

LABORATORY 8 - HARD CONNECTIVE TISSUE, continued - BONE

OBJECTIVES: LIGHT MICROSCOPY: In cancellous and compact adult bone recognize components including: matrix, trabeculae, osteons, resorption channels (canals), types of lamellae, cell types, etc. Recognize the two processes by which bone is formed: intramembranous bone formation and endochondral bone formation and understand the two processes.

ELECTRON MICROSCOPY: Recognize and distinguish between cell types that occur in mature bone and cells involved in bone formation as well as bone breakdown and remodeling.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

- [SL 38](#) (Dried, cross section, long bone) compact bone
- [SL 39](#) Cancellous bone
- [SL 40](#) (Cross section, long bone) compact bone (also [SL 40 - 40X](#))
- [SL 42](#) (Fetal pig snout) Intramembranous bone formation
- [SL 43](#) (Fetal pig foot) Endochondral bone formation
- [SL 41](#) (Growing long bone)

ELECTRON MICROGRAPHS

J. 8-1 Bone

POSTED ELECTRON MICROGRAPHS

S-27 Osteoblasts

S-29 Osteoclasts

[Lab 8 Posted EMs](#)

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 7. Supportive Connective Tissue

Frames: 373-408

EXTRA CREDIT CASE ON BLACKBOARD: OSTEOPOROSIS

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., [An Atlas of Histology](#)

Copies of this text are on reserve in the HSL.

Bone pp. 108 - 123

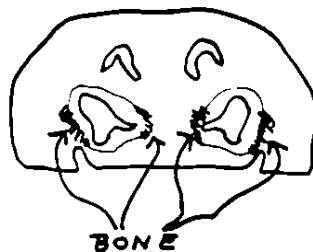
D. BONE

1. MATURE BONE

- a. COMPACT BONE [SL 38](#) ([low 1](#), [low 2](#), [med 1](#), [med 2](#)) (J. 8-8 to 8-11; W.10.9, 10.10)
This is a thin section of a piece of dried bone cut in cross section and then ground to a thickness that allows examination by LM. During the process of grinding, Haversian canals, lacunae and canaliculi accumulate debris and appear brown to black in the section. Identify the following: 1) Haversian Systems or Osteons, 2) interstitial lamellae (the lamellae between osteons), 3) lacunae and canaliculi (use high power and lower the condenser). What is the relationship between osteocytes and lacunae and canaliculi? (J. p. 141)
- b. CANCELLOUS (SPONGY) BONE [SL 39](#). H & E. (W. 10.14)
(1) Observe the acidophilic “network” of cancellous bone. cancellous bone is composed of thin plates formed of irregularly arranged lamellae of bone. Lacunae (sometimes containing osteocyte nuclei) can be seen, but in this section, canaliculi are not visible.
(2) The tissue found in the regions between the plates (trabeculae) of bone is bone marrow. It contains a variable amount of fat.
(3) How do osteocytes in cancellous bone exchange metabolites? (W. 10.14)
- c. BONE TISSUE ORGANIZED INTO AN ORGAN [SL 40](#) and [SL 40 – 40X](#) ([scan](#), [med](#)) Cross section of the decalcified, diaphysis of a long bone. (J. 8-6, 8-10 and W. 10.9 diagrams;). Staining of this section is unusual but is explained as follows. The whole section has been decalcified, however in some areas the ground substance has been removed leaving the blue staining collagen fibers of the lamellae; other areas show variations in the staining of the ground substance from orange to red that are of no significance. However, the general architecture of the bone can be demonstrated.
(1) Note grossly the outer ring of compact bone and the marrow cavity with trabeculae of cancellous bone.
(2) Under low power, within the compact bone numerous small openings of Haversian canals are visible. The Haversian canals are surrounded by indistinct circumferential lamellae making up the osteons. Larger openings are seen occasionally that may represent Resorption Channels (Canals) (usually irregular in outline). Interstitial lamellae fill the regions between the osteons.
(3) Note outer and inner circumferential lamellae are not distinct. Determine their position by referring to diagrams (J. 8-6; W. 10.8).
(4) On your slide Volkman's Canals may not be evident. Refer to (J. p. 147; W. 10.8) for a description.

2. DEVELOPING BONE

- a. INTRAMEMBRANOUS BONE FORMATION [SL 42](#) (J. 8-12; W. 10.17) - This is a section through the snout of a fetal pig. Bone tissue is present in the areas indicated in the diagram. The bone appears as three or four small, irregular, darkly stained regions. Several other tissues are present in this section including young hyaline cartilage.



Locate a region (spicule) of bone in the section. In the bone matrix find osteocytes within lacunae. Osteoblasts that are forming osteoid lie on the outer surface of the bone ([high 1](#), [high 2](#)). In most slides osteoclasts are evident, but better examples of osteoclasts appear on a later slide. Review the process of intramembranous bone formation (J. p. 148).

b. ENDOCHONDRAL BONE FORMATION

[SL 43](#) Fetal Pig Foot ([scan](#)) – While referring to your lecture handout notes, diagrams J. 8-13; W. 10.16 and figure J. 8-16 scan slide 43 with the [4X objective](#). Select a section of bone that has a marrow cavity present and identify the various zones of endochondral bone formation as shown in W. 10.19, 10.20, 10.21. If your slide does not show these stages, study earlier stages that are present as described (J. pp. 148-151). By exchanging slides with your neighbors, most of the stages in long bone development can be analyzed. The sequence of developmental stages of endochondral bone formation is depicted in the diagrams indicated. In your slide locate as many of these stages as possible. Identify [hyaline cartilage](#), [resting](#), [proliferative](#) and [hypertrophied cartilage](#) and [calcified cartilage](#) vs. [bone](#) ([med](#), [high](#)). In the higher mag. image ([high](#)) observe the projections of calcified cartilage that serve as scaffolding for the deposition of new bone. Regions of calcified cartilage ([blue arrow and circle](#)) and new bone ([red arrow and circle](#)) are indicated. On this slide and using the VLM images, identify [osteoid](#), [periosteum](#), [perichondrium](#), [bone collar](#), [osteoblasts](#), [epiphysis](#), [diaphysis](#), etc. Read the description of fracture repair in the text (J. 152, 8-21) and compare this process to embryonic development.

c. GROWTH AND REMODELING

[SL 41](#) Cross section of a growing bone ([scan](#)) - Review the process by which bones grow in length (J. pp. 148-152; W. 10.19, 10.20 10.21). Slide 41 remodeling of bone and growth in diameter of a long bone.

- (1) Observe the external surface of the [periosteum between blue arrows](#) (W. 10.12) with the fibrous ([red arrow](#)) and cellular ([green arrow](#)) layers
- (2) Observe the irregular appearance of the outer surface of the bone due to longitudinal grooves. These depressions are lined with osteoblasts (W. 10.12). The shaft of bone contains osteons in various stages of development. In general, the osteons have a central canal that is regular in outline ([blue arrows](#)), whereas the resorption channels are irregular in shape and have scalloped edges ([green arrows](#)). Both osteoblasts and [osteoclasts](#) ([blue arrow](#)) are evident in the wider channels. (J. 8-4, 8-14; W. 10.5, 10.6, 10.23).

3. JOINTS AND SYNOVIAL MEMBRANES - No slides. Study (J. 8-22 to 8-27; W. 10.24 to 10.28).
4. ELECTRON MICROSCOPY - Study (J. 8-1, 8-5) Relate the "ultrastructural" features of the various cell types to their role in bone formation, remodeling and maintenance.

OBJECTIVES FOR LABORATORY 8: BONE

1. Using the light microscope or digital slides, identify:

Cells of bone

- Osteoblasts
- Osteocytes
- Osteoclasts

Compact bone

- Haversian system (osteons)
 - Haversian canal
 - Lacunae (with osteocytes)
 - Canaliculi
- Lamellae
 - Interstitial
 - Outer circumferential
 - Inner circumferential

Cancellous (spongy) bone

Includes lacunae (with osteocytes), canaliculi

Bone as tissue

- Same structures as compact and spongy bone
- Diaphysis
- Epiphysis
- Resorption canals
- Volkman's canals
- Endosteum
- Periosteum

Bone formation

- Intramembranous
 - Spicule or trabecula
 - All cells of bone (see above)
 - Osteoid
- Endochondral
 - Hyaline cartilage
 - Calcified cartilage
 - Osteoid
 - Bone
 - All cells of bone (see above)
 - Metaphyseal plate
 - Zone of resting cartilage
 - Zone of proliferation
 - Zone of hypertrophy
 - Zone of calcification
 - Zone of bone deposition
 - Perichondrium
 - Periosteum
 - Bone collar

2. On electron micrographs, identify:

- Osteoblasts
- Osteoclasts
- Osteoid
- Bone

REVIEW QUESTIONS FOR CONNECTIVE TISSUES

1. How does interstitial growth of cartilage contribute to the growth of a long bone? What role does calcified cartilage matrix play in the growth of a long bone?
2. What is metachromasia? What components of connective tissues demonstrate metachromasia?
3. Define cancellous and compact bone.
4. What are some of the components of extracellular matrix (ground substance) that are found in various soft and hard connective tissues?
5. What types of fibers are found in connective tissue?
6. Briefly contrast white and brown fat in terms of distribution, function and histological appearance.