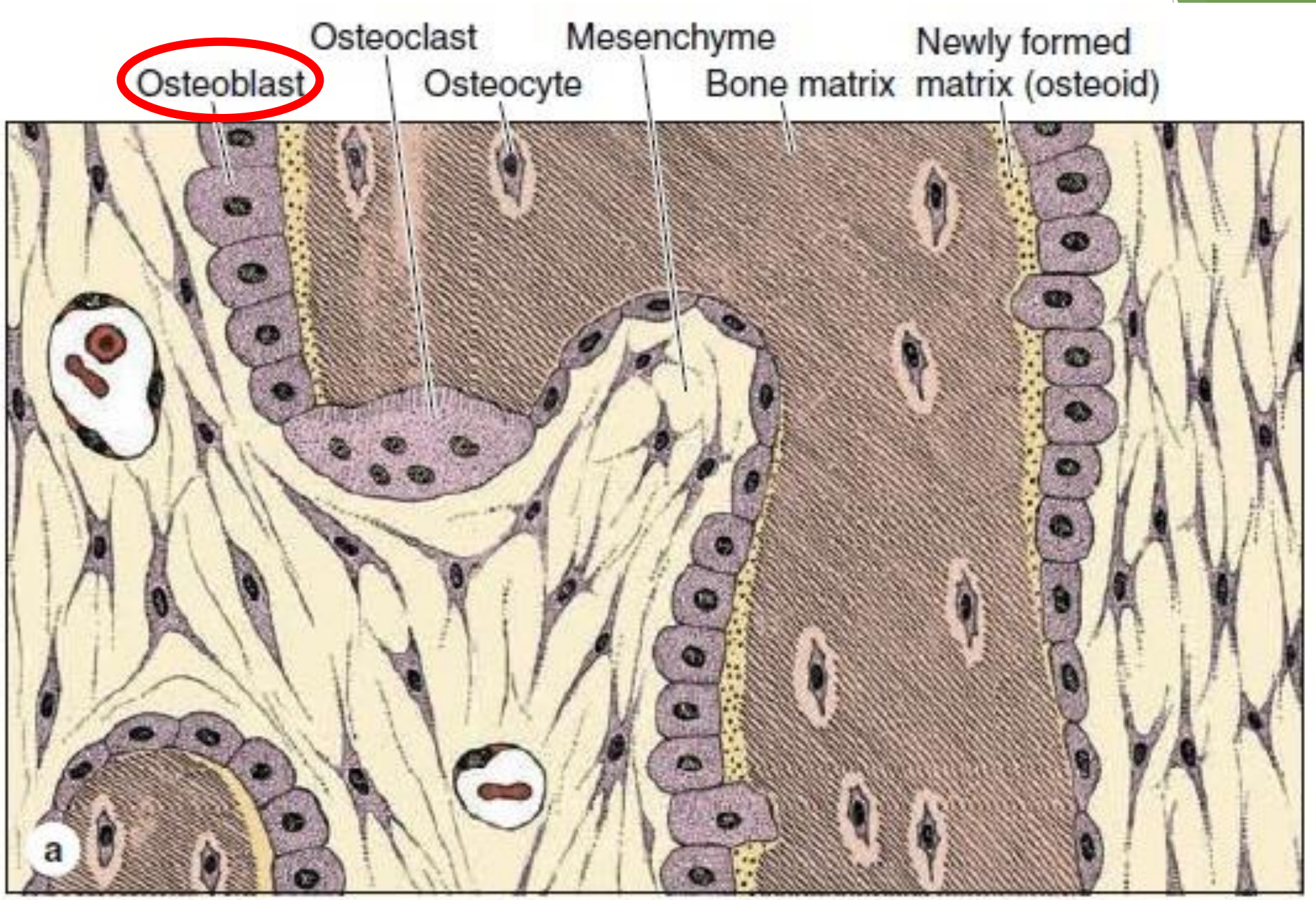
- Bone is a specialized connective tissue composed of calcified extracellular material, the **bone matrix**, and following three major cell types:
- **Osteocytes**, which are found in cavities (**lacunae**) between bone matrix layers (**lamellae**), with cytoplasmic processes in small **canaliculi** that extend into the matrix
- **Osteoblasts,** growing cells which synthesize and secrete the organic components of the matrix
- **Osteoclasts**, which are giant, multinucleated cells involved in removing calcified bone matrix and remodeling bone tissue


Diaphysis of humerus
Central canal
Osteon
External circumferential lamellae
Perforating fibers
Periosteum
Cellular layer
Fibrous layer
Internal circumferential lamellae
Interstitial lamellae
Inner circumferential lamellae
Trabeculae of cancellous bone
Perforating canals
Central canal
(a) Section of humerus
Concentric lamellae
Collagen fiber orientation
Nerve
Vein
Artery
Canaliculi
Central canal
Osteon
Lacuna
Osteocyte
Canaliculi
(b) Compact bone
Space for bone marrow
Trabeculae
Canaliculi opening at surface
Endosteum
Osteoclast
Lamellae
Osteocyte in lacuna
Osteoblasts aligned along trabecula of

Osteoblasts

- Originate from mesenchymal stem cells
- They produce the organic components of bone matrix, including type I collagen fibers, proteoglycans, and matricellular glycoproteins such as osteonectin.
- Active osteoblasts are located exclusively at the surfaces of bone matrix, forming a single layer of cuboidal cells joined by adherent and gap Junctions
- When their synthetic activity is completed, some osteoblasts differentiate as osteocytes entrapped in matrix-bound lacunae, and the majority undergo apoptosis.


Trabeculae of cancellous bone
Endosteum
Osteoclast
Lamellae
Osteocyte in lacuna
Space for bone marrow
Trabeculae
Canaliculi opening at surface
Osteoblasts
trabecula of

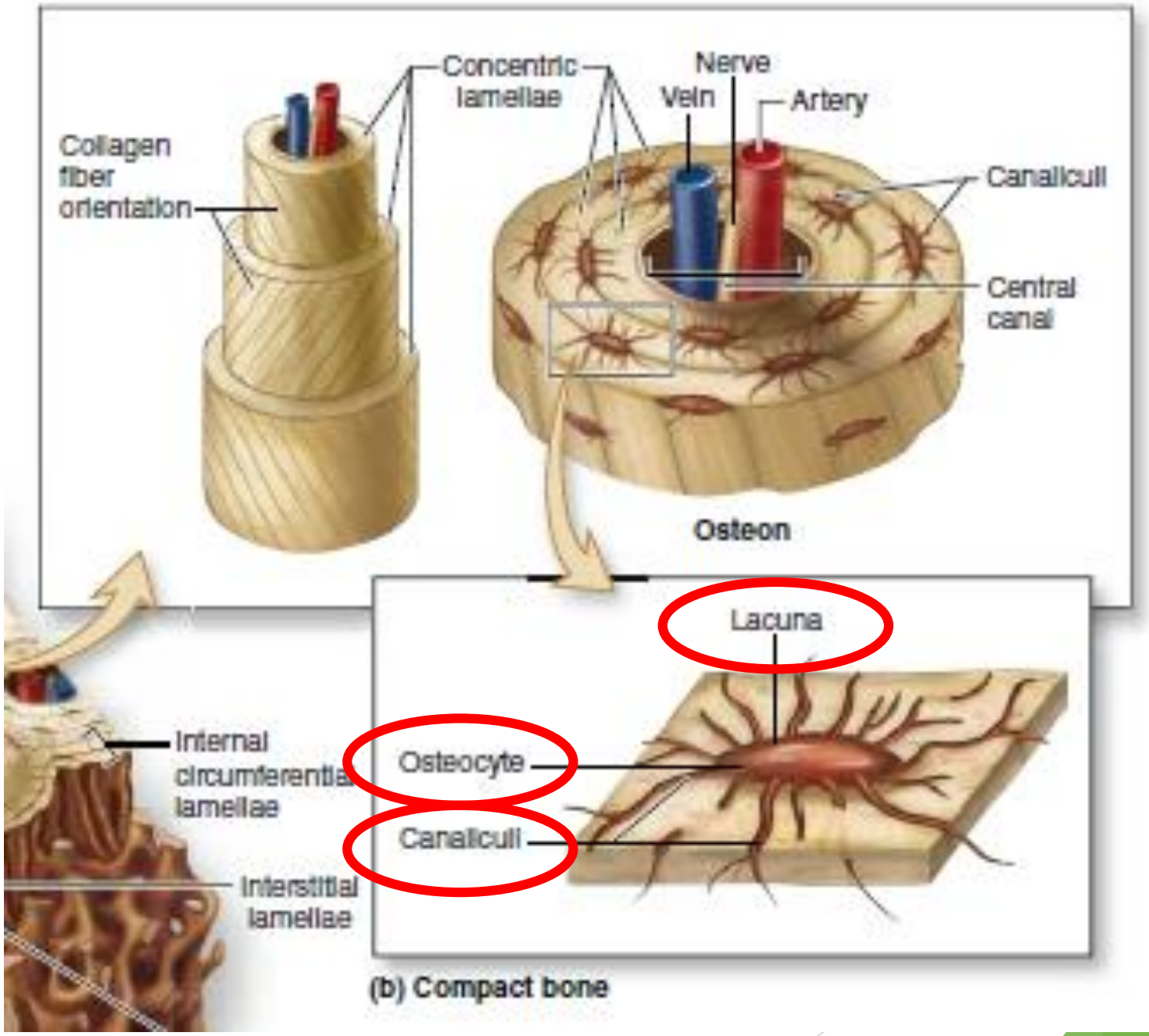

Osteoblast
Osteoclast
Osteocyte
Mesenchyme
Bone matrix
Newly formed matrix (osteoid)
a

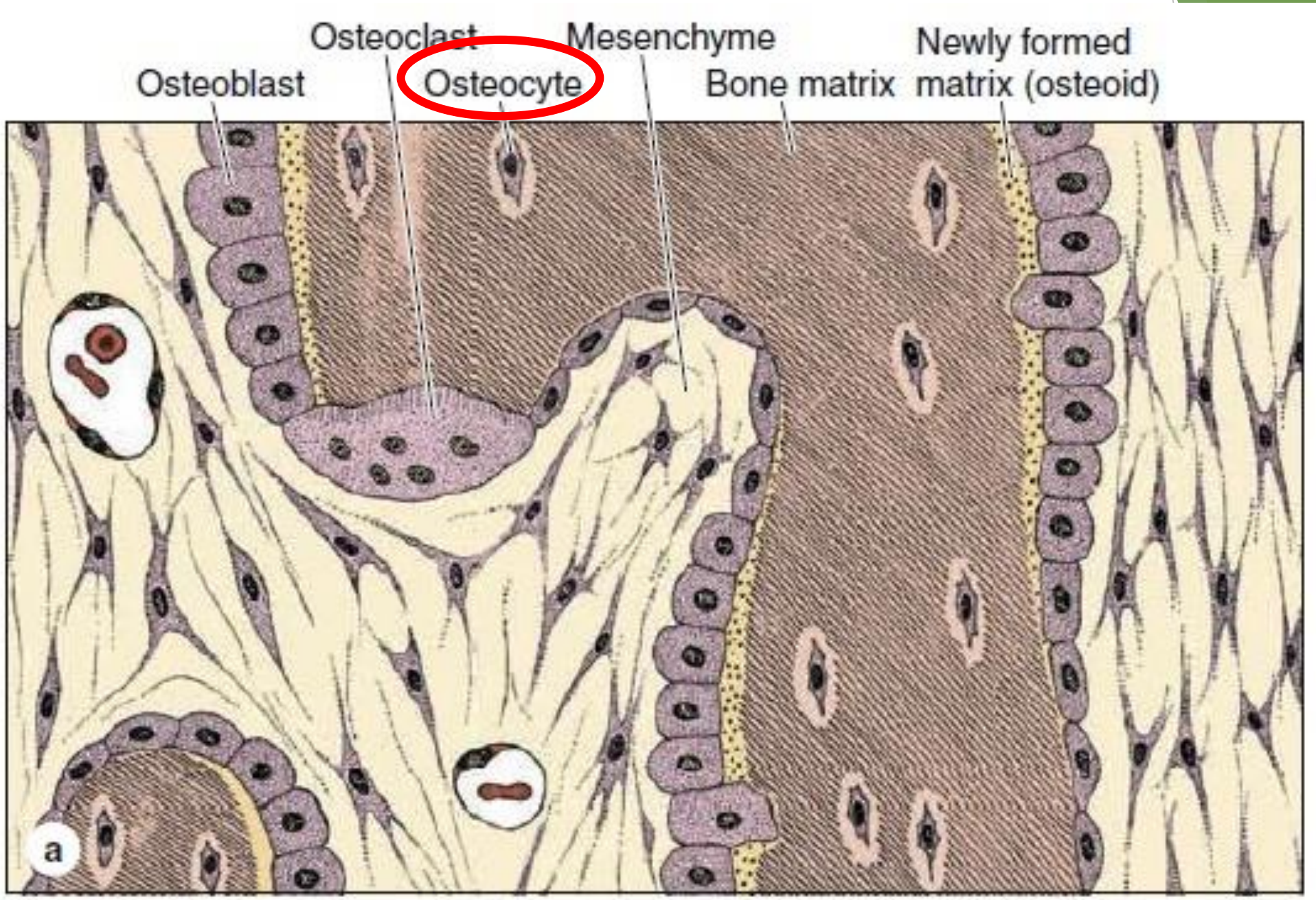
Bone formation

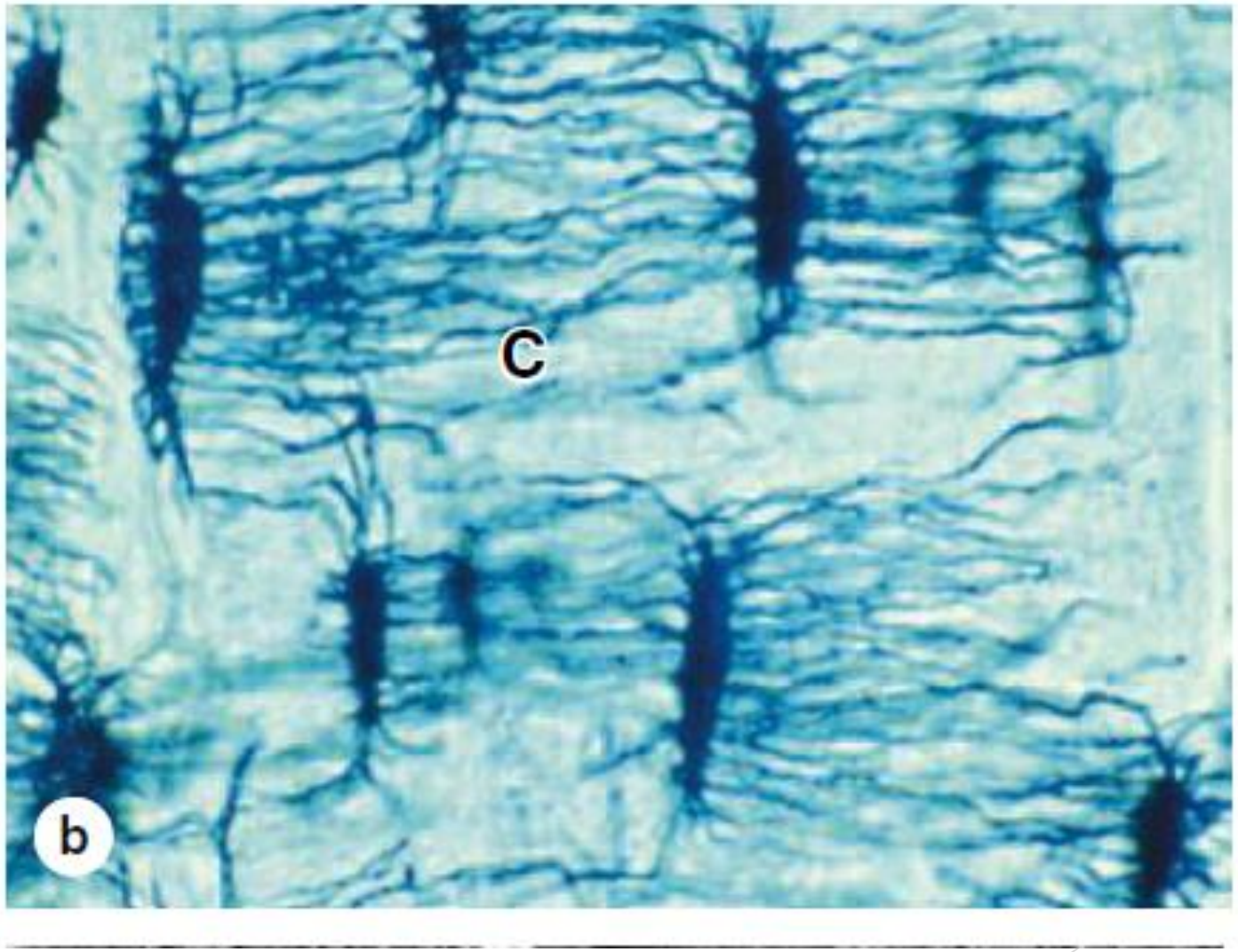
- Matrix components are secreted at the cell surface producing a layer of unique collagen-rich material called **osteoid**
- This process is completed by subsequent deposition of calcium salts into the newly formed matrix (mineralization)

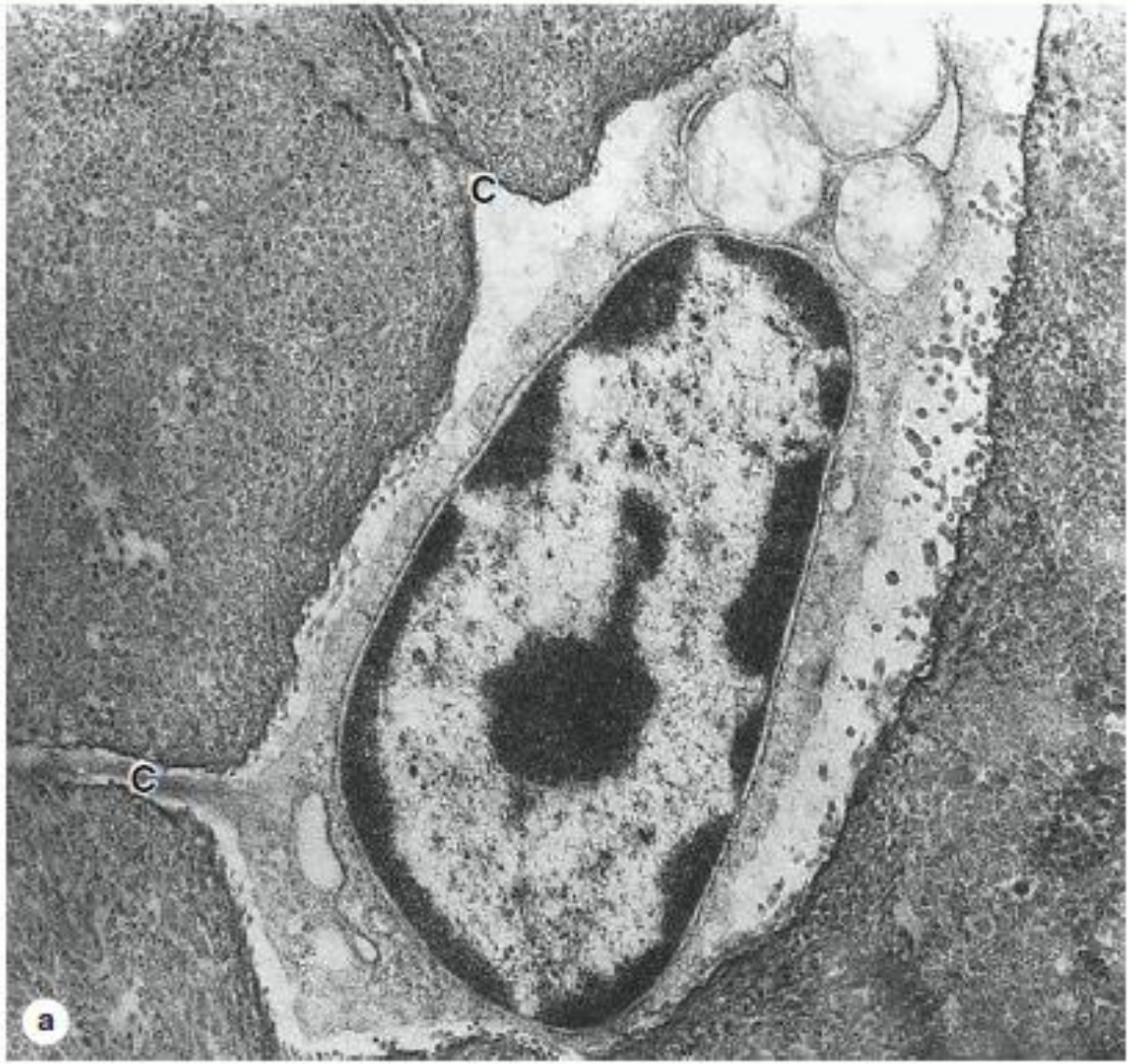
Osteocytes

- Some osteoblasts become surrounded by the material they secrete and then differentiate as **osteocytes** enclosed singly within the **lacunae** spaced throughout the mineralized matrix.
- During the transition from osteoblasts to osteocytes, the cells extend many long processes, which also become surrounded by calcifying matrix.
- The processes come to occupy the many canaliculi radiating from each lacuna


Concentric lamellae
Nerve
Vein
Artery
Collagen fiber orientation
Canaliculi
Central canal
Osteon
Lacuna
Internal circumferential lamellae
Osteocyte
Canaliculi
Interstitial lamellae
(b) Compact bone


Osteoclast
Mesenchyme
Newly formed
Osteoblast
Osteocyte
Bone matrix
matrix (osteoid)
a


C
b

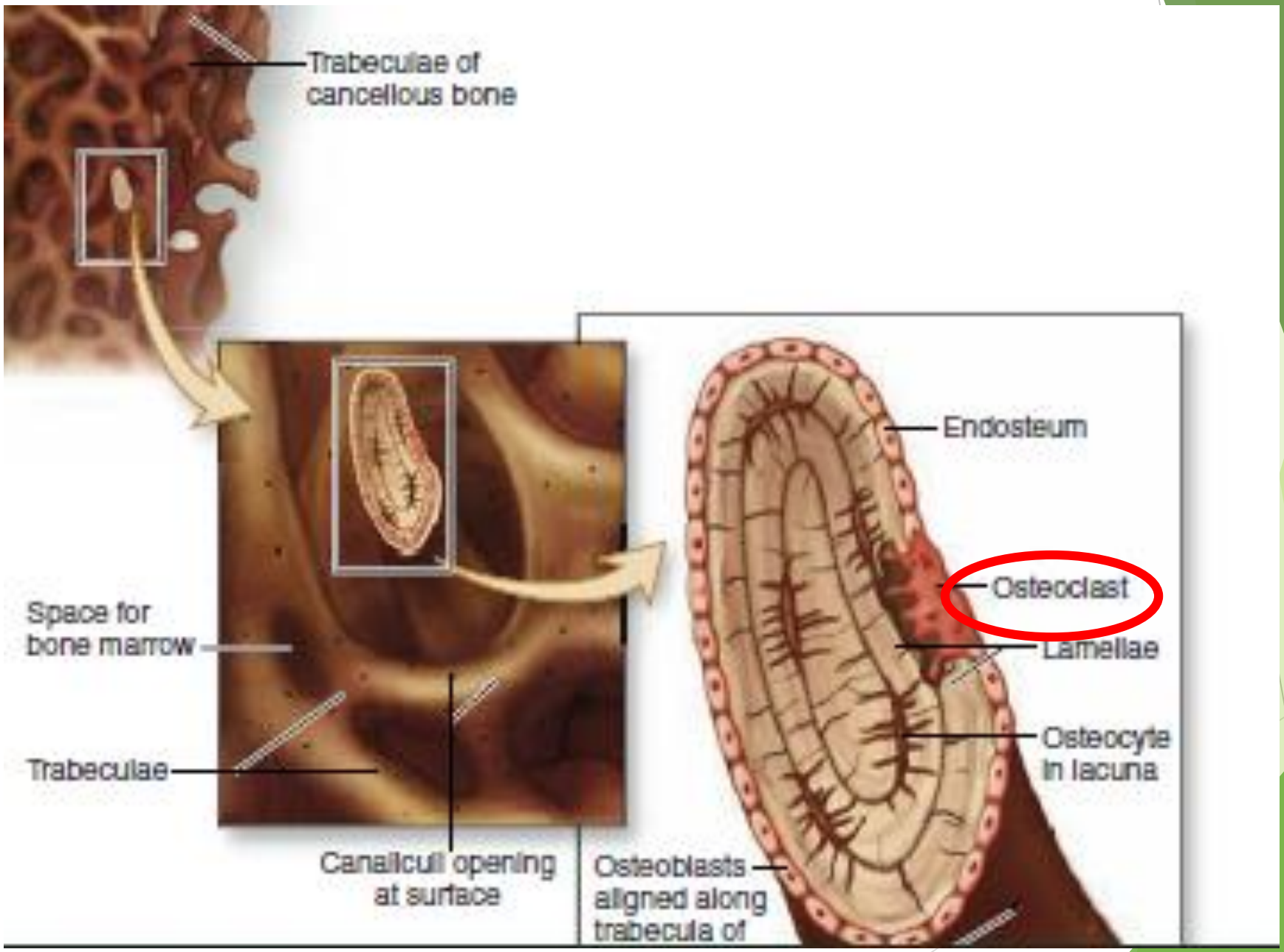

C
C
a

Osteoblasts vs osteocytes

- Osteocytes are the most abundant cells in bone.
- Osteocytes exhibit significantly less RER, smaller Golgi complexes, and more condensed nuclear chromatin than osteoblasts
- Osteocytes maintain the calcified matrix, and their death is followed by rapid matrix resorption

Osteoclasts

- **Osteoclasts** are very large, motile cells with multiple nuclei
- They are essential for matrix resorption during bone growth and remodeling.
- The large size and multinucleated condition of osteoclasts are due to their origin from the fusion of bone marrow–derived monocytes
- Osteoclasts on the bone surface lie within cavities in the matrix known as **resorption lacunae** (or **Howship lacunae**).


Trabeculae of cancellous bone
Endosteum
Osteoclast
Lamellae
Osteocyte in lacuna
Space for bone marrow
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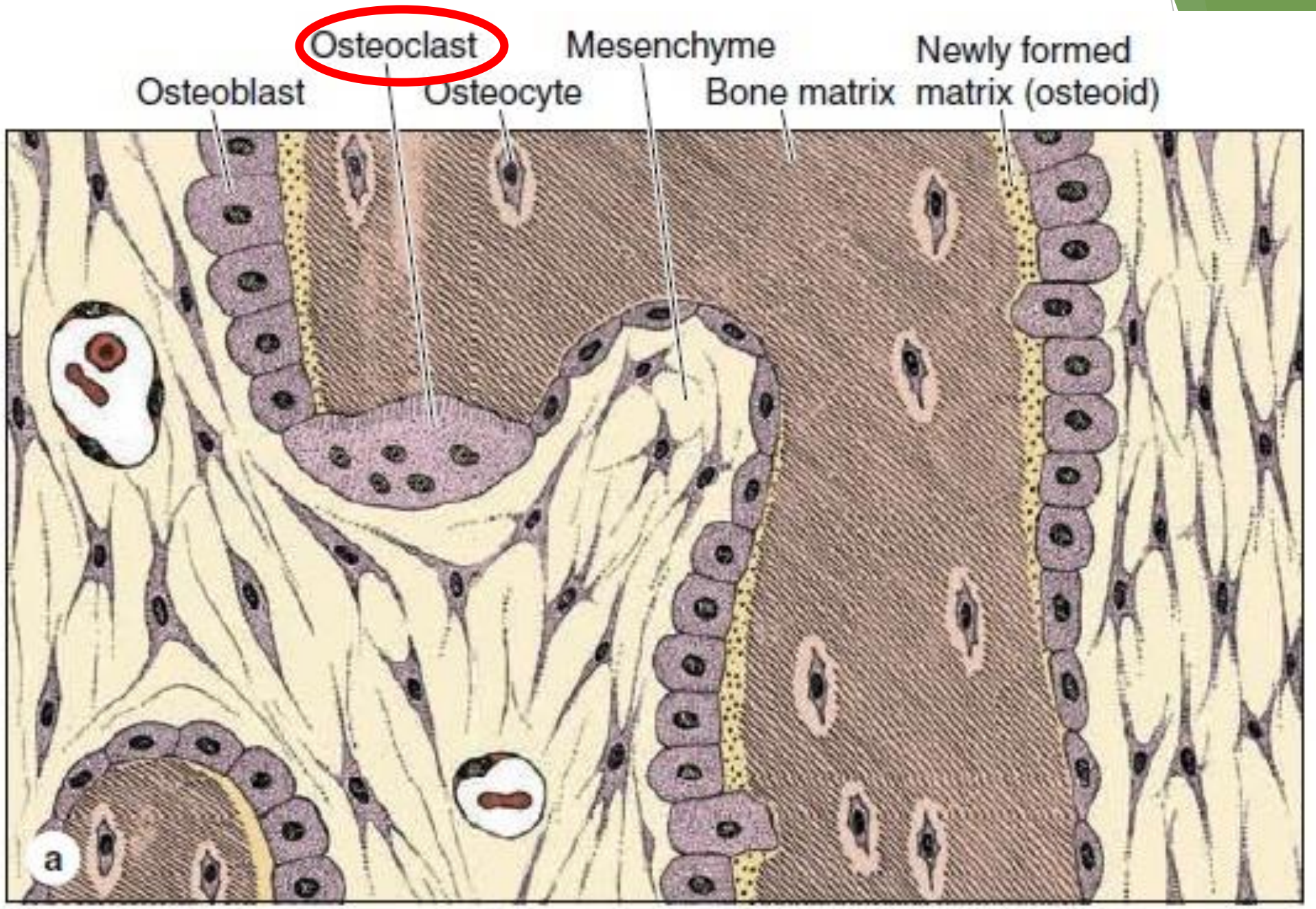
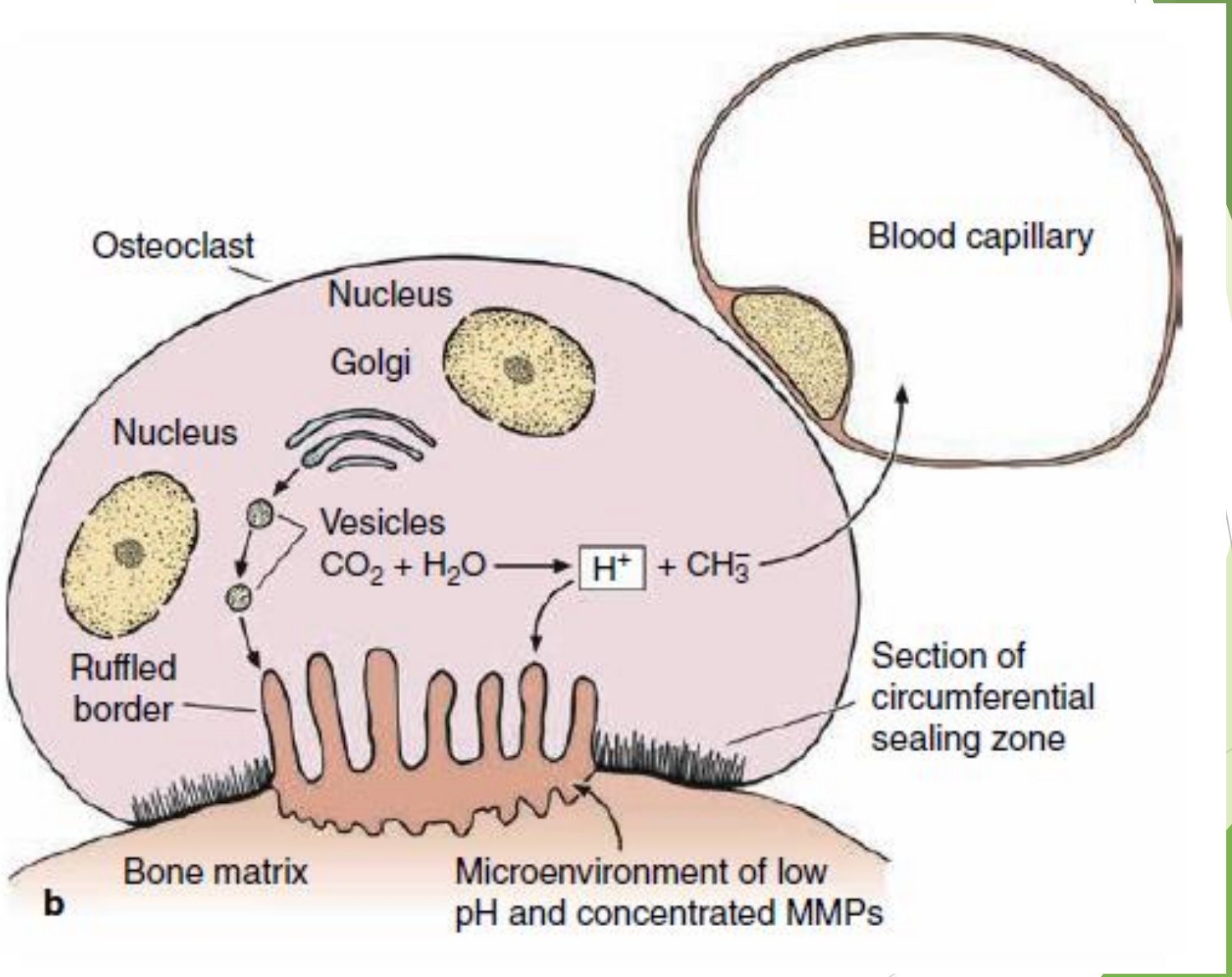
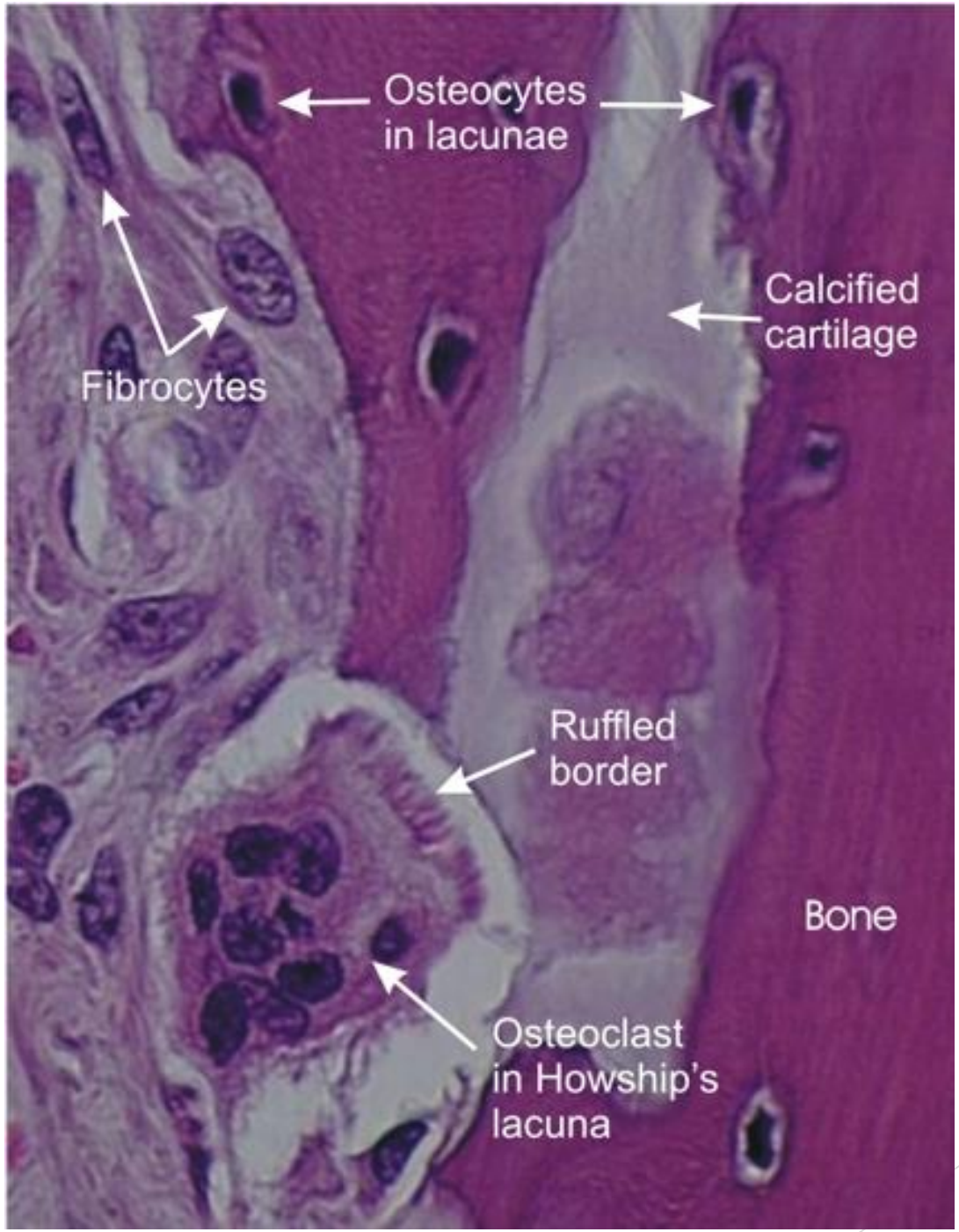

Osteoclast
Mesenchyme
Newly formed matrix (osteoid)
Osteoblast
Osteocyte
Bone matrix
a

Bone resorption

- In an active osteoclast the membrane domain that contacts the bone forms a circular **sealing zone** which binds the cell tightly to the bone matrix and surrounds an area with many surface projections, called the **ruffled border**.
- This circumferential sealing zone allows the formation of a specialized microenvironment between the osteoclast and the matrix in which bone resorption occurs
- Into this subcellular pocket the osteoclast pumps **protons** to acidify and promote dissolution of the adjacent hydroxyapatite, and releases matrix **metalloproteinases** and other **hydrolytic enzymes** from lysosome-related secretory vesicles for the localized digestion of matrix proteins


Osteoclast
Nucleus
Golgi
Nucleus
Vesicles
$CO_2 + H_2O \rightarrow H^+ + CH_3^-$
Blood capillary
Ruffled border
Section of circumferential sealing zone
Bone matrix
Microenvironment of low pH and concentrated MMPs
b


Osteocytes
in lacunae
Calcified
cartilage
Fibrocytes
Ruffled
border
Bone
Osteoclast
in Howship's
lacuna

Bone matrix

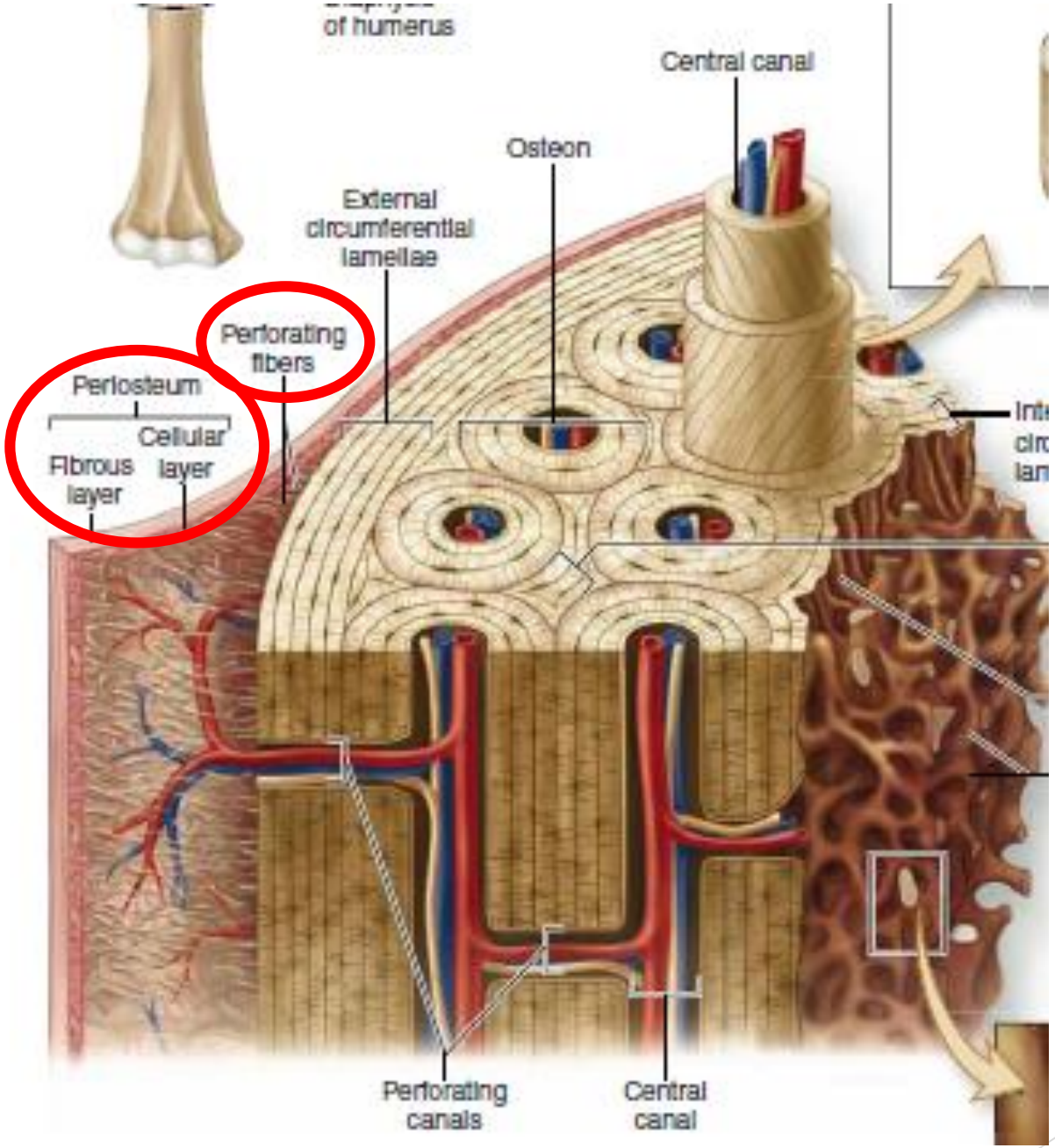
- About 50% of the dry weight of bone matrix is inorganic materials.
- Calcium hydroxyapatite is the most abundant inorganic material
- The organic matter embedded in the calcified matrix is 90% type I collagen, but also includes mostly small proteoglycans and multiadhesive glycoproteins such as **osteonectin**
- Calcium-binding proteins, notably **osteocalcin**, promote calcification of the matrix
- The association of minerals with collagen fibers during calcification provides the hardness and resistance required for bone function

Endosteum and Periosteum

- All bones are lined on their internal and external surfaces by layers of connective tissue containing osteogenic cells—**endosteum** on the internal surface surrounding the marrow cavity and **periosteum** on the external surface.

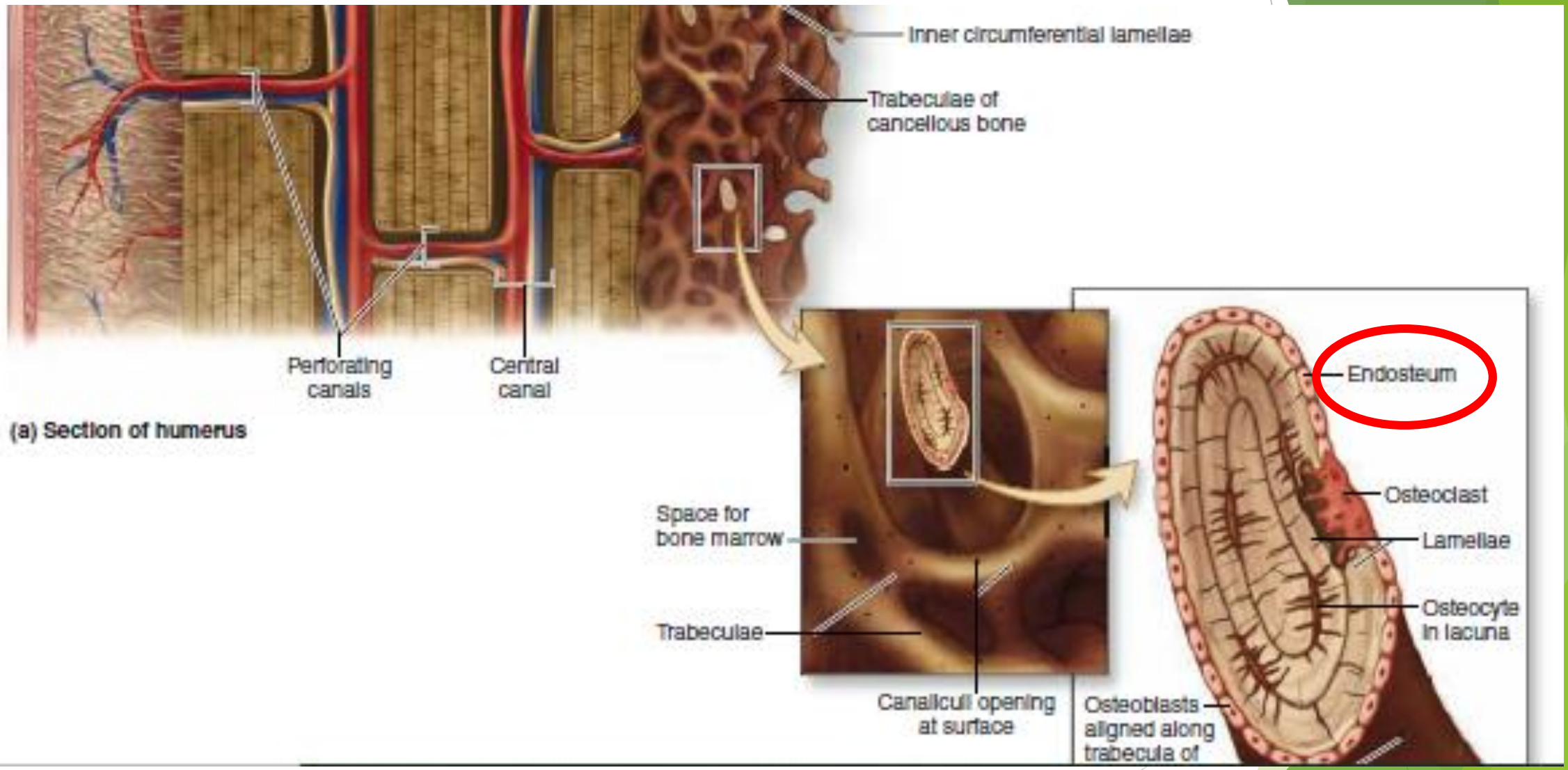
Periosteum

- The **periosteum** is organized much like the perichondrium of cartilage, with an outer fibrous layer of dense connective tissue, containing mostly bundled type I collagen, but also fibroblasts and blood vessels.
- Bundles of periosteal collagen, called **perforating** (or **Sharpey**) **fibers**, penetrate the bone matrix and bind the periosteum to the bone.
- The periosteum's inner layer is more cellular and includes osteoblasts, bone lining cells, and mesenchymal stem cells referred to as **osteoprogenitor cells**.
- With the potential to proliferate extensively and produce many new osteoblasts, osteoprogenitor cells play a prominent role in bone growth and repair.


of humerus
Central canal
Osteon
External circumferential lamellae
Perforating fibers
Periosteum
Cellular layer
Fibrous layer
Perforating canals
Central canal

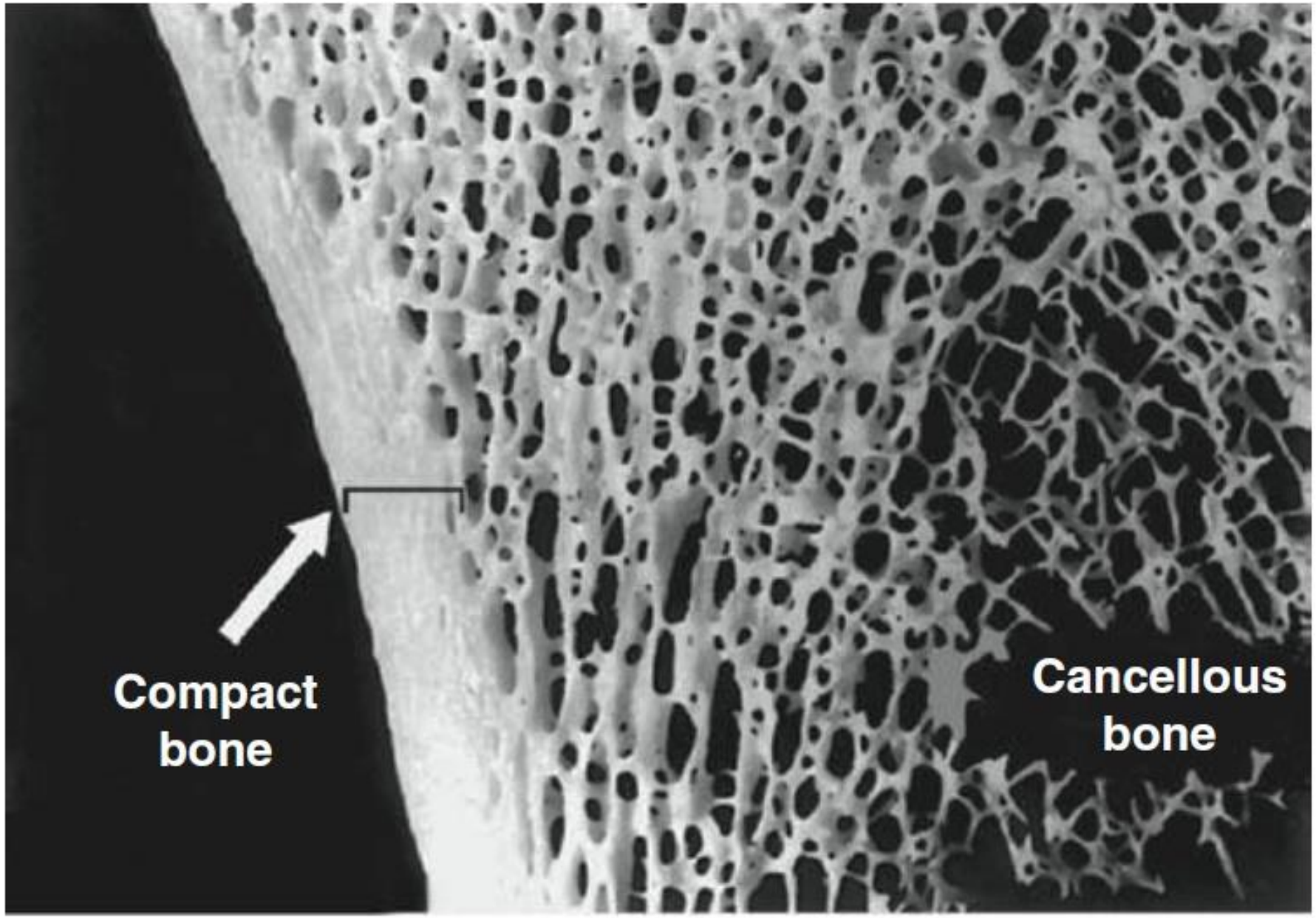
Endosteum

- Internally the very thin **endosteum** covers small **trabeculae** of bony matrix that project into the marrow cavities
- The endosteum also contains osteoprogenitor cells, osteoblasts, and bone lining cells, but within a sparse, delicate matrix of collagen fibers.


Inner circumferential lamellae
Trabeculae of cancellous bone
Perforating canals
Central canal
(a) Section of humerus
Space for bone marrow
Trabeculae
Canaliculi opening at surface
Endosteum
Osteoclast
Lamellae
Osteocyte in lacuna
Osteoblasts aligned along trabecula of

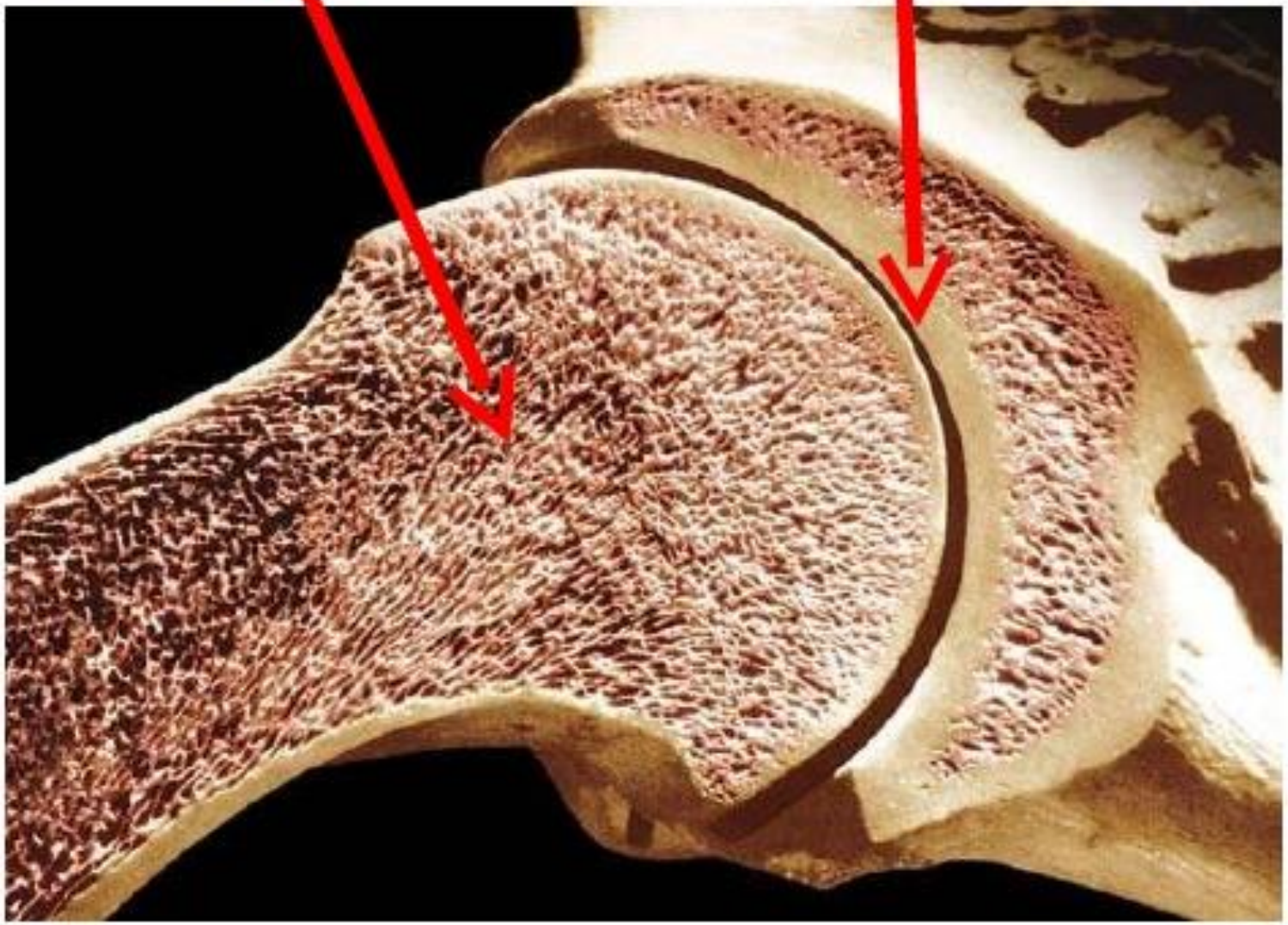
Types of Bone

- Gross observation of a bone in cross section shows a dense area near the surface corresponding to **compact (cortical) bone**, which represents 80% of the total bone mass, and deeper areas with numerous interconnecting cavities, called **cancellous (trabecular) bone**, constituting about 20% of total bone mass.


Compact bone
Cancellous bone

Spongy Bone

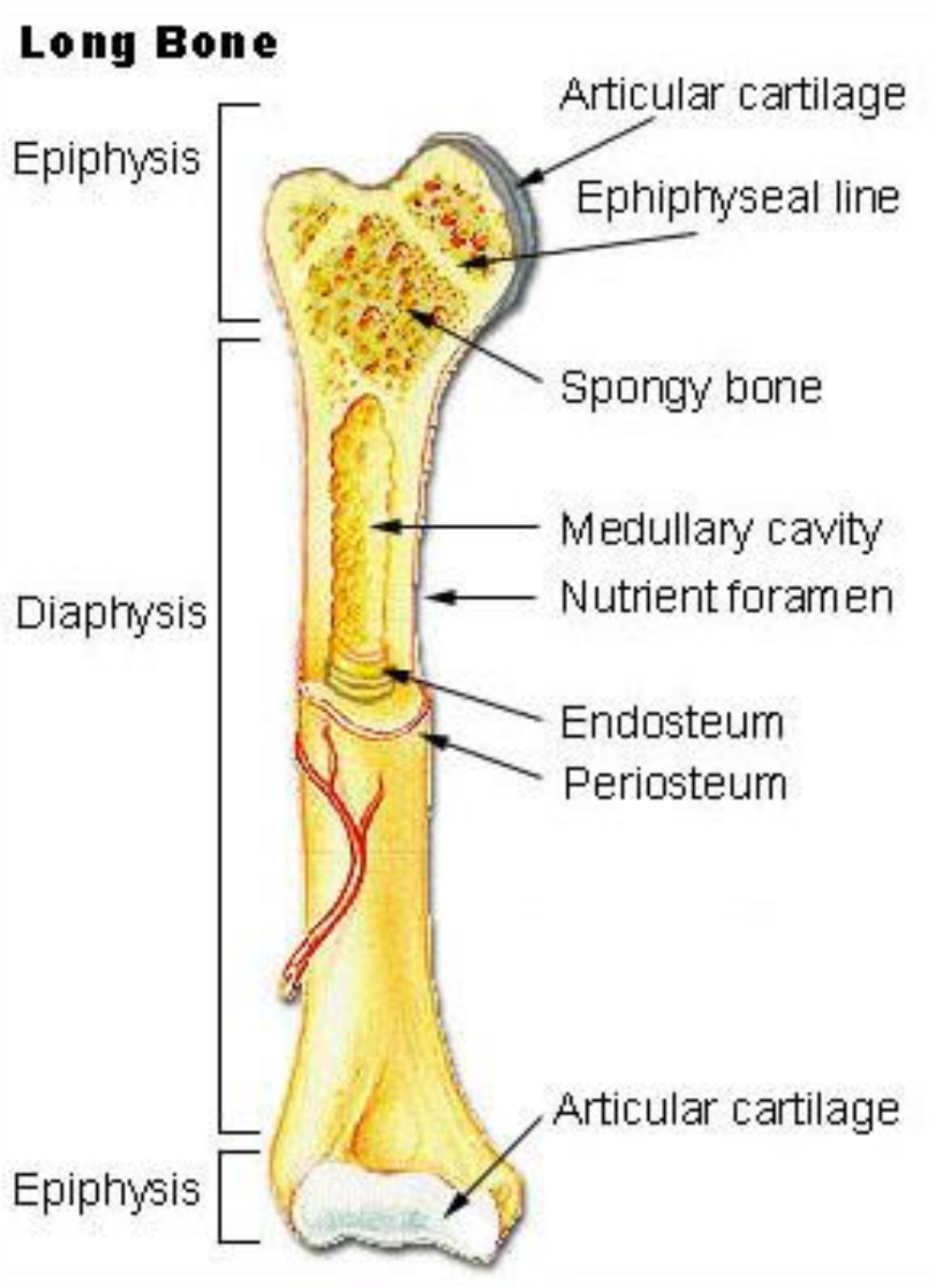
Compact Bone

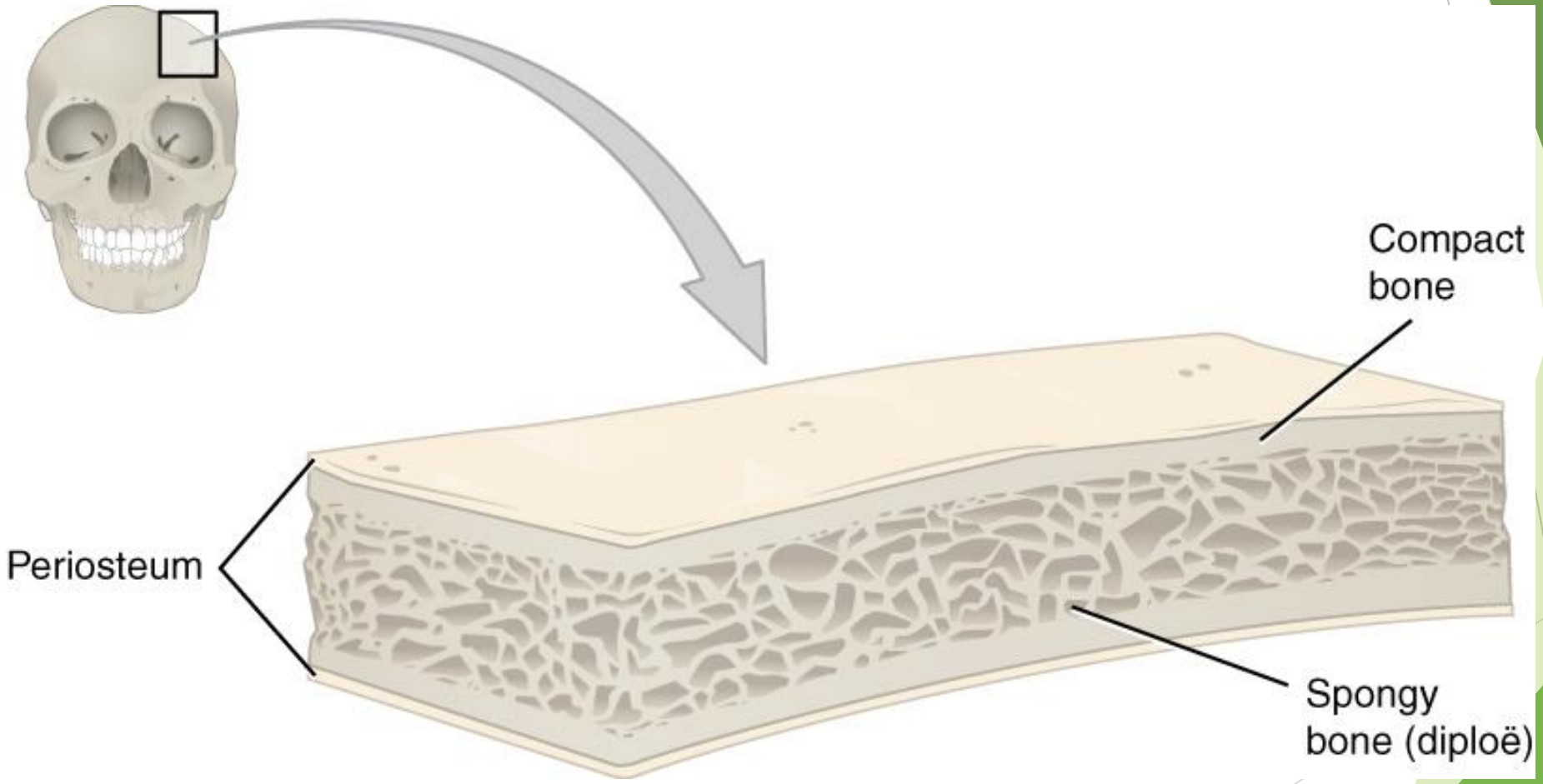


As can be seen from this picture, under normal magnification spongy bone looks porous, while compact bone looks solid.

Correlation between Anatomy and Histology

- In long bones, the bulbous ends—called **epiphyses** —are composed of cancellous bone covered by a thin layer of compact cortical bone.
- The cylindrical part—the **diaphysis**—is almost totally dense compact bone, with a thin region of cancellous bone on the inner surface around the central **marrow cavity**
- Short bones such as those of the wrist and ankle usually have cores of cancellous bone surrounded completely by compact bone.
- The flat bones that form the calvaria (skull) have two layers of compact bone called **plates**, separated by a thicker layer of cancellous bone called the **diploë**.


Long Bone
Articular cartilage
Epiphysis
Ephiphyseal line
Spongy bone
Medullary cavity
Diaphysis
Nutrient foramen
Endosteum
Periosteum
Articular cartilage
Epiphysis


Compact bone
Periosteum
Spongy bone (diploë)

Organization of Bone

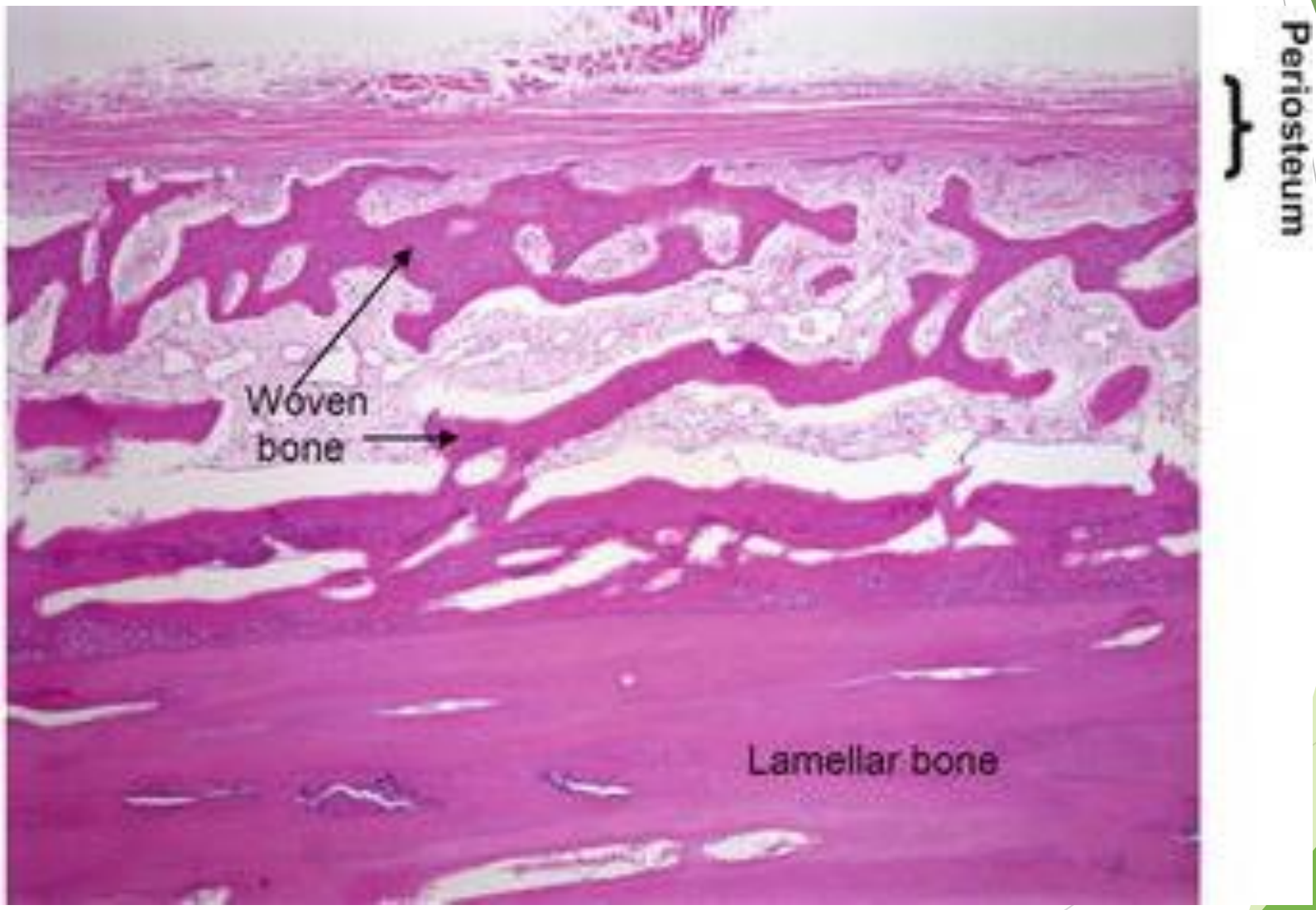
- At the microscopic level both compact and cancellous bone typically show two types of organization:
  - **lamellar bone**, which is mature with matrix existing as discrete sheets
  - **woven bone**, newly formed with randomly arranged components

Lamellar bone

- Most bone in adults (80%), compact or cancellous, is organized as **lamellar bone**, characterized by multiple layers or **lamellae** of calcified matrix
- The lamellae are organized as parallel sheets (cancellous) or concentrically around a central canal (compact)

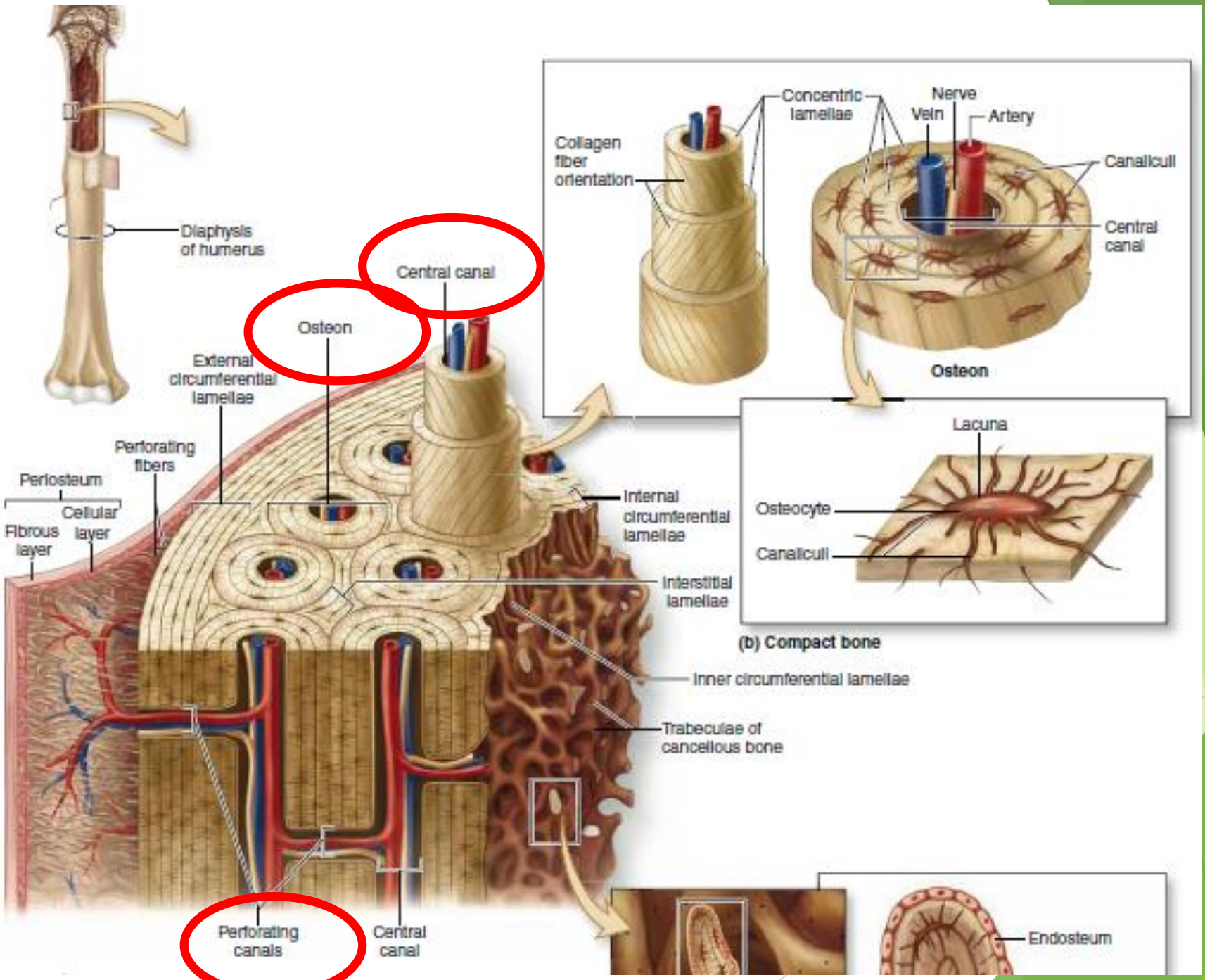
Woven bone

- **Woven bone** is nonlamellar and characterized by random disposition of type I collagen fibers and is the first bone tissue to appear in embryonic development and in fracture repair.
- Woven bone is usually temporary and is replaced in adults by lamellar bone, except in a very few places in the body, for example, near the sutures of the calvaria and in the insertions of some tendons.
- In addition to the irregular, interwoven array of collagen fibers, woven bone typically has a lower mineral content (it is more easily penetrated by x-rays) and a higher proportion of osteocytes than mature lamellar bone.
- These features reflect the facts that immature woven bone forms more quickly but has less strength than lamellar bone.


Woven
bone
Lamellar bone
Periosteum

Havarsian system

- An **osteon** (or **Haversian system**) refers to the complex of concentric lamellae, surrounding a central canal that contains small blood vessels, nerves, and endosteum
- Between successive lamellae are lacunae, each with one osteocyte, all interconnected by the canaliculi containing the cells' processes
- Processes of adjacent cells are in contact via gap junctions, and all cells of an osteon receive nutrients and oxygen from vessels in the central canal
- The outer boundary of each osteon is a layer called the cement line which includes many more non-collagen proteins in addition to mineral and collagen
- Haversian canals also communicate with one another through transverse **perforating canals** (or **Volkmann canals**)


Diaphysis of humerus
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Osteon
External circumferential lamellae
Perforating fibers
Periosteum
Cellular layer
Fibrous layer
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Central canal
Collagen fiber orientation
Concentric lamellae
Nerve
Vein
Artery
Canaliculi
Central canal
Osteon
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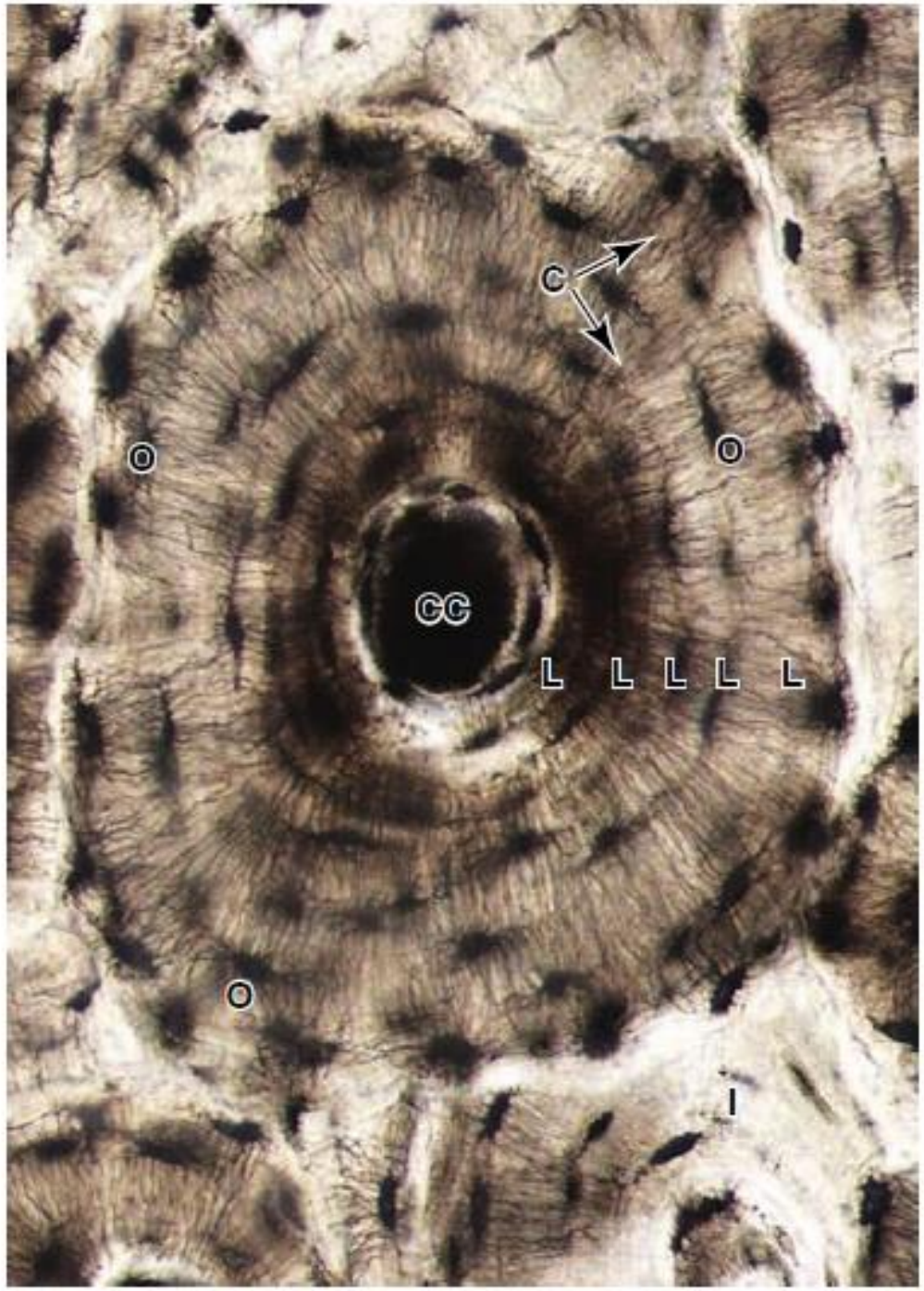
Osteocyte
Canaliculi
(b) Compact bone
Endosteum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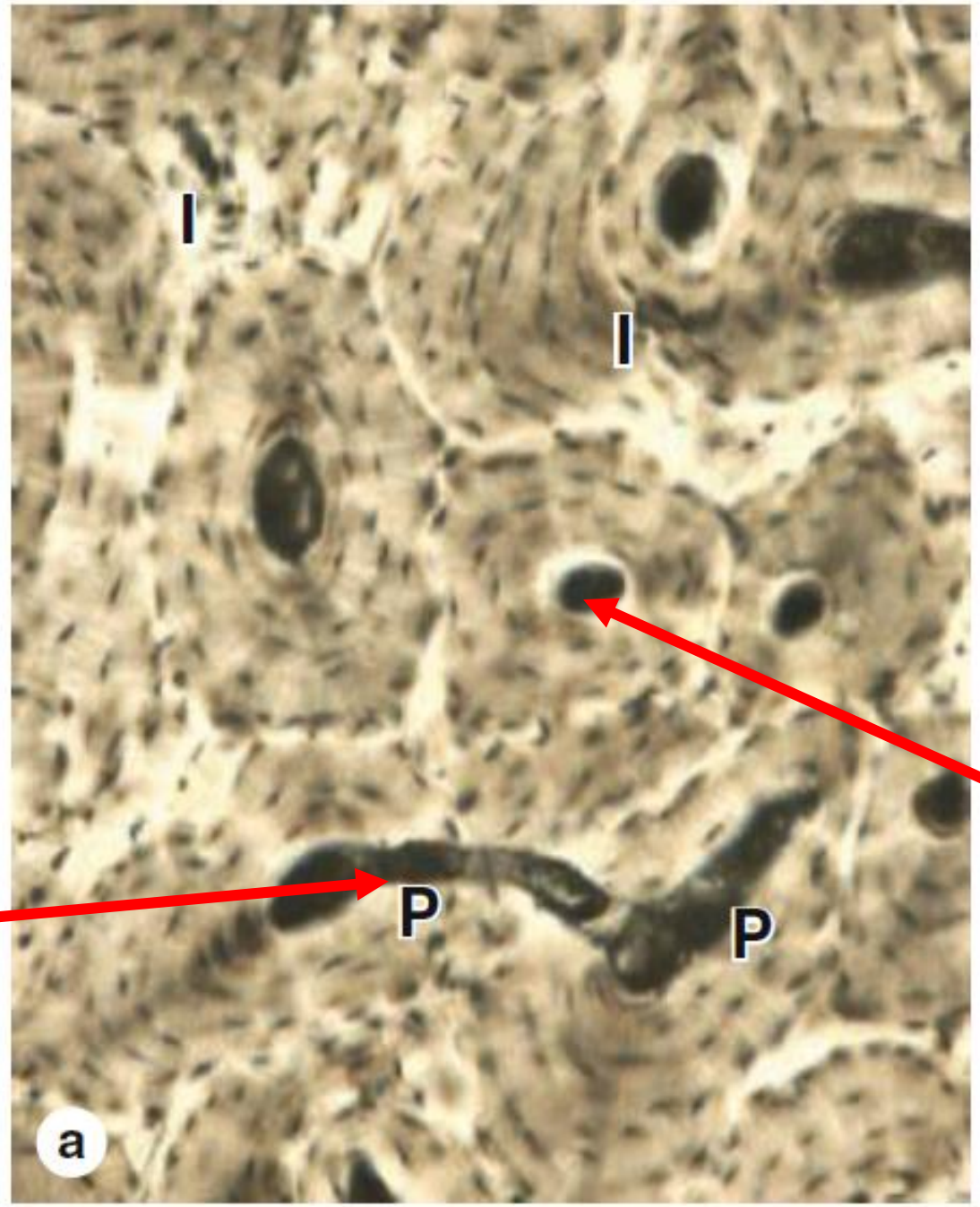


Volkman's canal

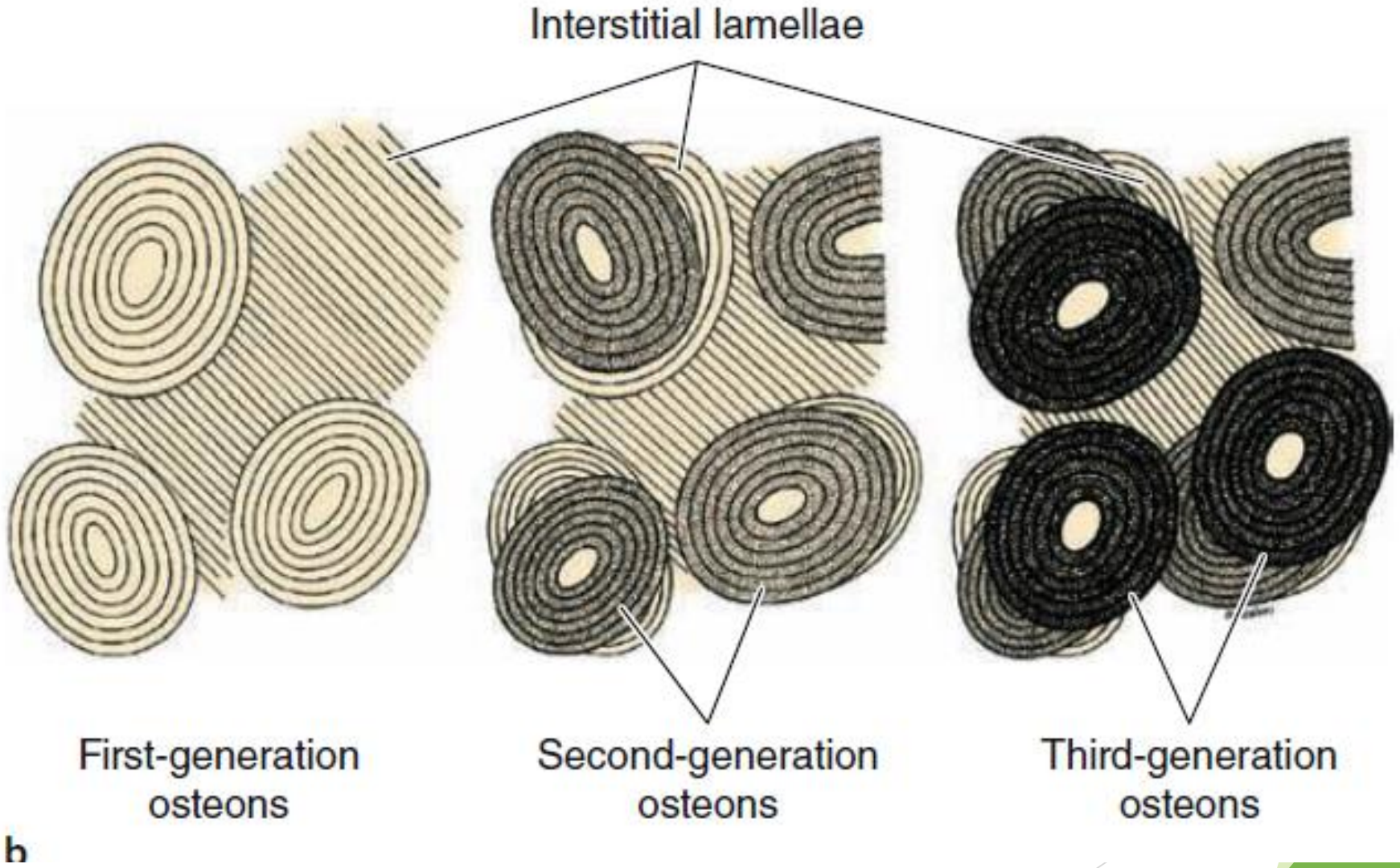
Havarsian canal

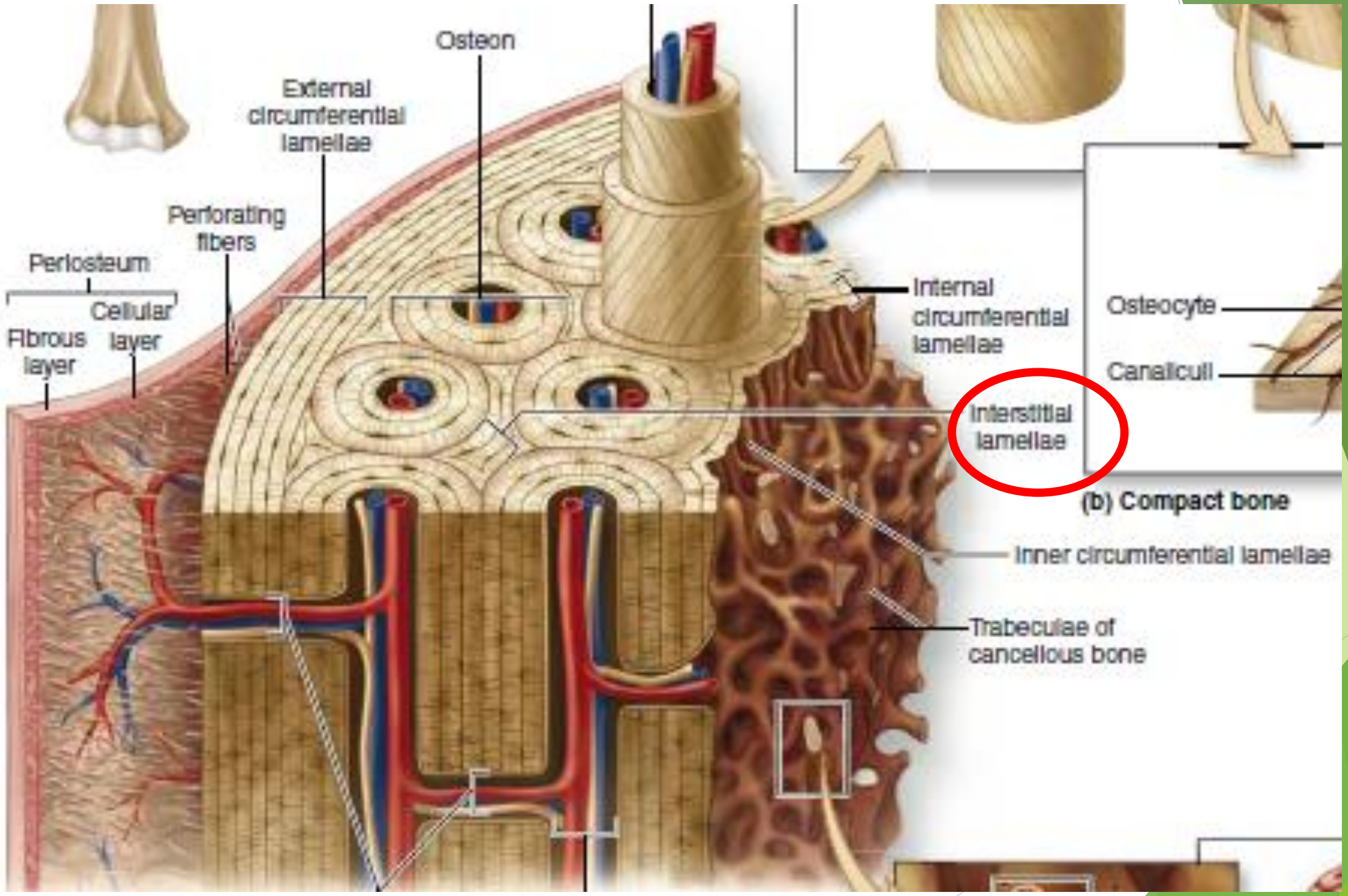
Table 8–1 Summary of bone types and their organization.

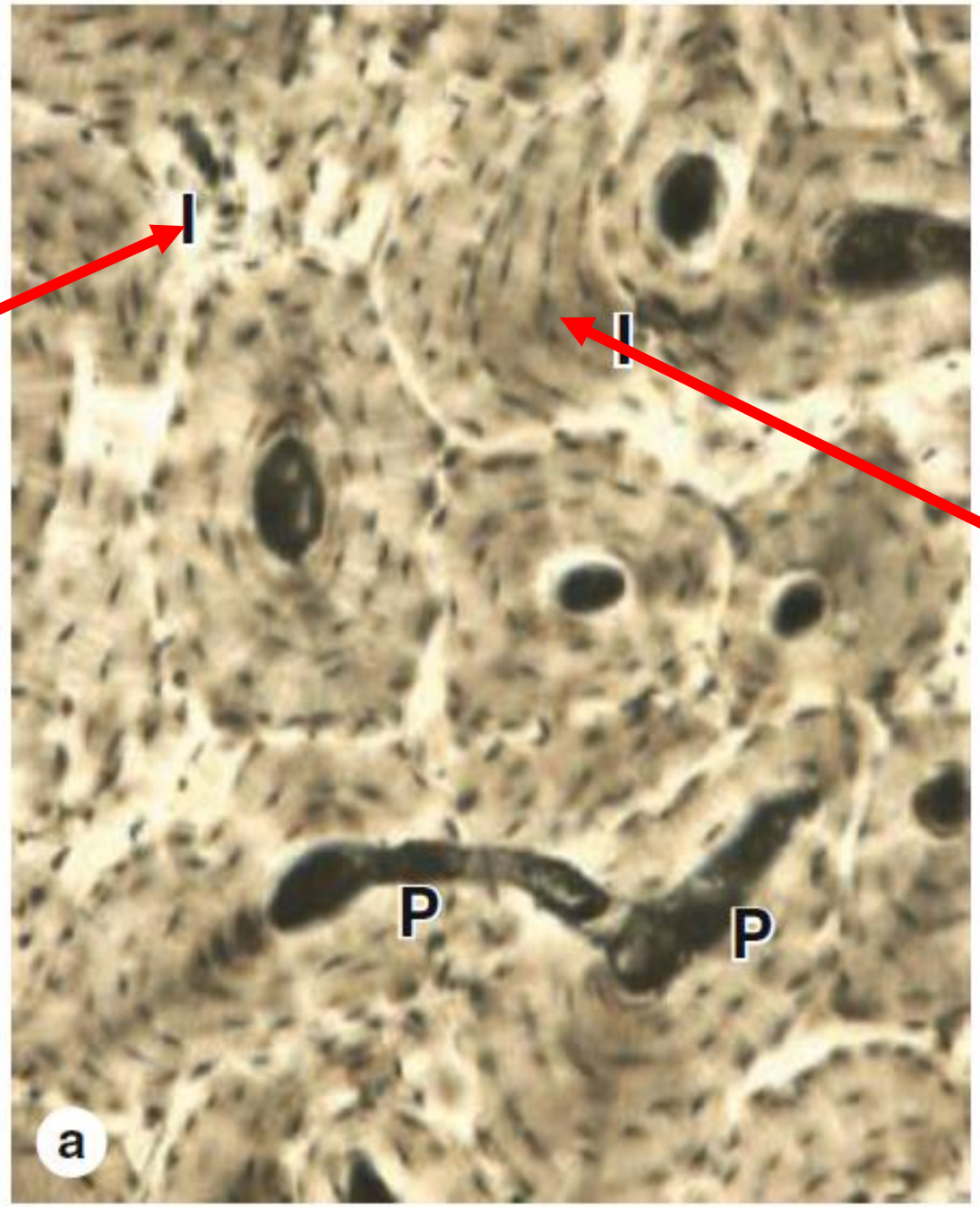
Type of Bone	Histological Features	Major Locations	Synonyms
Woven bone, newly calcified	Irregular and random arrangement of cells and collagen; lightly calcified	Developing and growing bones; hard callus of bone fractures	Immature bone; primary bone; bundle bone
Lamellar bone, remodeled from woven bone	Parallel bundles of collagen in thin layers (lamellae), with regularly spaced cells between; heavily calcified	All normal regions of adult bone	Mature bone; secondary bone
Compact bone, ~80% of all lamellar bone	Parallel lamellae or densely packed osteons, with interstitial lamellae	Thick, outer region (beneath periosteum) of bones	Cortical bone
Cancellous bone, ~20% of all lamellar bone	Interconnected thin spicules or trabeculae covered by endosteum	Inner region of bones, adjacent to marrow cavities	Spongy bone; trabecular bone; medullary bone

Interstitial lamellae

- Scattered among the intact osteons are numerous irregularlyshaped groups of parallel lamellae called **interstitial lamellae**.
- These structures are lamellae remaining from osteons partially destroyed by osteoclasts during growth and remodeling of bone

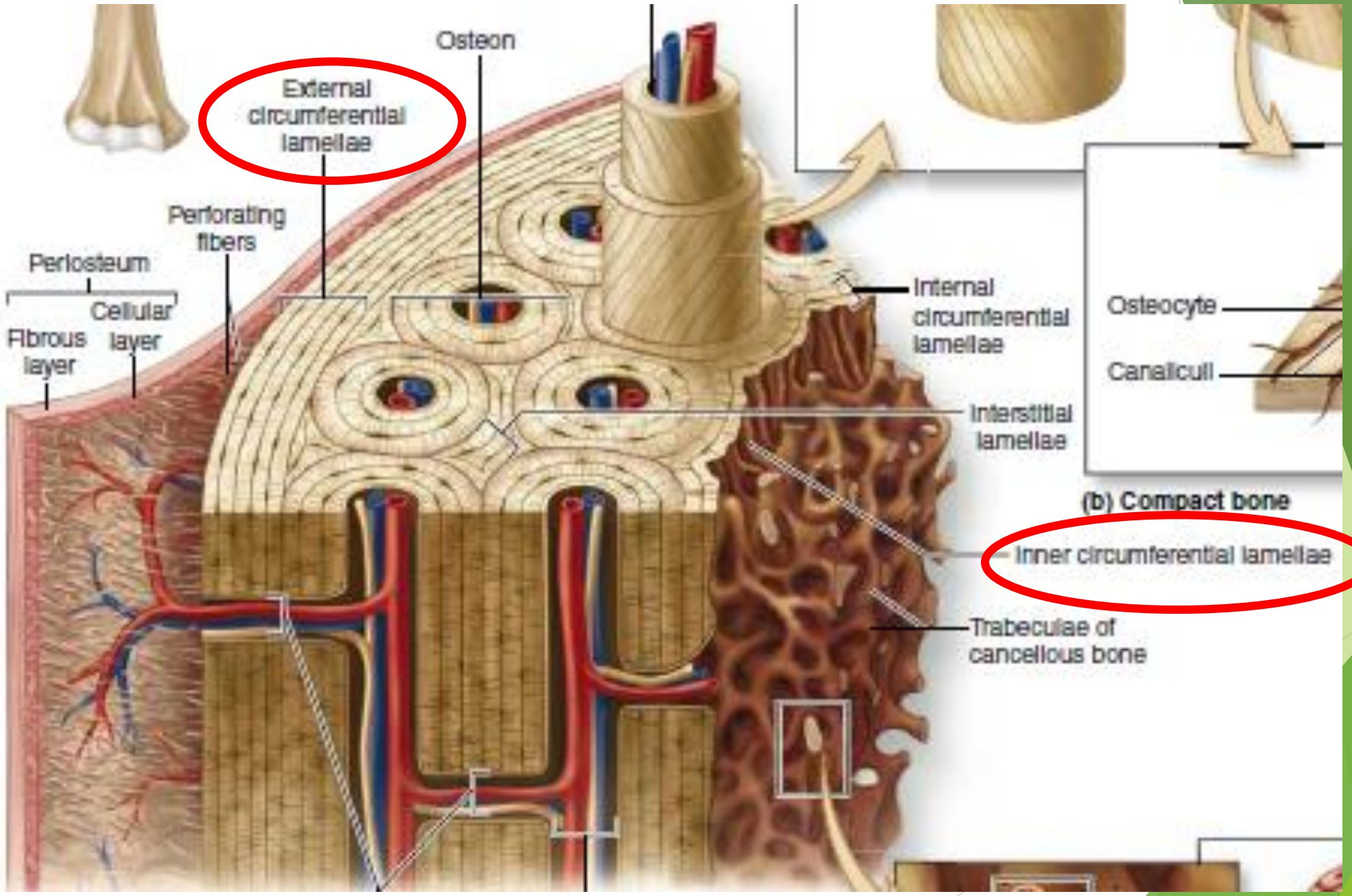

Interstitial lamellae
First-generation osteons
Second-generation osteons
Third-generation osteons
b


Osteon
External circumferential lamellae
Perforating fibers
Periosteum
Cellular layer
Fibrous layer
Internal circumferential lamellae
Interstitial lamellae
Osteocyte
Canaliculi
(b) Compact bone
Inner circumferential lamellae
Trabeculae of cancellous bone


I
I
P
P
a

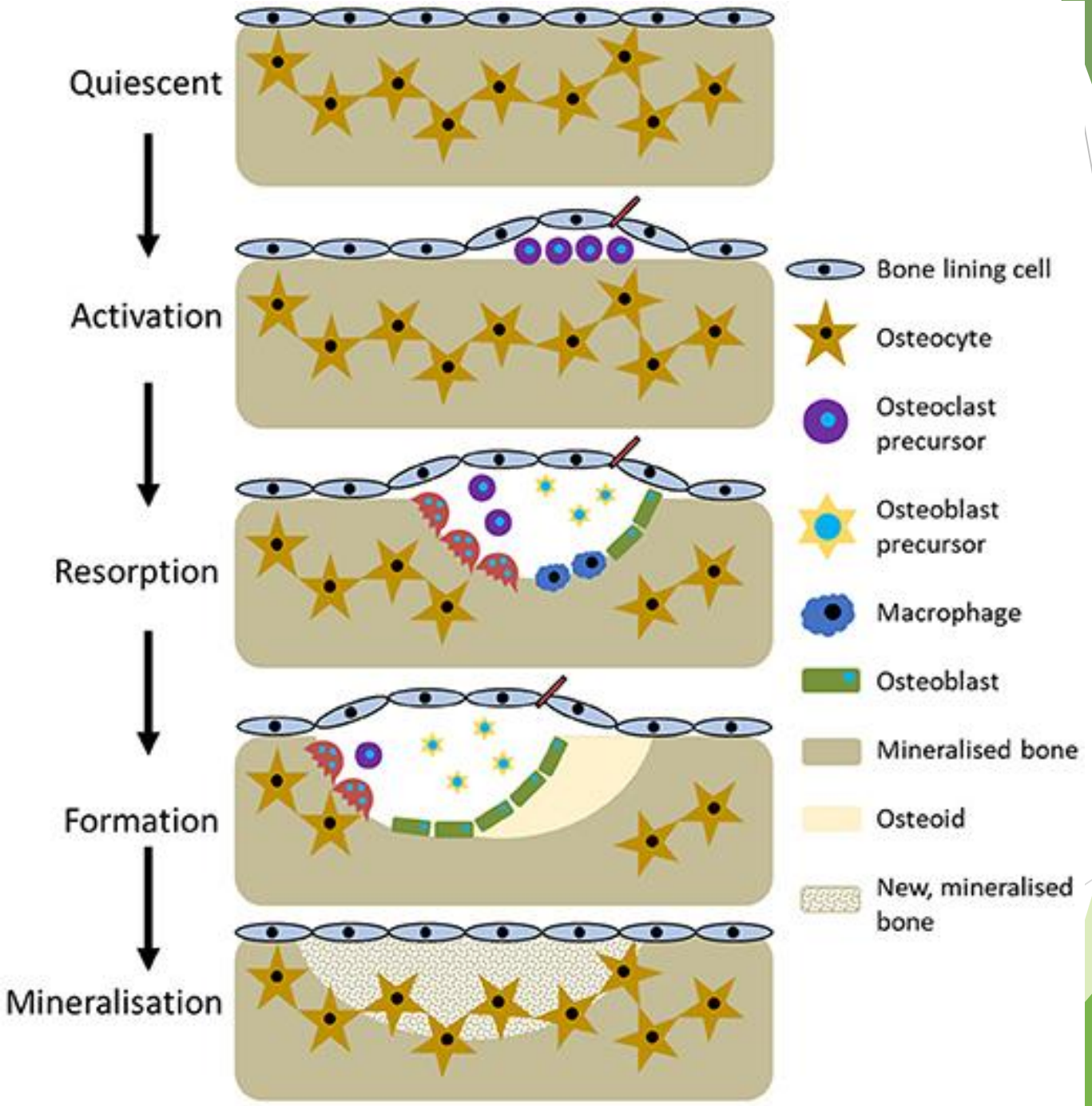
Circumferential lamellae

- Compact bone includes parallel lamellae organized as multiple **external circumferential lamellae** immediately beneath the periosteum and fewer **inner circumferential lamellae** around the marrow cavity
- The lamellae of these outer and innermost areas of compact bone enclose and strengthen the middle region containing vascularized osteons.


Osteon
External circumferential lamellae
Perforating fibers
Periosteum
Fibrous layer
Cellular layer
Internal circumferential lamellae
Interstitial lamellae
Osteocyte
Canaliculi
(b) Compact bone
Inner circumferential lamellae
Trabeculae of cancellous bone

Bone remodeling

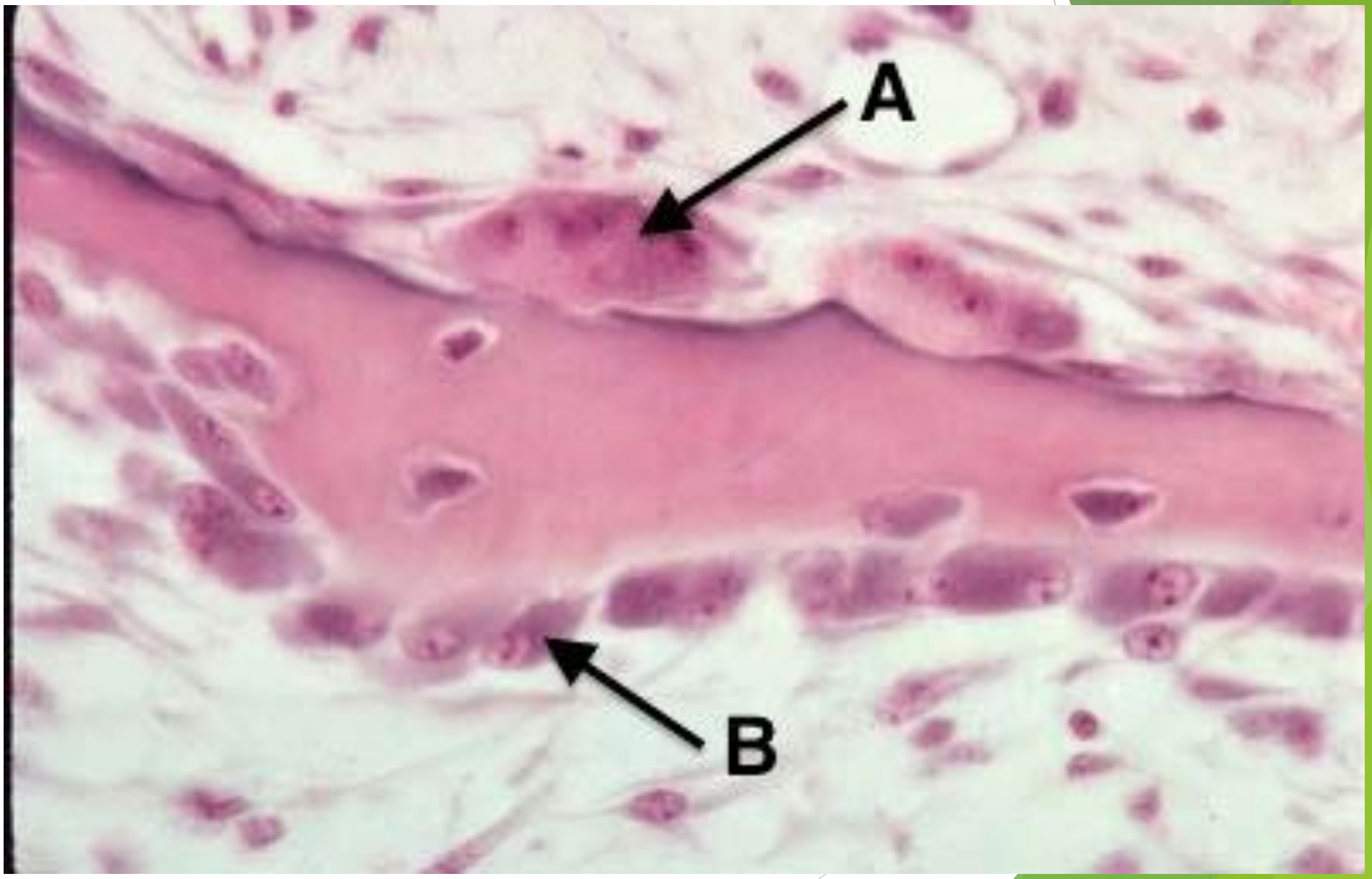
- **Bone remodeling** occurs continuously throughout life.
- In compact bone, remodeling resorbs parts of old osteons and produces new ones.
- Osteoclasts remove old bone and form small, tunnel-like cavities.
- Such tunnels are quickly invaded by osteoprogenitor cells from the endosteum or periosteum and sprouting loops of capillaries.
- Osteoblasts develop, line the wall of the tunnels, and begin to secrete osteoid in a cyclic manner, forming a new osteon with concentric lamellae of bone and trapped osteocytes.
- In healthy adults 5%-10% of the bone turns over annually.


Quiescent
Activation
Resorption
Formation
Mineralisation
Bone lining cell
Osteocyte
Osteoclast precursor
Osteoblast precursor
Macrophage
Osteoblast
Mineralised bone
Osteoid
New, mineralised bone

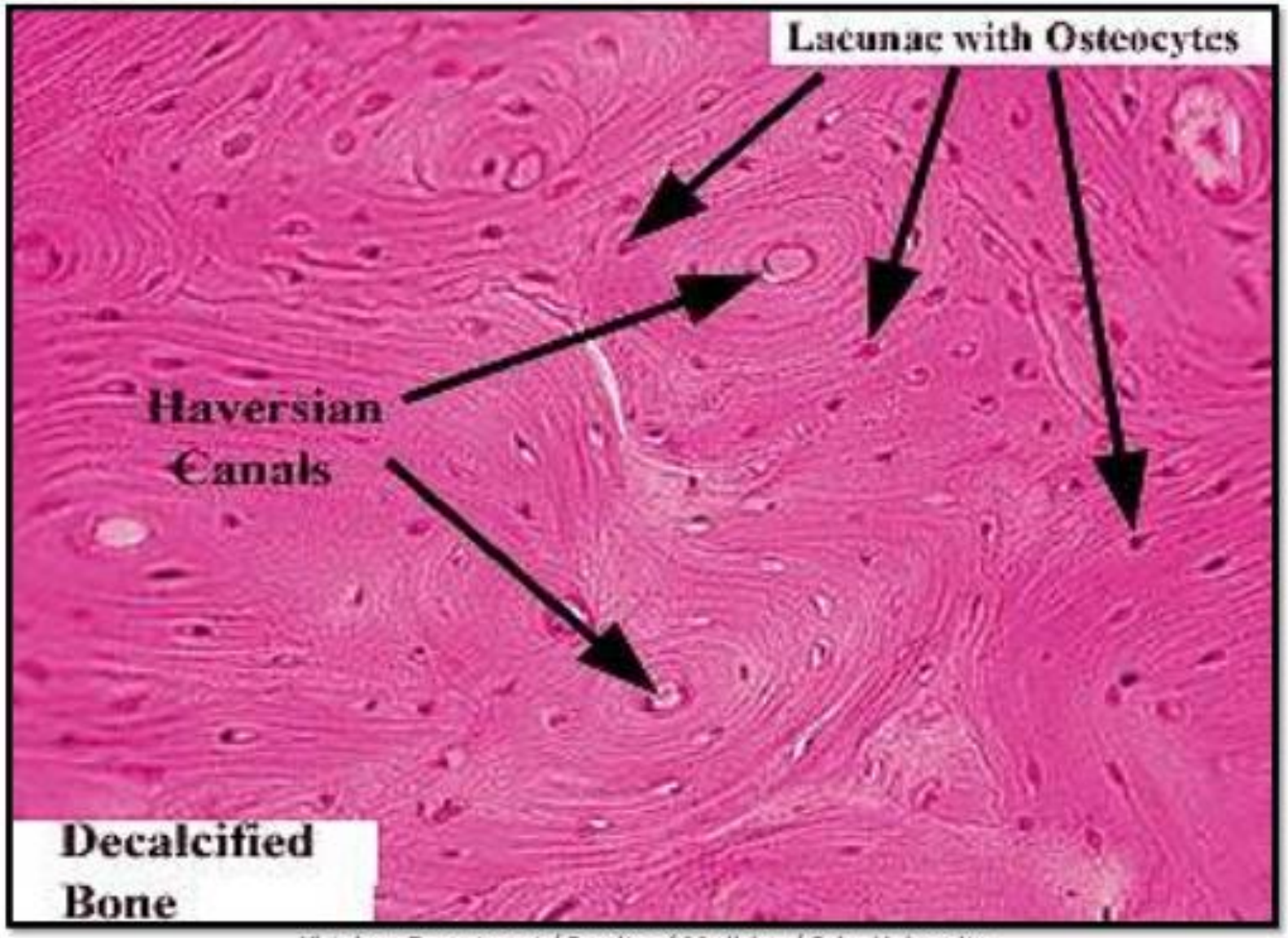
Preparation for histological examination

- Because of its hardness, bone cannot be sectioned routinely.
- Bone matrix is usually softened by immersion in a decalcifying solution before paraffin embedding (Decalcified section)
- If a bone is decalcified by a histologist, its shape is preserved but it becomes soft and pliable like other connective tissues. Because of its high collagen content, decalcified bone matrix is usually acidophilic
- Alternatively, bone can be embedded in plastic after fixation and sectioned with a specialized microtome (Ground section)

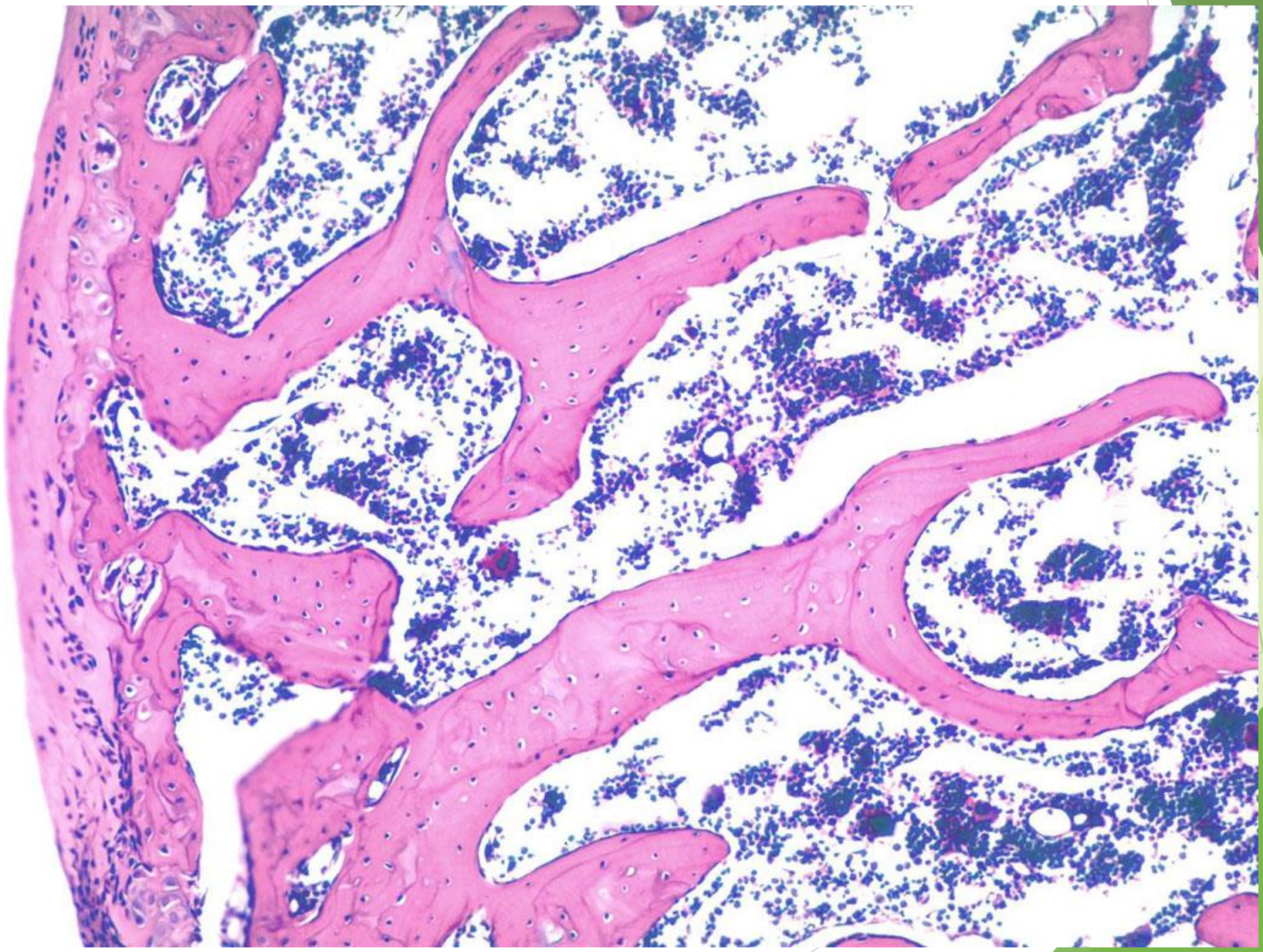
A: Osteoclast
B: Osteoblast



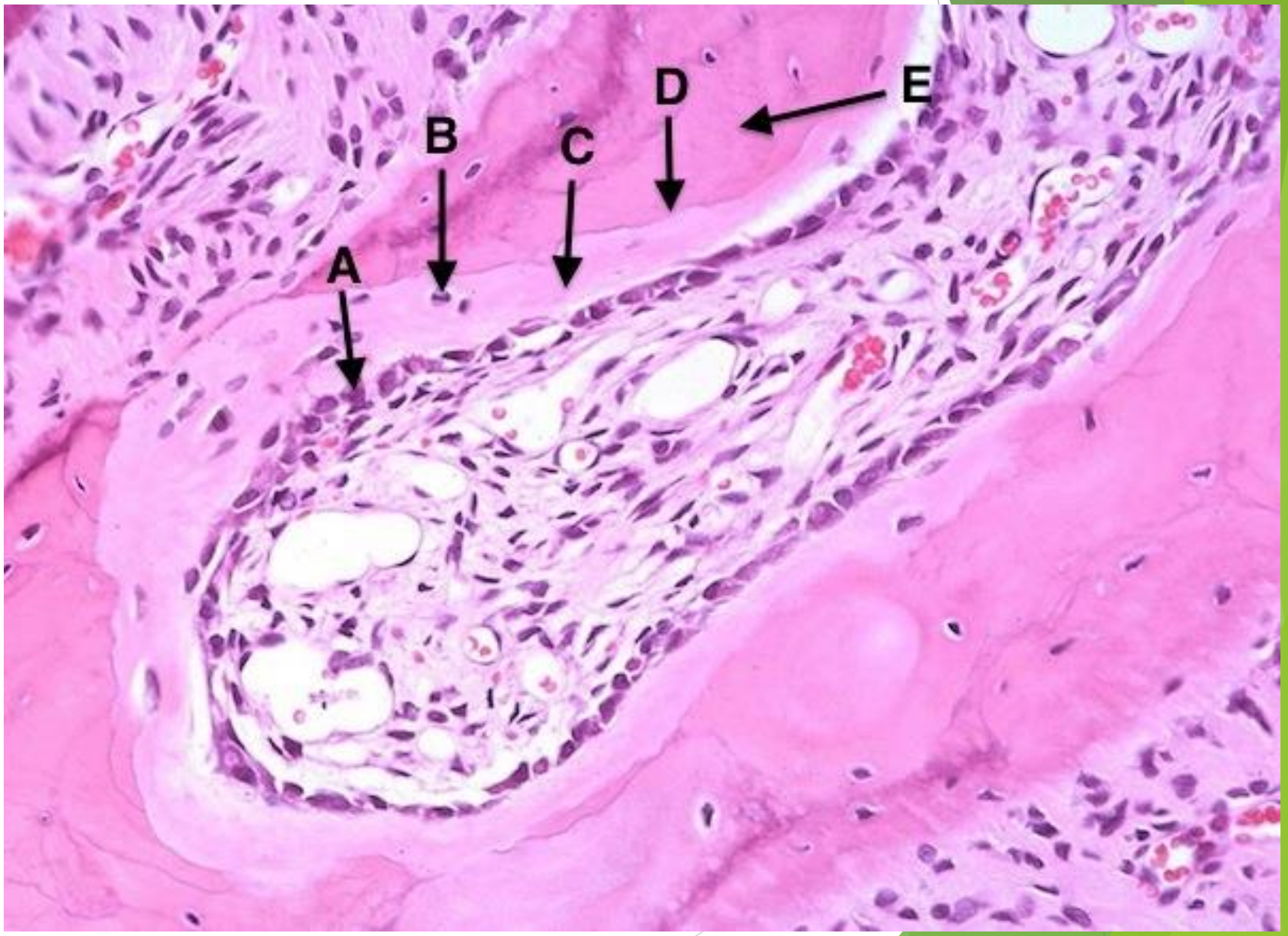
L.M. Decalcified Compact Bone

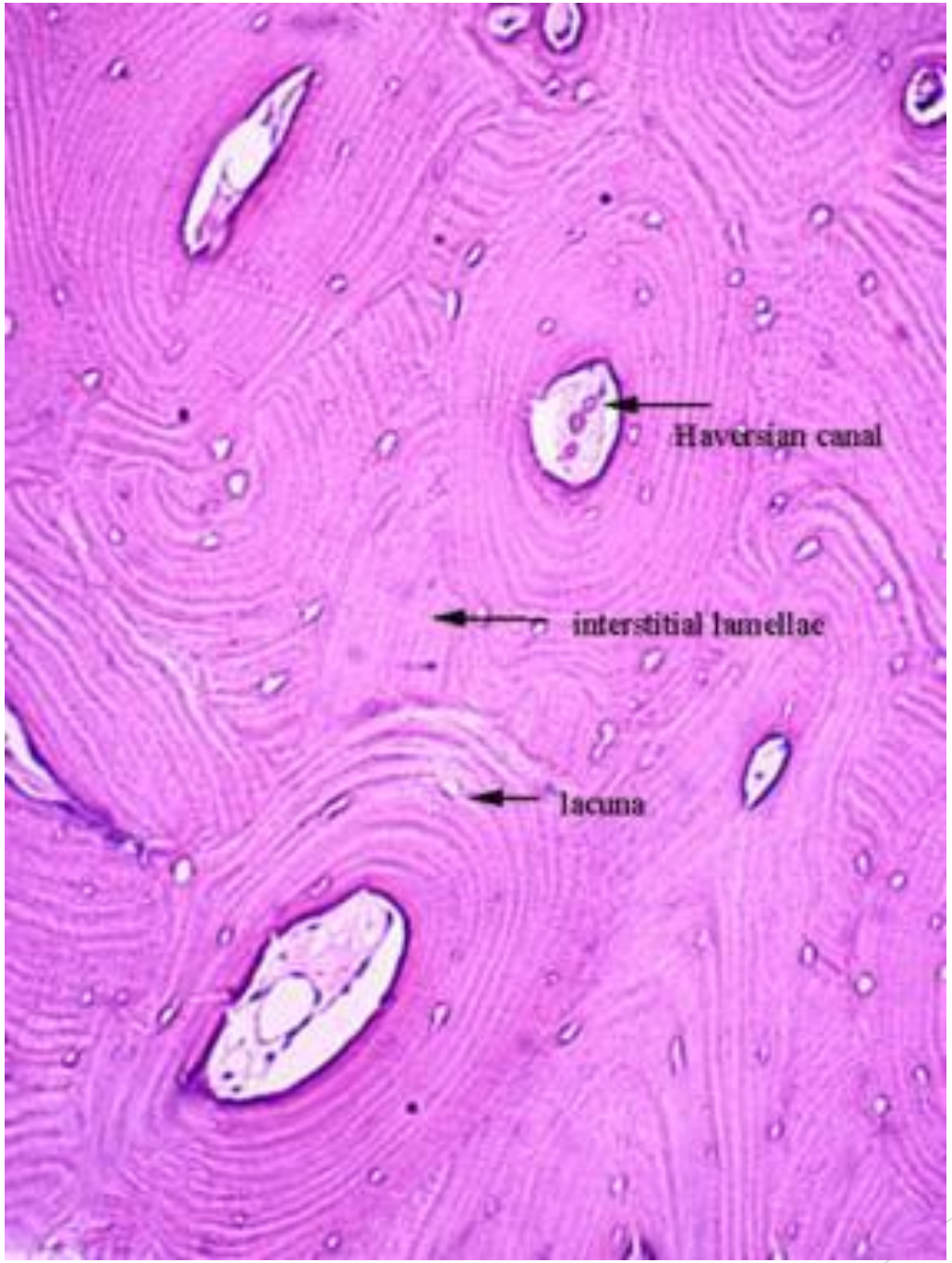


Decalcified trabecular bone



A: Osteoblast
B: Osteocyte
C: Osteoid
D: Cement line
E: Bone




Haversian canal
interstitial lamellae
lacuna