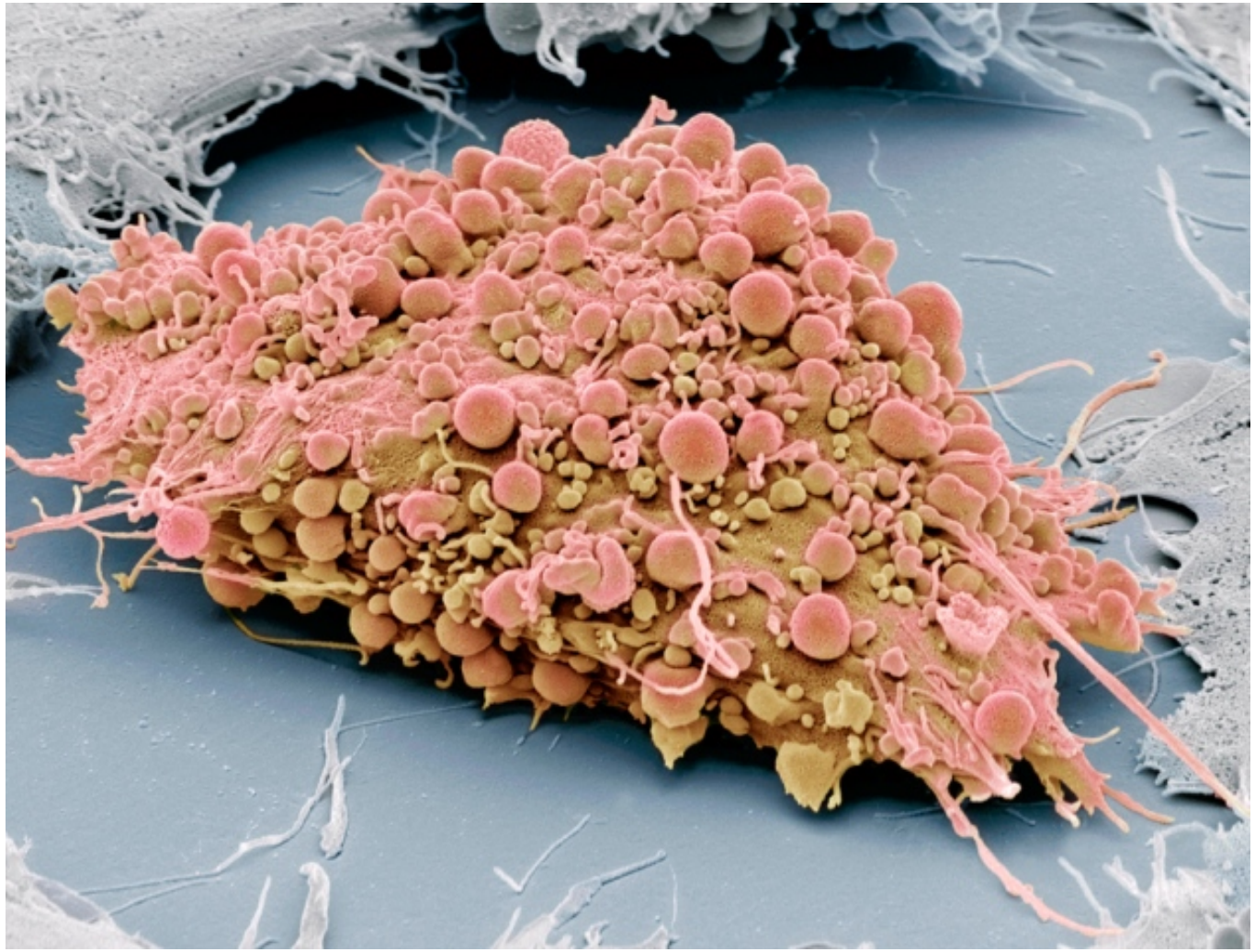
Lab 5 – Connective Tissue

IUSM – 2016

I. **Introduction**
II. Learning Objectives
III. Keywords
IV. Slides
    A. Types of Connective Tissue
        1. Mesenchyme
        2. Connective Tissue Proper
            a. Loose/Areolar
                i. Elastic fibers
                ii. Reticular fibers
            b. Dense
                i. Irregular
                ii. Regular
        3. Specialized CT
            a. Adipose
            b. Cartilage (Lab 6)
            c. Bone (Lab 6/7)
            d. Blood (Lab 8)
    B. Resident and Wandering Cells
        1. Lymphocytes
        2. Plasma cells
        3. Macrophages
        4. Mast cells
        5. Eosinophils
V. Summary

Connective Tissue

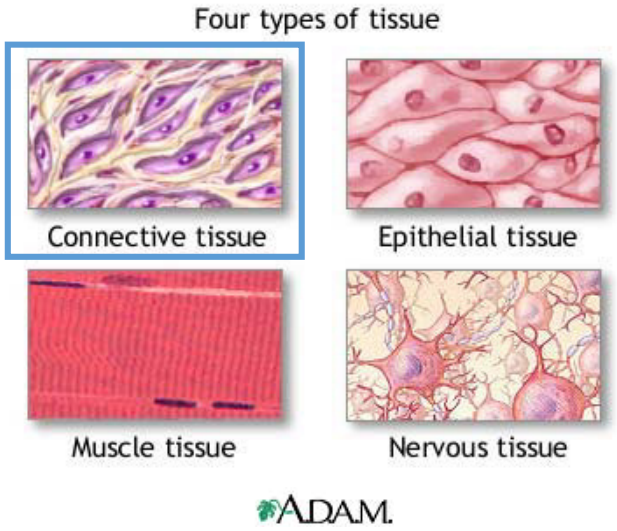


SEM of mesenchymal stem cell. Steve Gschmeissner.

Lab 5 – Connective Tissue

IUSM – 2016

I. **Introduction**
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. Macrophages
    4. Mast cells
    5. Eosinophils
V. Summary



Connective Tissue (CT)

1. Forms the **stroma** of most organs, serving to connect and support the other primary tissue types.

2. Derived from embryonic **mesenchyme.**

3. Unlike the other tissue types which are composed primarily of cells, CT consists of only a few dispersed, inconspicuous **cells** within a prominent **extracellular matrix** (ECM).

   - **Fibroblasts** are the principal resident cells of connective tissue, responsible for its synthesis and maintenance.

   - **ECM** is tissue-specific and composed of **protein fibers** (*collagen*, *reticular*, and *elastic*) and **ground substance** (amorphous gel-like substance).

4. Function and classification of CT is primarily based upon the composition and organization of the extracellular matrix and its functions.

5. Within connective tissue, several types of cells, primarily leukocytes (white blood cells), can be found; some are long-lived in the tissue (**resident cells**) while others are transient and short-lived (**wandering cells**).

Lab 5 – Connective Tissue

IUSM – 2016

Learning Objectives

1. Be able to identify the major types of connective tissue and understand how the structure of each reflects its function.

2. Understand how to distinguish the various cells found in connective tissue (fibroblasts, adipocytes, mast cells, plasma cells, macrophages, and undifferentiated mesenchymal cells) and to describe their functions and key features.

3. Know the composition, morphology, and variations in distribution of the ground substance and the three types of extracellular fibers and their functions.

Lab 5 – Connective Tissue

IUSM – 2016

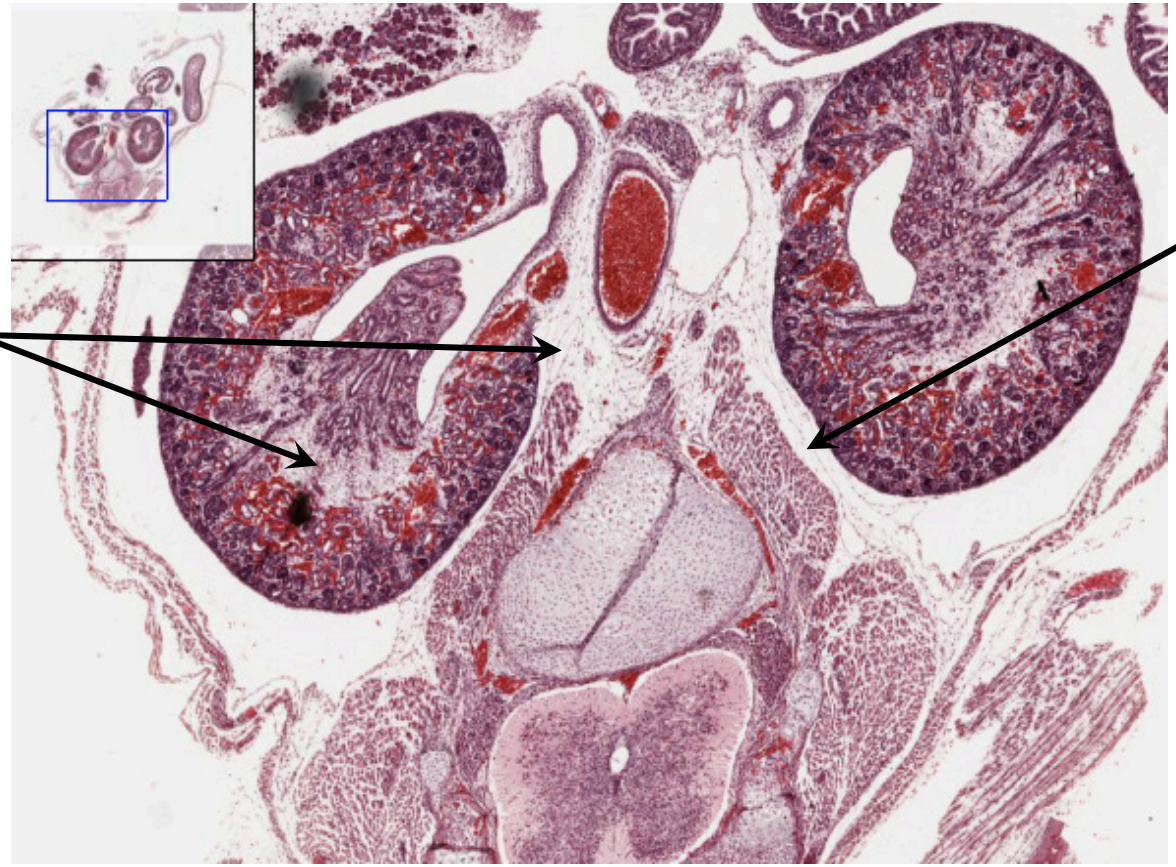
Keywords

Brown adipose tissue
Collagen fibers
Connective tissue proper
Dense irregular CT
Dense regular CT
Elastin (elastic) fibers
Fibroblasts
Loose/areolar CT
Macrophages
Mast cells
Mesenchyme
Plasma cells
Reticulin (reticular) fibers
White adipose tissue

Lab 5 – Connective Tissue

IUSM – 2016

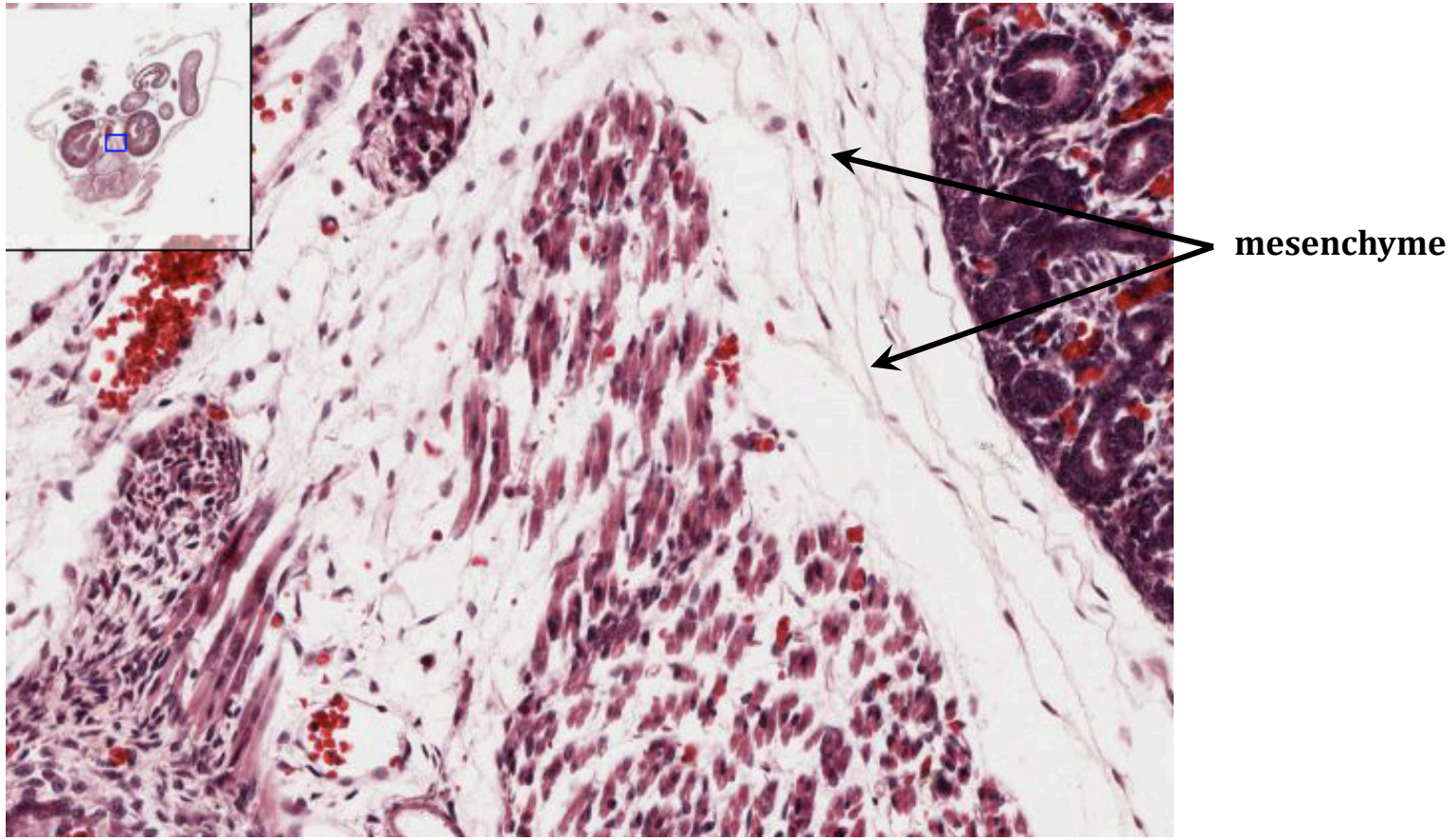
Slide 91: Hamster Embryo, H&E



Lab 5 – Connective Tissue

IUSM – 2016

Slide 91: Hamster Embryo, H&E



mesenchyme, or primitive connective tissue, derives from embryonic mesoderm and gives rise to the various connective tissues of the body; it contains spindle-shaped cells in an immature, loose extracellular matrix (ECM) containing reticular fibers, collagen, and ground substance; in general, its appearance is best described as “very loose” connective tissue

Lab 5 – Connective Tissue

IUSM – 2016

Slide 40a (464): Lower Fetal Jaw, H&E

look here for **mesenchyme**

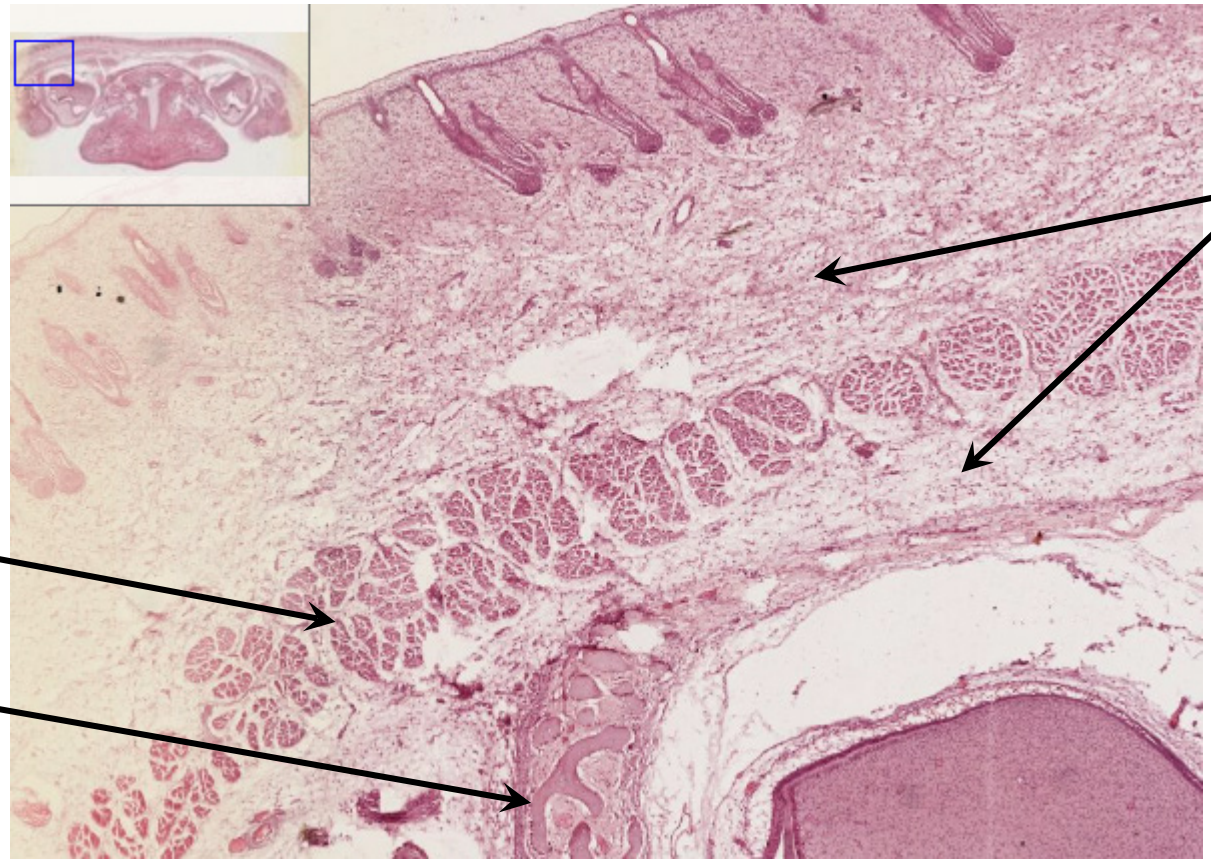
look here for **mesenchyme**

Lab 5 – Connective Tissue

IUSM – 2016

Slide 40a (464): Lower Fetal Jaw, H&E

Slide Overview

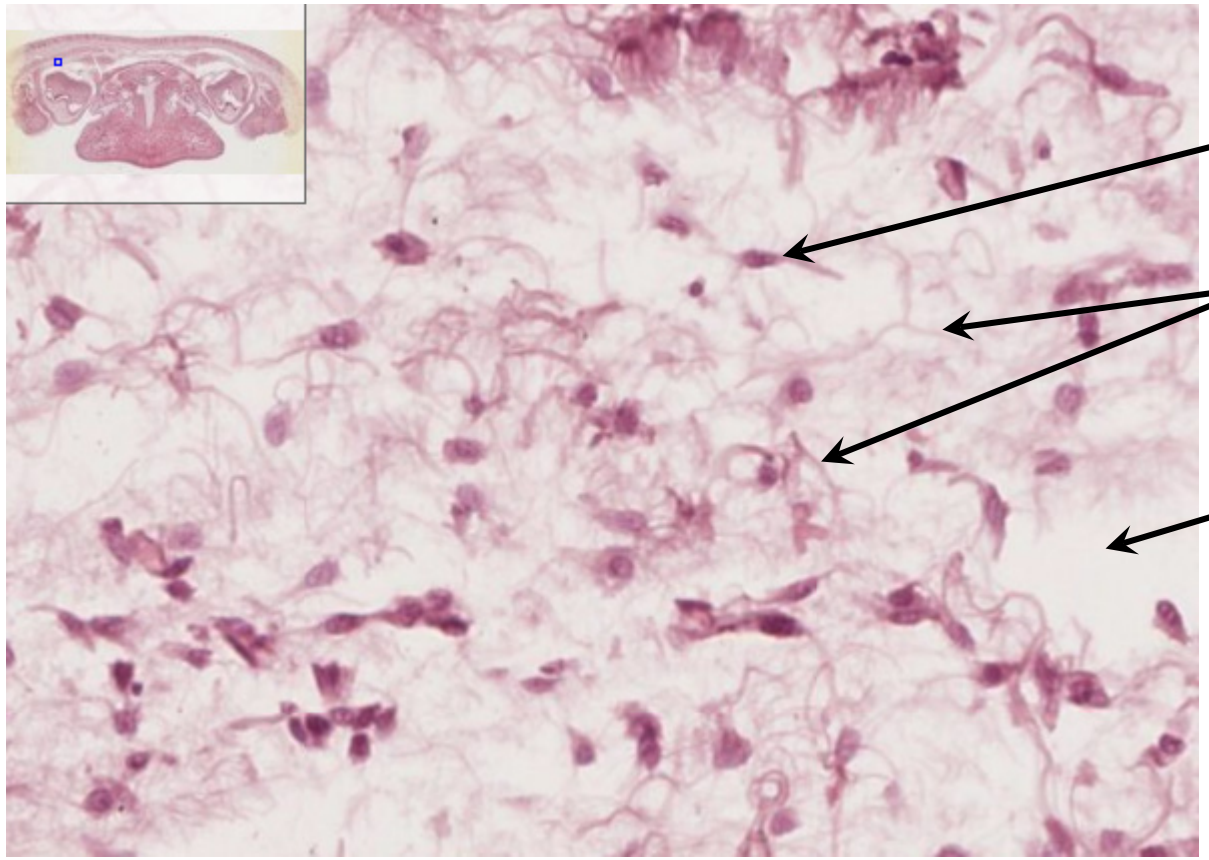


Lab 5 – Connective Tissue

IUSM – 2016

I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. **Mesenchyme**
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. Macrophages
    4. Mast cells
    5. Eosinophils
V. Summary

Slide 40a (464): Lower Fetal Jaw, H&E



spindle-shaped mesenchymal cell

extracellular matrix fibers (reticular and collagen)

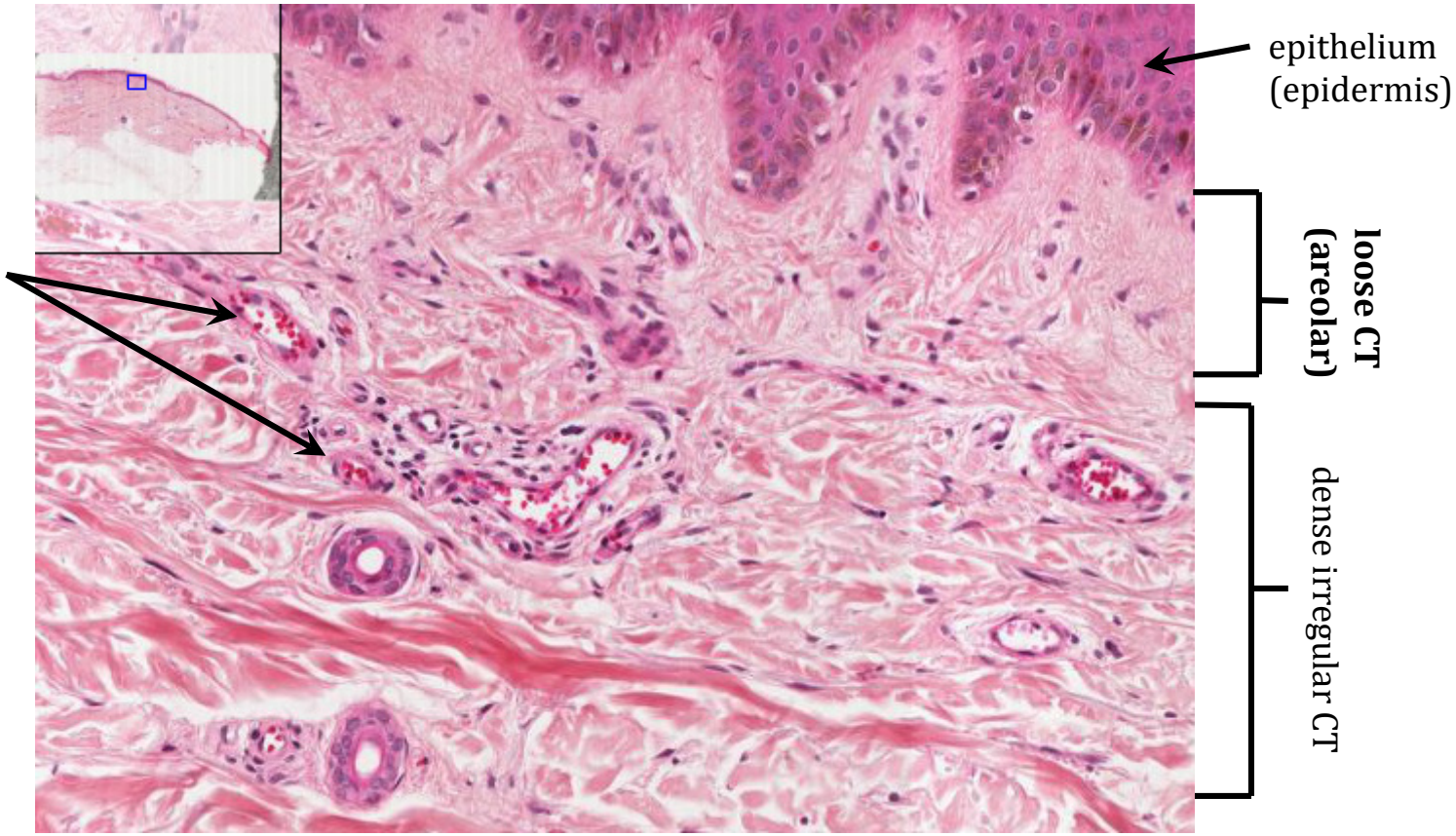
ground substance

the "stuff" between the fibers and cells

Lab 5 – Connective Tissue

IUSM – 2016

Slide 36: Thin Skin, H&E

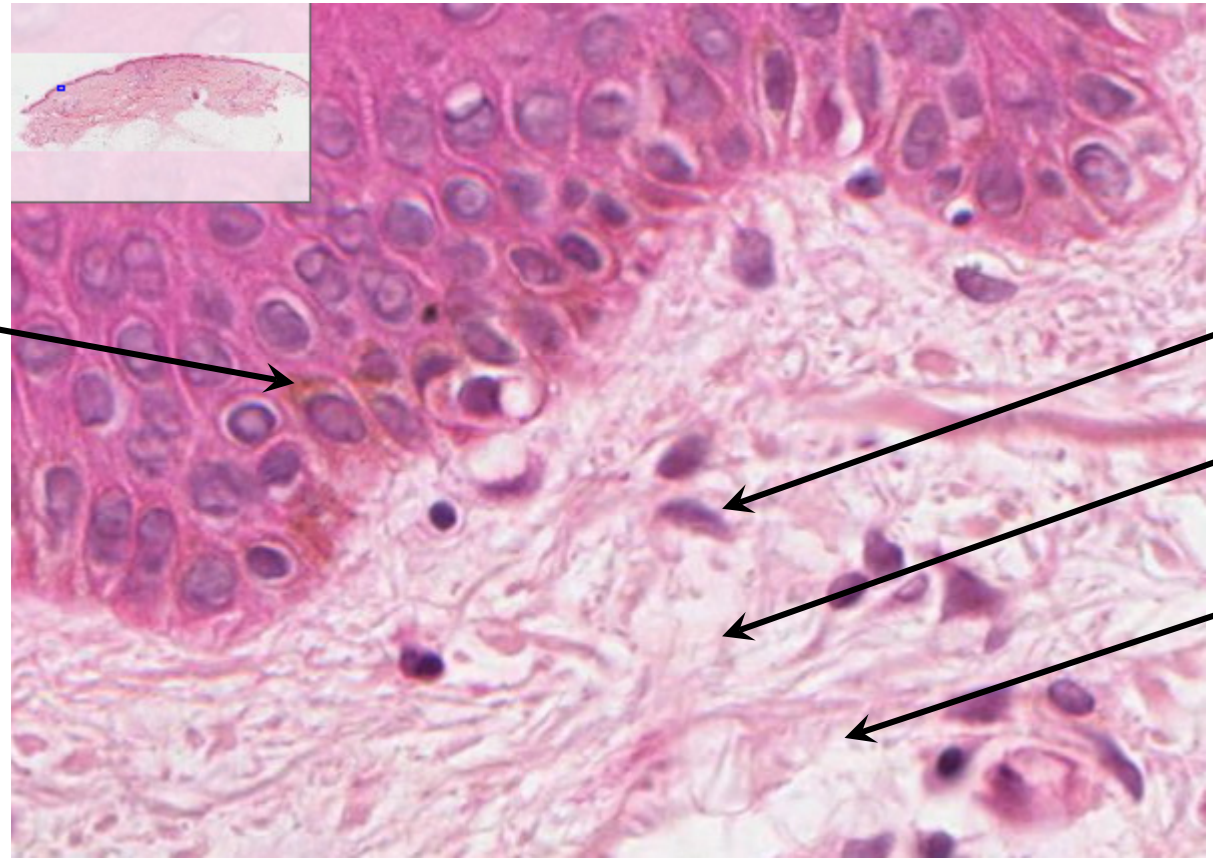


loose (or **areolar**) **CT** has loosely arranged, thin protein fibers (primarily type I collagen) and abundant ground substance, with a relatively large number of cells embedded within it, as compared to the underlying dense CT; loose CT is usually found beneath epithelia and surrounding glands and vessels; notice the gradual transition between the loose CT and the underlying dense irregular CT, making a distinct border between the two types of tissue arbitrary

Lab 5 – Connective Tissue

IUSM – 2016

Slide 36: Thin Skin, H&E

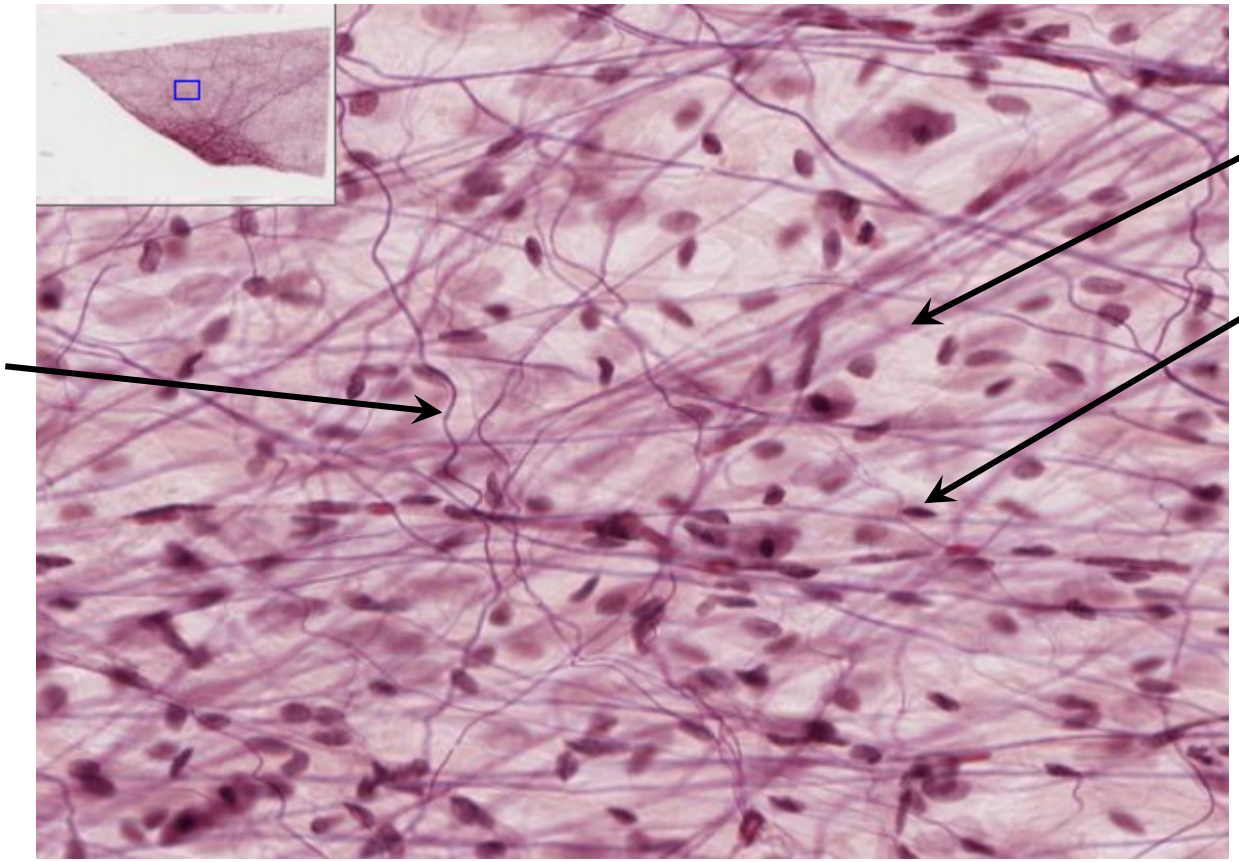


the principal cells of connective tissue proper are **fibroblasts** which synthesize and maintain the ECM components (both the fibers and ground substance); they generally appear elongated with an ovoid, condensed nucleus with one or two nucleoli (if visible); their thin cytoplasmic processes not readily seen; however, they may become "activated" and appear more ovoid with a more extensive basophilic cytoplasm (lots of rER) during periods of growth or wound repair (note: the term *fibrocyte* is sometimes used to refer to "inactive" fibroblasts)

Lab 5 – Connective Tissue

IUSM – 2016

Slide 4a (464): Areolar Connective Tissue



while collagen fibers provide strength to a tissue, **elastic** (or **elastin**) **fibers** are found interwoven in varying amounts in the ECM of most connective tissues providing stretch and recoil (e.g., in skin and lung); the fibers are produced by fibroblasts or smooth muscle cells and are eosinophilic but are usually only seen with specialized stains; they appears as fine, thin, and relatively straight fibers

Lab 5 – Connective Tissue

IUSM – 2016

Slide 100: Aorta, Van Gieson & Elastic

elastic fibers are the darker lines (sheets) within the middle layer (*tunica media*) of the wall of the aorta; they permit appropriate stretch and recoil of the aorta during systole and diastole of the heart in order to maintain a relatively smooth pulse pressure in the body

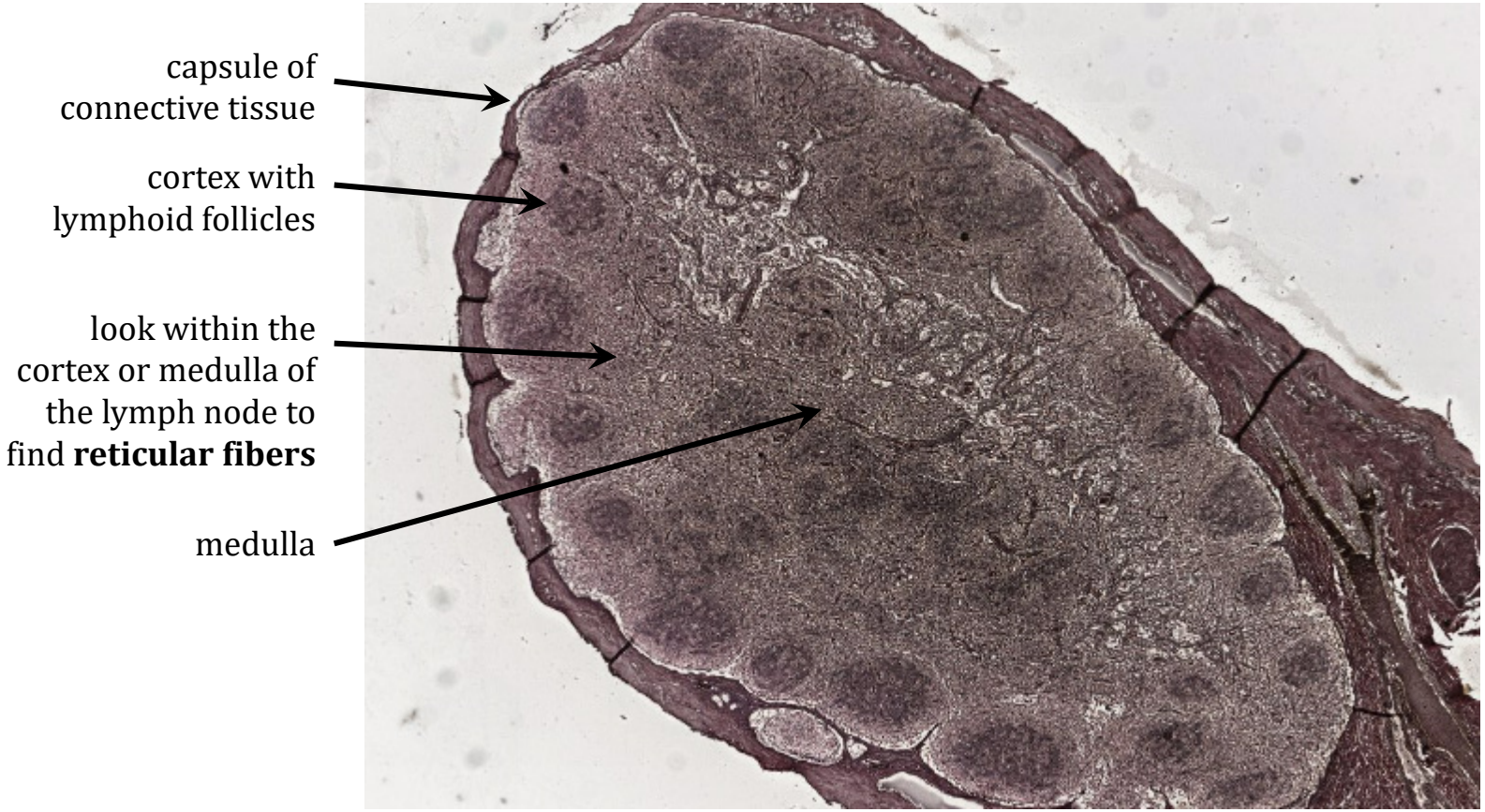
some tissues contain such a large amount of elastic fibers that they are at times classified as a specialized type of connective tissue known as **elastic tissue** (different than elastic cartilage), which generally has densely-packed, parallel bundles of elastic fibers; there are classically only a few examples of this tissue in the body, primarily in ligaments of the vertebral column, within the vocal cords/folds, and in the walls of large elastic arteries, best seen in the aorta above

Lab 5 – Connective Tissue

IUSM – 2016

Slide 99: Lymph Node, Silver

Slide Overview

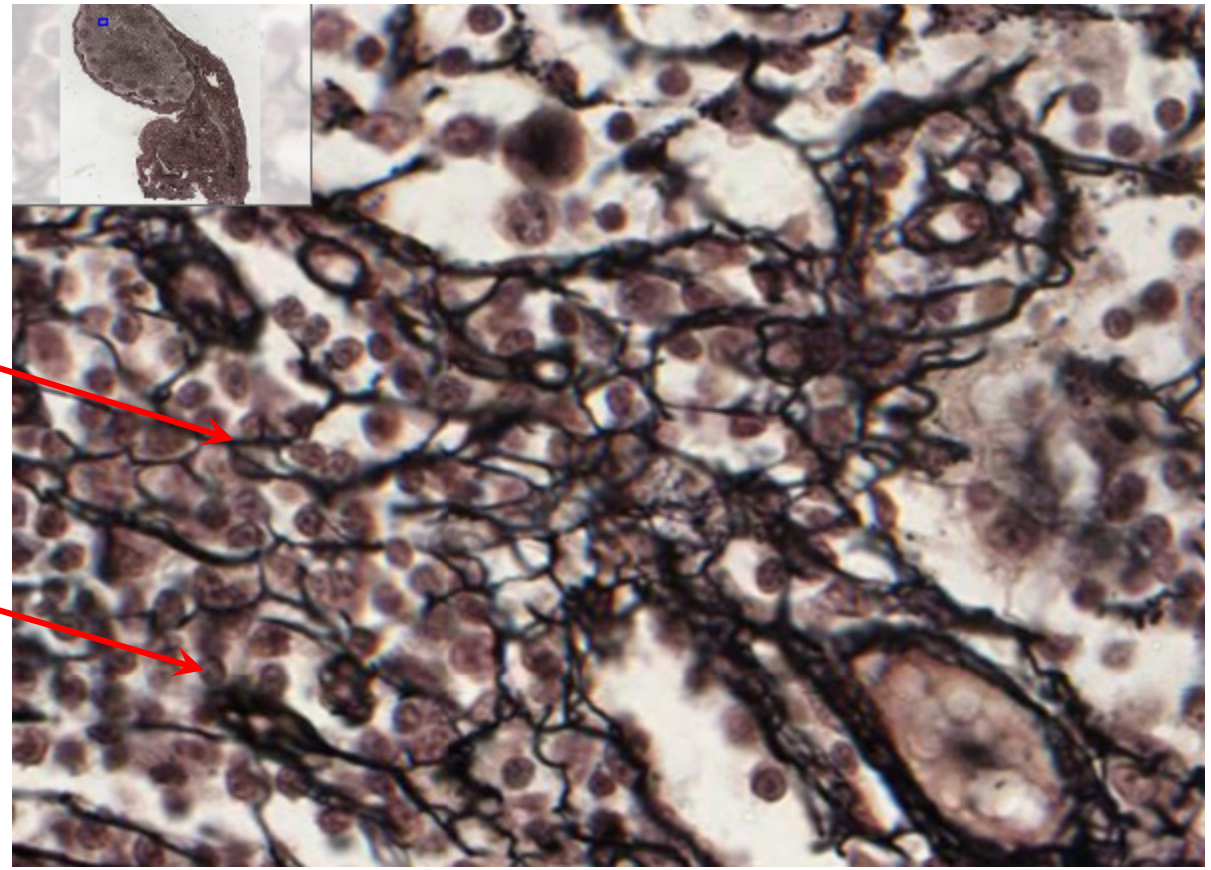


the stroma of certain organs – mainly in hematopoietic (e.g., bone marrow) and lymphatic tissues (excluding the thymus) – contains abundant **reticular fibers** and specialized reticular cells, instead of the fibroblasts or smooth muscle cells that typically make reticular fibers elsewhere; in these organs, the specialized stroma is sometimes referred to as **reticular connective tissue**

Lab 5 – Connective Tissue

IUSM – 2016

Slide 99: Lymph Node, Silver

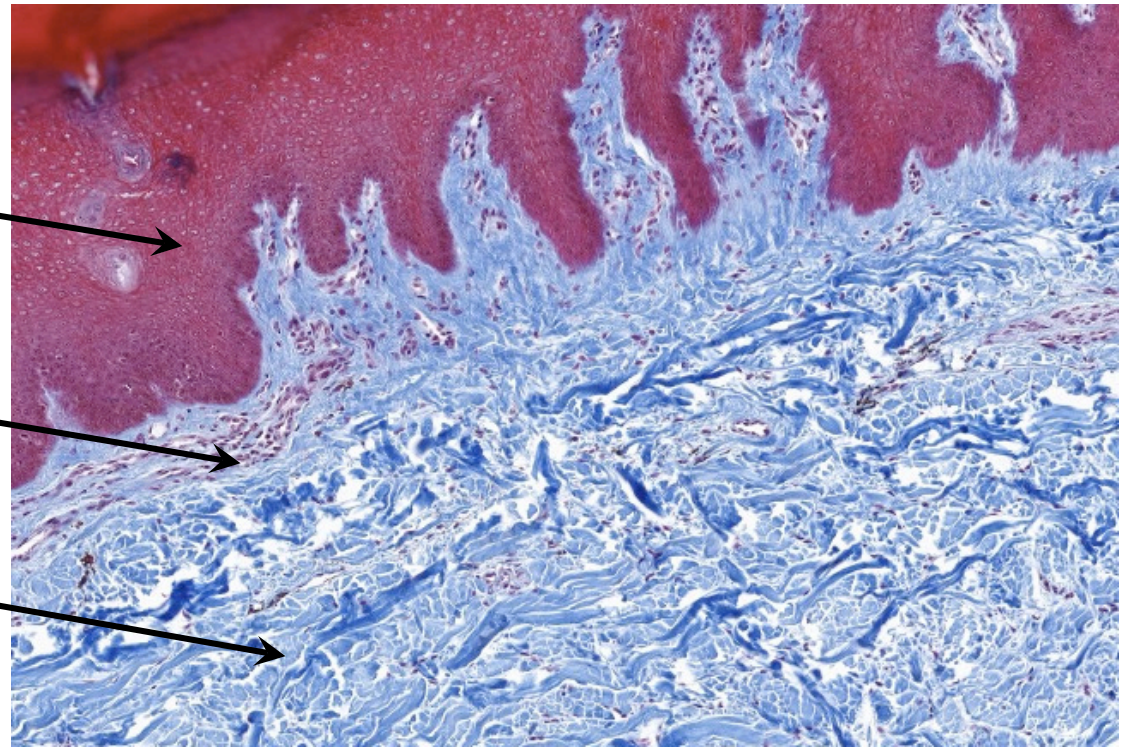


reticular fibers (type III collagen) (Lt. "net-like") are another type of connective tissue ECM fiber; they are thin, do not bundle, and may form a branching meshwork, providing a delicate, supporting scaffold for cells; they are not readily distinguishable in H&E staining but can be seen with specialized stains, such as the silver staining seen above; they are found at the borders of connective tissue, such as in the *reticular lamina* of the basement membrane between epithelium and the underlying connective tissue (the *basal lamina* layer is made by the epithelial cells); they are also the earliest type of collagen fiber produced, being found in mesenchyme and in wound healing and scar formation, before being replaced by stronger type I collagen fibers

Lab 5 – Connective Tissue

IUSM – 2016

Slide 89: Thick Skin, Trichrome



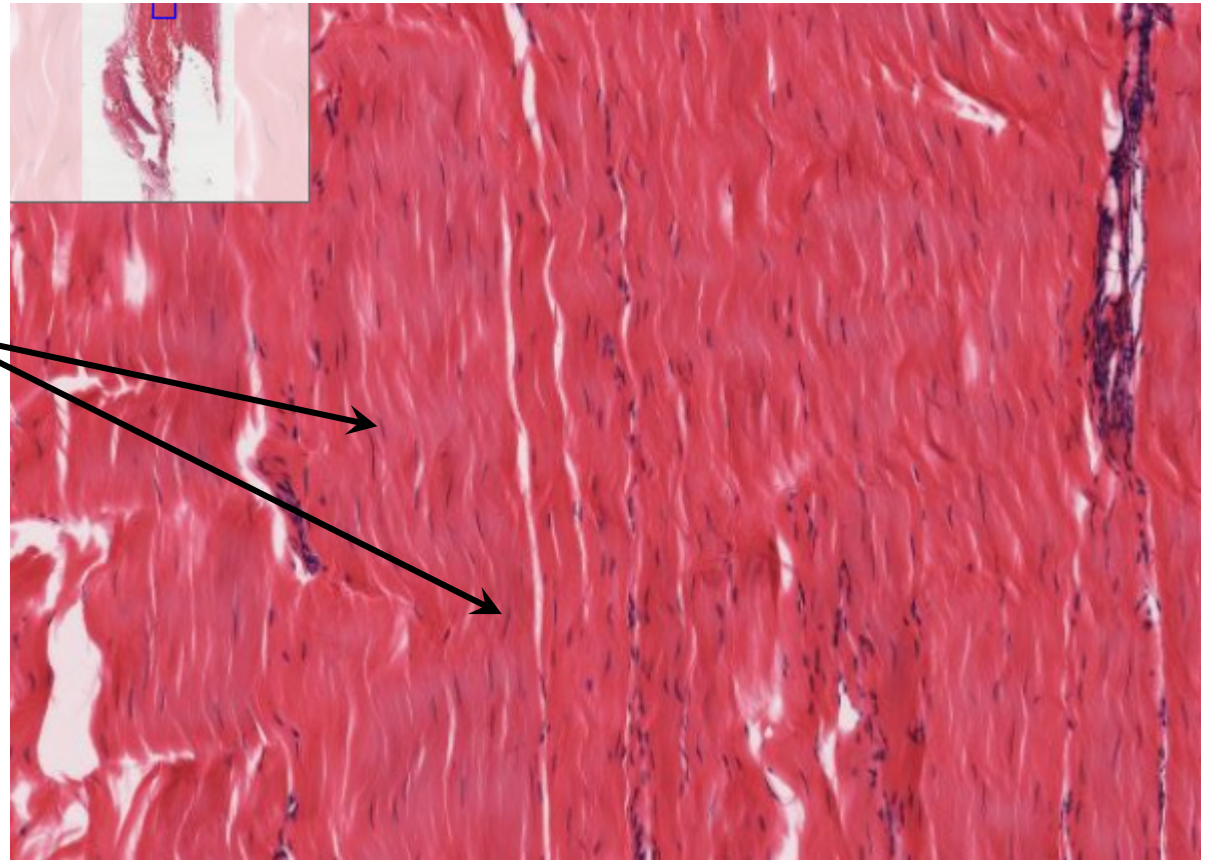
dense irregular CT has a lower cell density than loose/areolar CT and little ground substance, but it has a large amount of interwoven, large, variously-arranged (i.e., "irregular") bundles of collagen fibers; it is the density of the collagen in bundles that gives rise to the name for the tissue, and the various orientations of the fibers provide structural strength to the tissue in multiple directions; *notice that in Masson's trichrome collagen stains blue instead of pink as in H&E*

Lab 5 – Connective Tissue

IUSM – 2016

I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. **Regular**
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. Macrophages
    4. Mast cells
    5. Eosinophils
V. Summary

Slide 16 (NW): Tendon, H&E

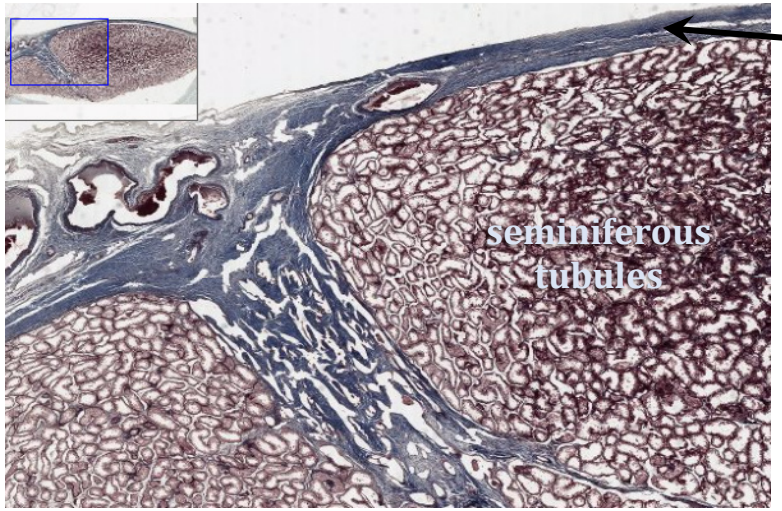


dense regular CT is found in tendons, ligaments, aponeuroses, and organ capsules; unlike the variously-arranged bundles of collagen seen in dense irregular CT, dense regular CT has densely-packed, parallel bundles of collagen with fibroblasts aligned between the fiber bundles; this arrangement of the fibers provides maximum strength for the tissue along the axis parallel to the fibers, but makes the tissue less resistant to perpendicularly-applied forces; *pay close attention to the appearance of the fibers and their relationship to the fibroblasts as dense regular CT can easily be confused with muscle or nervous tissue (discussed in future labs)*

Lab 5 – Connective Tissue

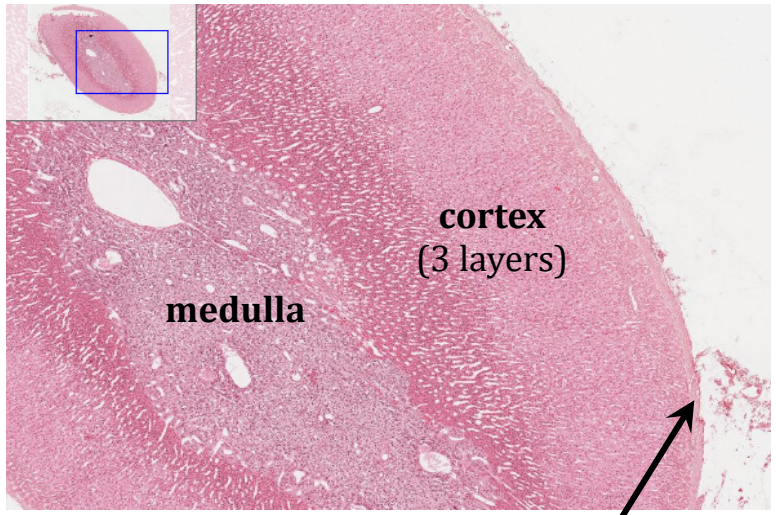
IUSM – 2016

Slide 55: Testis, Trichrome



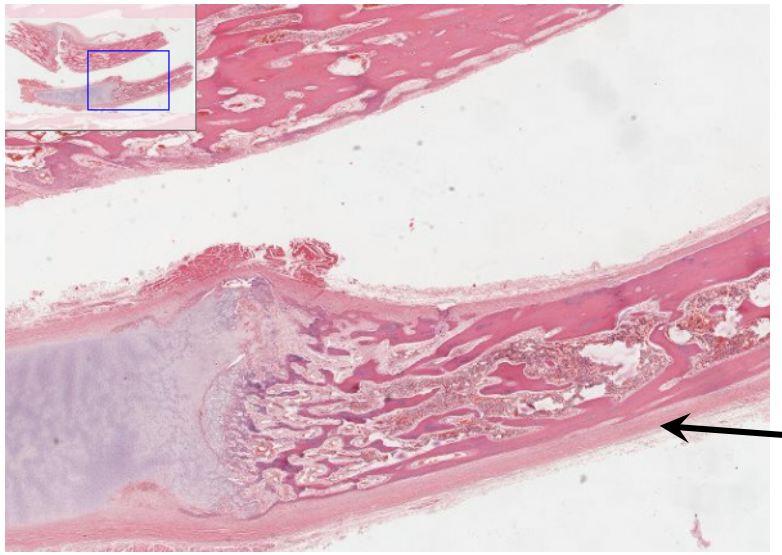
look here at the capsule (*tunica albuginea*) covering the testis to see **dense regular CT**

Slide 75: Adrenal, H&E



look here at the capsule to see **dense regular CT**

Slide 34: Bone, H&E

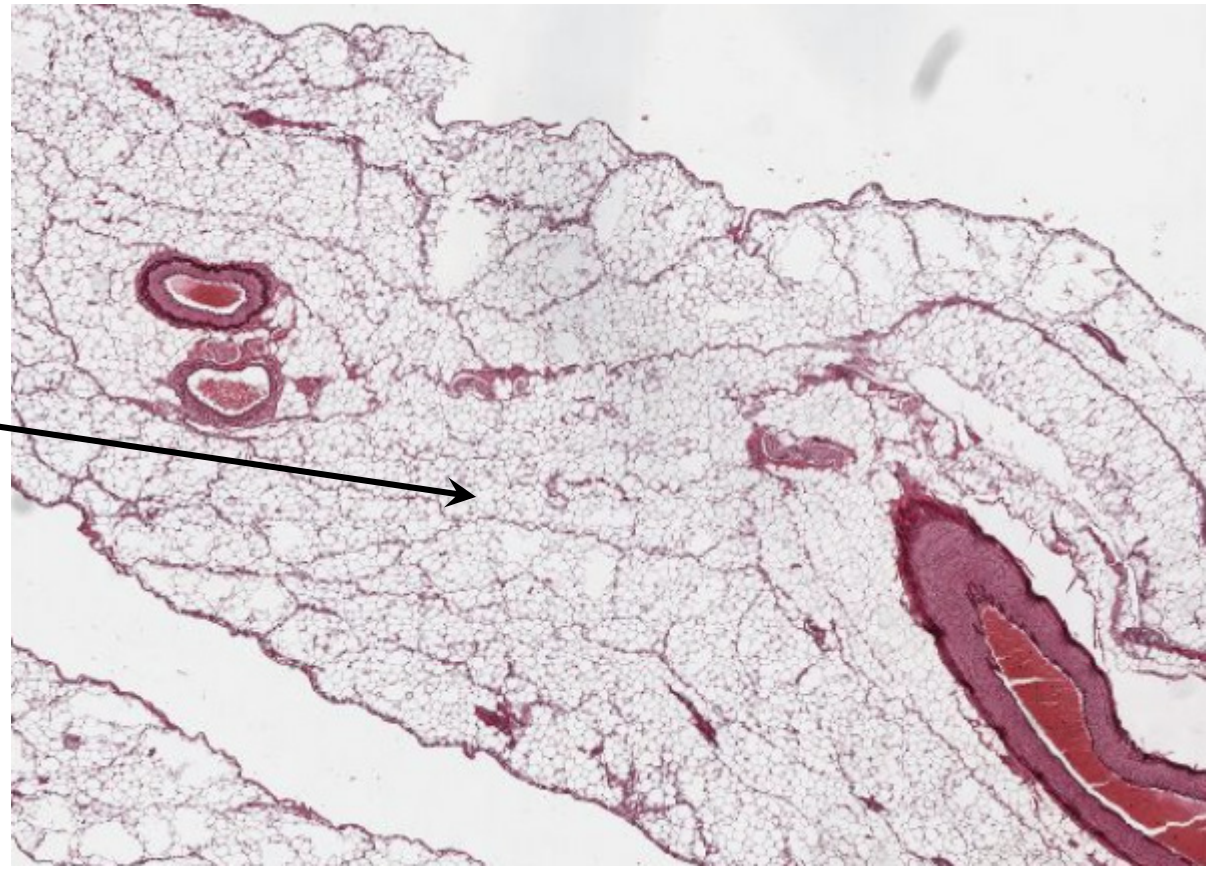


look here at the *periosteum* covering the bone to see **dense regular CT**
(see Lab 7 (Bone) for an overview of this slide)

Lab 5 – Connective Tissue

IUSM – 2016

Slide 92: Vessels, Elastic Stain

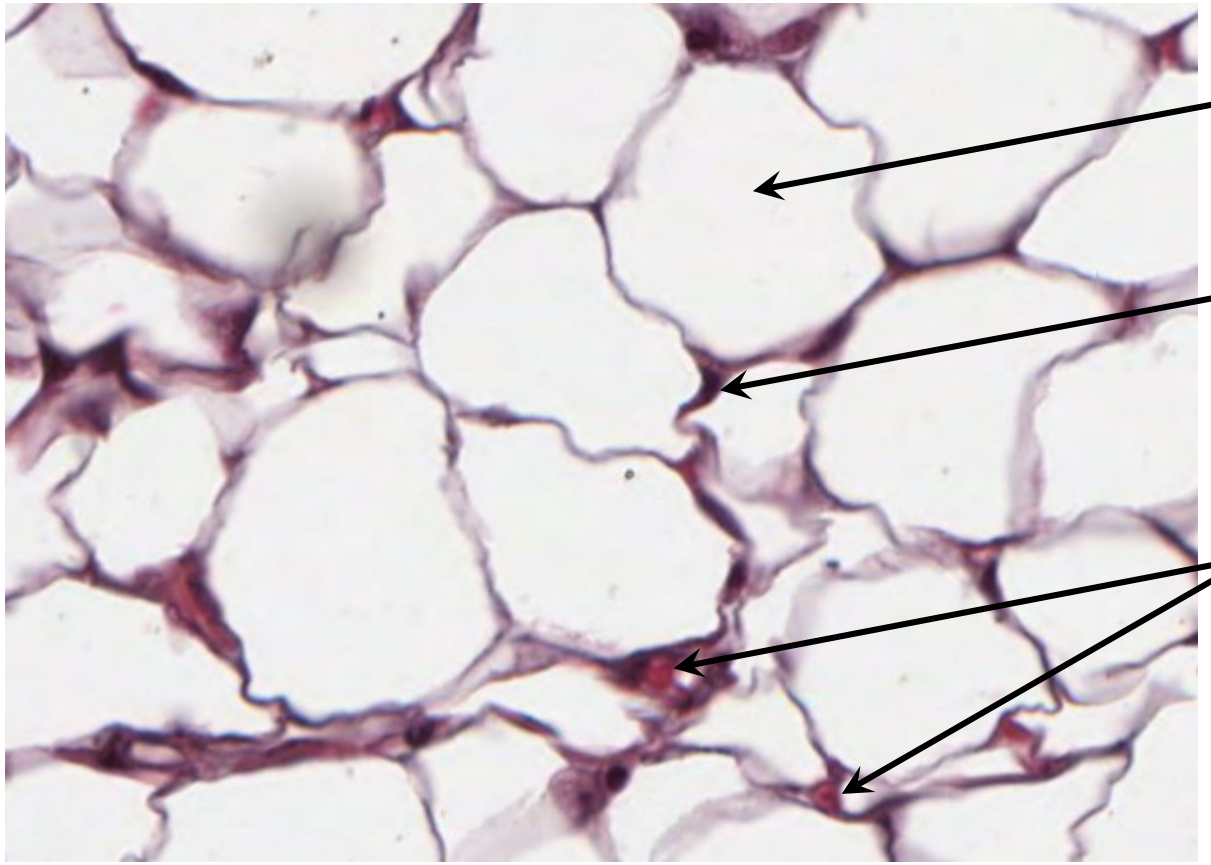


adipocytes (or **lipocytes**) are mesenchyme-derived cells specialized to store fat and produce hormones; they may be found in isolation or may accumulate as clumps of cells in loose CT; when adipocytes become the predominant type of cell present, the tissue is called **adipose**; during standard slide preparation, the lipid is cleared resulting in the empty appearance of the cells and an overall "chicken-wire" appearance for the tissue

Lab 5 – Connective Tissue

IUSM – 2016

Slide 92: Vessels, Elastic Stain



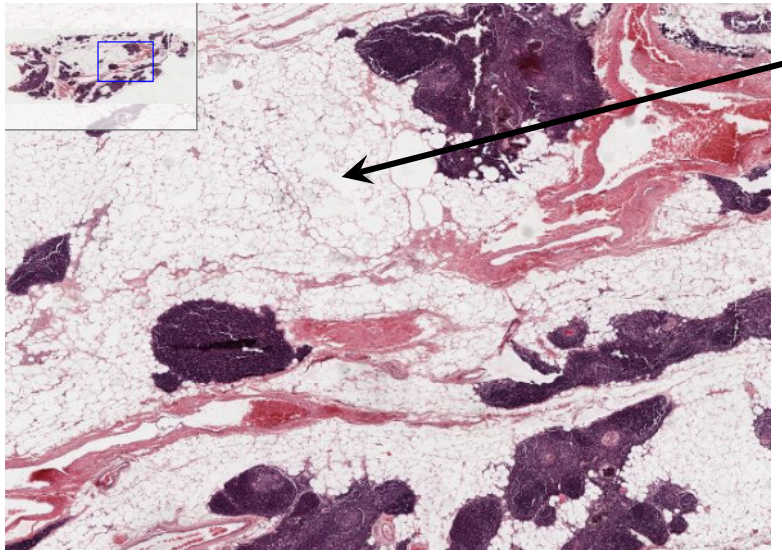
in **white adipose tissue**, adipocytes are unilocular with a single, centralized, large lipid droplet which is not membrane bound; the large lipid accumulation flattens and displaces the nucleus to the periphery of the cell, at times giving the cell a "signet-ring" appearance; adipocytes are the only cells that undergo dramatic physiologic changes in size, becoming up to 100µm or more in diameter, depending upon the amount of lipid present; research suggests the total number of adipocytes does not greatly vary after adolescence, rather it is the size of the cells that changes as persons gain or lose weight

Lab 5 – Connective Tissue

IUSM – 2016

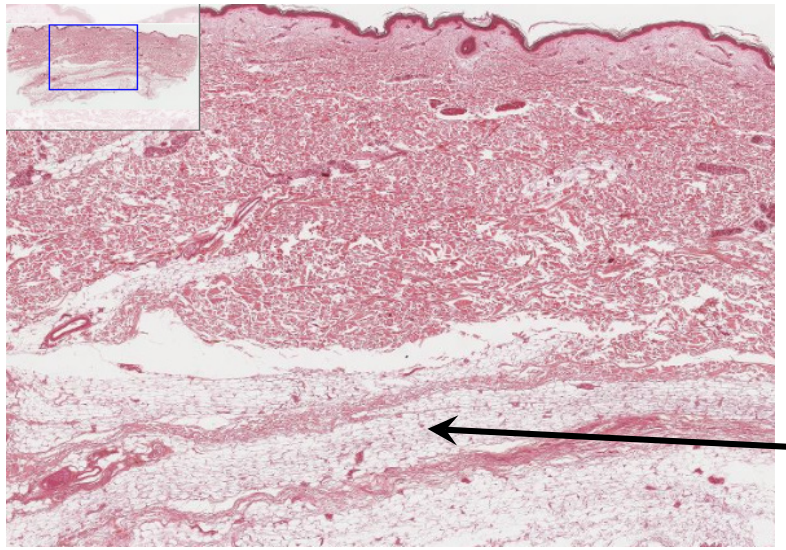
I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. **Adipose**
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. Macrophages
    4. Mast cells
    5. Eosinophils
V. Summary

Slide 123: Thymus, H&E



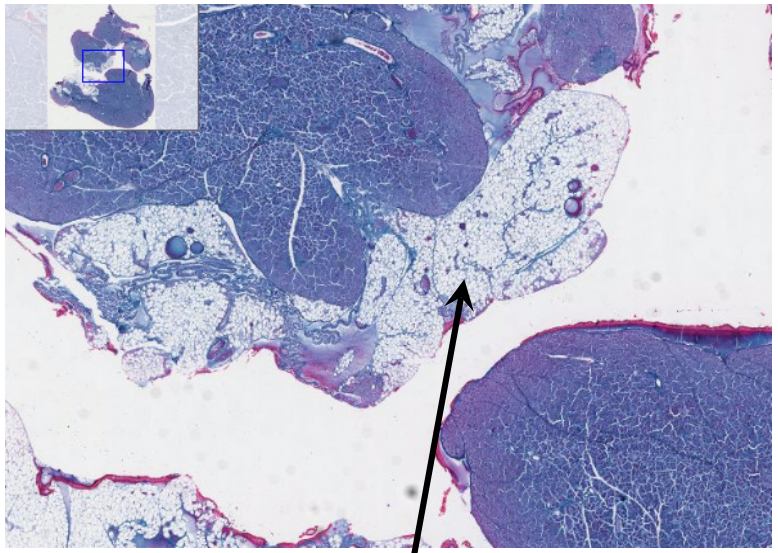
as a person ages, the original stroma of the thymus is replaced by **white adipose**

Slide 157: Skin, H&E



look here in the *subcutaneous layer* of the skin to see **white adipose**

Slide 62: Pancreas, AF



white adipose of the pancreas

Cells of Connective Tissue

1. Connective tissue cells are traditionally classified as either **resident** or **wandering** (transient) cells:

   a. **Resident cell populations** are relatively fixed, stable populations of cells within connective tissues; they include fibroblasts, macrophages, and mast cells; these cells serve to synthesize, maintain, and surveil the tissue.

   b. **Wandering cell populations** are all the other white blood cell types which leave the blood and enter loose CT, which is the principal site of their activity as part of immune surveillance and response; these cells are especially present in the *lamina propria* (loose CT underlying the epithelium of a *mucosa*) of the respiratory and GI tracts, where pathogens are readily encountered; wandering cells include lymphocytes, plasma cells, eosinophils, and neutrophils (however, neutrophils are rarely seen in connective tissue except during inflammatory conditions).

2. While white blood cells (leukocytes) will be explored more fully in Lab 8 (Blood), it is important to be familiar with those present in normal connective tissue and to appreciate the differences in appearance between leukocytes in the blood and those found in loose CT; these differences are due in part to differences in how slides are prepared for tissue samples vs. blood smears, but are also due to changes that leukocytes undergo as they leave the circulation and enter into tissues (e.g., as monocytes become tissue macrophages).

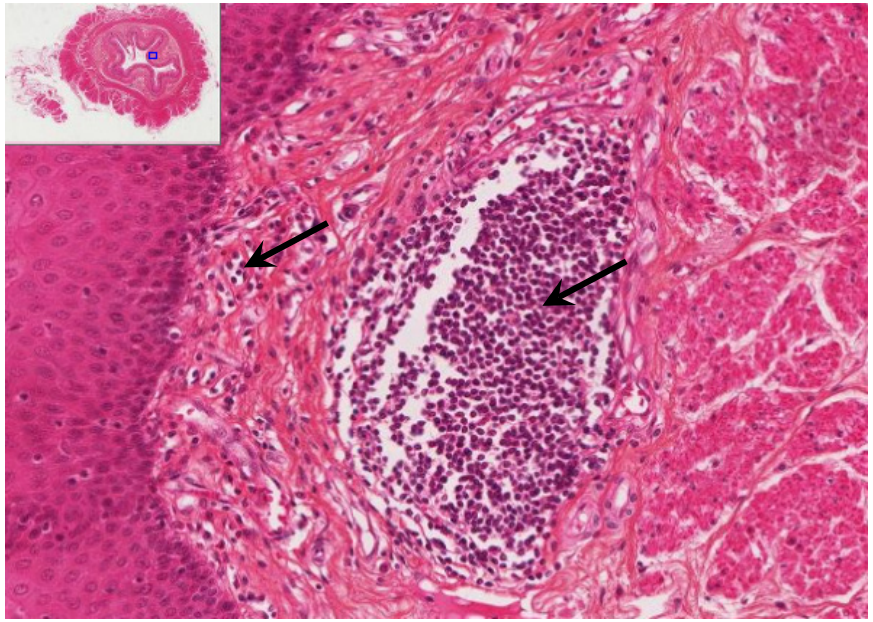
3. When examining the slides, many cells encountered in CT will be difficult – if not impossible – to identify with certainty; it is more important to know the characteristics for each of the respective cell types studied and to find examples than it is to attempt to identify every cell seen (a fruitful but ultimately futile endeavor).

Lab 5 – Connective Tissue

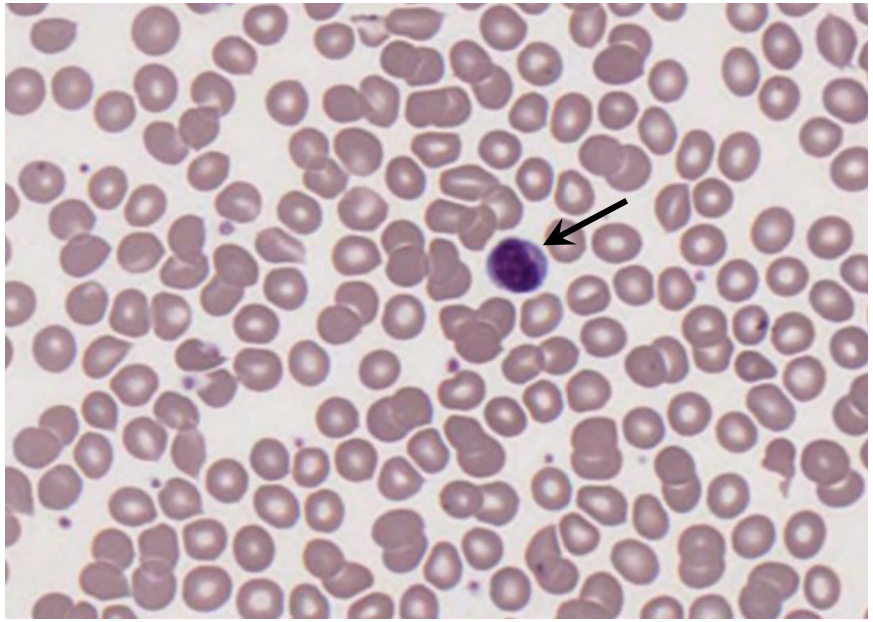
IUSM – 2016

I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. **Lymphocytes**
    2. Plasma cells
    3. Macrophages
    4. Mast cells
    5. Eosinophils
V. Summary

Slide 43: Esophagus, H&E



Slide 10: Blood Smear

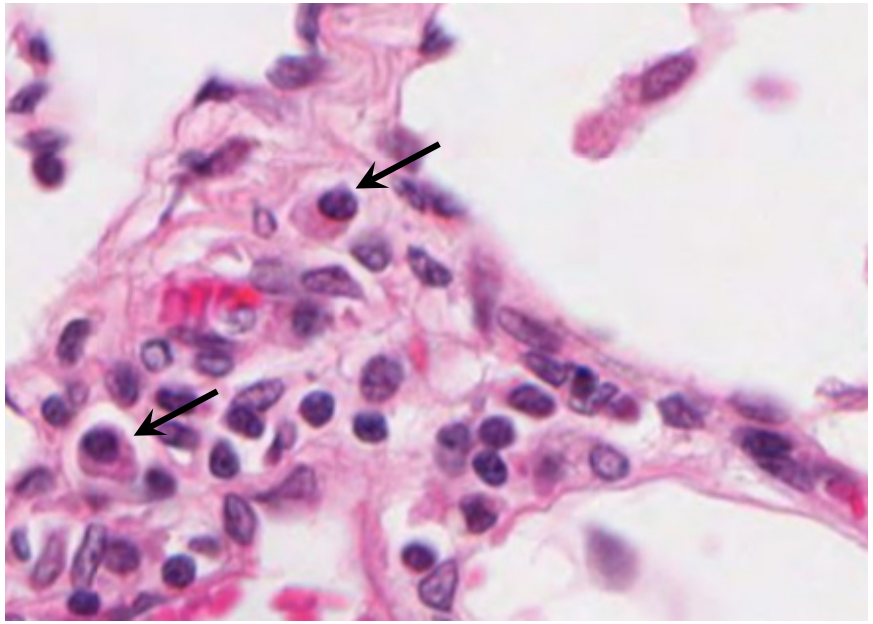


lymphocytes include a mixture of B-cells, T-cells, and NK cells; they are the smallest of the wandering cells (7µm in diameter), appearing about the same size as red blood cells, and providing a useful size reference for other cells; they are generally easily identified by their small, round, deeply-basophilic heterochromatic nucleus; the surrounding halo of cytoplasm is minimal and poorly-staining, and often it may not be seen; lymphocytes can occur as individual cells, in small clumps of cells, or in large clusters (e.g., the tonsils)

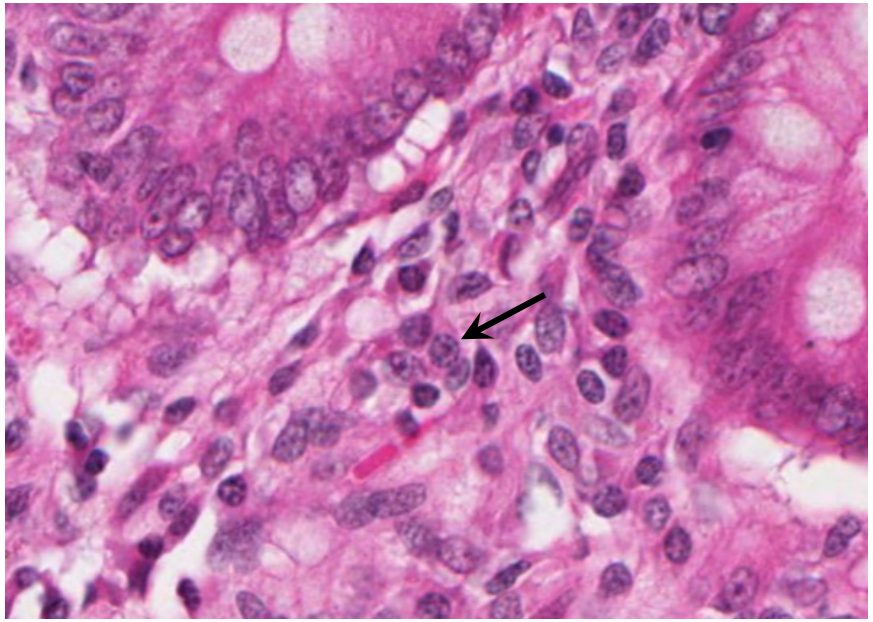
Lab 5 – Connective Tissue

IUSM – 2016

Slide 42: Lymph Node, H&E



Slide 41: Appendix, H&E

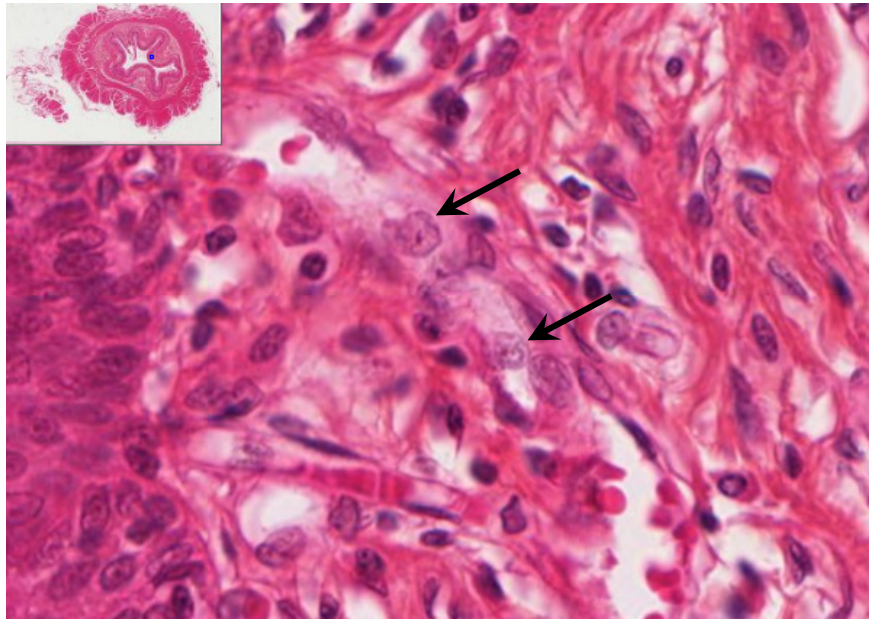


derived from antigen-exposed, active B lymphocytes, **plasma cells** are relatively large (20μm), ovoid cells, with a spherical, eccentrically-located nucleus ("fried egg"); the nucleus contains large clumps of heterochromatin along the periphery which tend to give a "clock-face" appearance, in addition to a small, central nucleolus; plasma cells are capable of releasing several thousand antibodies per second, requiring an abundant cytoplasm full of rER and Golgi; the rER is basophilic while the large number of antibody proteins are acidophilic, giving the cytoplasm an overall *amphophilic* (Gr. "both loving") staining pattern; typically a pale-staining region around the nucleus is present and represents the well-developed Golgi apparatus ("Golgi ghost")

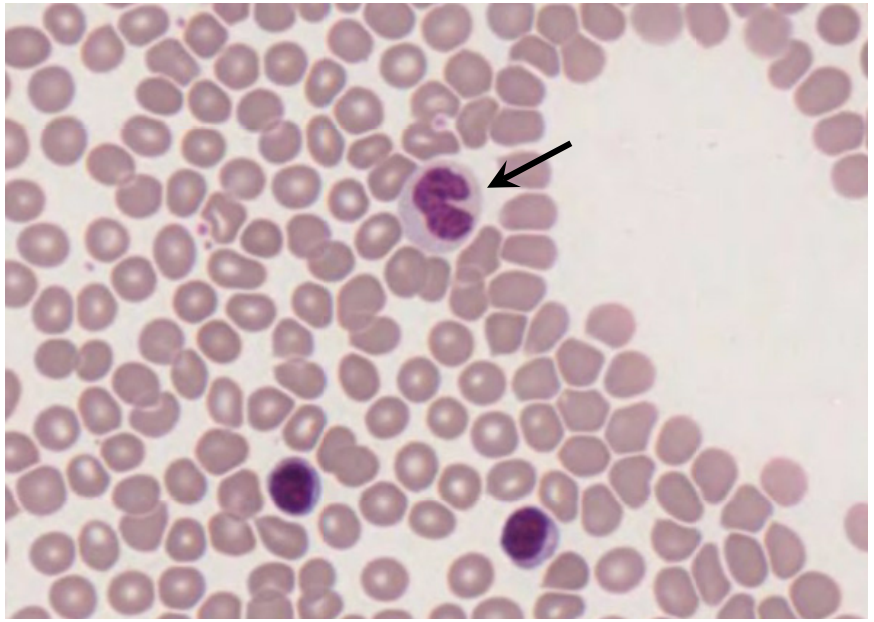
Lab 5 – Connective Tissue

IUSM – 2016

Slide 43: Esophagus, H&E



Slide 10: Blood Smear



macrophages (Gk. "large eater") (or **histiocytes** – a more ambiguous term) are large cells (20-80µm) derived from *monocytes* in the blood; they are phagocytic and involved in normal "housekeeping" and immune responses; they may be identified due to the presence of accumulated engulfed material (such as the inks of tattoos – which macrophages are responsible for maintaining), but overall they display a highly-variable appearance and may be difficult to distinguish; when active, they generally have an irregular, amoeboid shape for both the cell and nucleus, and are often poorly-staining providing an overall ghostly appearance; in the end, macrophages might best be identified by the exclusion of other possible cell types

it is worth being aware that macrophages are part of a mononuclear phagocyte system *which includes several different cell types but no great consensus on nomenclature (a malodorous potpourri of names exist based upon eponyms, historical names, ontogeny, cell location, cell surface markers, and cell function)*

Lab 5 – Connective Tissue

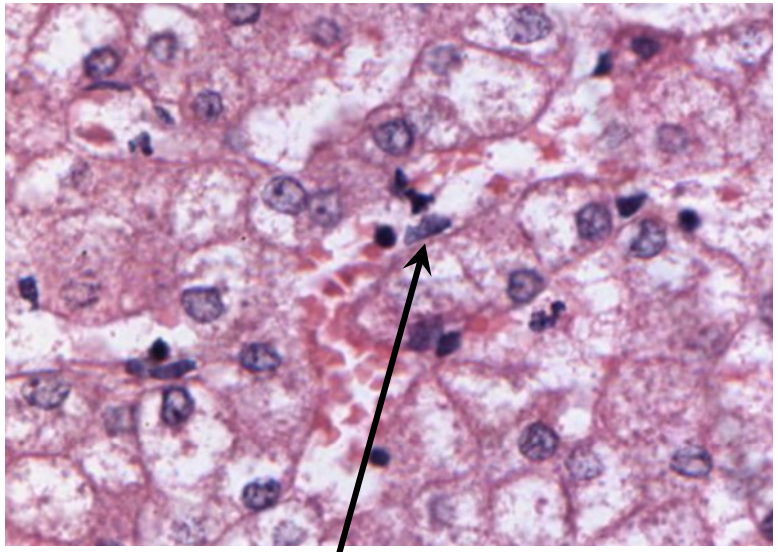
IUSM – 2016

I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. **Macrophages**
    4. Mast cells
    5. Eosinophils
V. Summary

Slide 97: Lung, H&E

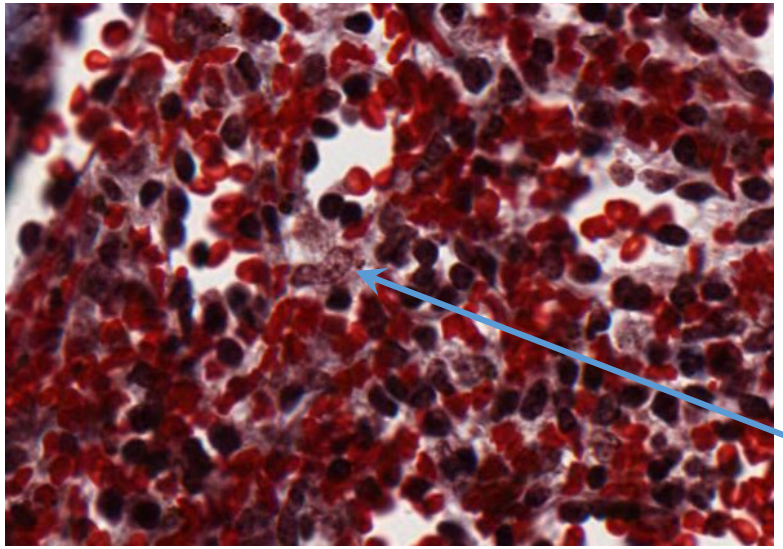
dust cells, found in the alveoli, are the resident **macrophages** of the lung

Slide 29: Liver, H&E



Kupffer cells, along the walls of vascular sinusoids, are the resident **macrophages** of the liver

Slide 18: Spleen, Trichrome



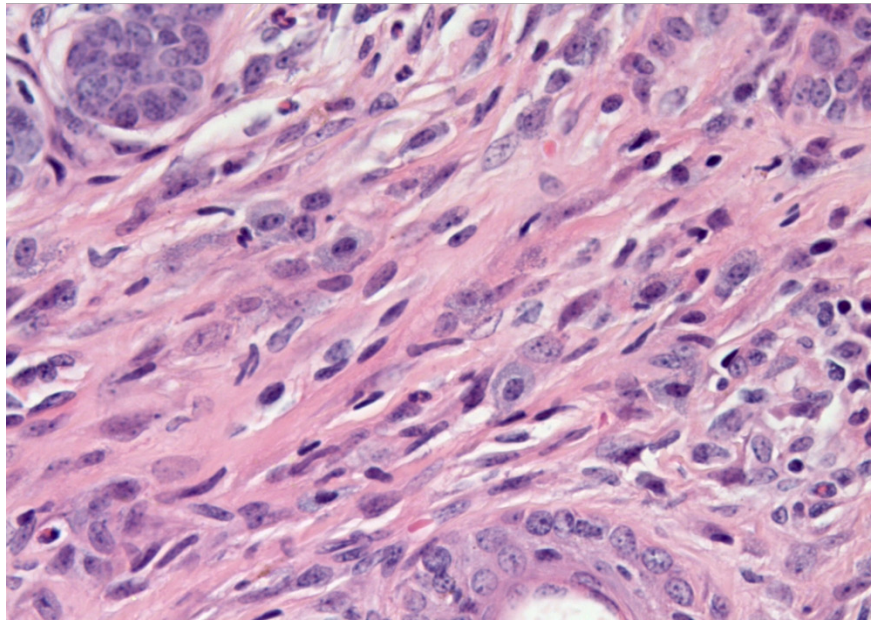
macrophages are commonly seen within the spleen

Lab 5 – Connective Tissue

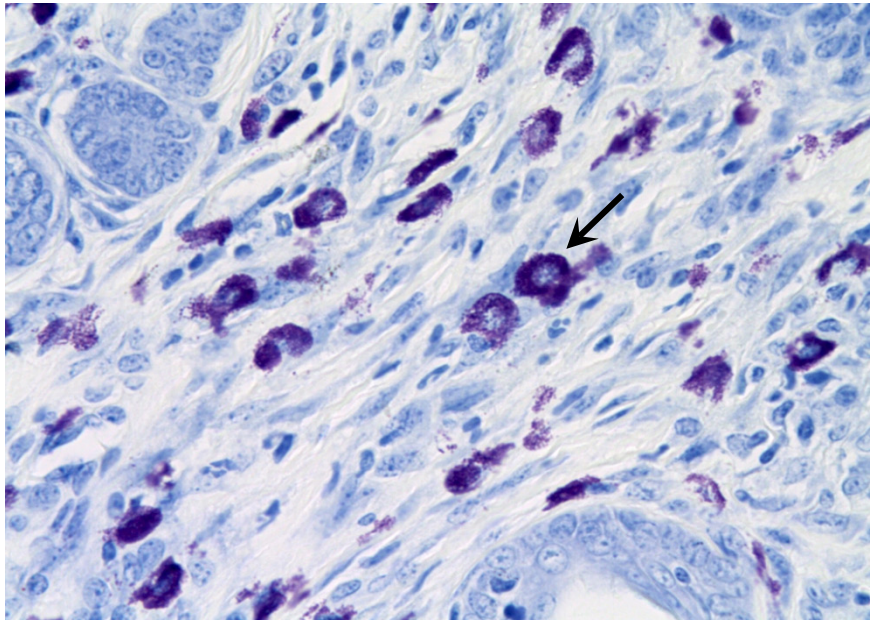
IUSM – 2016

I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. Macrophages
    4. **Mast cells**
    5. Eosinophils
V. Summary

Mouse Pinna, H&E



Mouse Pinna, Toluidine Blue



mast cells are resident cells present throughout CT but are not generally identifiable in routine H&E stains since their basophilic cytoplasmic granules are usually lost during slide preparation; mast cells are similar to basophils found in the blood, but they do not derive from them; when seen with appropriate basic stains such as Toluidine blue, they are large (20-30µm), ovoid cells with a small, central, spherical nucleus; the cytoplasm is packed with densely-staining granules of inflammatory mediators (e.g., histamine and heparin)

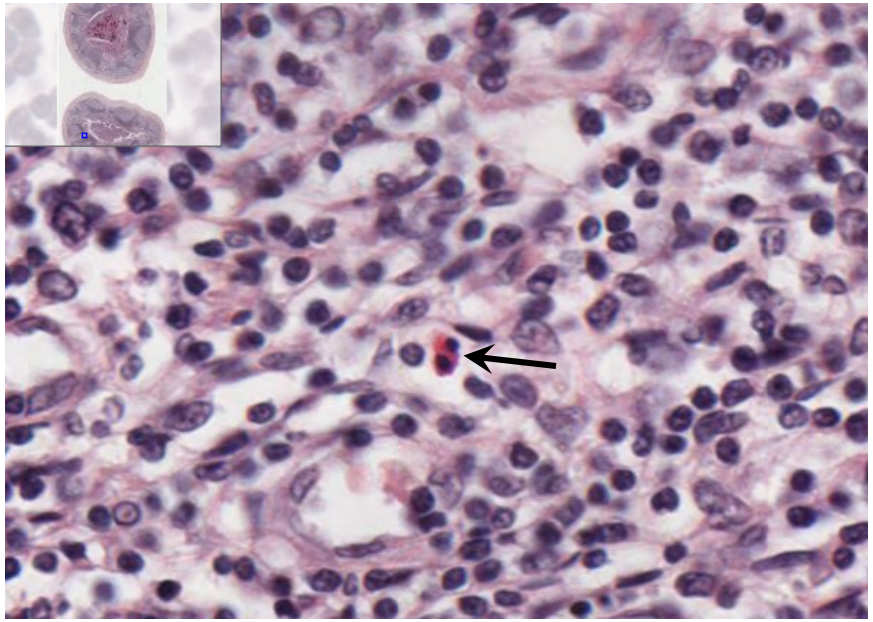
the slides above show corresponding sections stained with both H&E and Toluidine Blue; the cytoplasmic heparin granules of the mast cells are *metachromastic* (Gr. "color changing") and change the color of the blue basic dye to appear purple while all the rest of the slide stains blue

Images source: IUPUI – D502 (Histology) – Blood Pre-Lab

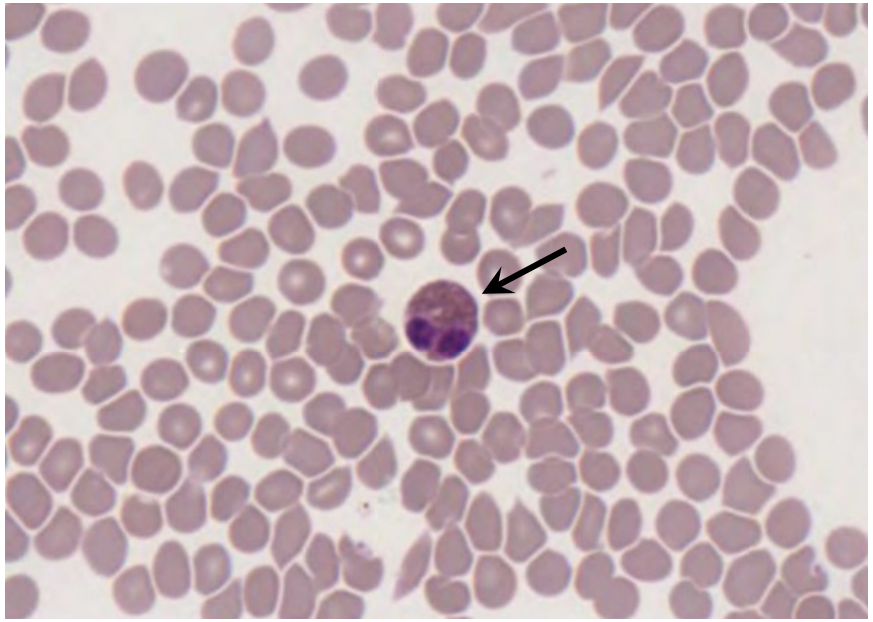
Lab 5 – Connective Tissue

IUSM – 2016

Slide 113: Appendix, H&E



Slide 10: Blood Smear



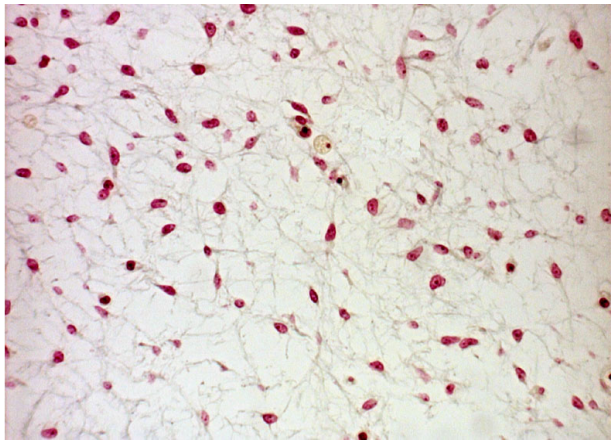
eosinophils are medium-sized wandering cells (12-15µm); they have a characteristic bi-lobed nucleus and, as the name implies, an intensely-eosinophilic, granule-filled cytoplasm; they are involved in modulating inflammatory conditions and responding to parasitic (e.g., helminth) infections; notice in the blood smear that the red blood cells and the cytoplasm of the eosinophil are both acidophilic so stain the same color

Lab 5 – Connective Tissue

IUSM – 2016

Common Confusion:

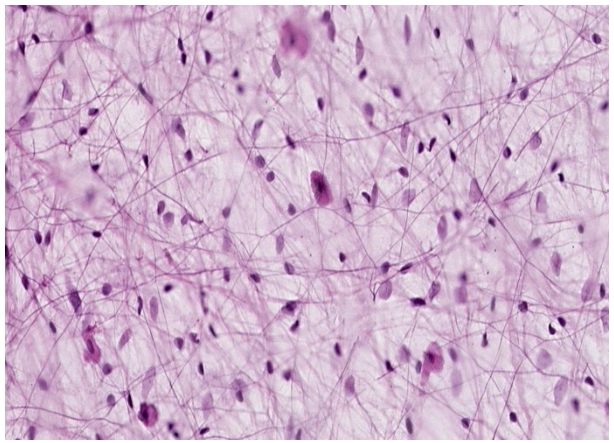
Mesenchyme vs. Loose CT



Mesenchyme

Mesenchyme: primitive connective tissue found primarily in embryonic tissue; mesenchymal cells are largely undifferentiated and capable of forming the cells typical of adult CT; can be considered "very loose" connective tissue

Look for: (1) angular or spindle-shaped cells with large, round/oval nuclei and prominent nucleoli; (2) ECM with abundant ground substance and very fine fibers; (3) mitotic figures may be visible



Loose CT

Loose/areolar connective tissue: connective tissue with protein fibers of the ECM occurring singly, not in bundles as in dense CT; occurs in mucosal membranes and often seen as "filler" tissue between other tissues such as muscle and epithelia

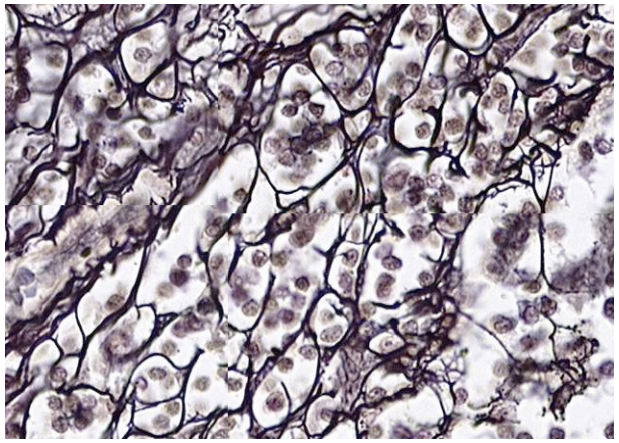
Look for: (1) nuclei of fibroblasts tend to be more condensed and elongated; (2) ECM contains clearly visible, individual protein fibers; (3) other cells, such as macrophages, mast cells, lymphocytes, and plasma cells, may be present; (4) blood vessels, lymphatic vessels, and nerves may be seen

Lab 5 – Connective Tissue

IUSM – 2016

I. Introduction
II. Learning Objectives
III. Keywords
IV. Slides
  A. Types of Connective Tissue
    1. Mesenchyme
    2. Connective Tissue Proper
      a. Loose/Areolar
        i. Elastic fibers
        ii. Reticular fibers
      b. Dense
        i. Irregular
        ii. Regular
    3. Specialized CT
      a. Adipose
      b. Cartilage (Lab 6)
      c. Bone (Lab 6/7)
      d. Blood (Lab 8)
  B. Resident and Wandering Cells
    1. Lymphocytes
    2. Plasma cells
    3. Macrophages
    4. Mast cells
    5. Eosinophils
V. **Summary**

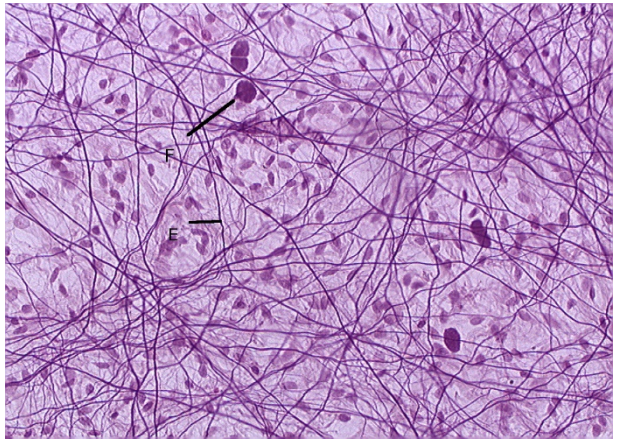
Common Confusion:
Reticular vs. Elastic Fibers



Lymph Node with Reticular Fibers

Reticular fibers: provide a delicate, supporting framework (scaffolding) for cells in tissues (very common in hematopoietic and lymphatic tissue); generally only seen with specialized stains (e.g. silver) which tend to stain black

Look for: (1) branching and spreading network of fibers; (2) fibers appear to "cradle" or surround cells they support



Loose CT with Elastic Fibers

Elastic fibers: provide ability for stretch and recoil to tissue; usually seen with specialized stains (e.g., Verhoeff's)

Look for: (1) fine, thin, straight, unbranching (relatively) fibers; (2) fibers do not appear to "cradle" the adjacent cells

Summary

1. Connective tissue (CT) is one of the four primary tissue types; it forms the *stroma* that supports and connects the other types of tissues (*parenchyma*) to form organs: epithelium overlays it and muscle and nervous tissue are surrounded by it.

2. CT is composed primarily of a few dispersed, inconspicuous **cells** within a prominent **extracellular matrix** (ECM), which is primarily responsible for the properties and functions of CT; the ECM is composed of **protein fibers** (collagen, reticular, and elastic) and **ground substance** (amorphous gel-like substance rich in proteoglycans, with the specific composition varying between types of CT (e.g., the ECM of CT proper differs from the ECM of cartilage or bone).

3. **Connective tissue proper** is categorized as loose or dense depending upon the relative abundance of bundles of collagen protein fibers in the ECM:

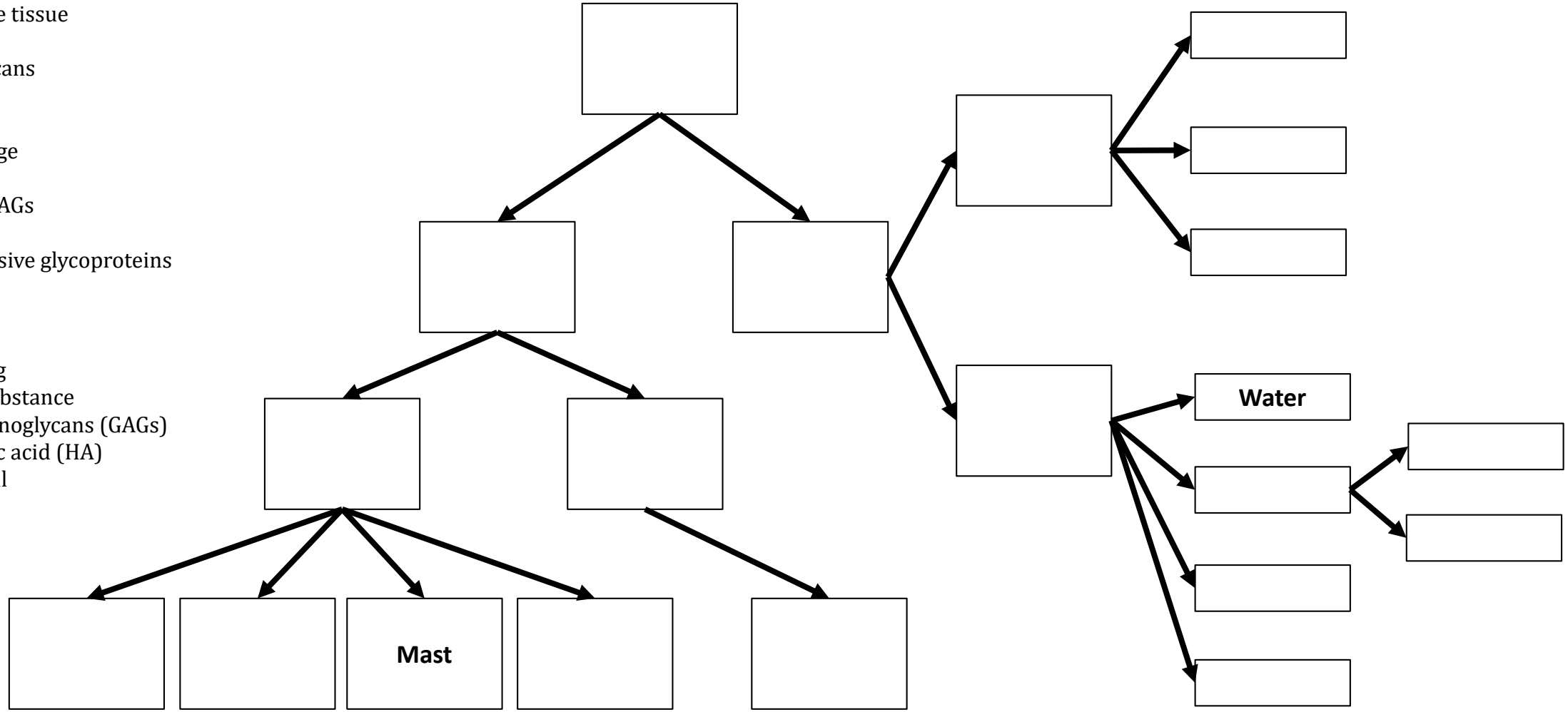
   - **Loose CT** has loosely-arranged fibers with a relatively large numbers of cells and ground substance present.
   - **Dense CT** has large bundles of fibers (collagen) with few cells and little ground substance.

4. Connective tissue cells are classified as **resident cells** (relatively stable, non-migratory; includes: fibroblasts, macrophages, adipocytes, mast cells, and stem cells) or **wandering cells** (transient cells that have migrated from blood vessels; includes: plasma cells and other white blood cells).

Connective Tissue Concept Map

Terms:

Extracellular matrix (ECM)
Connective tissue
Fibroblast
Proteoglycans
Elastic
Resident
Macrophage
Adipocyte
Sulfated GAGs
Fibers
Multiadhesive glycoproteins
Reticular
Cells
Collagen
Wandering
Ground substance
Glycosaminoglycans (GAGs)
Hyaluronic acid (HA)
Plasma cell



Objectives: (1) fill-in each box with a term from the list given above which demonstrates the appropriate relationship/hierarchy between the connected boxes (two terms have been given); (2) after all the boxes have been filled-in, go back and write definitions/distinguishing characteristics for each of the terms and provide a label for the arrows between boxes, such as “is composed of…” or “includes…”