

PCB 4023 – Cell Biology

Lab 4: Connective Tissue - Part II

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N.B. Since this document is in “pdf” format, the URLs (web addresses) cannot be linked. To use them, simply highlight and copy the address and paste it into the address box of your browser.

Preparation assignment (to be completed before lab (1/25/02):

- 1) Kerr (1999) Chapter 2. Blood (pp. 25-37).
- 2) Kerr (1999) Chapter 9. Skeletal Tissues (pp. 184)

Web resources:

N.B. Again, you may wish to examine the modules pertaining to connective tissue at the University of Florida College of Medicine histology tutorial. For connective tissue, there are two review modules and one on-line quiz. As before, these modules may contain information that is not covered in this course and you will not be held responsible for any information presented there. However, you may wish to consider using them as additional preparation and/or review. The URL is as follows:

<http://www.medinfo.ufl.edu/year1/histo/index.html>

Know of any other web sites pertaining to connective tissue that you have found helpful or interesting? E-mail me the link at condon@fiu.edu and let me know what and why you found it informative and/or interesting.

I. INTRODUCTION

This is the second of two labs devoted to connective tissue. In Part I you examined the general connective tissues (mesenchyme, adipose, areolar, dense) and one of the special connective tissues, cartilage. In Part II, you will examine the remaining special connective tissues (bone, blood and hemopoietic tissue).

As before, the laboratory assignment will consist of microscopic observation of slides containing sections or smears of cells, tissues, or organs. The laboratory directions include the number of the slides in your collection that you are to observe. The slides have cells and tissue types which correspond to many of the photographs in your texts. The text may help you locate and identify particular structures; it also describes and correlates the cells and tissues seen under the microscope.

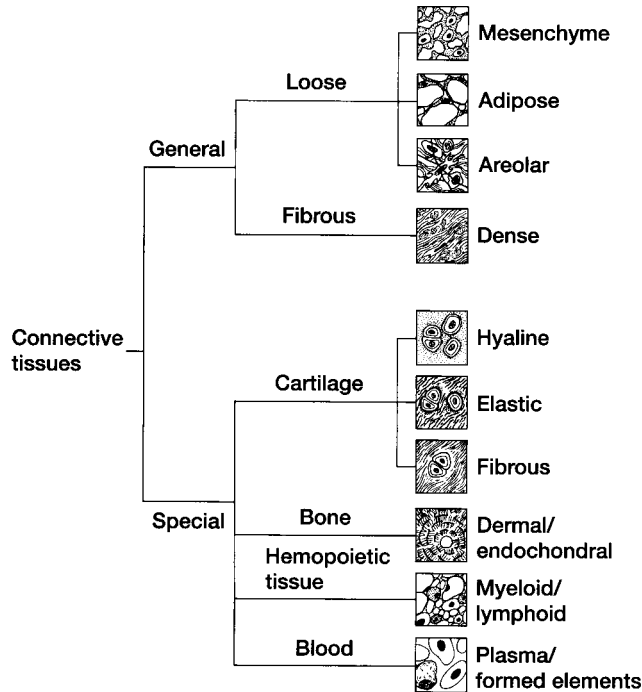
II. CONNECTIVE TISSUE

You will recall that connective tissues are defined as tissues primarily composed of intercellular material (or extracellular matrix; ECM) with varying densities of cells. The intercellular matrix (or ECM) typically consists of (1) fibers (either collagen or elastin) and/or (2) a relatively amorphous “ground substance” (it’s molecular composition is better described in your text; obviously this characterization does not apply to “fluid” connective tissues, one of which, blood, we will examine today).

As seen in the accompanying figure, connective tissues can be broadly divided into two major groups, general and special. The general tissues (basically those produced by fibroblasts and/or

adipocytes) were examined last week. The special connective tissues include the two skeletal tissues, cartilage (examined previously) and bone, blood (a fluid connective tissue), and hemopoietic tissue. As noted previously, examination of hemopoietic cells is extremely challenging and beyond the scope of this course.

figure: 3K5-219.tif



[For reasons which are not clear to me, dentin is never included in classifications of connective tissue although it is always described as such (*i.e.*, a connective tissue) and clearly meets both the morphological (cells embedded within an extracellular matrix; specifically a densely mineralized, collagen-rich matrix) and developmental (derived from neural crest (ectomesenchyme)) criteria. Since we hope to put together an oral tissues lab, this tissue will be examined at a later date.]

Cartilage and bone form the skeleton which provides a rigid framework to which all other tissues/organs attach. The term “bone” is used to refer to both an organ (comprised of multiple tissue types) and a type of connective tissue. It is the latter which is of interest today but some discussion of bone the organ is warranted.

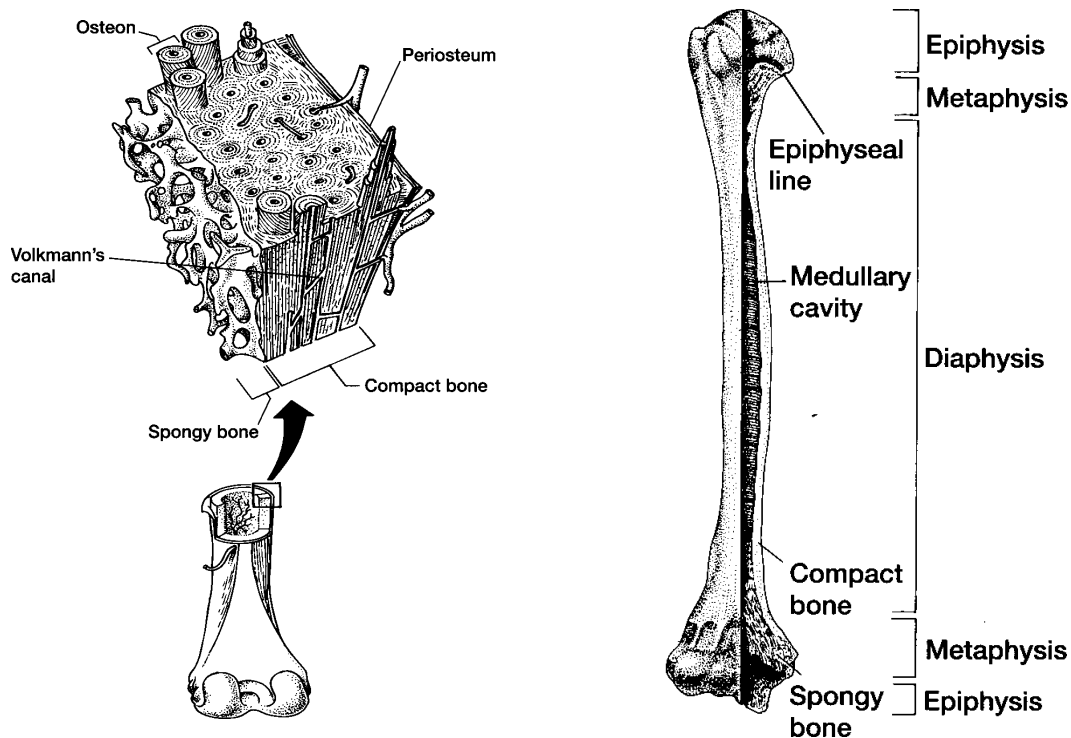
Bone the organ consists of (1) skeletal connective tissue (bone and/or cartilage), (2) marrow, (3) nervous tissue, and (4) vascular tissue. Marrow is a soft, pulpy tissue found in the medullary (or central) cavities of a bone. It comes in two flavors: (1) red marrow, which consists of a connective tissue stroma with hemopoietic cells, and (2) yellow marrow, a connective tissue stroma with fat cells. Bone, and particularly its periosteum (the fibrous covering of a bone), is densely innervated. You can demonstrate this on your lab partner by delivering a hefty kick to their “shin”, which is the anteromedial surface of the tibia immediately below the skin of your leg, and observing their response. Bone is also a highly vascular tissue which partially accounts for its ability to mend itself (in contrast to the avascular cartilage). Observe the fibula with a fracture callus on the demonstration table.

As an organ, bone has multiple functions: As a skeletal tissue it supports and protects soft tissues (e.g., heart, lungs, brain, etc.), and muscles use bones as mechanical levers or links to produce movement. Bone is also an important storage site of lipids (in yellow marrow) and minerals (specifically calcium and phosphate ions stored in the bone matrix). Finally, bone is an important site of hemopoiesis (red marrow).

Bone the tissue is a mineralized connective tissue. As such it consists of cells embedded within an extracellular matrix. The composition of the extracellular matrix in bone consists of approximately 5% proteoglycans, 10-20 % water, 30-40% collagen fibrils and 60-70 percent inorganic (mineral) salts (predominately calcium and phosphate. The shape of the bone is actually determined by its organic components as can be demonstrated by the demineralized rib on the demonstration table. The inorganic salts serve simply to rigidify the material.

As noted above, bone is a highly vascular tissue and it is riddled with vascular canals (nutrient, *Haversian*, *Volkman's*) through which vessels run and reflecting its high metabolic rate and capacity for remodeling. Bone is deposited or formed in layers whose collagen fibers can be oriented parallel to one another (lamellar bone) or randomly arranged (woven bone). The latter is characteristic of newly formed bone, such as is found in developing tissues or in healing fractures (see the fracture callus on the fibula at the demonstration table).

figure: 3K5-21.tif, 3K5-22.tif



Surrounding the bone, both externally and internally are fibrocellular layers which contain the osteogenic cells, the osteoblasts. The periosteum is the fibrocellular layer covering the entire outer surface except the articular surfaces which are covered by a thin layer of articular (hyaline) cartilage. The endosteum covers the inner surface (marrow cavity).

Osteoprogenitor cells are pluripotent stem cells that are important in both bone growth and remodeling (= non-growth related changes in bone). Two distinct cell lineage's gives rise to osteoblasts and osteoclasts. Osteoblasts are the bone forming cells and are responsible for the synthesis, deposition and mineralization of bone. These cells secrete collagen fibrils and osteoid (bone's "ground substance") which subsequently mineralizes to form bone. In mature tissue, osteoblasts can be found in all bone forming areas: periosteum, endosteum and osteon canals.

When an osteoblast has been enclosed within a bony matrix it becomes an osteocyte. These reside within lacuna which are interconnected by numerous cellular extensions via the canaliculi. Since these cells are no longer secretory, their exact function is unknown but they may play a role in mineral metabolism.

Osteoclasts are bone resorbing cells, and not surprisingly, shared a lineage/ancestry with the immune system, particularly the macrophages.

Macroscopically, two types of osseous tissue can be observed (see longitudinally split humerus at the demonstration table): (1) compact and (2) cancellous bone. Compact bone is dense and typically limited to the periphery or cortex of mature bone. In long bones it forms the shaft and in dermal bones (such as the cranial vault) it forms the inner and outer tables. Along articular surfaces it forms the thin layer of subchondral bone beneath the articular cartilage.

Cancellous (or trabecular or spongy) bone is honey-combed by large cavities to form a lattice. In mature bone it fills the articular ends of bone and surround the medullary (marrow) cavity. It is also characteristic of new formed (woven) bone. Cancellous bone in mature tissue is usually arranged to aid in the transmission of forces, thus serving to strengthen the cortex.

As noted in your text (Kerr, 1999) bone growth is strictly appositional, i.e., new layers are added to pre-existing surfaces (either cartilage, existing bone or fibrous membrane), and no interstitial growth is possible (in contrast to cartilage). Bone growth (changes in size and shape associated with general body size increase) typically involves both deposition (by osteoblasts) and removal (by osteoclasts).

As also was noted in your text, there are two mechanisms of ossification, (1) intramembranous and (2) endochondral, which differ only in the nature of the precursor tissue. In intramembranous ossification bone forms within a highly vascular dense connective tissue (membrane) derived from a condensation of mesenchymal cells. Ossifications appear as small rods of bone (L. trabeculae – small beams) which subsequently coalesce. Intramembranous ossification is found in the (1) dermal bones of the skull and pectoral girdle (where the dermis provides the membrane and forming an exoskeleton), (2) the sesamoid bones found within tendons (e.g., the patella), and (3) the bone laid down by the fibrous periosteum and endosteum.

In endochondral ossification, bone forms around and within cartilage, gradually replacing it. This process accounts for the majority of bone found in the endoskeleton. Endochondral ossification takes advantage of cartilage's ability to grow interstitially, thus allowing the skeletal element to increase its length (by producing cartilage) with significantly increasing its width. Thus, during development three regions of a bone can be distinguished: (1) the diaphysis, the ossifying shaft representing the primary (or first) center of ossification (bone formation); (2) the epiphysis, the ossifying articular ends (representing secondary or later forms centers of ossification); and (3) the metaphysis, the cartilaginous growth plates which connects the diaphysis to the epiphysis.

Blood

The second special connective tissue we will examine today is blood. That blood is a connective tissue strikes many, both students and professors, as odd but it clearly meets the morphological criteria of cells embedded within an extracellular matrix. It's just that in this case the extracellular matrix is a fluid (plasma) rather than protein fibrils and/or "ground substance.". Similarly, its origin from mesenchymal cells is also consistent with its classification.

The plasma of mammalian blood forms roughly 60% of the blood volume and is an aqueous medium containing a diverse array of products including nutrients, metabolic gas, respiratory gases, hormones, heat, ions (Na^+ , Cl^- , HCO_3^- , Cu^+ , K^+ , Zn^{2+} ; Ca^{2+} , Mg^{2+} , etc.) and proteins. Among the latter include antibodies (immune response), fibrinogen (important in blood clotting), and serum albumin.

Blood cells (approximately 40% of blood volume) are formed by the cells of hemopoietic tissue (a special connective tissue found in the medullary cavities of some bones and other loci) which enter the circulatory system. Blood "cells" are divisible into three major groups: red (Gr, erythrocytes), white (Gr, leukocytes) and platelets.

Erythrocytes, or red blood cells (RBCs), comprise the vast majority of blood cells (>99%) and are filled with hemoglobin for the transport of oxygen. They also contain the enzyme which converts CO₂ into bicarbonate for transport within the plasma.

Leukocytes, or white blood cells (WBCs), are an integral part of the immune system although they comprise only about 1% of all blood cells. Unlike erythrocytes, they routinely migrate out of the blood stream. Leukocytes can be divided into (1) granulocytes (named for the staining properties of their granules: neutrophils, eosinophils and basophils), (2) monocytes, and (3) lymphocytes. Granulocytes are an important part of the inflammatory response; neutrophils are the most common (60-70% of WBCs) whereas eosinophils (3%) and basophils (0.5%) are relatively scarce. Monocytes are the precursor cells to macrophages which are phagocytic cells and account for 3-7% of WBCs. Lymphocytes mediate both the humoral and cellular immune response and account for 20-30% of WBCs.

Platelets are not true cells but separate with the cellular component of blood when it is centrifuged. They are bits of cytoplasm produced by the megakaryocytes cells found in bone marrow and function in blood clotting.

Blood cells are usually studied in preparations called smears. Two stains, (1) Wright's (methylene blue and eosin) and (2) Giesma (methylene azure and eosin) are routinely used to differentiate blood cell types and both give similar staining patterns which are summarized below:

| | |
|-------------------------------------|--|
| Erythrocytes | cytoplasm is orange to pink; nuclei (if present) deep blue; enucleated with bi-concave shape; 5-7 μm dia. |
| Monocytes | cytoplasm is gray-blue with light red granules; nuclei, lilac with reticular chromatin; 12-18 μm dia. |
| Lymphocytes | cytoplasm is pale "robin's egg" blue; nuclei, purple blue; cytoplasm reduced to narrow rim; 7-12 μm dia. |
| Polynuclear neutrophilic leukocytes | cytoplasm has red lilac granules; nuclei, blue; 2-5 lobed nucleus, 10-13 μm dia. |
| Eosinophilic leukocytes | cytoplasm is blue with red granules; nuclei, blue; 2-3 lobed nucleus, 11-14 μm dia. |
| Basophilic leukocytes | cytoplasm has dense purple granules; nuclei, purple; 2-3 lobed nucleus, 9 - 12 μm dia. |

Lymphocytes vs monocytes

Lymphocytes (1) have a round, darker nucleus, (2) are small cells (10-12 μm ; approximately 1.5x the diameter of a RBC), and (3) contain a thin rim of cytoplasm. Monocytes (1) have an indented, lighter, flocculent (fluffy) nucleus, (2) are larger cells (12-15 μm ; approximately 2x the diameter of a RBC), and (3) contain more cytoplasm.

Lab Assignment: Connective Tissue – Part II

Work through the following sections using your atlas as a guide. Make sure to answer the questions (marked by “?”) at the end of the lab; these will be evaluated when you turn in your handout next week. A list of structures which will form the basis for next week’s quiz is given at the end of the handout.

To learn how to identify the structures, write down criteria which will assist you in your identification (e.g., simple squamous epithelium: single layer, flat cells with flattened nuclei). Your text is a good source for such material as well as your own observations. Some students find it helpful to make rough sketches of the structure to assist in their learning.

N.B. Due to [unprogrammed] slide death, your slide box may not contain the required slide. If this is the case, notify an instructor and they will provide a replacement or suggest an alternative. If you end up borrowing a slide from one of your colleagues’, please don’t forget to return it to them.

I. Bone

A. Mineralized ground section of compact bone

bone cross-section, ground: HB 804

In this slide you seen a piece of mineralized, dried compact bone. The chief characteristic is the presence of longitudinal Haversian systems, consisting of concentric lamellae or sheets of bony matrix laid down around a central canal (Haversian canal) containing a blood vessel. The osteocytes, which are now dried out, were embedded within the lamellae in lacunae. The lacunae and the thin, spidery, interconnecting canaliculi can still be seen. Look for Volkman’s canals connecting adjacent Haversian systems

Identify: osteons (Haversian systems)
 Haversian canals
 lamellae
 lacunae
 osteocytes
 canaliculi
 Volkmann’s canals

B. Decalcified

tail: H800 (best), 93W3321 (faded)

The inorganic salts of the matrix have been removed, leaving the fibrous constituents and the cells. The center of these vertebra are filled with hemopoietic tissue. Look for Haversian systems (scarce; young rodent), lamellae (matrix deposited in layers), osteocytes and lacunae. The bright pink color of this tissue is due to the collagen. Pale areas of calcified cartilage can be observed, a remnant of the process of endochondral ossification

osteons (Haversian systems)
osteocytes
lacunae
lamellae
osteoclasts (look for resorbtion pits)
periosteum
endosteum
osteoblasts

II. Osteogenesis

A. Intramembranous

intramembranous ossification: H730 (scalp; H&E); 93W3282 (skull; trichrome; extras available)

This is the process by which mesenchymal cells differentiate directly into osteoblasts and lay down a dense intercellular substance. In this instance bone formation is not preceded by cartilage. In this slide of a scalp or fetal skull such ossification is occurring. Observe the formation of bony spicules (woven bone), periosteum, osteoblasts, osteocytes, and osteoclasts.

Identify: woven bone
periosteum
osteocytes
osteoblasts

B. Endochondral

bone, endochondral: HB 8-1

In this process hyaline cartilage is replaced by cancellous bone. This slide shows the active process consisting of areas of reserve cartilage, cartilage cell multiplication, lacunar enlargement, and calcification of the cartilaginous matrix.

Identify: hyaline cartilage
calcified cartilage
woven bone
osteocytes
lacunae
osteoblasts
osteoclasts (difficult)
periosteum / perichondrium

At the epiphyseal growth plate identify the following zones:

resting
proliferative
maturation
hypertrophic
ossification.

III. Blood

A. Mammalian Dried Blood Smear (Wright's or Giesma Stain)

blood smear (mammalian): 93W6541

This is a smear of normal mammalian peripheral blood. In studying it learn to identify all types of leucocytes and observe the shape of the erythrocytes. Also, identify blood platelets.

Identify: erythrocytes
neutrophils
basophils
eosinophils
monocytes
lymphocytes
platelets

Optional exercise: After you are certain of the identification of the cell types, make a "differential" white cell count. Identify and record by means of the data sheet how many of each type of leukocyte you observe in 200 successive white cells encountered. Move your slide in a systematic fashion up and down across the most uniform part of the smear. After making your counts, calculate the percentage of each cell-type and record it on the data sheet.

| | <u>Actual Count</u> | <u>Percent</u> |
|-------------|---------------------|----------------|
| Neutrophils | | |
| Eosinophils | | |
| Basophils | | |
| Lymphocytes | | |
| Monocytes | | |

B. Non-Mammalian Dried Blood Smear (Wright's or Giesma Stain)

blood smear (frog): 92W3640 (DEMONSTRATION)

The erythrocytes of mammals (approximately 4,000 species) are unusual in that they are enucleated cells. Of course, like all mammalian cells they are "born" with a nucleus but this is usually (but not always) shed during the differentiation process. [Embryos and fetuses, for example, often have nucleated red blood cells.] In contrast, all other craniates (approximately 50,000 species) have nucleated red blood cells, so you are the freak. Examine this blood smear from a frog and confirm that its erythrocytes are nucleated.

C. Sickle cell anemia

blood smear (human): HC 1-44 (DEMONSTRATION)

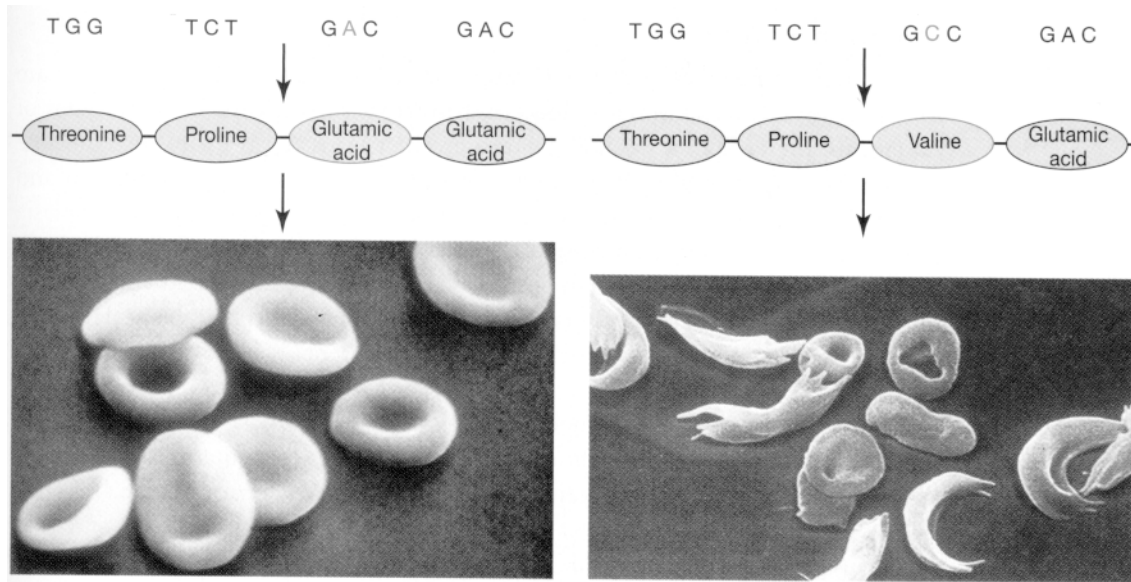
This blood smear is prepared from an individual who has (or had) sickle cell anemia. Observe that many of the erythroblasts (red blood cells; RBCs) have a collapsed or sickle-shaped appearance rather than the normal biconcave disk shape.

Sickle cell anemia is a genetic disease inherited as an autosomal recessive. Individuals who are homozygous for the recessive allele express the disease whereas heterozygotes and homozygous dominants do not, although the former may have a small percentage of sickle cells in their blood smears (sickle cell trait).

Sickle cell anemia results from a point mutation in the hemoglobin (Hb) gene. Specifically, in this allele (Hb S) an adenine has mutated to a cytosine resulting in a coding change from the amino acid glutamic acid to valine. The resulting hemoglobin is unstable and crystallizes under the conditions of low partial pressures of oxygen. The crystallization of hemoglobin and the subsequent polymerization of these crystals distorts the shape of the erythrocytes producing a characteristic sickle-shape. Such cells become lodged in the capillaries, producing a host of complications (ischemia, anemia, etc.) that typically result in premature death, if left untreated.

Sickle cell anemia was the first identified "molecular disease", a term coined by Linus Pauling (Nobel Laureate 1954 and 1962) who in 1949, along with his colleagues Harvey Itano, Seymour Singer and Ibert Wells, demonstrated using electrophoresis that Hb S differed from normal hemoglobin in its molecular composition. Prior to 1949, all diseases were thought to result either from an infectious agent (e.g., viruses, bacteria, protozoa, etc.) or from environmental agents (e.g., lead, alcohol, etc.). Sickle-cell anemia was the first disease demonstrated to have a genetic ("molecular") basis.

figure: f&h4-5.tif



Sickle cell is one of the myriad of anti-malarial mutations found in human populations (others include the thalassemias and glucose 6-phosphate dehydrogenase (G6PD) deficiency). Malaria, contrary to its name, results not from bad air but from the parasitic infection of the erythrocytes by the sporozoan *Plasmodium falciparum* which is transmitted by the bite of an infected female mosquito of the genus *Anopheles*. In areas where malaria is endemic and the sickle cell allele is common, individuals who are heterozygous for the mutation (*i.e.*, those with sickle cell trait) are at a selective advantage because the *Plasmodium* will die if it enters a cell containing the mutant hemoglobin. Individuals without the trait (homozygous dominants) potentially carry a heavier malarial infection load and thus are more likely to be sick. Individuals with sickle cell anemia (homozygous recessive) don't have to worry about malaria but, as noted above, typically die prior to reproductive age. Thus, sickle cell mutation is a classic example of heterozygote superiority. For the quantitative genetics buffs among you, the fitness (τ) for heterozygotes, homozygous dominants and homozygous recessives are estimated to be 1.0, 0.83 and 0, respectively.

IV. Questions (due Week 5):

As always, these and a list of structures for which you will be responsible will be distributed in class.