CONNECTIVE TISSUE

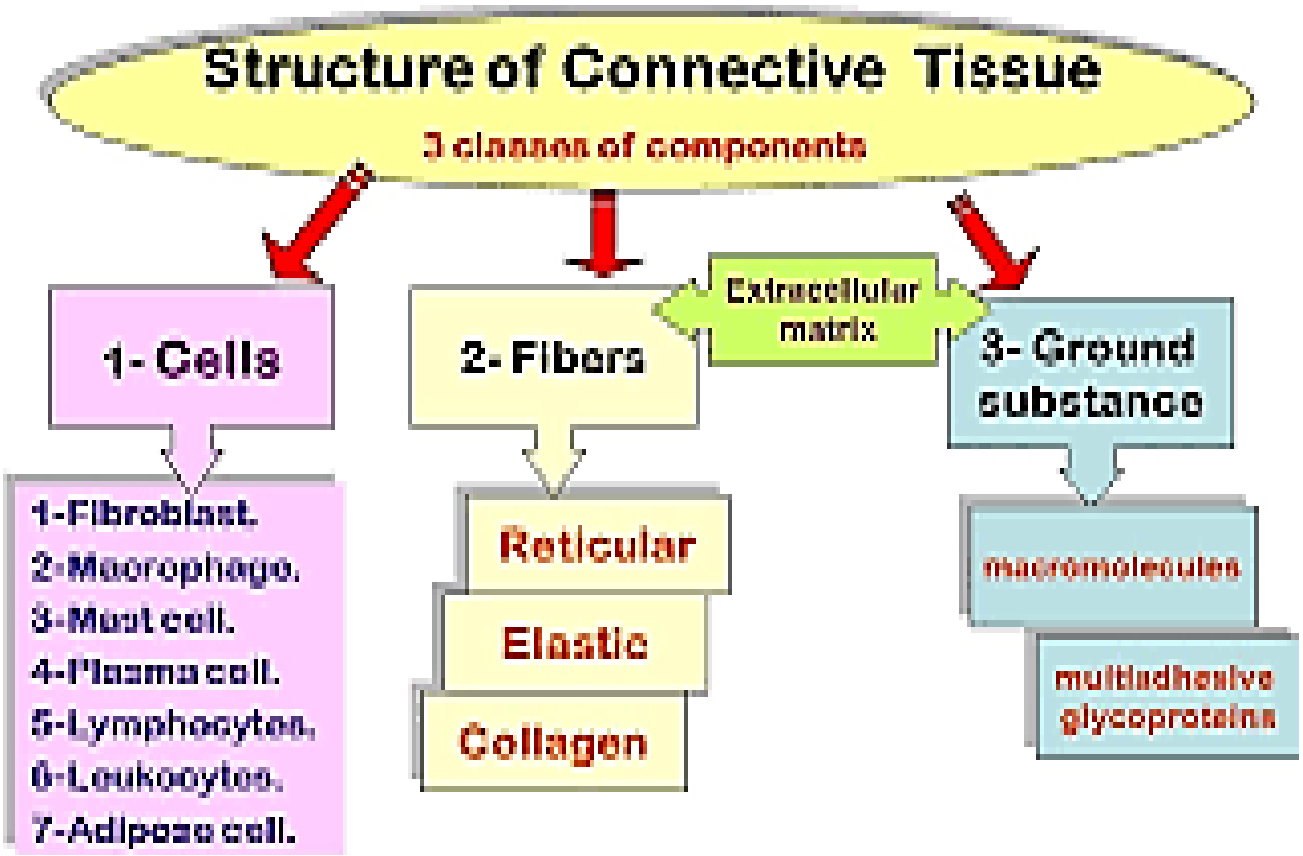
Connective tissue provides support, binds together, and protects tissues and organs of the body. consists of three main components: **cells**, **protein fibers**, and an amorphous **ground substance**. The cells separated by varying amounts of extracellular substance. In connective tissues cells typically account for only a small fraction of the tissue volume. The extracellular substance consists of fibres which are embedded in ground substance containing tissue fluid. Fibres in connective tissue can be divided into three types: collagen fibres, reticular fibres and elastic fibres.

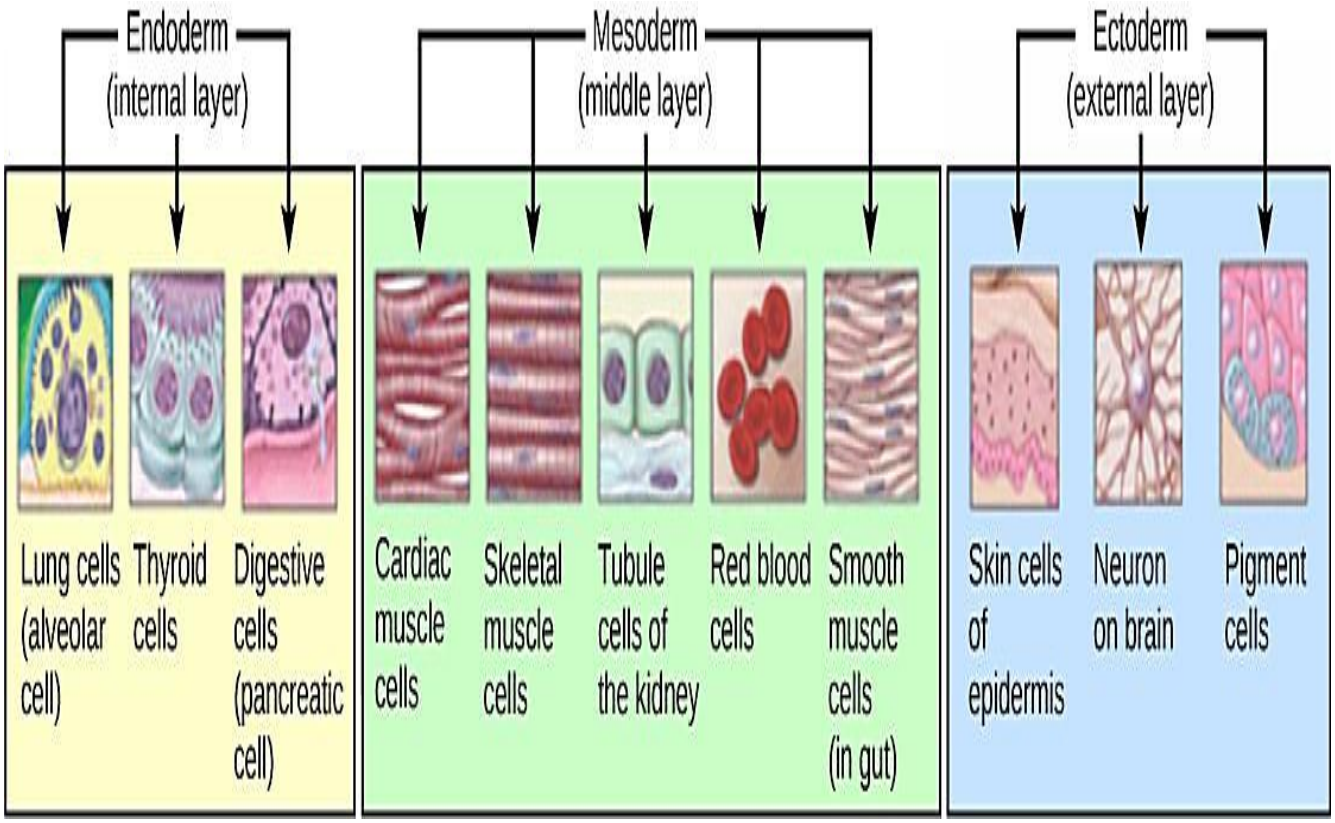
Connective Tissue Fibers

The three types of connective tissue fibers are:

- **Collagen fibers** - most are type I collagen (most abundant protein in the body)
  - Tensile strength - resistance to stretching
- **Elastic fibers** - contain elastin and fibrillin
  - Elasticity - can be stretched, yet still, return to its original length
- **Reticular fibers** - contain type III collagen
  - Support - network of thin fibers

Different stains can be used to visualize each type of fiber.





Classification of Connective Tissues

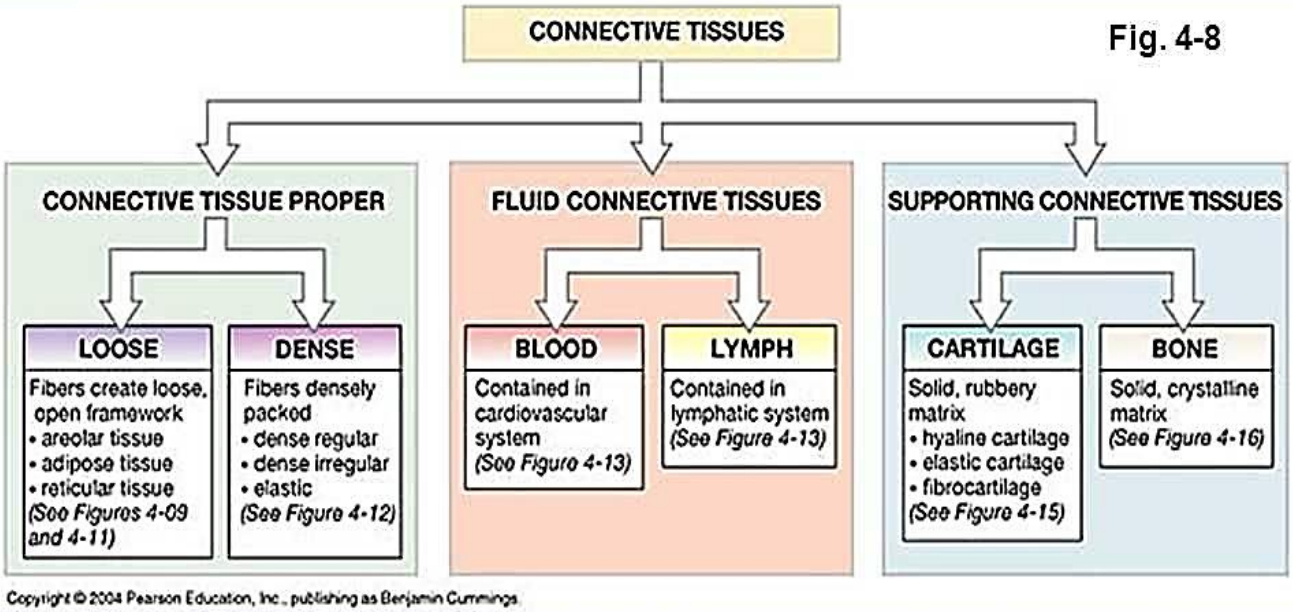


Fig. 4-8

Many cell types — Distinct cell types — Less diverse cell types

Syrupy ground substance — Watery matrix — More fibrous matrix

	Types of connective tissue proper					
	Loose connective tissue proper			Dense connective tissue proper		
Constituents	Areolar connective tissue	Reticular connective tissue	Adipose	Regular dense connective tissue	Irregular dense connective tissue	Elastic dense connective tissue
Cells	Fibroblasts, w/some macrophages, other white blood cells	Fibroblasts, w/many white blood cells	Adipocytes, w/some macrophages, other white blood cells.	Fibroblasts, w/ some macrophages, other white blood cells	Fibroblasts, w/ some macrophages, other white blood cells	Fibroblasts, w/ some macrophages, other white blood cells
Protein fibers	Collagen, elastic	Reticular	Collagen, some reticular	Thick collagen fibers, in parallel	Thick collagen fibers, no consistent arrangement	Elastic mainly, some collagen
Ground substance	Jelly-like, abundant	Jelly-like abundant	Smaller amounts due to abundant cells	Smaller amounts due to abundant fibers	Smaller amounts due to abundant fibers	Smaller amounts due to abundant fibers
Locations	Under epithelia of skin, mucous membranes, capillaries, organs	Spleen, lymph nodes, bone marrow	Under skin, surrounding organs, between muscle fibers, in pericardium.	Tendons, ligaments, aponeuroses	Capsules around joints and organs, dermis of skin	Walls of large arteries, walls of bronchial tubes, vertebral ligaments

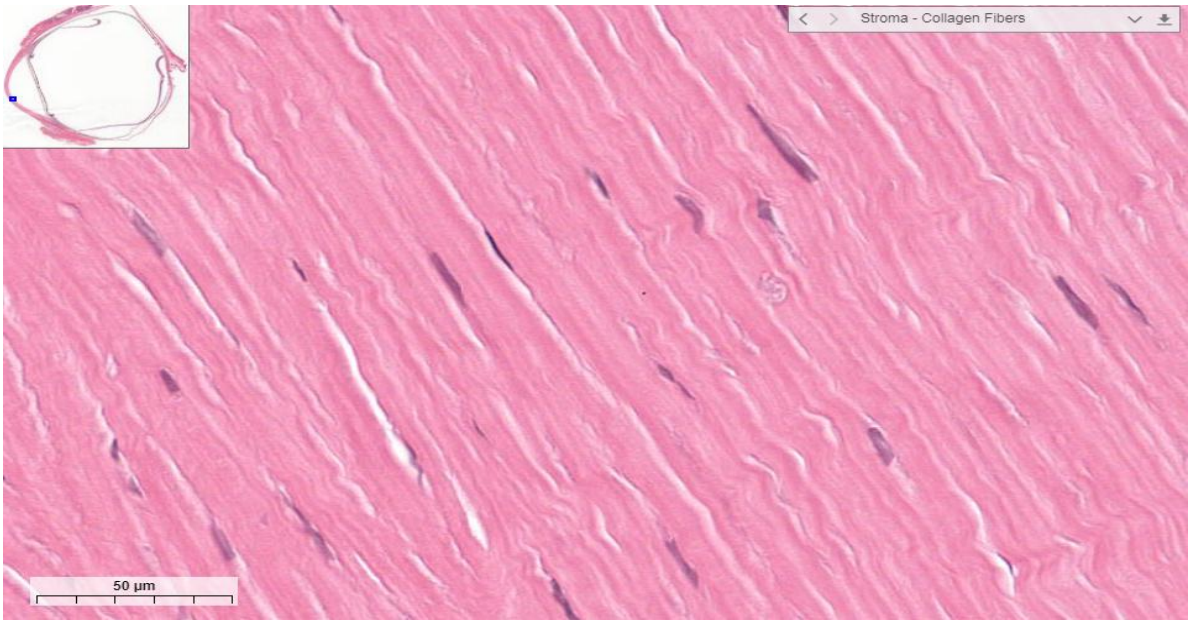
Extracellular Substance

Collagen fibres

Collagen fibres are the dominant fibre type in most connective tissues. The primary function of collagen fibres is to add strength to the connective tissue.

The thickness of the fibres varies from ~ 1 µm to 10 µm. Longitudinal striations may be visible in thicker fibres. These striations reveal that the fibres are composed of thinner collagen fibrils (0.2 to 0.5 µm in diameter). Each of these fibrils is composed of microfibrils, which are only visible using electron microscopy.

Microfibrils are assemblies of tropocollagen, which, in turn, is an spiral-like assembly of three collagen molecules (triple helix). The organisation of the tropocollagen within the microfibrils is highly regular. A small gap (60 nm wide) is found between the subsequent tropocollagens forming the microfibrils. Staining solutions used in electron microscopy tend to fill in these gaps, and the aligment of the gaps gives the microfibrils a cross-striated appearance (with 68 nm intervals) in EM images .

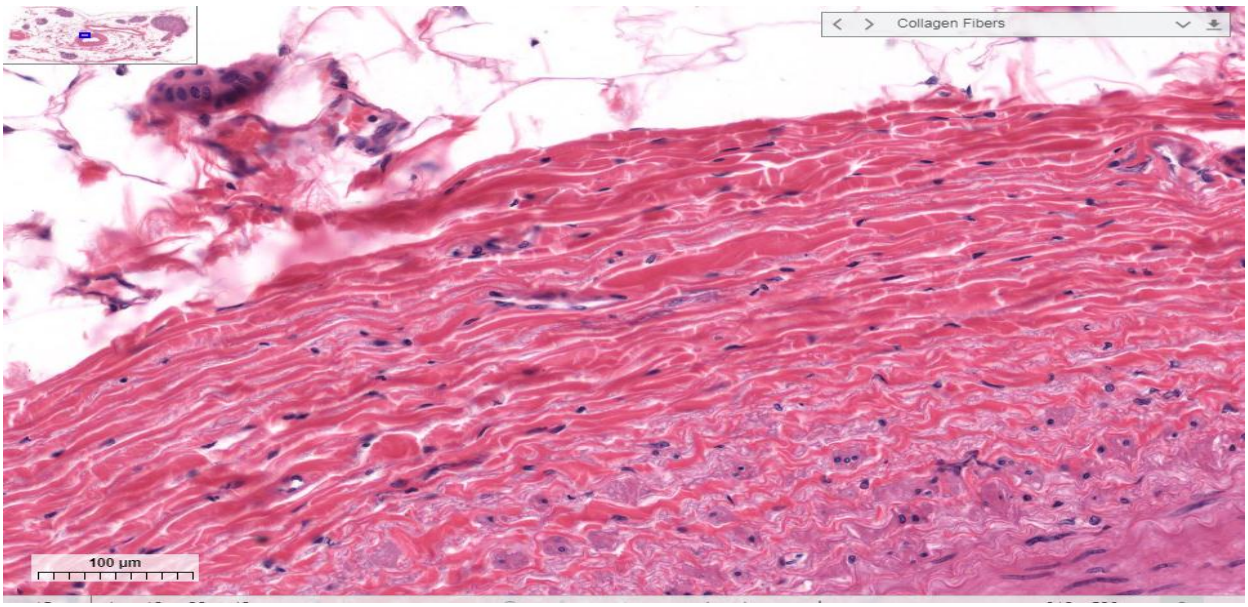


Fibroblasts

The cornea is dense regular connective tissue. It is covered by a stratified squamous epithelium on the anterior surface and a simple squamous epithelium on the posterior surface.

The stroma primarily consists of densely packed, parallel collagen fibers. Fibroblasts produced these fibers and are mostly inactive in adults. Their flattened nuclei of condensed chromatin are visible between the fibers. Their sparse cytoplasm (pink) is not visible largely because it blends in with the collagen fibers.

The ultrastructure of fibroblasts is shown in EM 006 Fibroblast and EM 109 Fibroblast as seen by transmission electron microscopy.



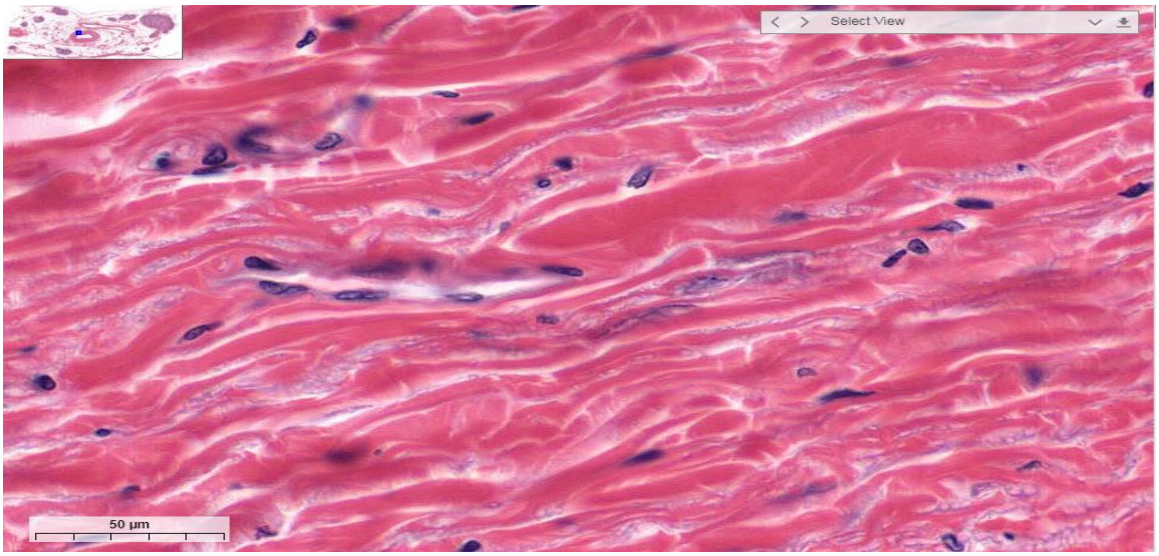
Hematoxylin & Eosin

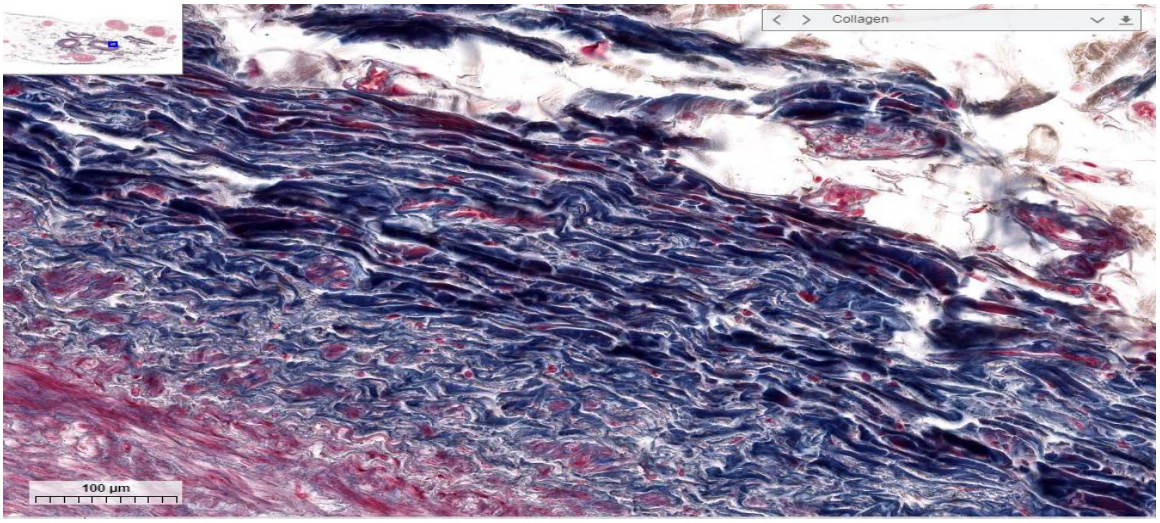
Of the fibrous components in connective tissue, **hematoxylin & eosin** (H&E) stains both collagen and muscle.

Examine the staining of a blood vessel in this section of mesentery:

- **Collagen - the outer wall is composed of collagen fibers stained pink/red**
- Elastic Fibers - the crenulated structure in the inner wall is composed of elastic fibers that are unstained (or light pink)
- Reticular Fibers - unstained
- Muscle - the middle region of the wall is composed of smooth muscle stained pink

Although both collagen and muscle are stained pink/red by H&E, they can still be distinguished from each other based on their morphology and location.





Azan

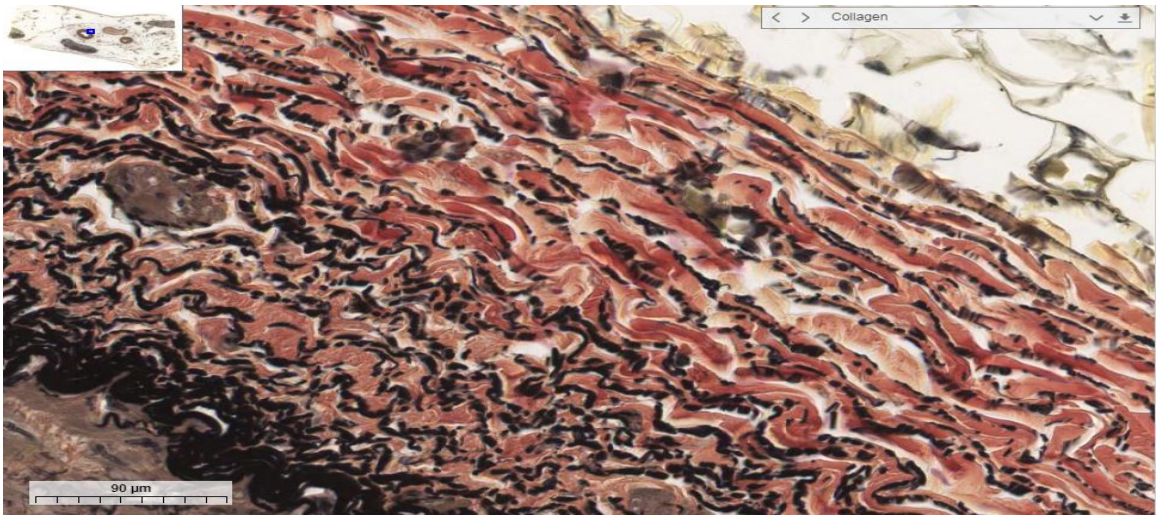
Of the fibrous components in connective tissue, **azan** is used to distinguish collagen from muscle.

Examine the staining of a blood vessel in this section of mesentery:

- **Collagen - the outer wall is composed of collagen fibers stained blue**
- Elastic Fibers - the crenulated structure in the inner wall is composed of elastic fibers that are unstained (or red)
- Reticular Fibers - unstained

- Muscle - the middle region of the wall is composed of smooth muscle stained pink/red

Unlike H&E, collagen (blue) and muscle (red) are stained different colors by azan.

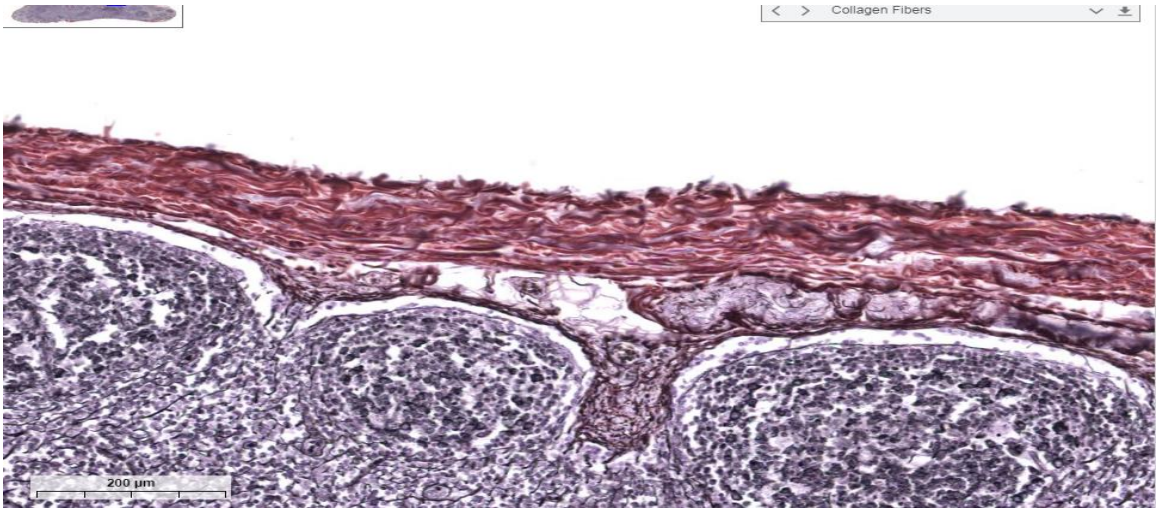


Verhoeff Stain

Of the fibrous components in connective tissue, **Verhoeff** stain is used to identify elastic fibers.

Examine the staining of a blood vessel in this section of mesentery:

- **Collagen - the outer wall is composed of collagen fibers stained red**
- Elastic Fibers - the crenulated structure in the inner wall is composed of elastic fibers stained black. Another band of elastic fibers is found between the layers of muscle and collagen.
- Reticular Fibers - unstained
- Muscle - the middle region of the wall is composed of smooth muscle that is unstained (or brown) Unlike H&E and azan, elastic fibers (black) are stained by Verhoeff.



Reticular Fibers

Reticular fibers are abundant in lymphoid organs (lymph nodes, spleen), bone marrow and liver.

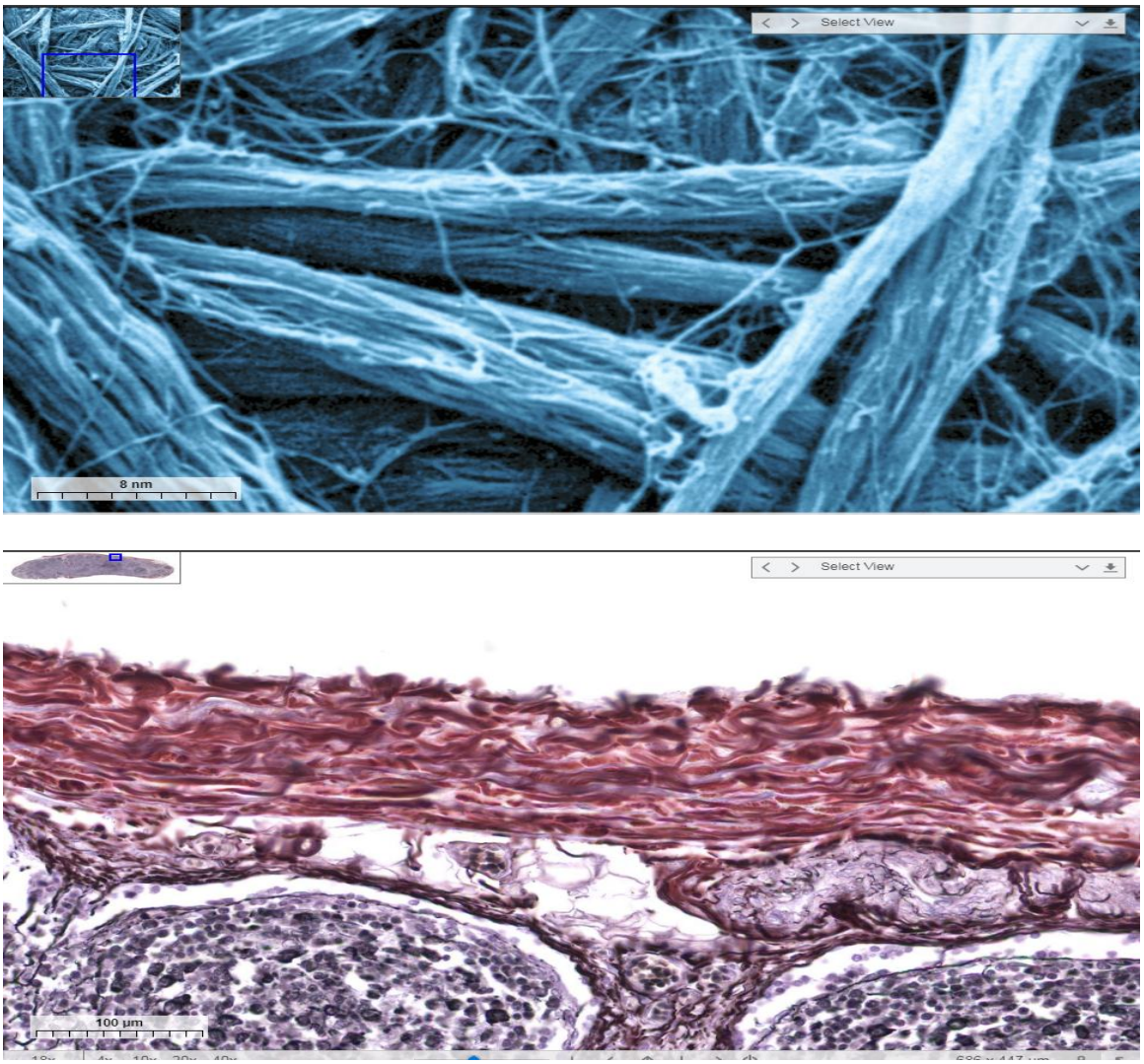
Reticular fibers are composed of thin and delicately woven strands of type III collagen. They are not visible with hematoxylin & eosin (H&E), but are specifically stained by silver. This renders

them black and makes them easily distinguishable from type I collagen fibers that are stained red/brown.

Lymph nodes are examples of tissues with a reticular framework that supports its cellular constituents.

- **Surrounding the lymph node is a capsule of collagen fibers (type I collagen) stained red.**
- The outer portion of the lymph node contains aggregations of lymphocytes organized in nodules. Reticular fibers (type III collagen) stained black surround each nodule.
- Reticular fibers also support clusters of cells (gray) in the stroma.

The fibers form attachment sites for lymphocytes and other immune cells.



Reticular Fibers

Reticular fibers are abundant in lymphoid organs (lymph nodes, spleen), bone marrow and liver.

Reticular fibers are composed of thin and delicately woven strands of type III collagen. They are not visible with hematoxylin & eosin (H&E), but are specifically stained by silver. This renders them black and makes them easily distinguishable from type I collagen fibers that are stained red/brown.

Lymph nodes are examples of tissues with a reticular framework that supports its cellular constituents.

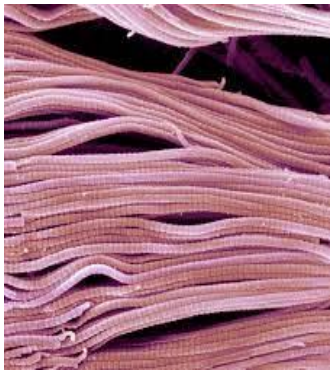
- **Surrounding the lymph node is a capsule of collagen fibers (type I collagen) stained red.**

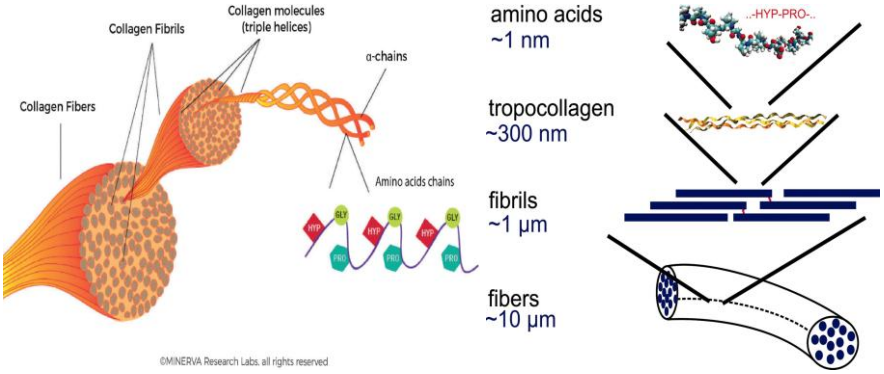
- The outer portion of the lymph node contains aggregations of lymphocytes organized in nodules. Reticular fibers (type III collagen) stained black surround each nodule.
- Reticular fibers also support clusters of cells (gray) in the stroma.

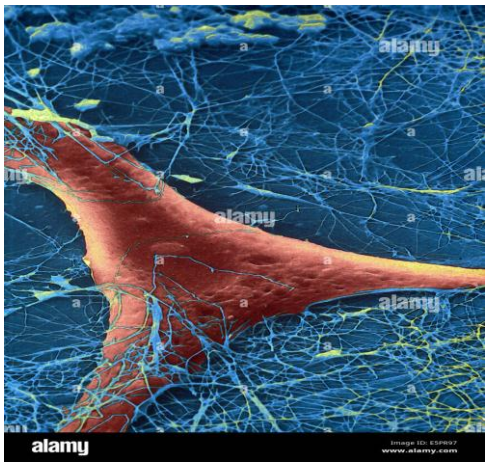
The fibers form attachment sites for lymphocytes and other immune cells.

Coarse collagen fibres are formed by type I tropocollagen.

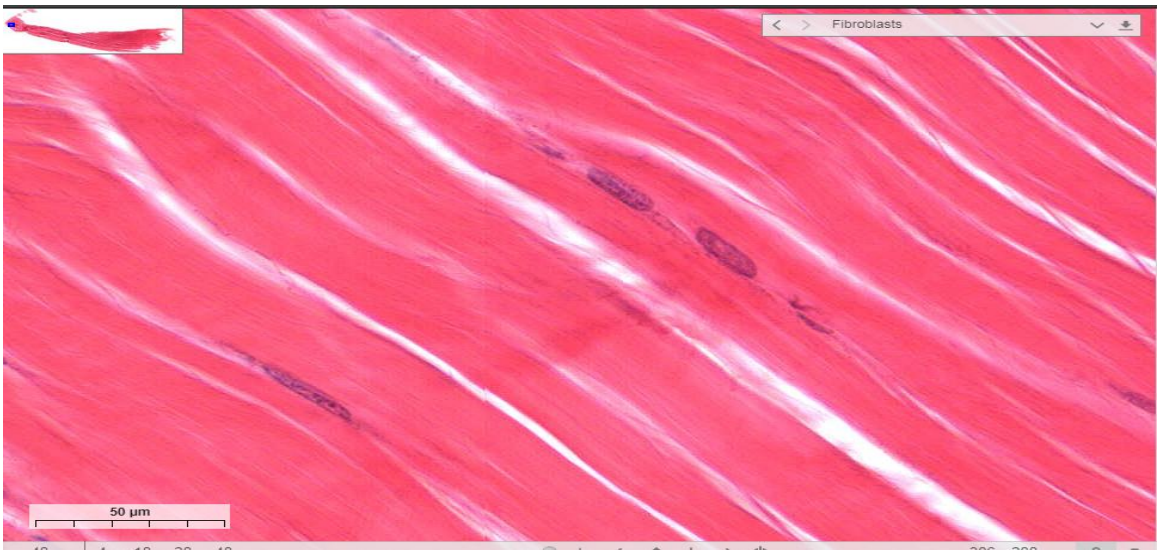
There are many different tropocollagen types around (currently named type I to XX). These types differ in their content of the amino acids hydroxyproline and hydroxylysine. They also differ in the amount of carbohydrates attached to the collagen molecules. The different types of tropocollagen give the fibres the structural and functional features which are appropriate for the organ in which the fibres are found. Types I, II and III are the major fibre-forming tropocollagens. Tropocollagen type IV is an important structural component of the basal lamina. A tensile force of several hundred kg/cm^2 is necessary to tear or break human collagen fibres. The fibres stretch by only 15-20%.







Fibroblast and Collagen fibres

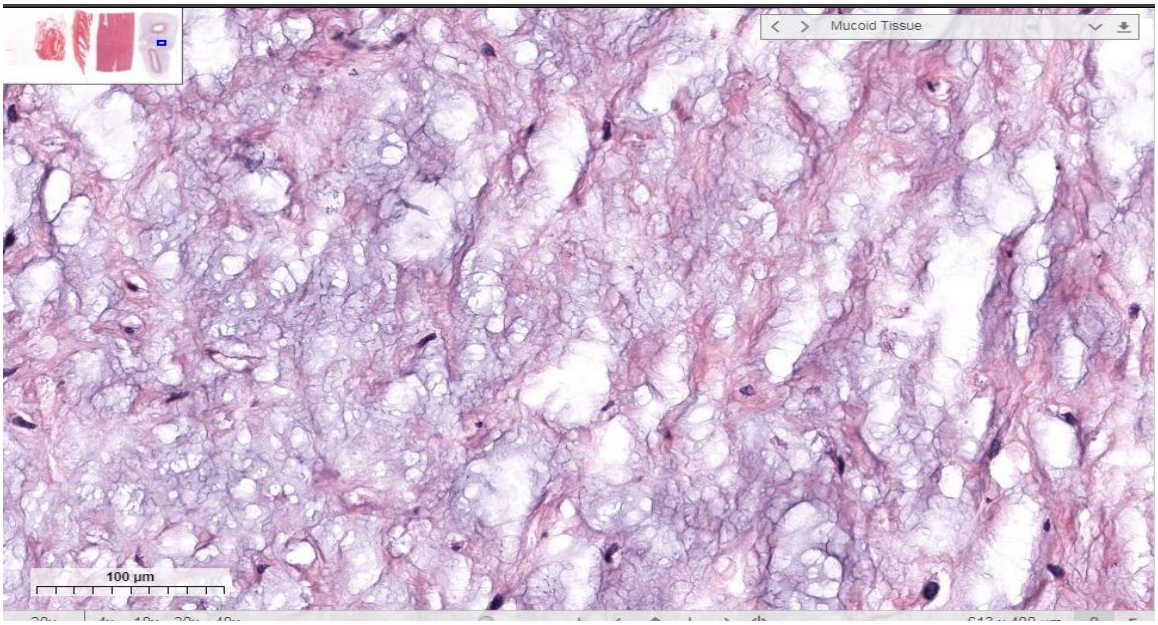


Dense Regular Connective Tissue

Dense regular connective tissue contains densely packed collagen fibers arranged in parallel bundles.

It is found in tendons (joins muscles to bone or cartilage) and ligaments (bone to bone). The primary function of the collagen fibers is to provide tensile strength to tissues.

The fibroblasts that produced the fibers are relatively inactive in adults. They reside in close proximity to the collagen fibers and often only their flattened nuclei are visible. Their sparse cytoplasm is not visible largely because it blends in with the collagen fibers.



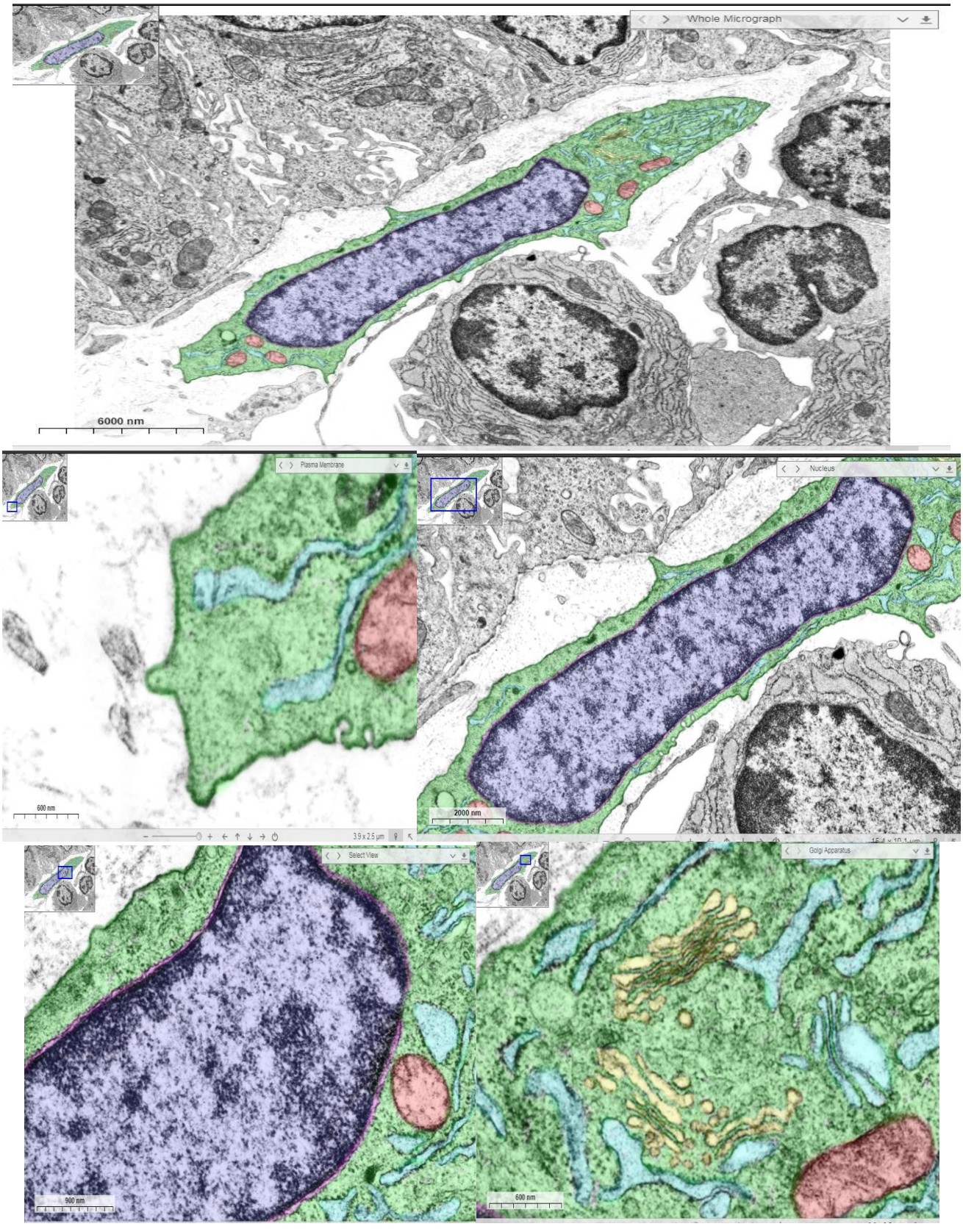
Fibroblasts

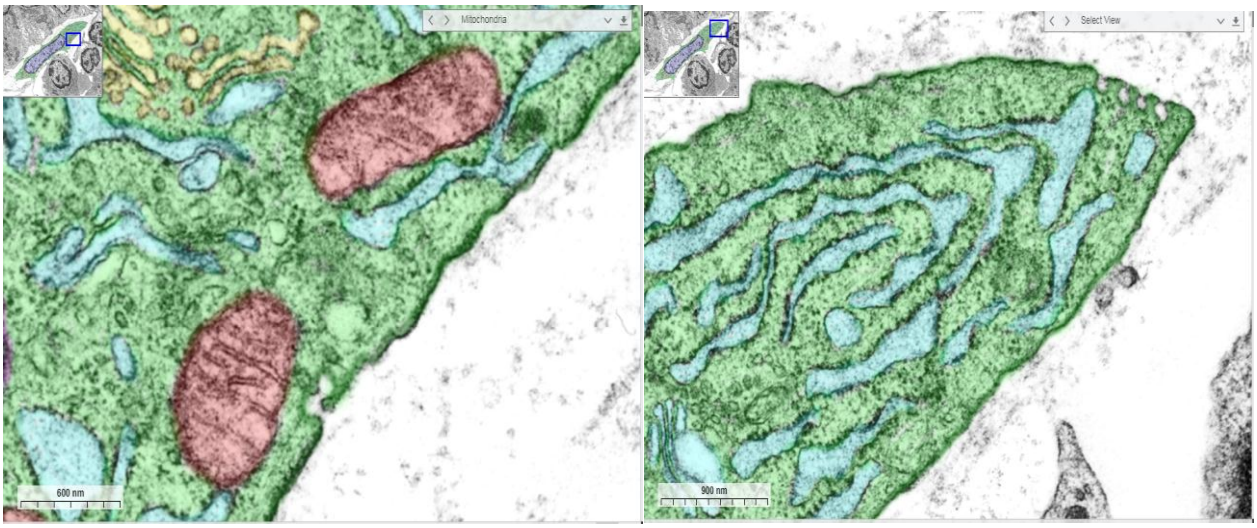
The umbilical cord connects the developing fetus and the placenta. It consists mostly of mesenchyme (embryonic connective tissue). Its bluish-pink color is from the abundant ground substance (blue) and sparse collagen fibers (pink).

Fibroblasts produced this extracellular matrix and are dispersed in the connective tissue. Only their nuclei can be identified. Some appear round with heterochromatin, while

others are flattened with mostly euchromatin. This suggests some of the fibroblasts are active, while others are inactive.

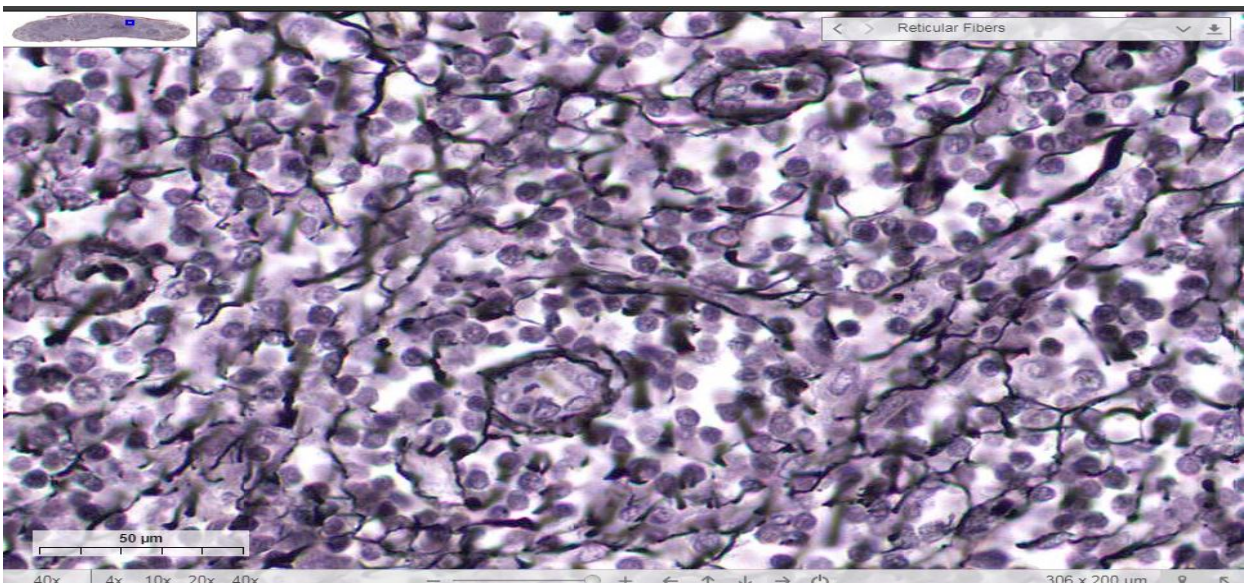
The ultrastructure of fibroblasts is shown in EM 006 Fibroblast and EM 109 Fibroblast as seen by transmission electron microscopy.





Reticular fibres

Reticular fibres are very delicate and form fine networks instead of thick bundles. They are usually not visible in histological sections but can be demonstrated by using special stains. In such preparations they look like fine, black threads - collagen fibres appear reddish brown in the same type of preparation. Because of their different staining characteristics, reticular fibres were initially thought to be completely different from collagen fibres. Cross-striations with the same periodicity as in collagen fibres are however visible using electron microscopy. We now know that reticular fibres consist of collagen - although the main type of tropocollagen found in reticular fibres (type III) is different from that of the coarse collagen fibres. Reticular fibres give support to individual cells, for example, in muscle and adipose tissue.



Reticular Fibers

Reticular fibers are abundant in lymphoid organs (lymph nodes, spleen), bone marrow and liver.

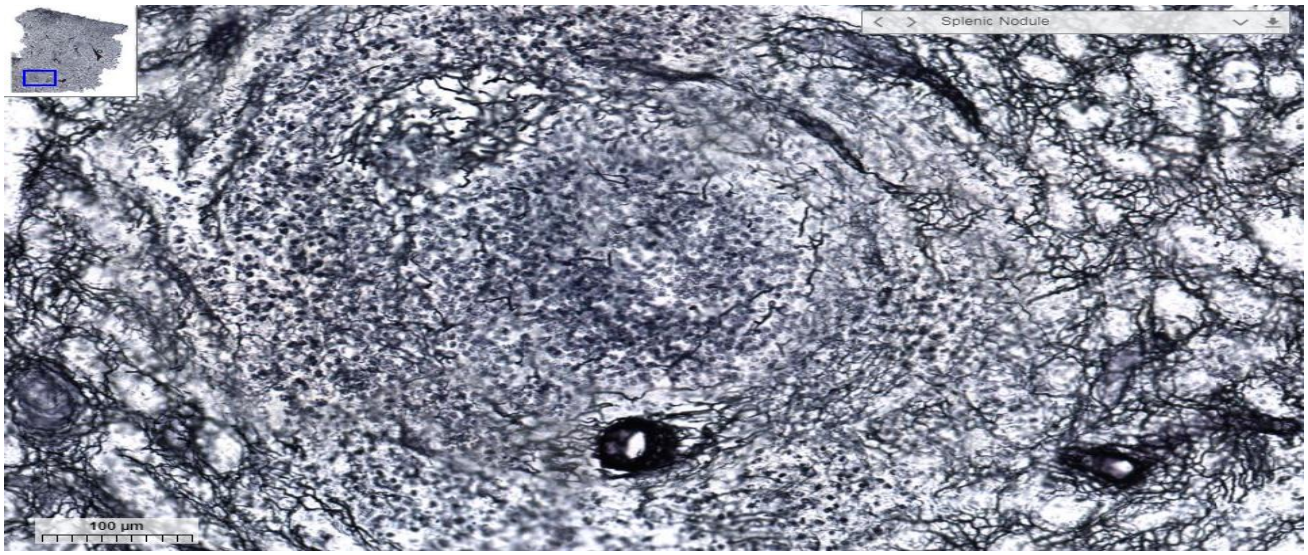
Reticular fibers are composed of thin and delicately woven strands of type III collagen. They are not visible with hematoxylin & eosin (H&E), but are specifically stained by silver. This renders

them black and makes them easily distinguishable from type I collagen fibers that are stained red/brown.

Lymph nodes are examples of tissues with a reticular framework that supports its cellular constituents.

- Surrounding the lymph node is a capsule of collagen fibers (type I collagen) stained red.
- The outer portion of the lymph node contains aggregations of lymphocytes organized in nodules. Reticular fibers (type III collagen) stained black surround each nodule.
- **Reticular fibers also support clusters of cells (gray) in the stroma.**

The fibers form attachment sites for lymphocytes and other immune cells.



Spleen

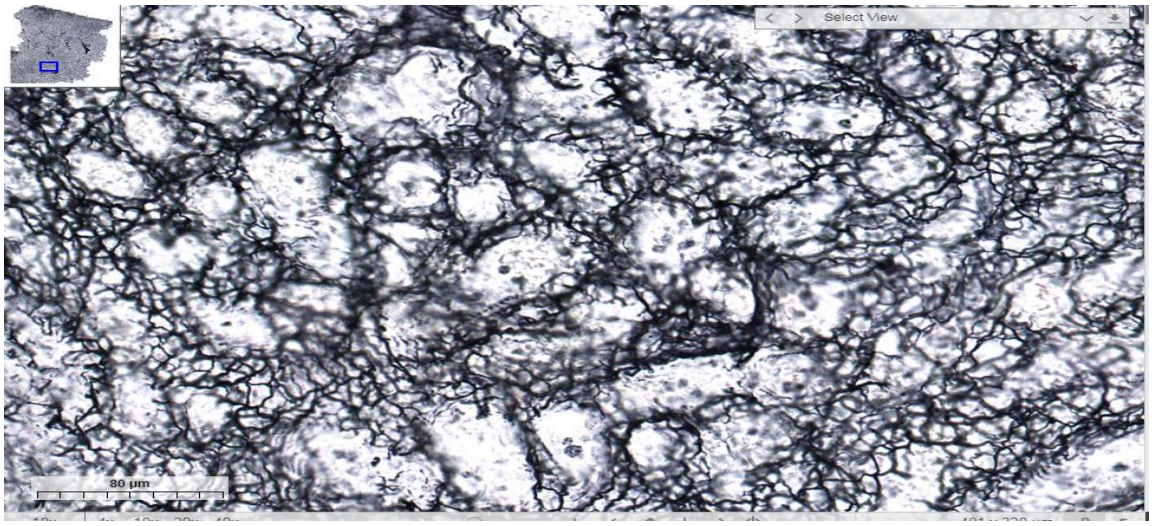
Reticular fibers are abundant in lymphoid organs (lymph nodes, spleen), bone marrow and liver.

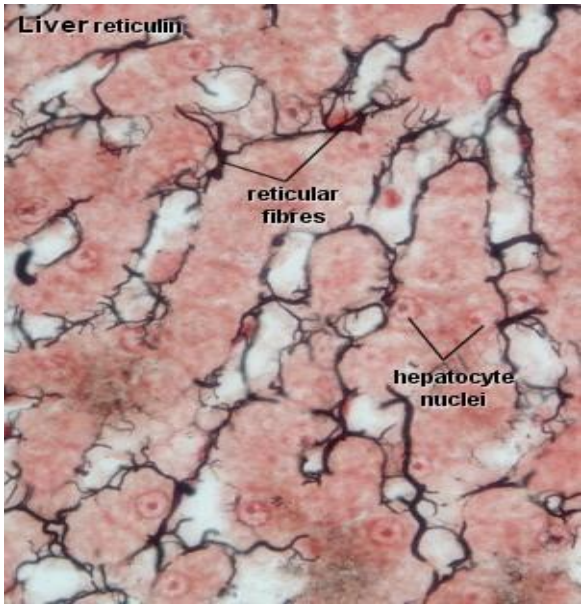
Reticular fibers are composed of thin and delicately woven strands of type III collagen. They are not visible with hematoxylin & eosin (H&E), but are specifically stained black by silver.

The spleen is an example of an organ with a reticular framework that supports its cellular constituents.

- **Aggregations of lymphocytes are organized into nodules that are loosely surrounded by reticular fibers.**
- Much of the spleen is organized into cords of loose connective tissue supported by a meshwork of reticular fibers.
- Blood vessels are also surrounded by a network of reticular fibers.

The fibers form attachment sites for lymphocytes and other immune cells.

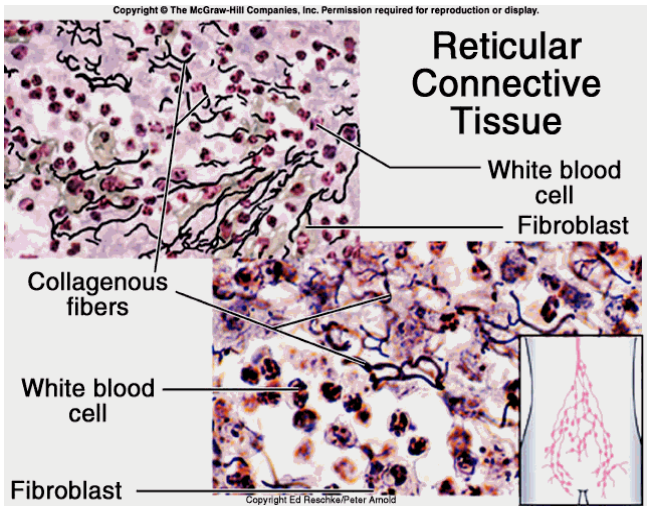


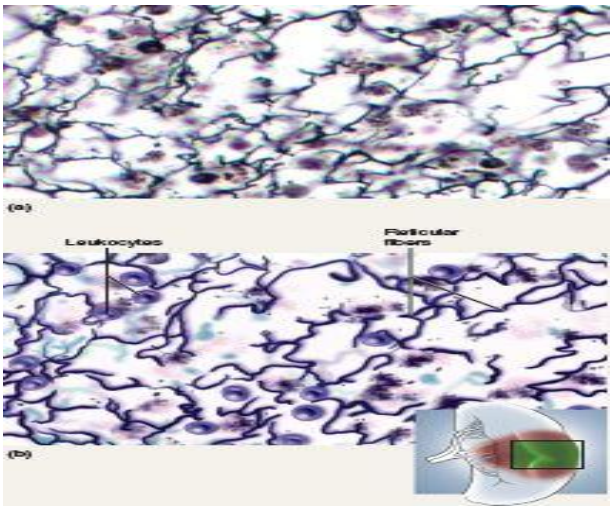


Suitable Slides

sections of liver, spleen or lymph nodes – reticulin The liver is one of the organs in which the cells are supported by a network of reticular fibres. They appear as fine black lines in this silver stained preparation. The fibres surround the individual sheets of liver cells (hepatocytes) and are the only fibrous connective tissue component supporting the cells. While providing support, the fine, open meshwork of reticular fibres facilitates the exchange of substances between the hepatocytes and the blood, which circulates in the irregularly shaped blood vessels (sinusoids) between the hepatocytes. Reticular fibres are also present in the connective tissue surrounding the larger vessels, which penetrate the parenchyma of the liver.

Draw reticular fibres as they surround a nice piece of a row of liver cells at high magnification - include a suitable scale and label your drawing.
Blood will not be visible in some types of preparations and the sinusoids appear empty.

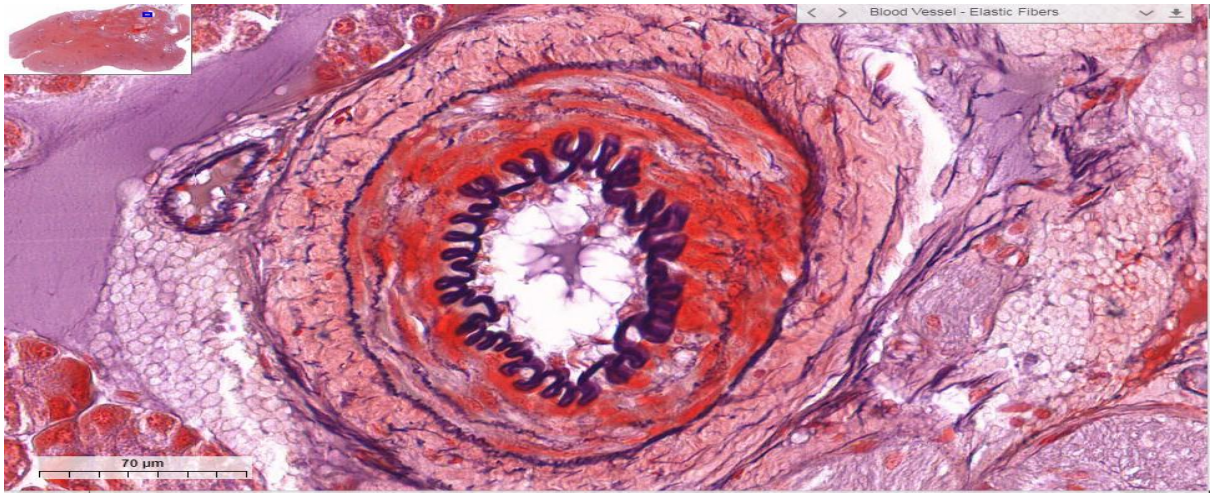


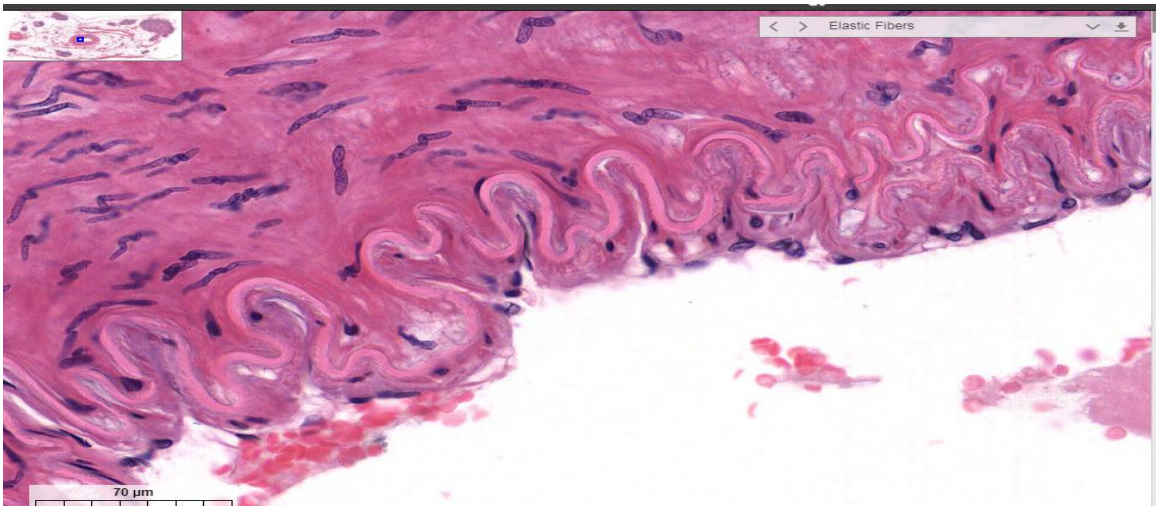


Elastic fibres

Elastic fibres are coloured in fresh tissues - they are light yellow - but this colouration is only visible if large amounts of elastic fibres are present in the tissue, for example, in the elastic ligaments of the vertebral column. Special stains are necessary to show elastic fibres in tissue sections (resorcin fuchsin is one of these stains, which gives the elastic fibres a dark violet colour).

Light microscopy does not reveal any substructure in the elastic fibres. Electron microscopy shows that elastic fibres consist of individual microfibrils, which are embedded in an amorphous matrix. The matrix accounts for about 90% of the fibre and is composed of the protein elastin. Neither the elastin nor the microfibrils are collagens.
Elastic fibres can be stretched to about 150% of their original length. They resume their original length if the tensile forces applied to the elastic fibres are relaxed.
Elastin is a somewhat odd protein in that its amino acid sequence does not determine a specific three-dimensional structure of the molecule. Instead, elastin remains unfolded as a "random coil". Elastin molecules are cross-linked to each other by desmosin and isodesmosin links, which are only found between elastin molecules. Tensile forces straighten the cross-linked mesh of elastin coils.





Hematoxylin & Eosin

Of the fibrous components in connective tissue, **hematoxylin & eosin** (H&E) stains both collagen and muscle.

Examine the staining of a blood vessel in this section of mesentery:

- Collagen - the outer wall is composed of collagen fibers stained pink/red
- **Elastic Fibers - the crenulated structure in the inner wall is composed of elastic fibers that are unstained (or light pink)**
- Reticular Fibers - unstained
- Muscle - the middle region of the wall is composed of smooth muscle stained pink

Although both collagen and muscle are stained pink/red by H&E, they can still be distinguished from each other based on their morphology and location.

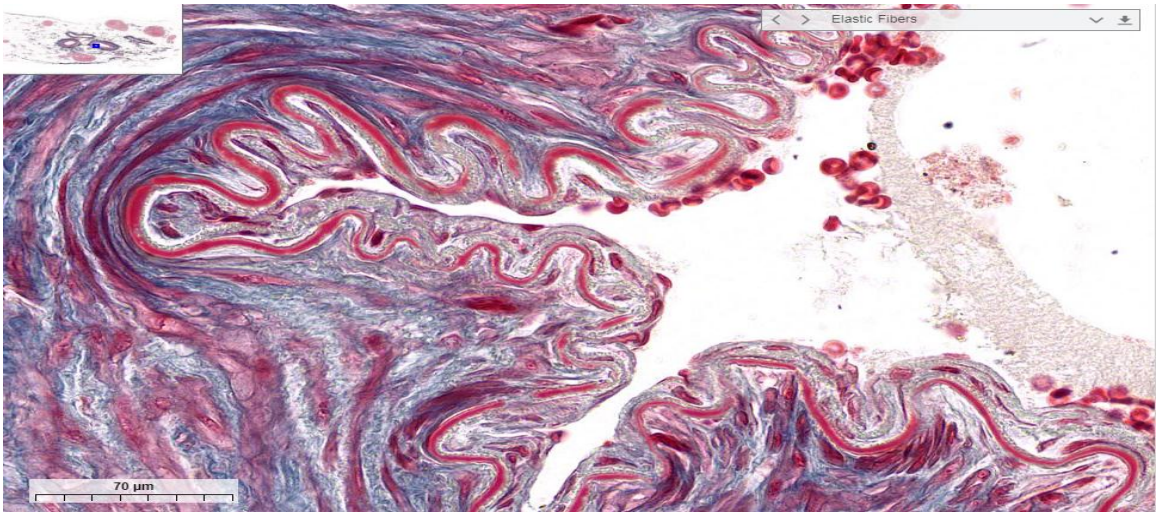
Verhoeff Stain

Of the fibrous components in connective tissue, **Verhoeff** stain is used to identify elastic fibers.

Examine the staining of a blood vessel in this section of mesentery:

- Collagen - the outer wall is composed of collagen fibers stained red
- **Elastic Fibers - the crenulated structure in the inner wall is composed of elastic fibers stained black. Another band of elastic fibers is found between the layers of muscle and collagen.**
- Reticular Fibers - unstained
- Muscle - the middle region of the wall is composed of smooth muscle that is unstained (or brown)

Unlike H&E and azan, elastic fibers (black) are stained by Verhoeff.



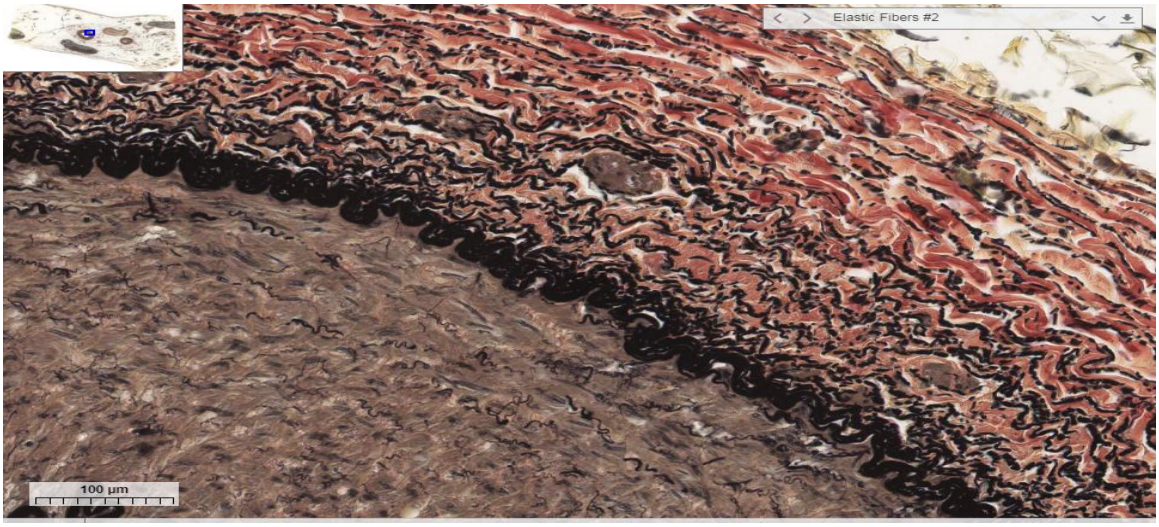
Azan

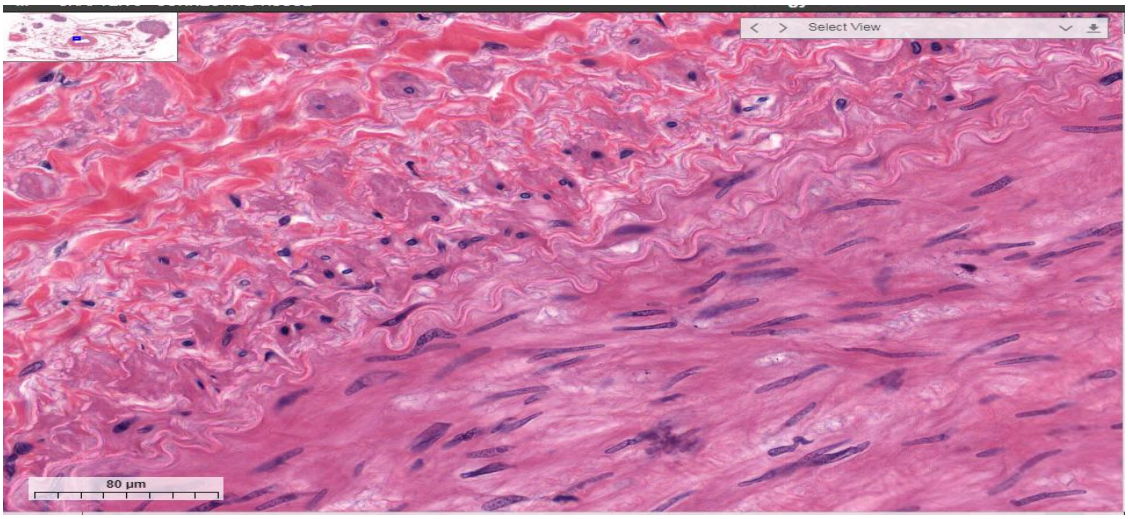
Of the fibrous components in connective tissue, **azan** is used to distinguish collagen from muscle.

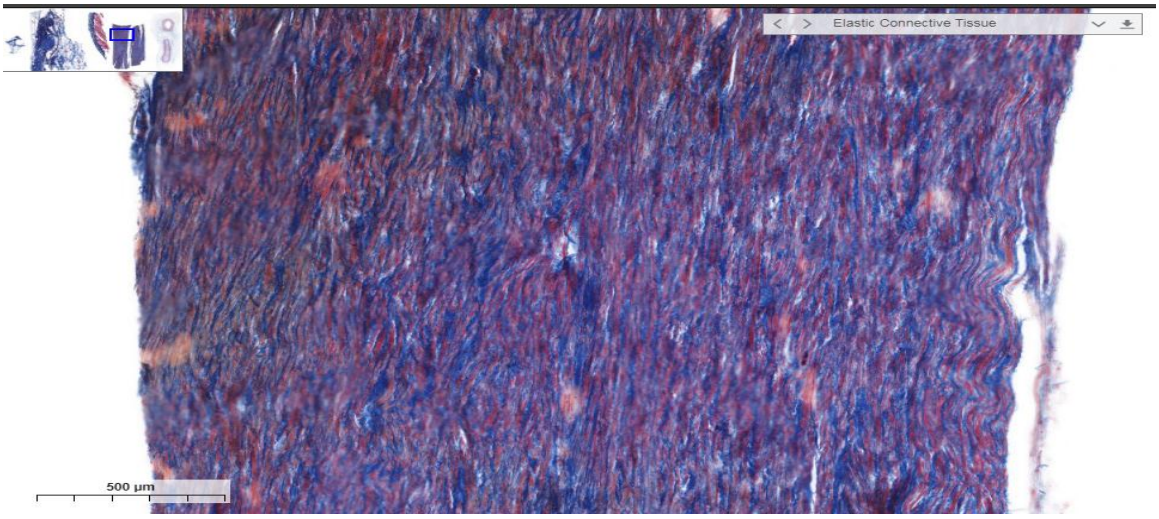
Examine the staining of a blood vessel in this section of mesentery:

- Collagen - the outer wall is composed of collagen fibers stained blue
- **Elastic Fibers - the crenulated structure in the inner wall is composed of elastic fibers that are unstained (or red)**
- Reticular Fibers - unstained
- Muscle - the middle region of the wall is composed of smooth muscle stained pink/red

Unlike H&E, collagen (blue) and muscle (red) are stained different colors by azan.



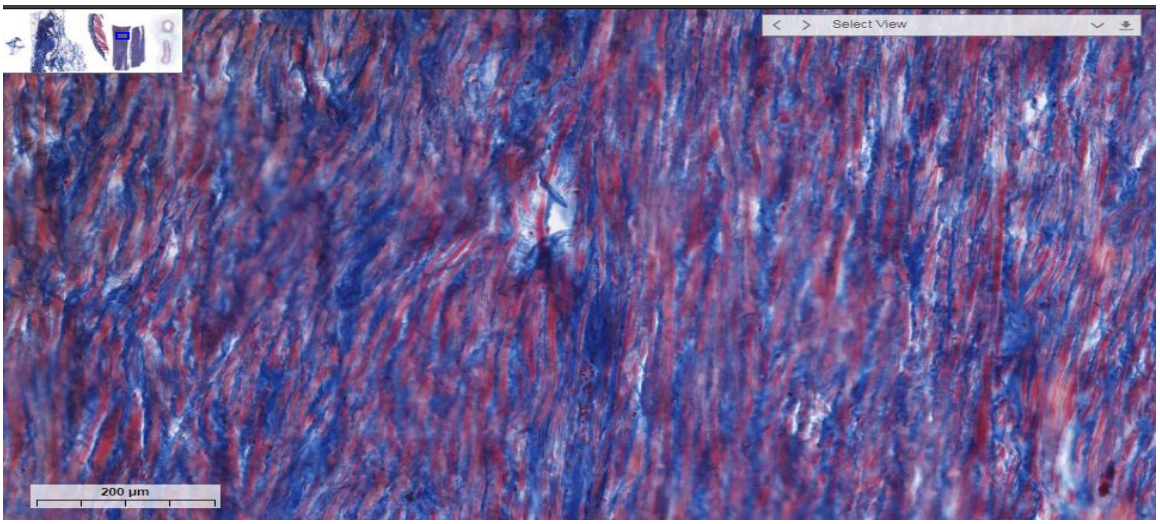


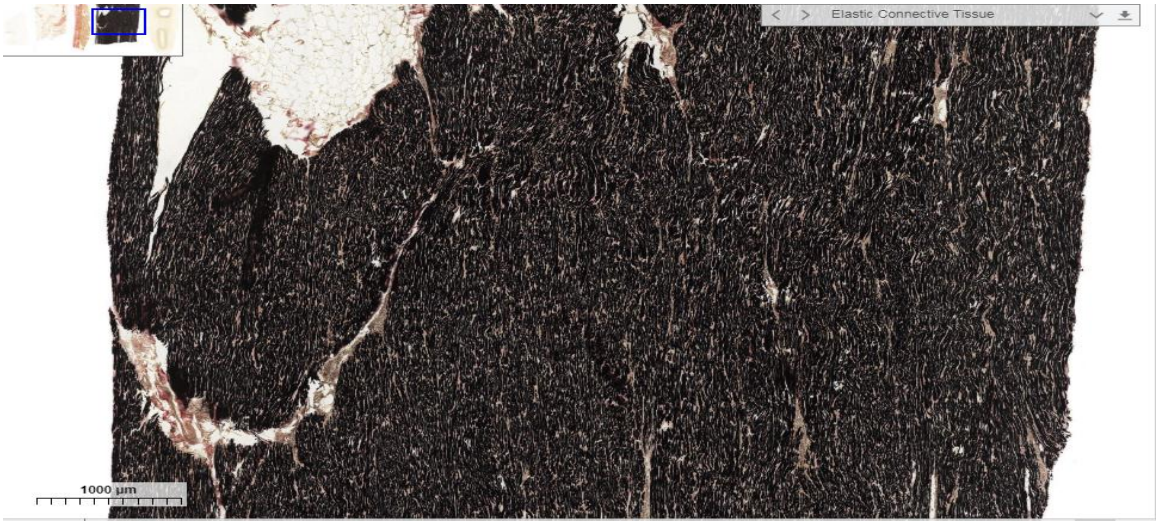


Azan

Different types of connective tissues stained with azan. Azan stains **collagen** (blue) and **muscle** (red).

- Adipose Tissue - a specialized, loose connective tissue that contains large numbers of adipocytes. The cells are empty because lipids are extracted during tissue preparation. IT also contains small amounts of collagen fibers stained blue.
- Loose Connective Tissue - mesentery is an example of loose connective tissue. Mesentery is folds of connective tissue that attach organs to the walls of the abdomen. It is composed of collagen fibers interspersed with adipocytes, blood vessels and nerves.
- Dense Connective Tissue - fascia lata is an example of dense connective tissue. The fascia lata is connective tissue that encloses thigh muscles. Collagen fibers are located to the left are stained blue and muscle fibers to the right are stained red.
- **Elastic Connective Tissue - the ligamentum nuchae is an example of elastic connective tissue. The ligamentum nuchae is a ligament at the back of the neck. It is dense regular connective tissue with both collagen and elastic fibers. The collagen fibers are stained blue and the elastic fibers appear red in this specimen. (Verhoeff stain can be used to distinguish elastic fibers.)**
- Embryonic Connective Tissue - the umbilical cord is an example of embryonic connective tissue. The umbilical cord connects the developing fetus and the placenta. The bluish color is from the sparse collagen fibers. The muscle of the blood vessels is stained red.

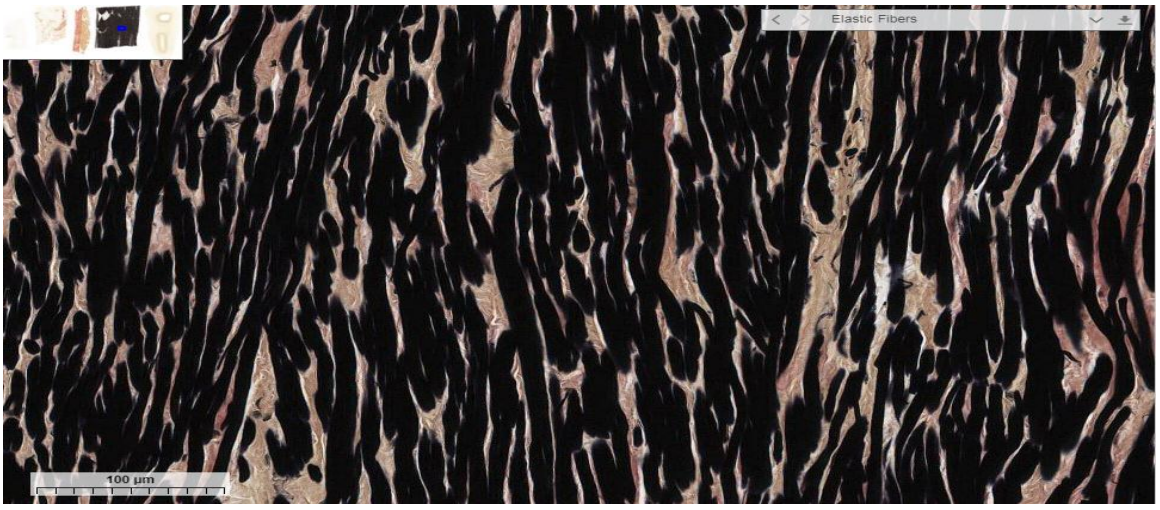




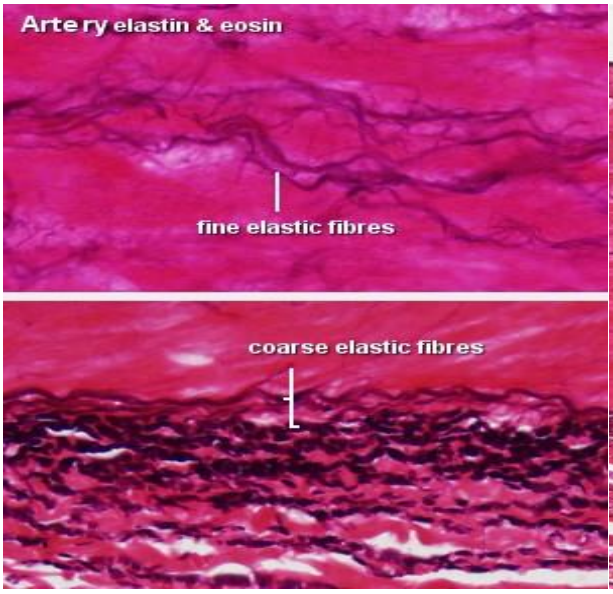
Different types of connective tissue stained with Verhoeff stain. It intensely stains **elastic fibers** (black) and lightly stains **collagen** (red).

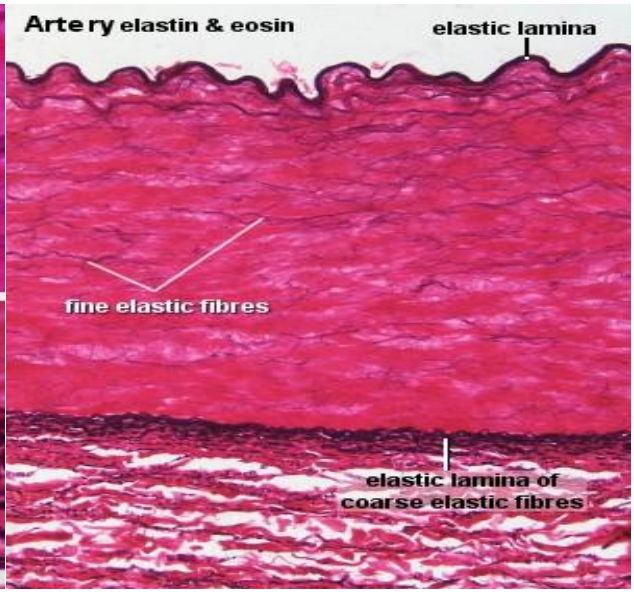
Elastic connective tissue is the only specimen that contains significant amounts of elastic fibers.

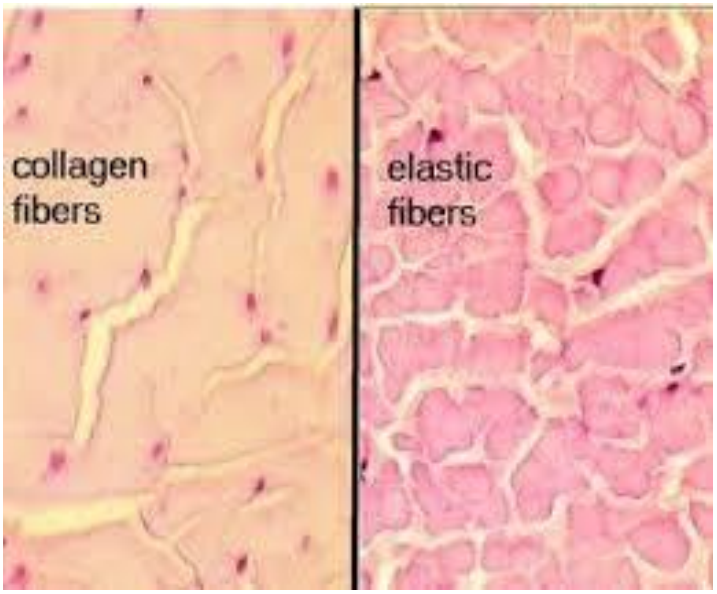
- **Elastic Connective Tissue - the ligamentum nuchae is an example of elastic connective tissue. The ligamentum nuchae is a ligament at the back of the neck. It is dense regular connective tissue with both collagen and elastic fibers. The abundant, compact masses of elastic fibers are intensely stained black.**

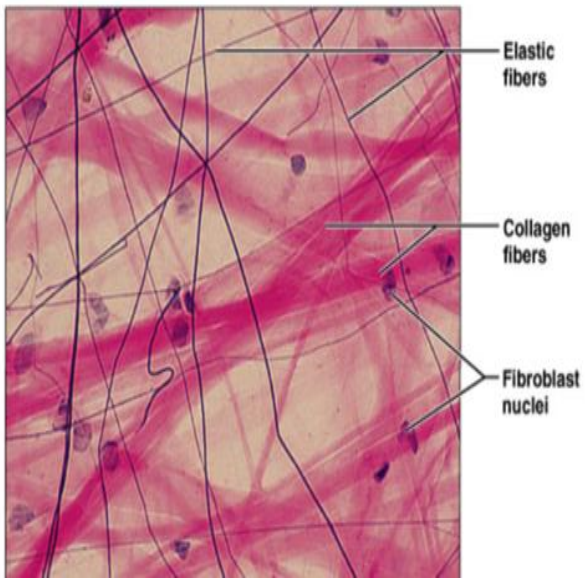


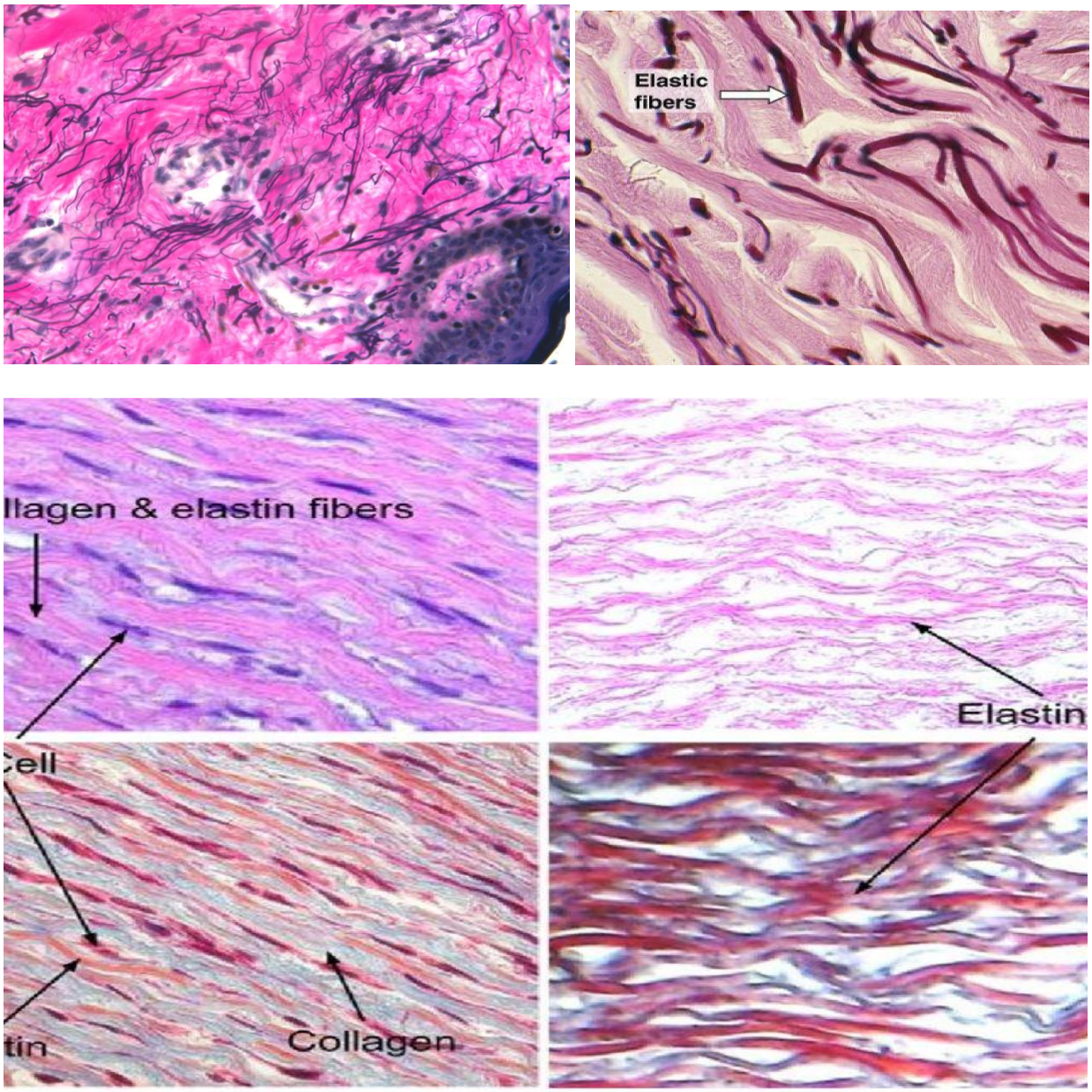
Suitable Slides
sections of blood vessels or skin – elastin Artery, human - elastin & eosinLike reticular fibres, elastic fibres require special stains to be visualized. Typically elastic fibres will appear as fine, dark violet and gently undulating fibres in the tissue. Elastic fibres can form membranes - not unlike the collagen membrane in the basal lamina of epithelia. This is the case at some levels in the walls of blood vessels. A combination with a second stain is necessary to visualize other tissue components. Colours visible in the sections will depend on the method used in combination with the elastin stain. Eosin gives an even pink or red colour to many tissue components. Nuclei of cells remain unstained without the inclusion of the haematoxylin in the staining solutions.
Identify the artery and the vein in the section. Their walls contain large amounts of elastic fibres.
Which one contains more elastic fibres - artery or vein?
Draw a small section of the wall of a vessel, preferably an artery, at high magnification. Identify elastic laminae, fine and coarse elastic fibres in your drawing.











Ground substance

Ground substance is found in all cavities and clefts between the fibres and cells of connective tissues. Water, salts and other low molecular substances are contained within the ground substance, but its main structural constituent are proteoglycans. Ground substance is soluble in most of the solvents used to prepare histological sections and therefore not visible in ordinary sections.

Proteoglycans are responsible for the highly viscous character of the ground substance. Proteoglycans consist of proteins (~5%) and polysaccharide chains (~95%), which are covalently linked to each other. The polysaccharide chains belong to one of the five types of glycosaminoglycans, which form the bulk of the polysaccharides in the ground substance.

Hyaluronan (or hyaluronic acid) is the dominant glycosaminoglycan in connective tissues. The molecular weight (MW) of hyaluronic acid is very high (~ MW 1,000,000). With a length of about 2.5 µm hyaluronan is very large. Hyaluronan serves as a "backbone" for the

assembly of other glycosaminoglycans in connective and skeletal tissue, which results in even larger molecule complexes (MW 30,000,000 - 200,000,000). Hyaluronan is a major component of the synovial fluid and the vitreous body of the eye.

The remaining four major glycosaminoglycans are chondroitin sulfate, dermatan sulfate, keratan sulfate and heparan sulfate. These glycosaminoglycans attach via core- and link-proteins to a backbone formed by the hyaluronic acid. The coiled arrangement of the hyaluronan and other attached glucosaminoglycans fills a roughly spherical space with a diameter of ~0.5 µm. This space is called a domain. Neighbouring domains overlap and form a more or less continuous three-dimensional molecular sieve in the interstitial spaces of the connective tissues.

The large polyanionic carbohydrates of the glycosaminoglycans bind large amounts of water and cations. The bound water in the domains forms a medium for the diffusion of substances of low molecular weight such as gases, ions and small molecules, which can take the shortest route, for example, from capillaries to connective tissue cells. Large molecules are excluded from the domains and have to find their way through the spaces between domains.

The restricted motility of larger molecules in the extracellular space inhibits the spread of microorganisms through the extracellular space. A typical bacterium (0.5 x 1 µm) is essentially immobilised in the meshwork formed by the domains. The pathogenicity of a bacterium is indeed to some extent determined by its ability to find its way through the mesh, and some of the more invasive types produce the enzyme hyaluronidase, which depolymerises hyaluronic acid.

Components of the ground substance, collagen and reticular fibres are synthesised by cells of the connective tissues, the fibrocytes. Elastic fibres are synthesised by both fibrocytes and smooth muscle cells.

Connective Tissue Cells

Connective tissue cells are usually divided into two groups based on their ability to move within the connective tissue. Fibrocytes (or fibroblasts) and fat cells are fixed cells. Macrophages, monocytes, lymphocytes, plasma cells, eosinophils and mast cells are wandering cells.

Fibrocytes

Fibrocytes are the most common cell type in connective tissues. They are the "true" connective tissue cells. Usually only their oval, sometimes flattened nuclei are visible in LM sections. The cytoplasm of a resting (i.e. inactive) fibrocyte does not contain many organelles. This situation changes if the fibrocytes are stimulated, for example, by damage to the surrounding tissue. In this case the fibrocyte is transformed into a fibroblast, which contains large amounts of the organelles which are necessary for the synthesis and excretion of proteins needed to repair the tissue damage (Which ones?). Fibrocytes do not usually leave the connective tissue. They are, however, able to perform amoeboid movement.

The terms fibrocyte and fibroblast refer here to the inactive and active cells respectively - at times you will see the two terms used as synonyms without regard for the state of activity of the cell.

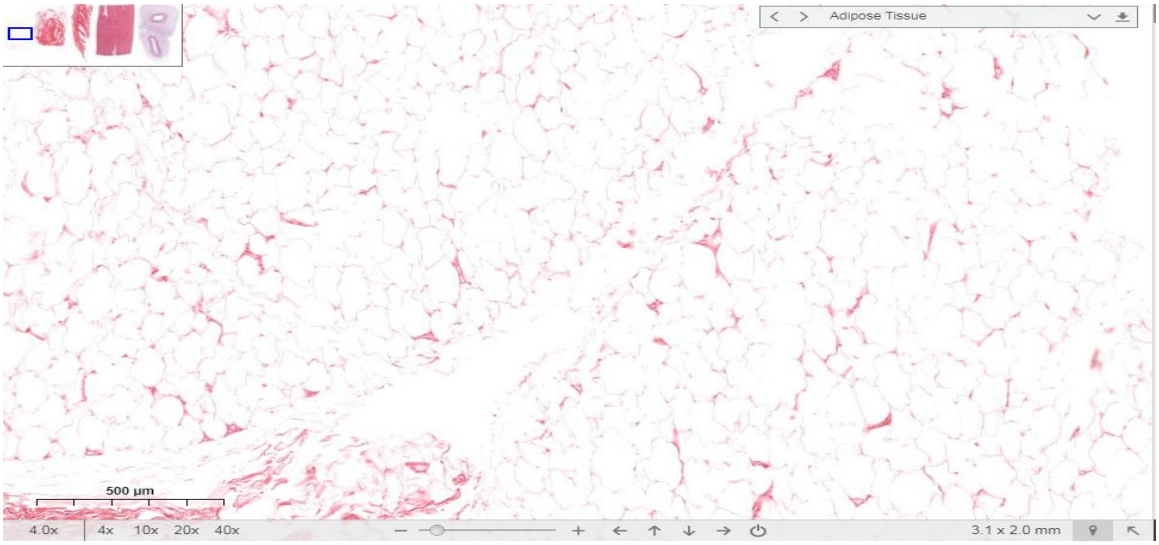
Reticular cells

Reticular cells are usually larger than an average fibrocyte. They are the "fibrocytes" of reticular connective tissue and form a network of reticular fibres, for example, in the lymphoid organs. Their nuclei are typically large and lightly stained (H&E) and the cytoplasm may be visible amongst the cells which are housed within the network of reticular fibres.

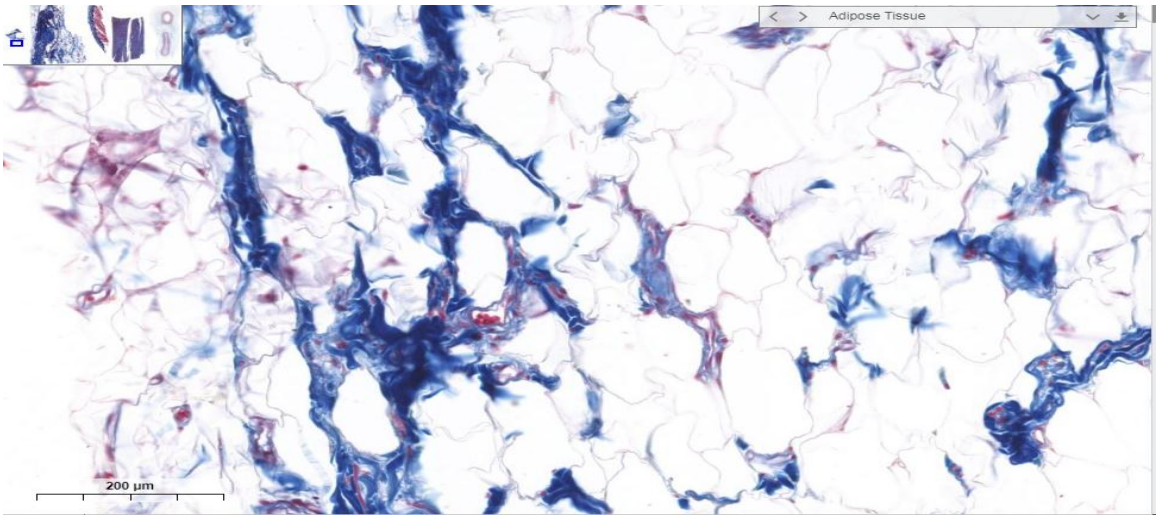
Adipocytes

Fat cells or adipocytes are fixed cells in loose connective tissue. Their main function is (what surprise!) the storage of lipids. If "well fed" the cytoplasm only forms a very narrow rim around a large central lipid droplet. The flattened nucleus may be found in a slightly thickened part of this cytoplasmic rim - if it is present in the section, which may not be the case since the diameter of an adipocyte (up to 100 µm) is considerable larger than the thickness of typical histological sections. A "starving" adipocyte may contain multiple small lipid droplets and gradually comes to resemble a fibrocyte.

Adipocytes are very long-lived cells. Their number is determined by the number of preadipocytes (or lipoblast) generated during foetal and early postnatal development.



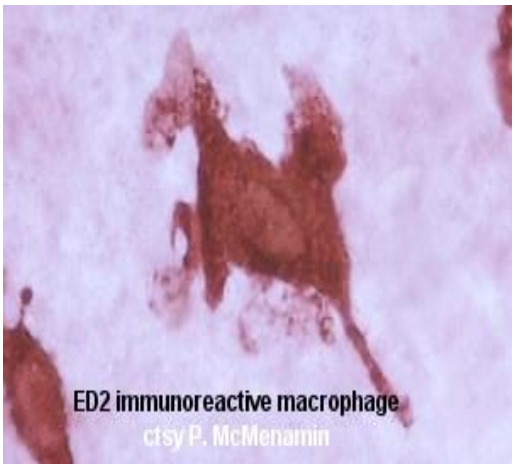
- **Adipose Tissue - a specialized, loose connective tissue that contains large numbers of adipocytes. The cells are empty because lipids are extracted during tissue preparation. It also contains small amounts of collagen fibers stained pink/red.**



Azan

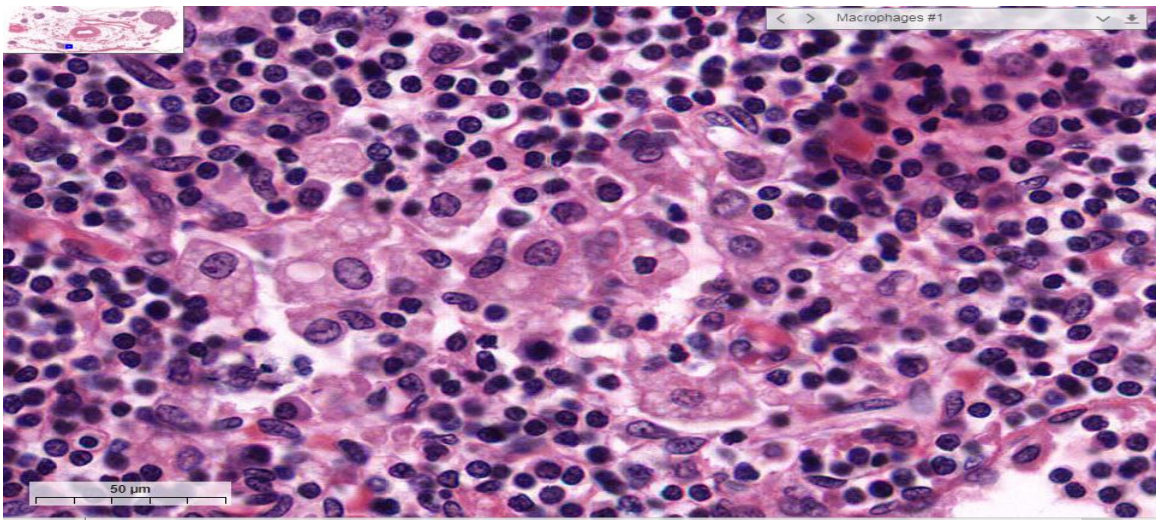
Different types of connective tissues stained with azan. Azan stains collagen **(blue) and** muscle **(red).**

- **Adipose Tissue - a specialized, loose connective tissue that contains large numbers of adipocytes. The cells are empty because lipids are extracted during tissue preparation. IT also contains small amounts of collagen fibers stained blue.**



Macrophages

Macrophages arise from precursor cells called monocytes. Monocytes originate in the bone marrow from where they are released into the blood stream. They are actively mobile and leave the blood stream to enter connective tissues, where they differentiate into macrophages. Macrophages change their appearance depending on the demand for phagocytotic activity. Resting macrophages may be as numerous as fibrocytes. Resting macrophages are difficult to distinguish from fibrocytes in H&E stained sections.



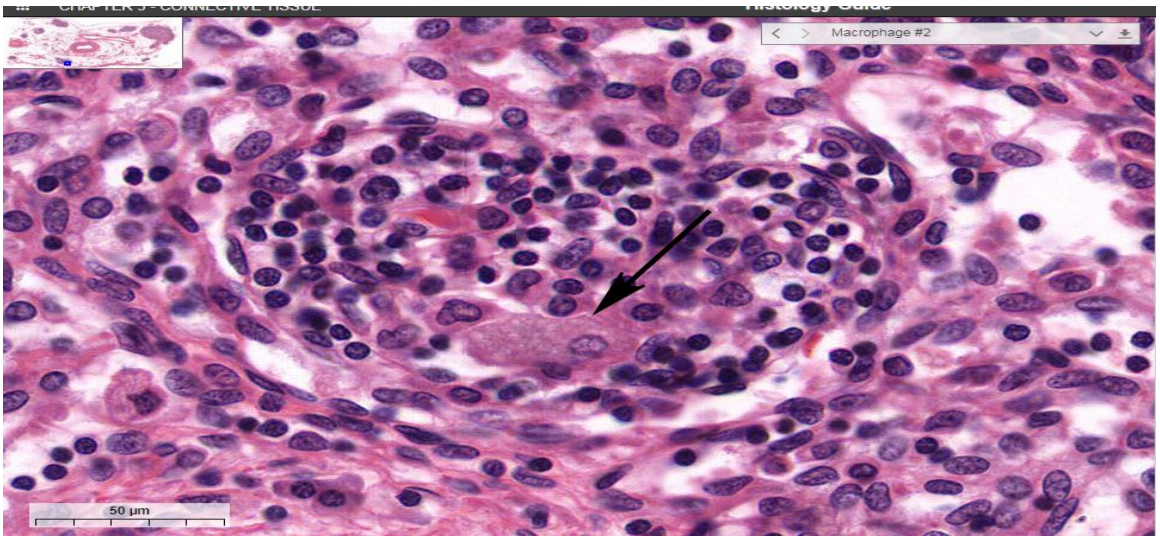
Macrophage (H&E)

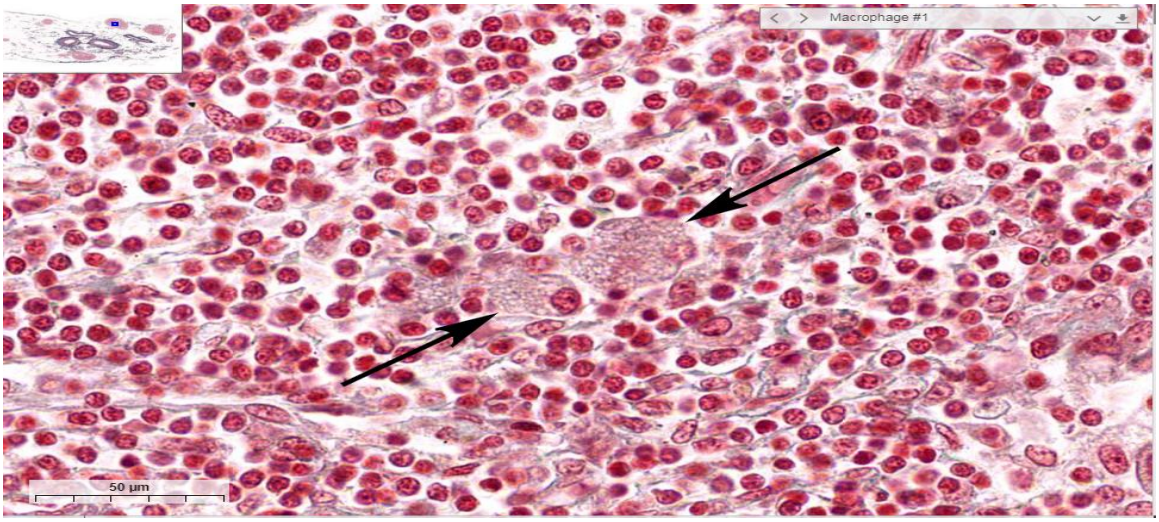
Macrophages are phagocytic cells that are common in connective tissue.

Macrophages are round to oval in shape (10-30 µm in diameter), and an eccentrically located, oval or indented nucleus. The cytoplasm appears to be "foamy" (because of numerous secondary lysosomes).

Macrophages can be identified in lymph nodes stained with H&E:

- Lymph Node #1
- A cluster of macrophages
- Lymph Node #2
- A large macrophage





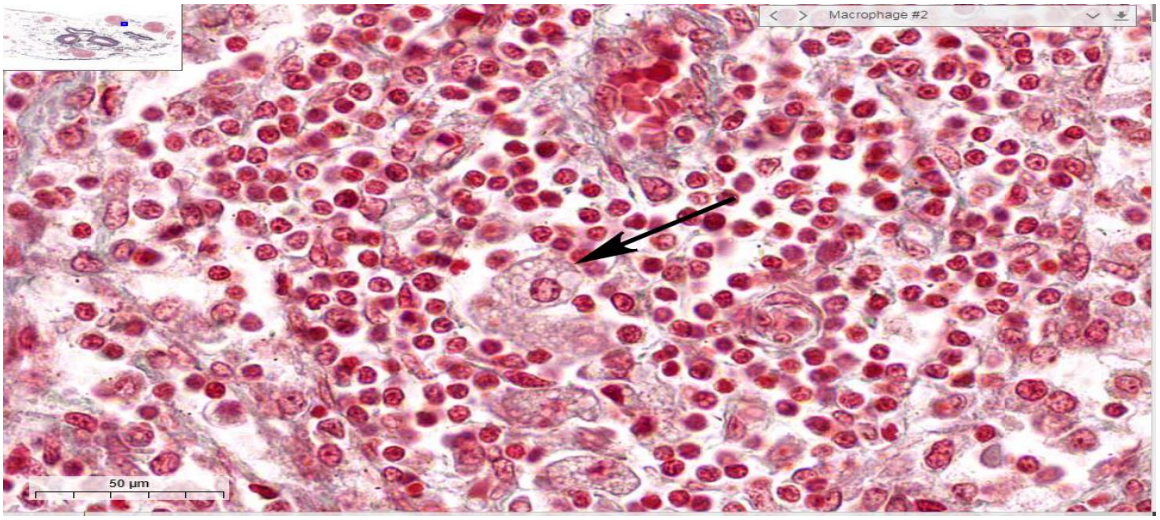
Macrophage (Azan)

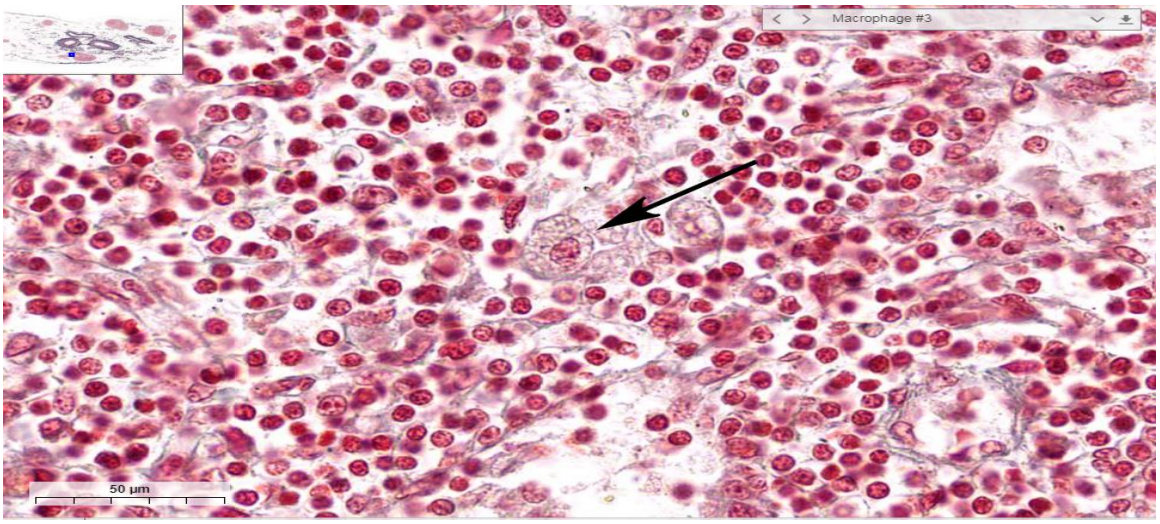
Macrophages can also be identified in lymph nodes stained with azan:

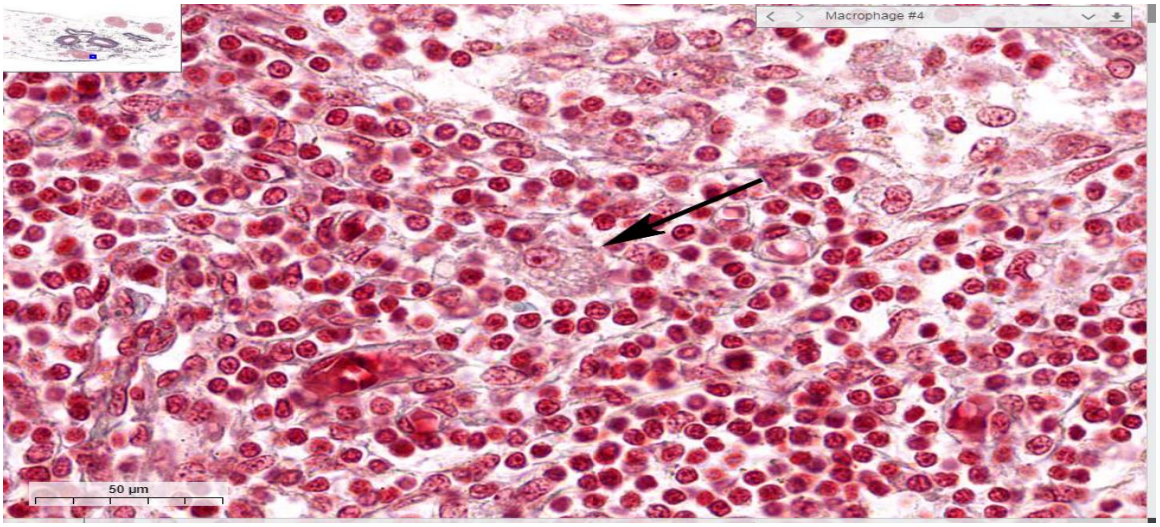
- Lymph Node #1
- Macrophages #1
- Macrophage #2
- Lymph Node #2

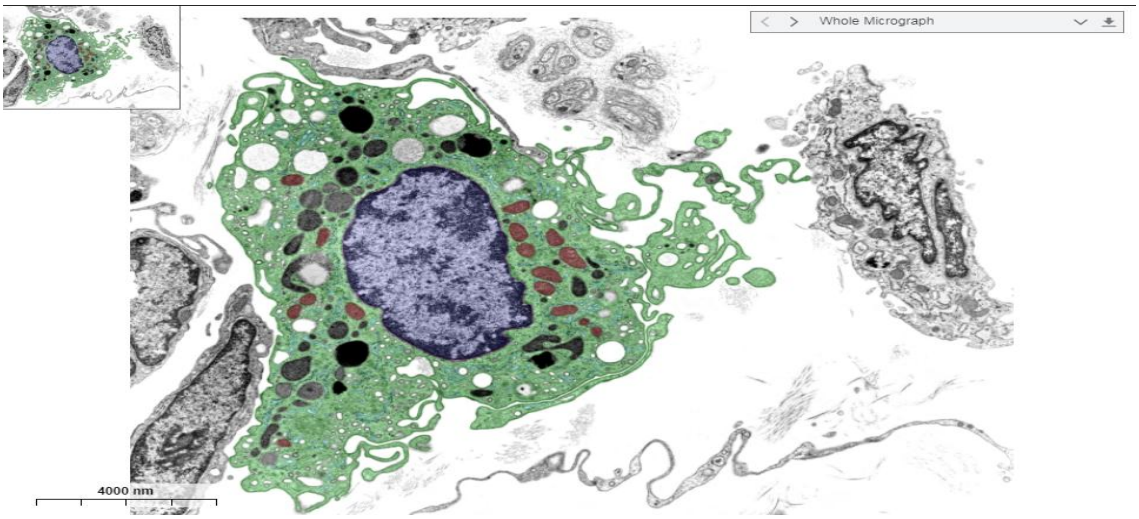
- Macrophage #1
- Macrophage #2

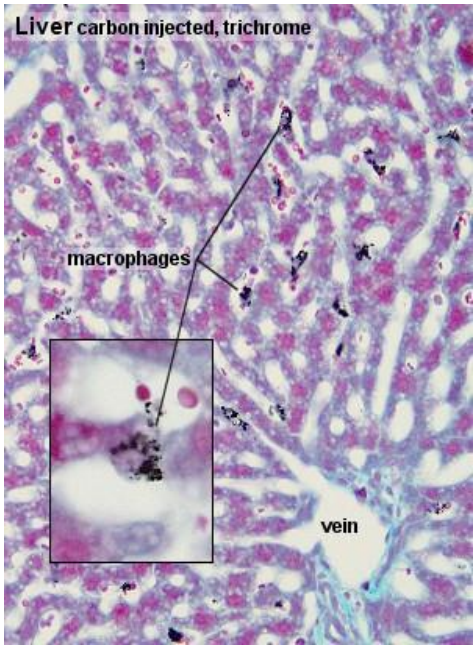
Macrophages in connective tissue can be identified by their large numbers of lysosomes, phagosomes, and residual bodies by transmission electron microscopy. See EM 103 Macrophage.











Suitable Slides

sections of liver or lymph nodes - carbon injected animal - trichrome or H&E sections of lung - H&E sections containing irregular connective connective tissues - Alcian blue & van Gieson

Liver, rabbit - carbon injected, trichrome Macrophages are usually difficult to distinguish from other cell types in connective tissues. One way to visualize them is to inject an experimental animal with very fine carbon particles. Macrophages which come into contact with the circulating particles will phagocytose some of them. In sections the particles will be visible as dark, black-brown accumulations in the cytoplasm of the macrophages.

Draw a few macrophages in situ.

Macrophages found in the liver are also called Kupffer cells. They adhere to the epithelial lining of the liver sinusoids, i.e. blood filled spaces between the liver cells. Blood will not be visible in some types of preparations and the sinusoids appear empty.

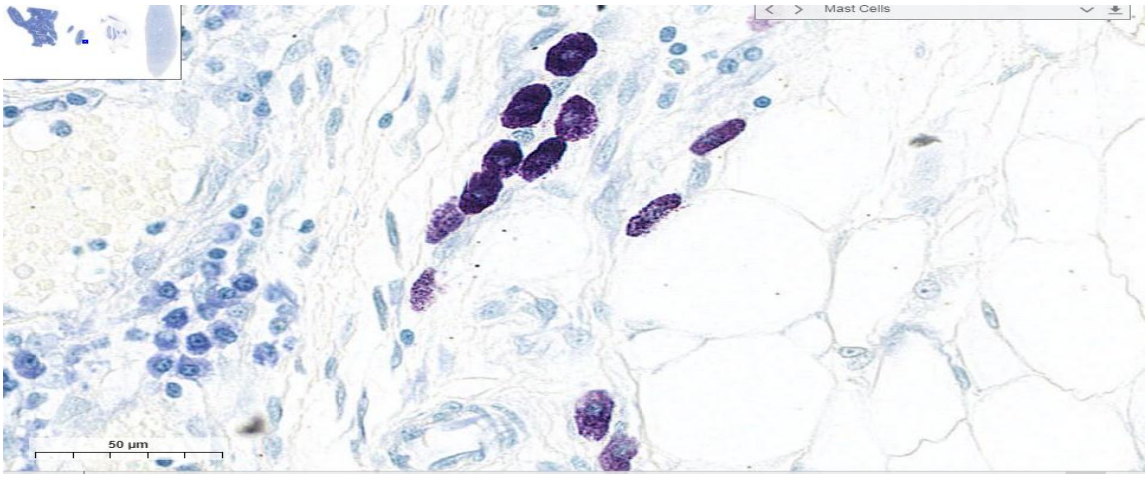
Once you have identified macrophages, go hunting for some good collagen - in trichrome stains the collagen fibres will often appear green(ish). Typically you will see them only in the connective tissue surrounding larger blood vessels. Improve your knowledge on epithelia and look out for ducts lined by a simple cuboidal or columnar epithelium. What is flowing in these ducts?

In the lung, macrophages "patrol" the respiratory surfaces and ingest airborne particles which settle there. They "retire" to the connective tissue of the lung were they can be identified by the accumulations of fine particles in their cytoplasm. In Alcian blue & van Gieson stained sections macrophages may appear as intensely green, roundish cells with a dark nucleus.

Mast cells

Mast cells are - like macrophages, lymphocytes and eosinophils - in demand when something goes wrong in the connective tissue. Quite a few of them are present in healthy connective tissue as they stand on guard and monitor the local situation. The cytolasm of mast cells is filled by numerous large vesicles. Mast cells discharge the contents of these vesicles if they come in contact with antigens, for example, proteins on the surface of an invading bacterium or, in allergic reactions, in response to antigens found, for example, on the surface of pollen grains.

The most prominent substances contained in the vesicles are heparin and histamine. They increase blood flow in close by vessels and the permeability of the vessel walls to plasma constituents and other white blood cells. By facilitating access to the area, mast cells facilitate an immune response to the antigen which triggered the release histamine and heparine.



Mast Cells

Toluidine blue is a basic dye that binds nucleic acids, but preferentially stains RNA. It also intensely stains some substances leading to a change in color. This effect known as **metachromasia**.

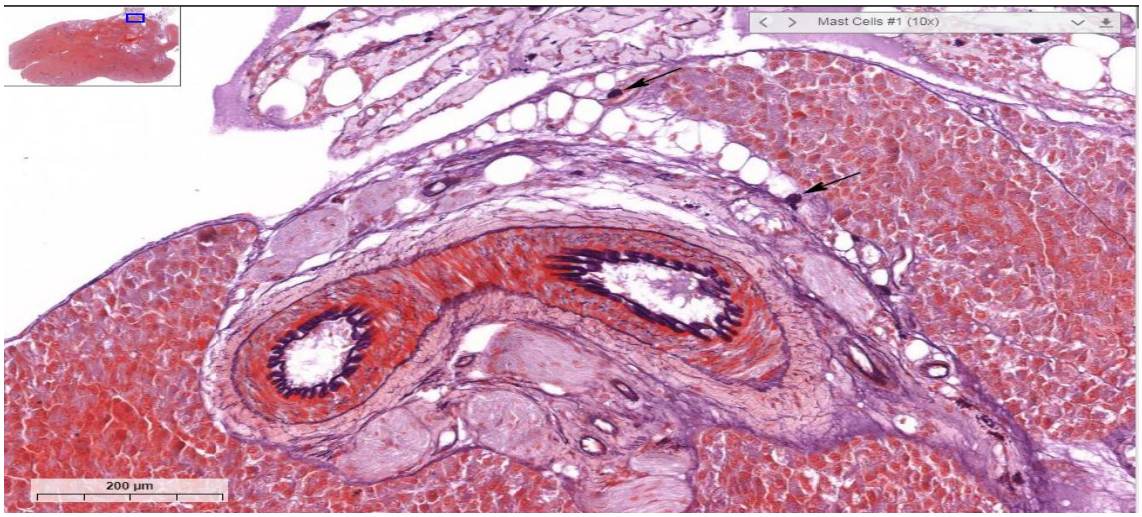
- **Orthochromatic Staining** - structures stained blue
- **Metachromatic Staining** - structures stained dark purple from polymeric aggregates formed when binding to high concentrations of polyanions

Toluidine blue is often used to identify mast cells by virtue of heparin (which is very polyanionic) found in their secretion granules.

Mast cells are scattered in the connective tissue surrounding this lymph node.

- **Mast Cells - metachromatic staining (dark purple). Oval-shaped cells with intensely stained cytoplasm.**
- Lymphocytes - orthochromatic staining (blue).

Mast cells in connective tissue can be identified by their abundant, large secretion granules by transmission electron microscopy. See EM 108 Mast Cells and EM 110 Mast Cell.



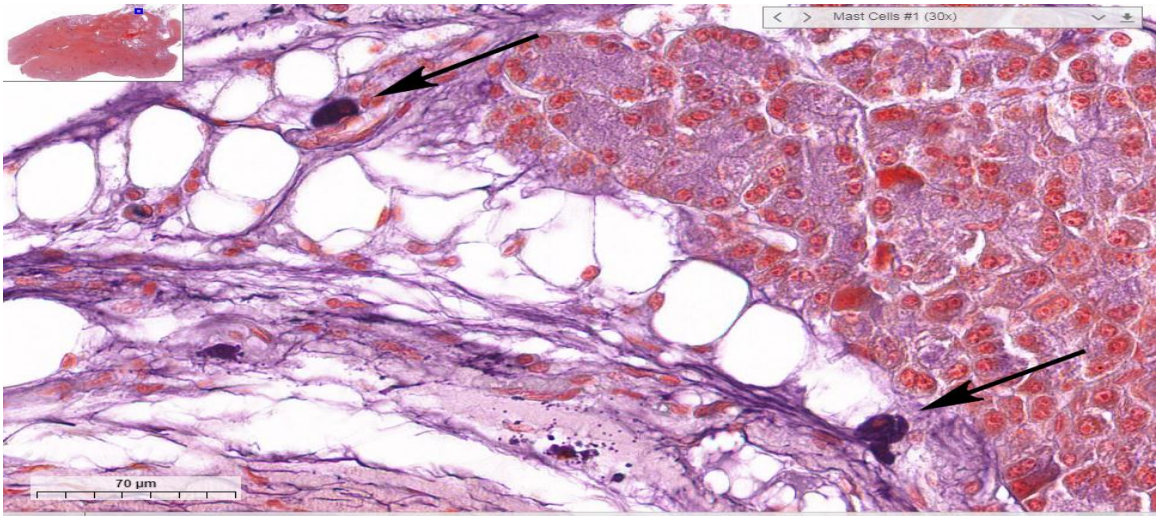
Mast Cells (Aldehyde Fuchsin)

Mast cells are involved in inflammation and immune responses. They release histamine, heparin and other inflammatory mediators.

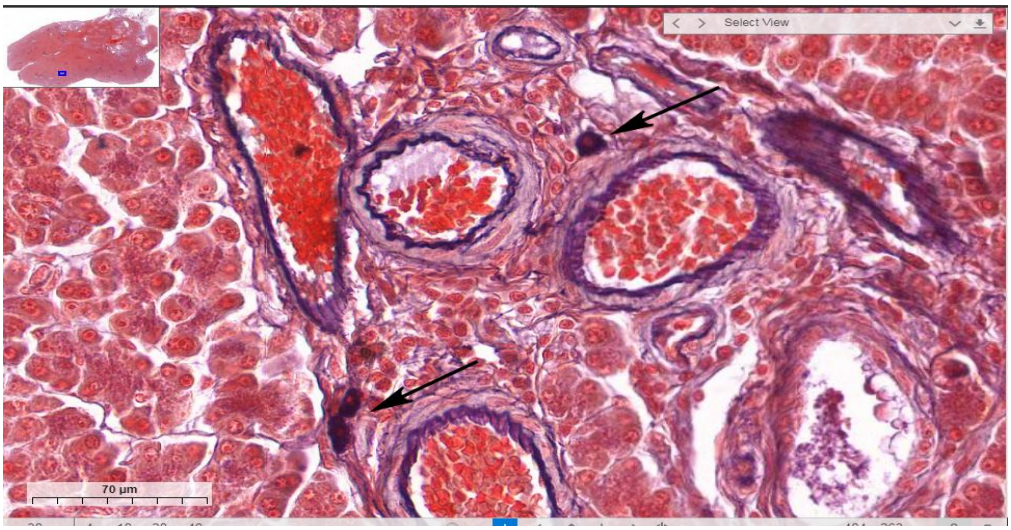
Aldehyde fuchsin produces violet staining of mast cell granules, elastic fibers, and islets of Langerhans.

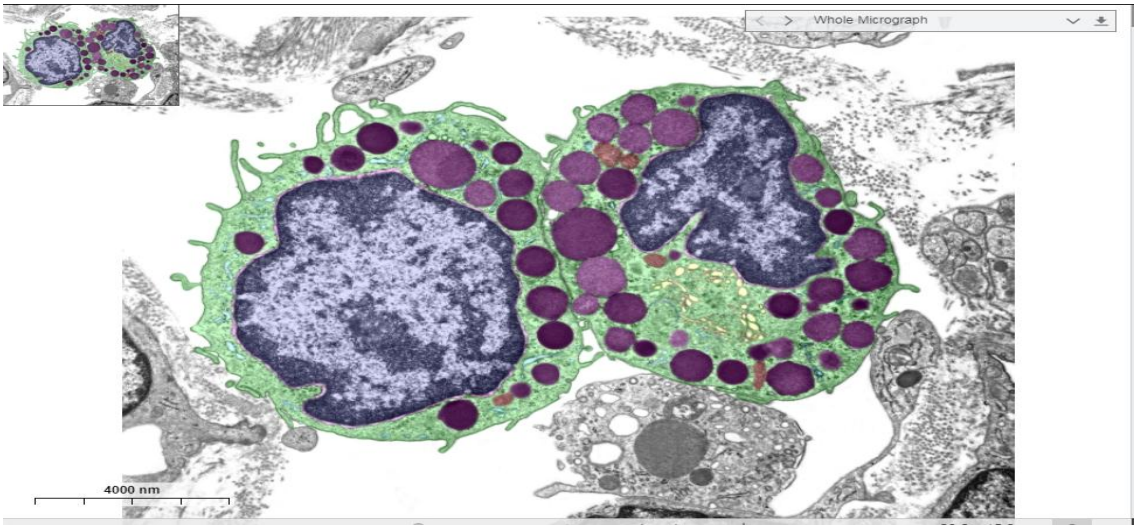
- Mast Cells - oval-shaped cells with intensely stained purple cytoplasm.
- **Mast Cells #1 - individual cells scattered in the connective tissue around blood vessels. Compare the mast cells to adjacent adipocytes.**
- Mast Cells #2 - individual cells in connective tissue around blood vessels.
- Elastic Fibers - stain purple in blood vessels.
- Islets of Langerhans - numerous islands ("islets") of beta cells with purple stained insulin-containing secretion granules. These endocrine cells are unrelated to mast cells.

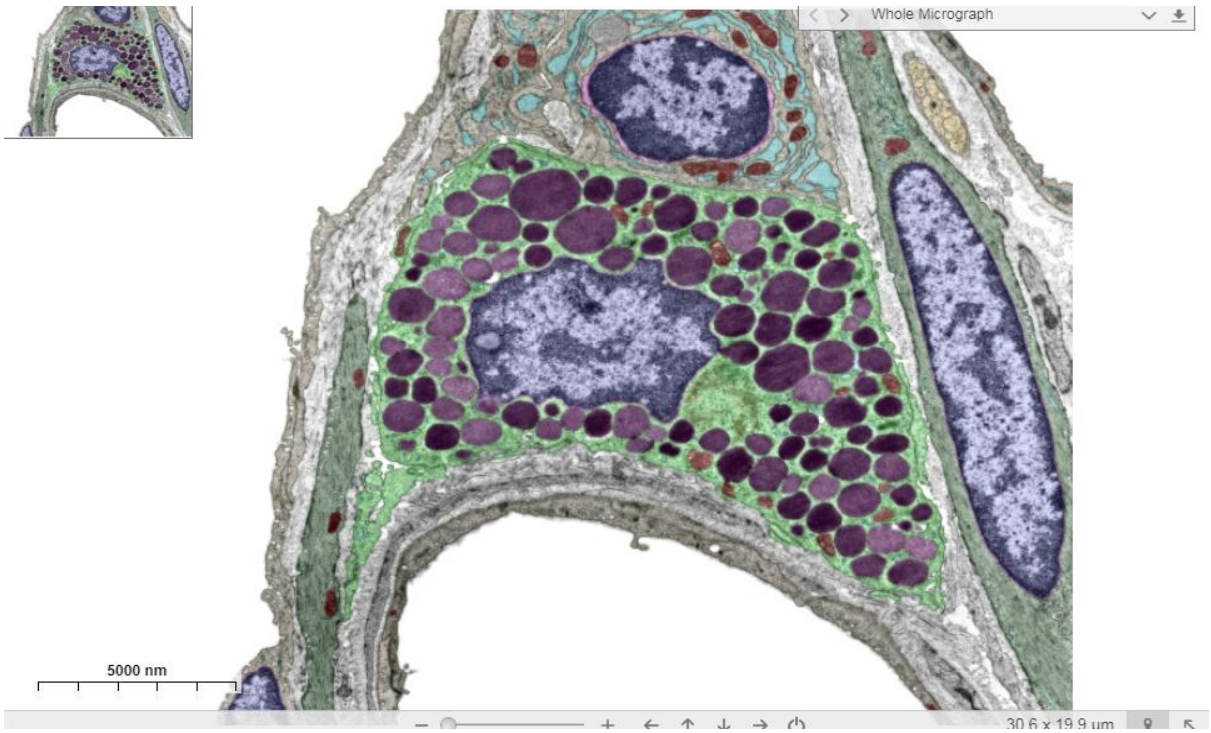
Mast cells in connective tissue can be identified by their abundant, large secretion granules by transmission electron microscopy. See EM 108 Mast Cells and EM 110 Mast Cell.



- **Mast Cells #1 - individual cells scattered in the connective tissue around blood vessels. Compare the mast cells to adjacent adipocytes.**


Select View
70 µm


Whole Micrograph
4000 nm


Whole Micrograph
5000 nm
30.6 x 19.9 µm

Mast Cell

Mast cells are immune cells that can migrate from blood into connective tissue. They engage in inflammatory responses by releasing molecules that dilate blood vessels and recruit other cells. They play a key role in allergic reactions.

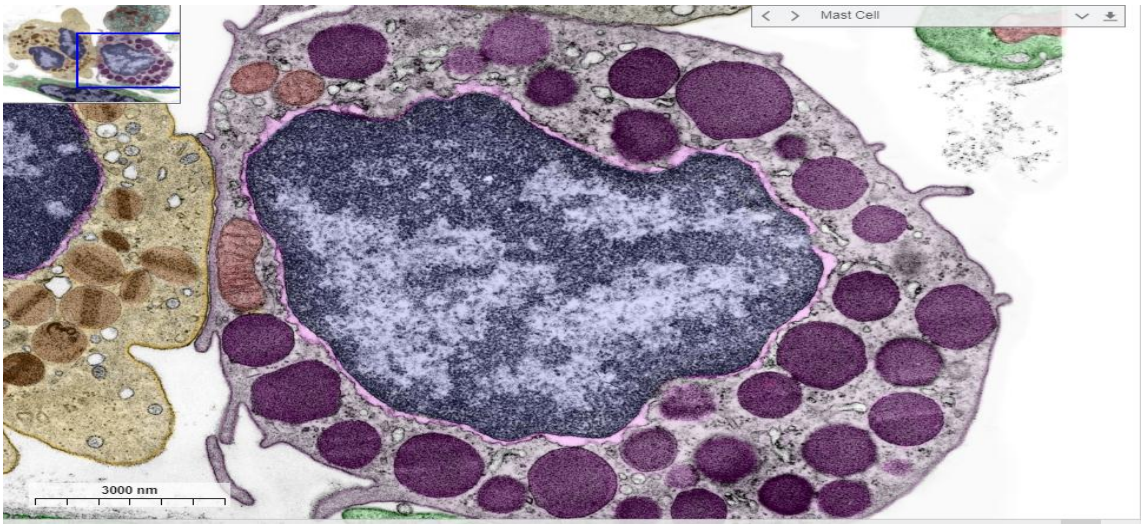
A mast cell contains abundant, large granules that contain inflammatory substances (histamine and heparan). This one is in the connective tissue enclosed by several simple squamous epithelia.

Cell Types:

- Mast cell (green) - degranulation upon stimulation by an antigen.
- Granules (purple) - heterogeneous in size, contents, and function.
- Plasma cell (tan) - abundant rough endoplasmic reticulum dilated with newly synthesized proteins (antibodies).
- Smooth muscle cells (dark green) - cytoplasm contains densely packed filaments with scattered dense bodies. Many caveolae are found on its surface.
- Simple Squamous Epithelia (tan)
- Transcytosis - many small vesicles budding from their surface.
- Small Nerve Fiber (orange) - bundle of unmyelinated axons enclosed by a Schwann cell.

Subcellular Structures:

- Nuclei (blue) / Nuclear Envelope (purple)
- Golgi Apparatus (yellow)
- Mitochondria (red)
- Endoplasmic Reticulum (cyan)



Other connective tissue cells

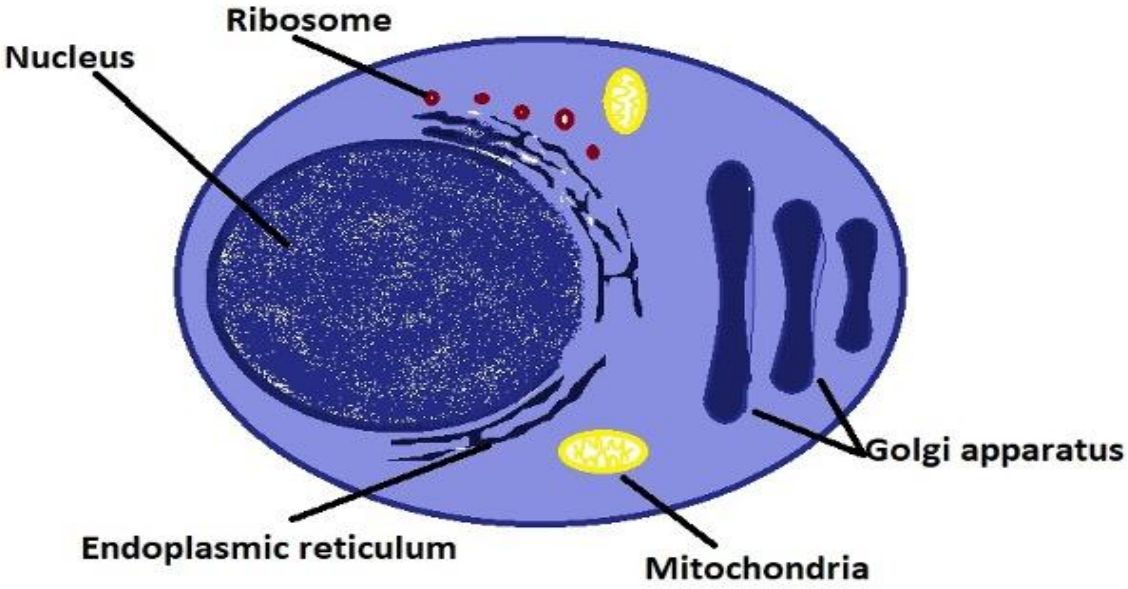
Lymphocytes and plasma cells

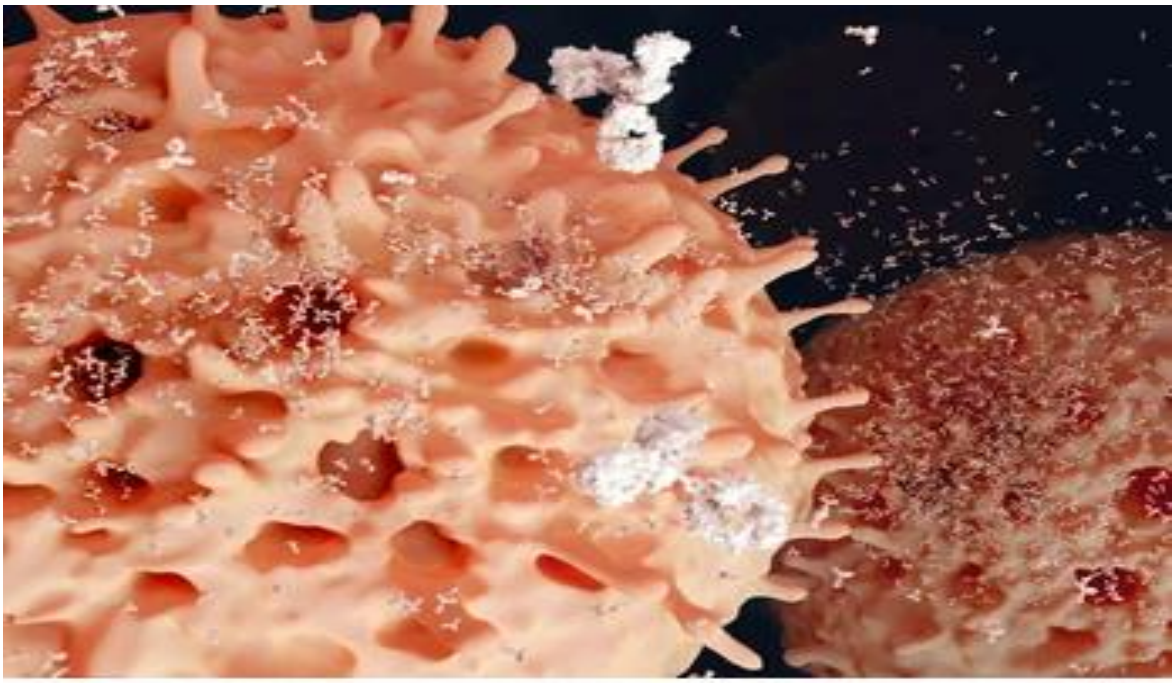
Lymphocytes are usually small cells (6 - 8 µm). Their nuclei are round and stain very dark. The cytoplasm forms a narrow rim around the nucleus and may be difficult to see. There are many of them in the connective tissue underlying the epithelia of the gastrointestinal tract but usually much fewer in other connective tissues. Again, this situation may change - in this case with immunological reactions. Some lymphocytes may differentiate into plasma cells. Plasma cells are lymphocytes which produce antibodies. To accommodate the necessary organelles for this function the size of the cytoplasm increases dramatically and the cells become basophilic. Plasma cells can occasionally be spotted in the loose connective tissue present in

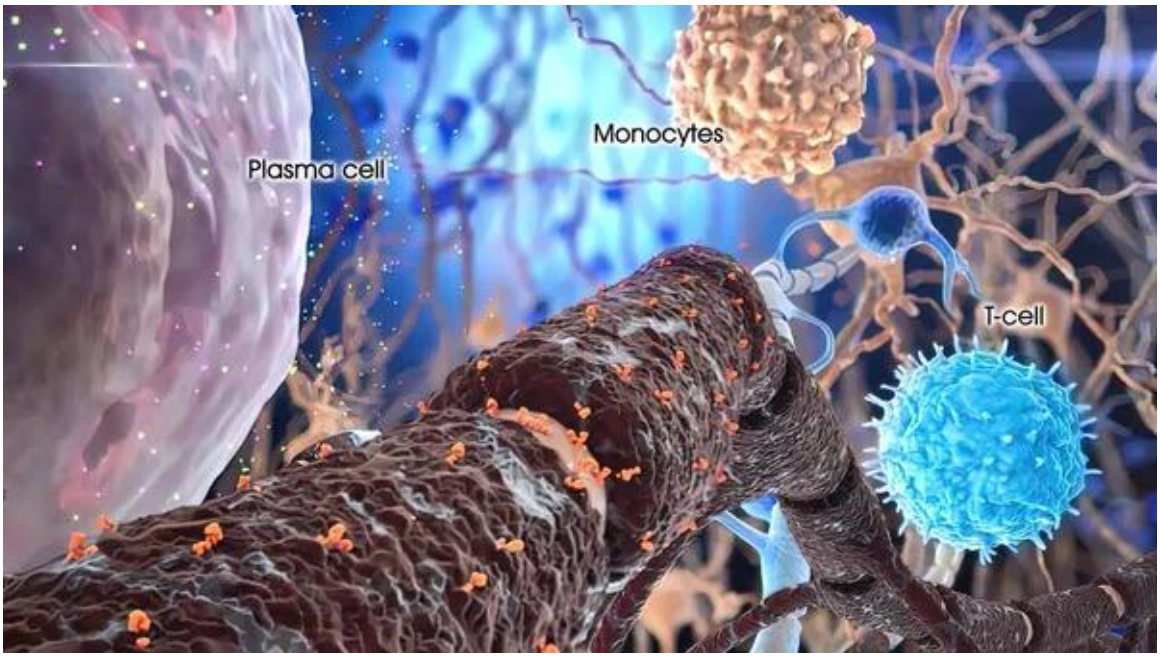
sections.
Like eosinophilic cells and monocytes, lymphocytes are white blood cells. More information about these cell types can be found on the HA235 Histology - Blood page

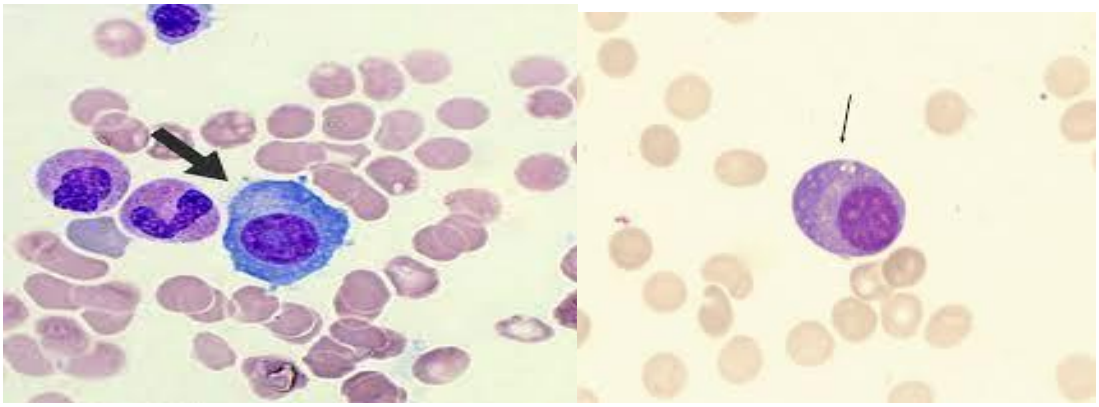
Plasma cell

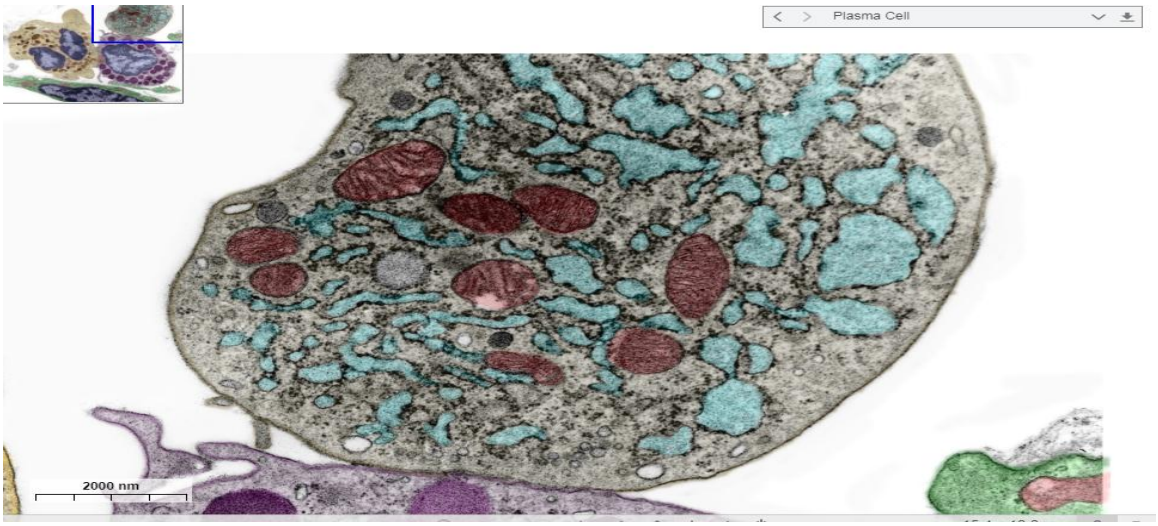
.

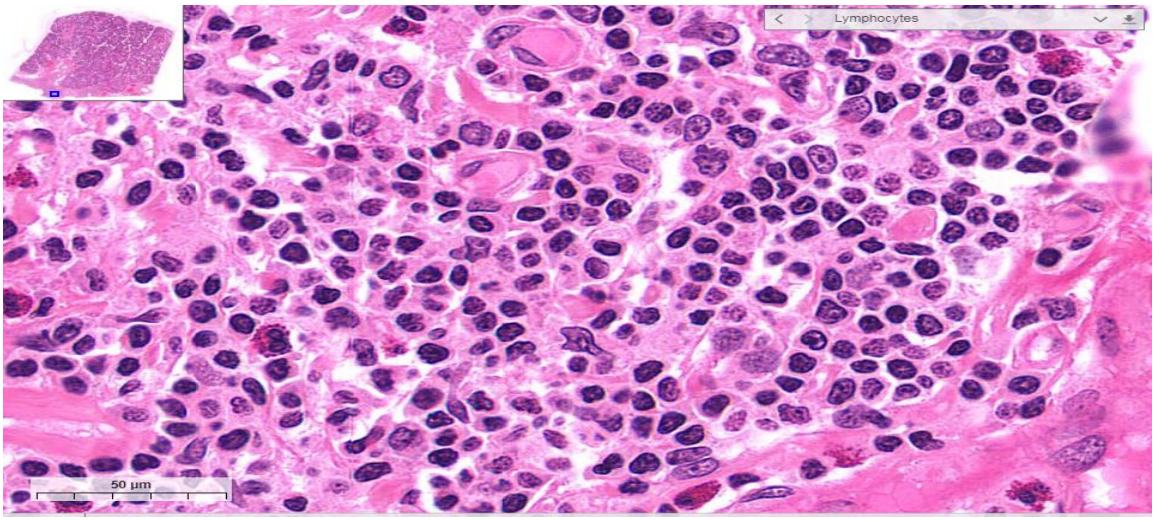




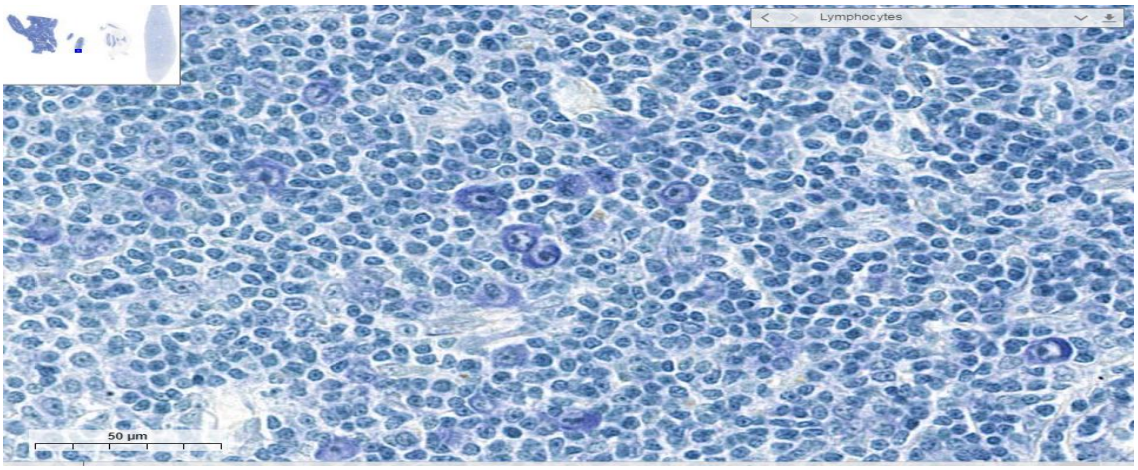

Plasma cell
Monocytes
T-cell




Plasma Cell
2000 nm



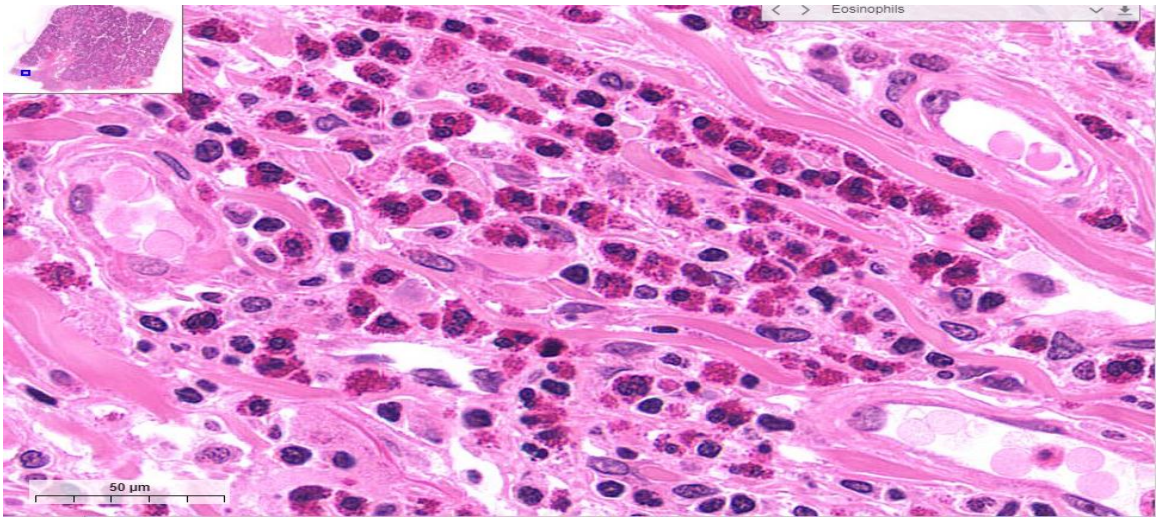
- **Lymphocytes - small cells with nuclei of mostly heterochromatin (dark purple)**



- **Lymphocytes - orthochromatic staining (blue).**

Eosinophilic cells

Eosinophilic cells are tyically rounded or oval, large cells, which contain large amounts of bright red granules in their cytoplasm. They originate, like the monocytes, in the bone marrow. They enter connective tissues early in inflammatory reactions, where they phagocytose antigen-antibody complexes. Their numbers in healthy connective tissue vary with location, but a few of them can usually be found.



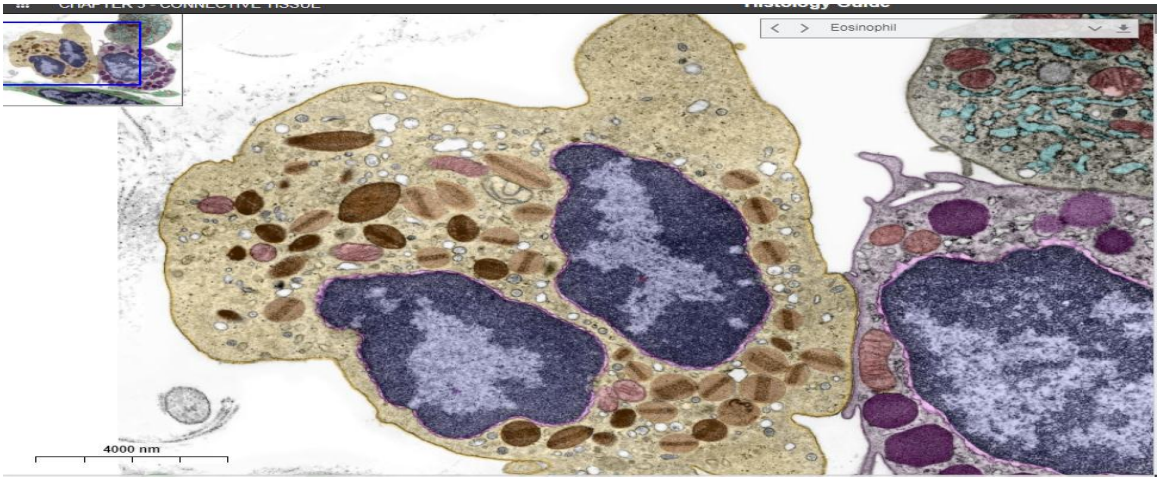
Eosinophils

Eosinophils trigger or maintain inflammatory and immune responses. They contribute to combating infections by multicellular parasites and inflammatory reactions.

This parotid has immune infiltration in the capsule of connective tissue.

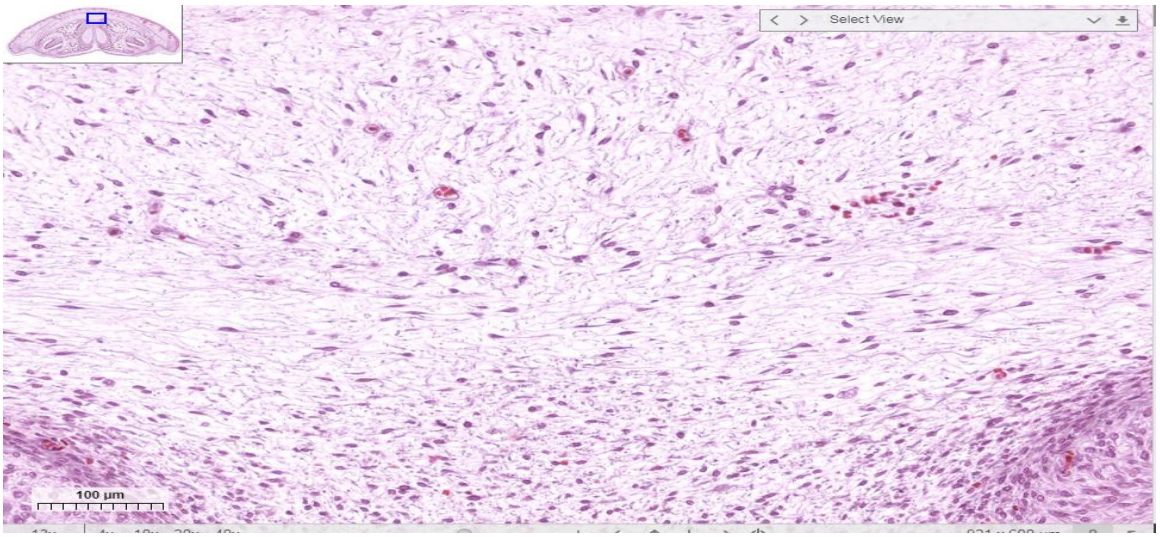
- **Eosinophils - oval-shaped cells with eosinophilic secretion granules (bright pink/magenta) in their cytoplasm**
- Lymphocytes - small cells with nuclei of mostly heterochromatin (dark purple)

Eosinophils in connective tissue can be identified by their large, ellipsoid granules with a linear, crystalline core by transmission electron microscopy. See EM 161 Eosinophils.



Mesenchymal cells

During development, mesenchymal cells give rise to other cell types of the connective tissue. A small number of them may persist into adulthood. Mesenchymal cells are smaller than fibrocytes and difficult to detect in histological sections. They may regenerate blood vessels or smooth muscle which have been lost as a consequence of tissue damage.



Mesenchyme

This fetal pig snout contains excellent examples of mesenchyme. It is an early form of connective tissue found in embryos.

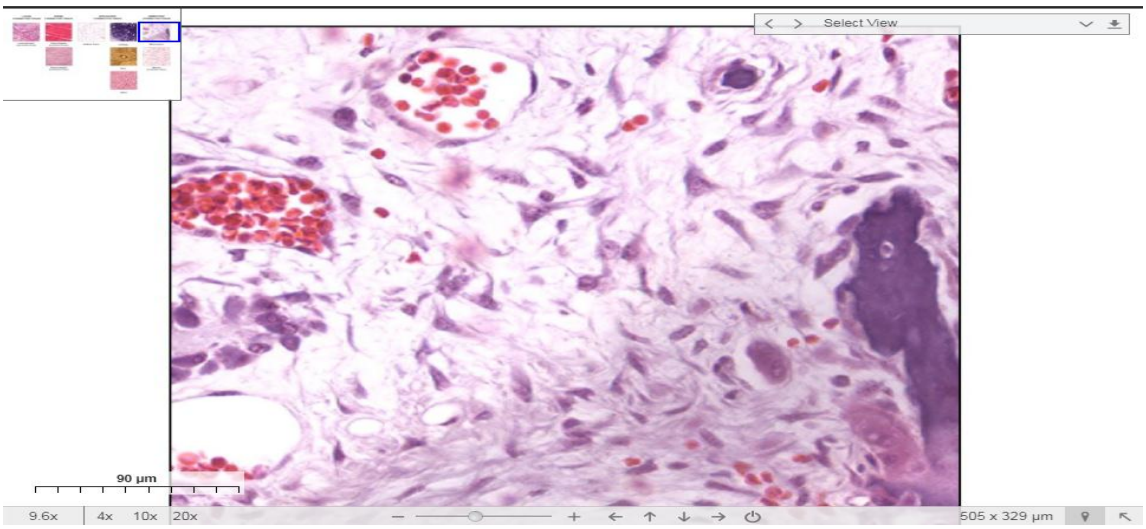
Mesenchyme is composed mainly of ground substance with few cells or fibers. Mesenchymal cells differentiate into other cell types such as fibroblasts, fat, cartilage and bone.

Mesenchyme is found between the differentiating tissues.

- **Mesenchymal Cells - stellate or spindle-shaped (fusiform) cells with round to oval nuclei. Easy to identify when three or more processes are visible.**
- Dental Papilla - condensed mesenchymal cells within the developing tooth..
- Fibroblasts - spindle-shaped (fusiform) cells with elongated nuclei.

Other structures:

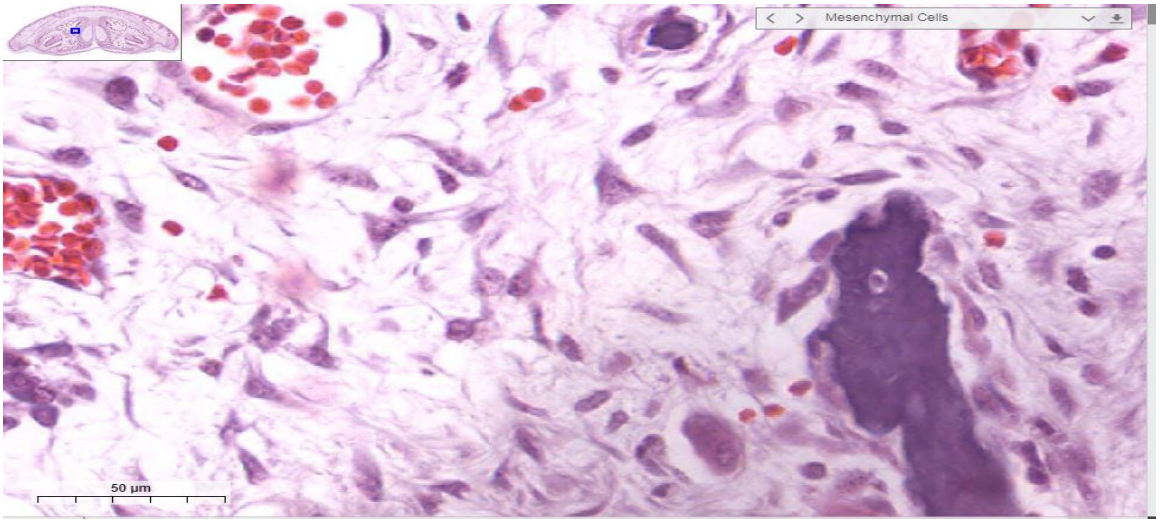
- Skin - a stratified squamous epithelium that covers the specimen. This fetal epithelium is very hydrated giving it an unusual, "fluffy" appearance.
- Cartilage - the two large, oval structures near the midline with sparse cells.
- Bone - basophilic spicules often with cells on their surface.
- Tooth - a protrusion of tissue that will develop into a tooth.



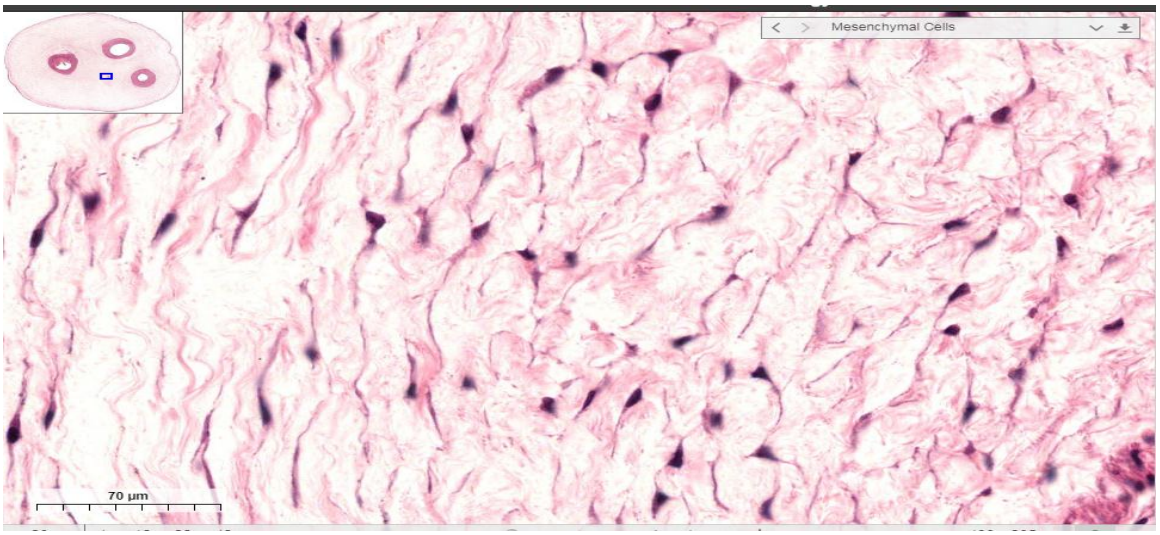
Connective Tissue

Connective tissue is classified based on the type of cells, arrangement and type of fibers, and composition of the extracellular matrix.

- **Connective tissue proper**
- Loose (areolar) connective tissue - highly cellular with a sparse, random arrangement of collagen fibers (and some elastic fibers)
- Dense connective tissue - collagen fibers with little ground substance
- Dense regular connective tissue - collagen fibers oriented in the same direction
- Dense irregular connective tissue - collagen fibers woven in multiple directions
- **Specialized connective tissues**
- Adipose tissue - storage of fats in adipocytes (more cells than extracellular matrix)
- Cartilage - extracellular matrix of type II collagen fibers and a highly-hydrated ground substance
- Bone - calcified extracellular matrix
- Blood - fluid matrix containing cells
- **Embryonic connective tissue**
- **Mesenchyme - forms connective tissue between developing organs**
- **Mucous connective tissue - found in the umbilical cord**



- **Mesenchymal Cells - stellate or spindle-shaped (fusiform) cells with round to oval nuclei. Easy to identify when three or more processes are visible.**



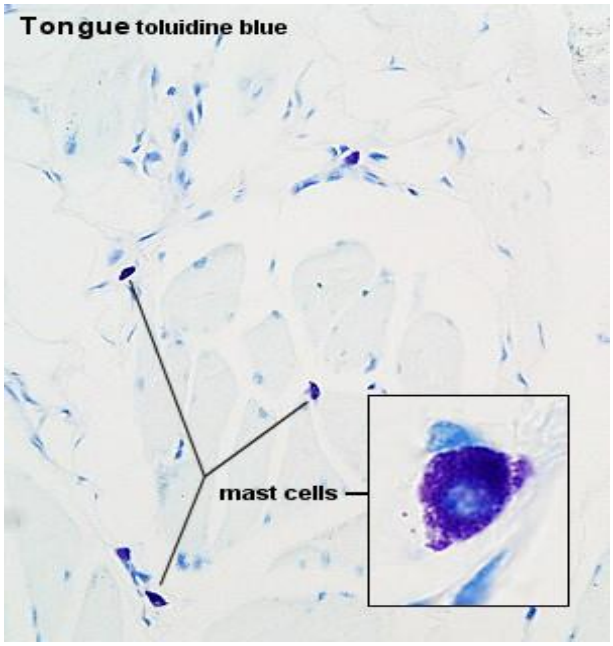
Mucoid Connective Tissue

The umbilical cord normally contains two arteries (umbilical arteries) and one vein (umbilical vein) embedded within connective tissue named Wharton's jelly.

Mucoid connective tissue (or Wharton's jelly) is a gelatinous substance that is mostly ground substance (primarily hyaluronic acid and chondroitin sulfate) with a low abundance of collagen or reticular fibers.

Near the remnant of the allantois are many stellate and spindle-shaped (fusiform) cells. Most of these are **mesenchymal cells. However, spindle-shaped mesenchymal cells are indistinguishable from resting fibroblasts (which also may be spindle-shaped).**

Suitable Slides



sections of tongue, skin or other tissues or organs containing epithelia and / or loose connective tissue - toluidine blue

Tongue, Mast Cells - toluidine blue Mast cells are relatively frequent beneath the stratified squamous epithelium which covers the surface of the tongue, where they are scattered among the other cells found in the connective tissue which supports the epithelium. In most connective tissue cells only the nucleus is stained by the toluidine blue. The cytoplasm of the mast cells is however filled with dark, blue / violet grains which represent their secretory vesicles. At low magnification mast cells stand out as large, dark dots among smaller and lighter stained nuclei and among the very weakly stained remaining connective tissue components.

Draw a few mast cells in situ and label both the mast cells and some of the surrounding tissue components.

Connective Tissue Types

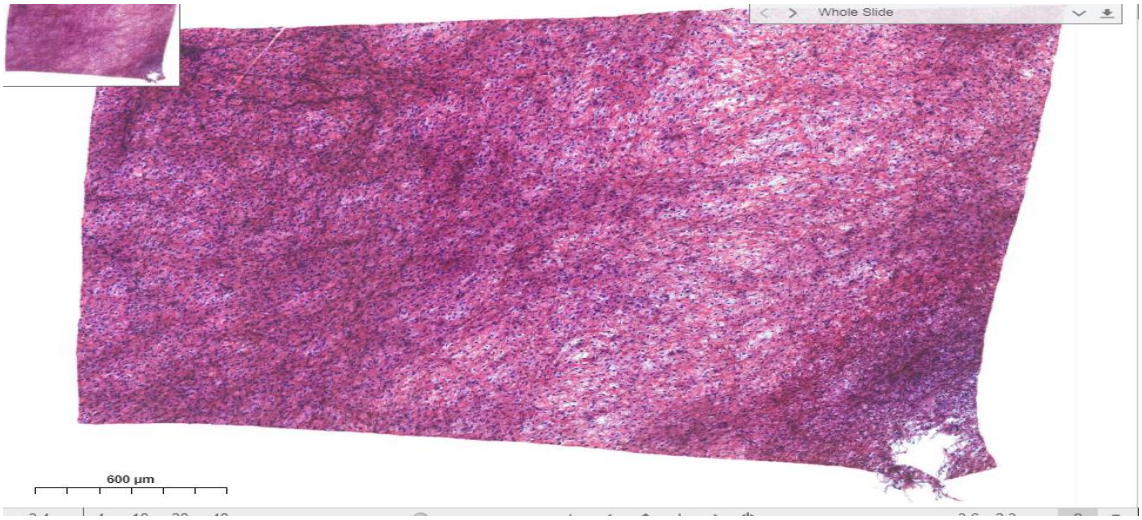
Loose connective tissue and dense connective tissues

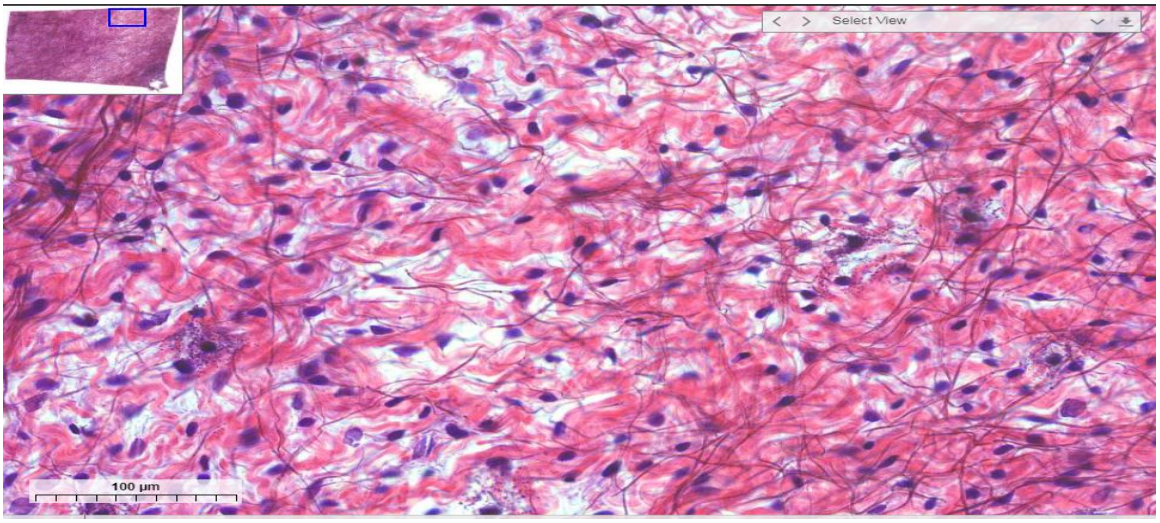
These two tissues are distinguished according to the relative amounts of fibres they contain. Dense connective tissues are completely dominated by fibres. They are subdivided according to the spatial arrangement of the fibres in the tissue.

In dense irregular connective tissue the fibres do not show a clear orientation within the tissue but instead form a densely woven three-dimensional network. A good example is the dermis of the skin.

We talk about regular dense connective tissue if the fibres run parallel to each other. Good examples of regular dense connective tissue are tendons, ligaments and the fasciae and aponeuroses of muscles.

Loose connective tissue is relatively cell rich, soft and compliant. It is also rich in vessels and nerves. It is best understood as a kind of generalised connective tissue in which all connective tissue cell types may occur. Loose connective tissue may occur in some special variants: mucous connective tissue, reticular connective tissue and adipose tissue.





Loose Connective Tissue

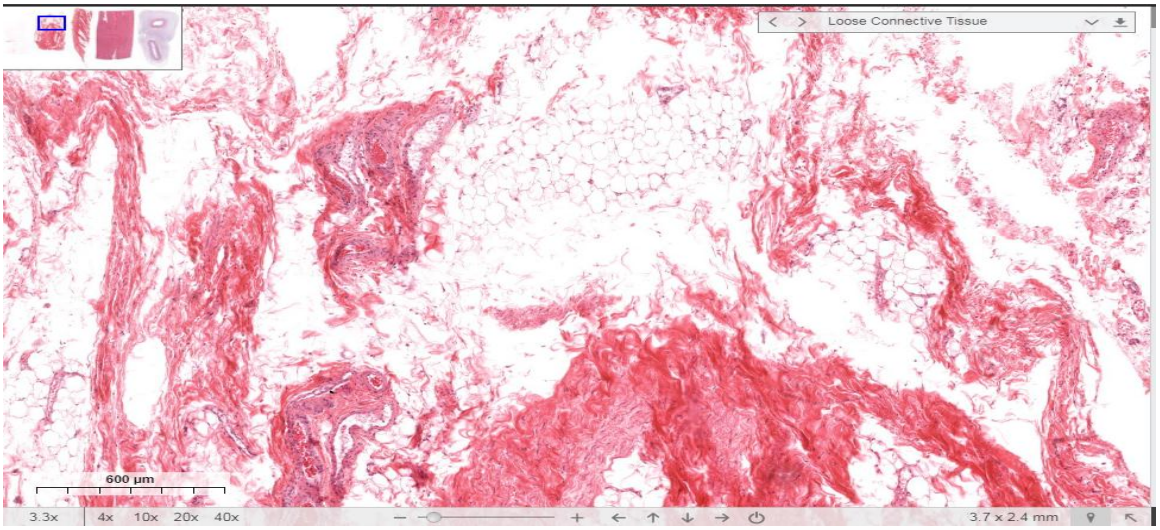
Mesentery is an example of loose (areolar) connective tissue. This whole mount is from the layer of collagen fibers on its surface.

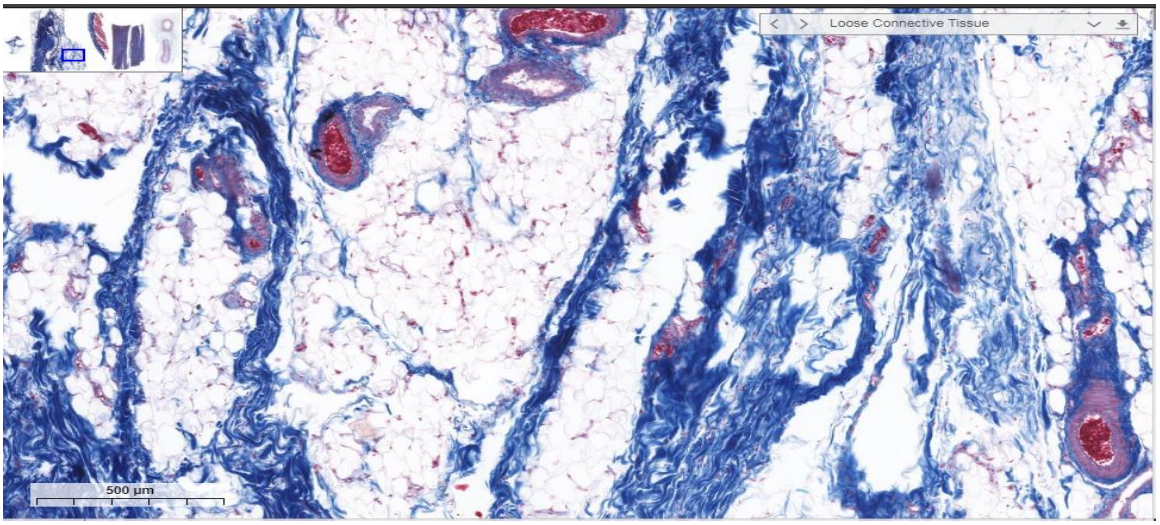
Fibrous components:

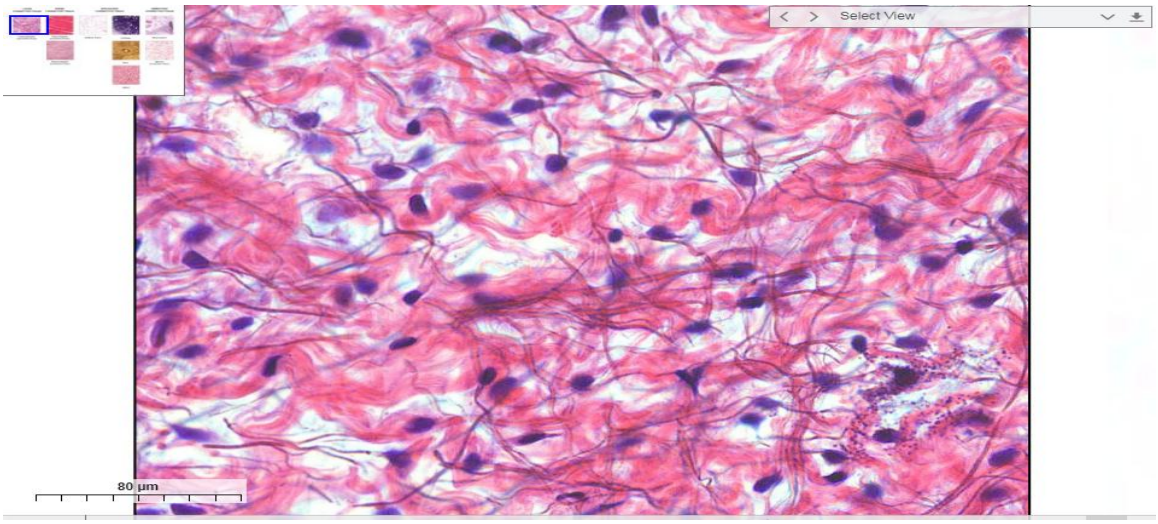
- Collagen Fibers - abundant, irregular bundles stained pink/red
- Elastic Fibers - scattered, thin dark strands stained blue/purple

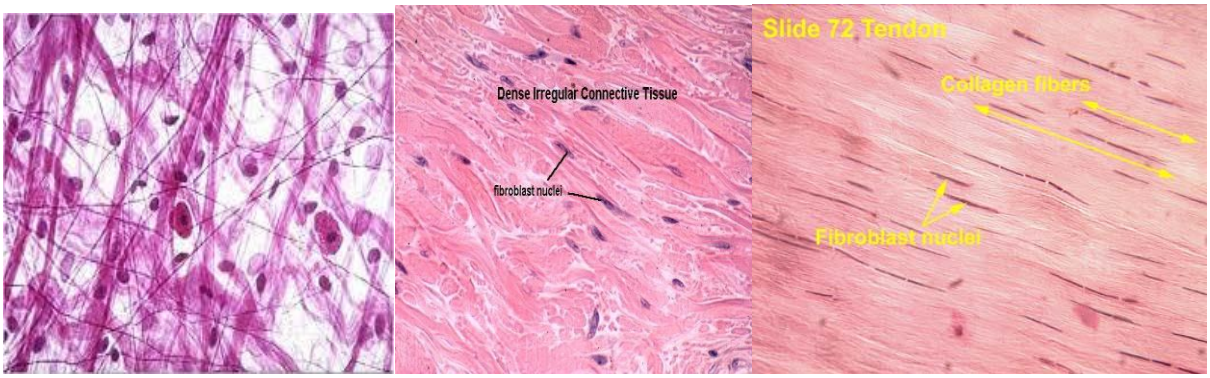
Cells:

- Fibroblasts - most of the nuclei are from fibroblasts. Their thin processes (stained pink) are not visible largely because they blend in with the collagen fibers.



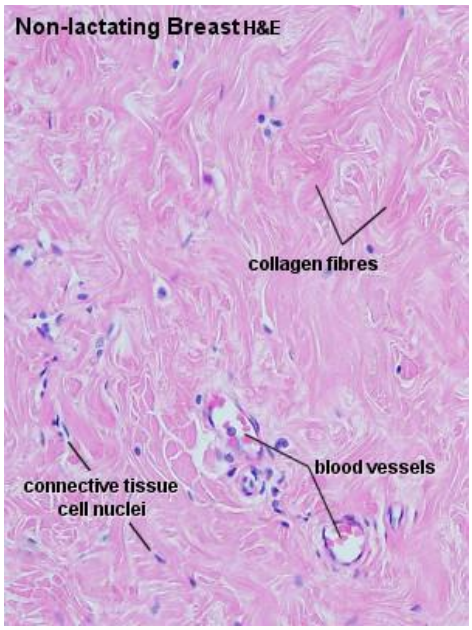






Suitable Slides

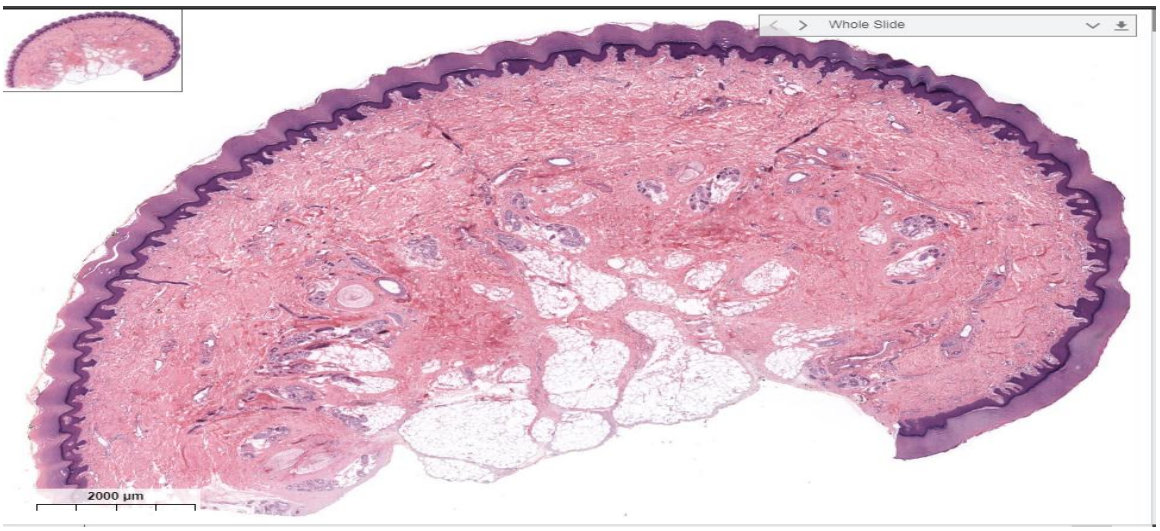
sections of tendons or ligaments - van Gieson, H&E Muscle-Tendon Junction, rat - van Gieson . In van Gieson stained preparations collagen stains dark red while other tissue components appear in varying shades of grey and yellow. Areas of dense regular connective tissue are usually easy to identify in these preparations. Coarse collagen fibres are aligned with each other with only narrow opens spaces between them. Like in most other connective tissues, there will be only a few cells between the fibres. Their cytoplasm will be difficult to identify but the nuclei can be seen scattered among the collagen fibres. Nuclei are often elongated. Their long axis runs parallel to the course of the collagen fibres Sketch part of the regular dense connective tissue.



Suitable Slides

sections of skin or non-lactating mammary gland - H&E, van Gieson, trichrome Non-lactating Breast - H&E Dense irregular connective tissue forms the dermis of the skin, i.e. the layer of connective tissue immediately below the epithelium lining the surface of the skin. Beneath the skin forming the mammae (nipples), dense connective tissue areas are very extensive. This tissue surrounds the resting mammary gland. Both the high density of collagen fibres and the their irregular distribution are easily visible. Again, only a very small

fraction of the tissue is taken up by cells. Like in van Gieson stained preparations, their cytoplasm is often not visible in H&E stained sections. Dark spots scattered between the collagen fibres represent the nuclei of the cells. **Draw part of the connective tissue including some fibrocytes.**



Dense Irregular Connective Tissue

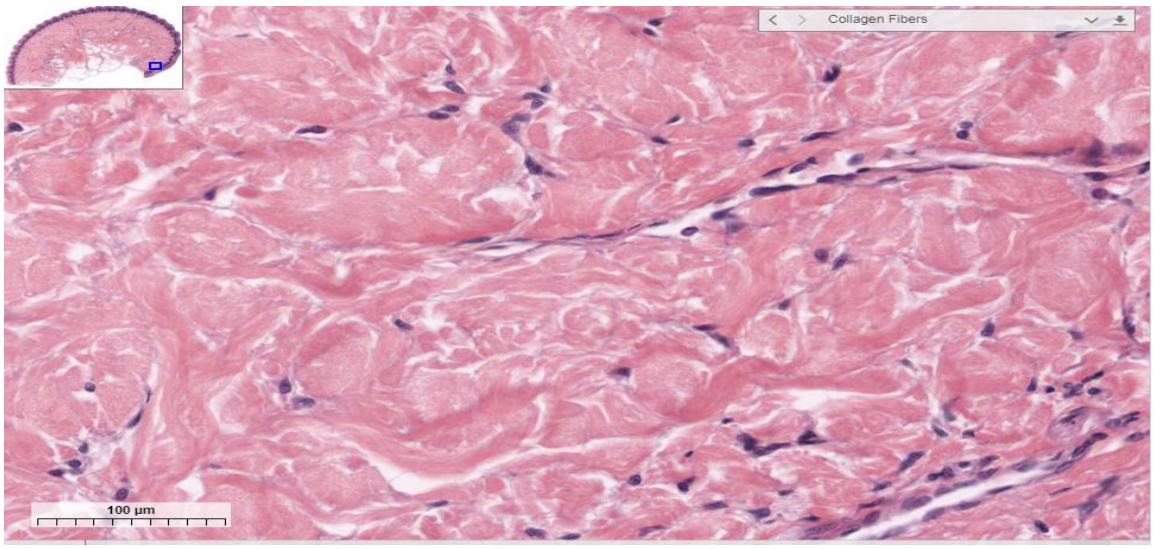
Dense irregular connective tissue contains collagen fibers arranged into irregular, interwoven bundles. (Some elastic fibers are also present.)

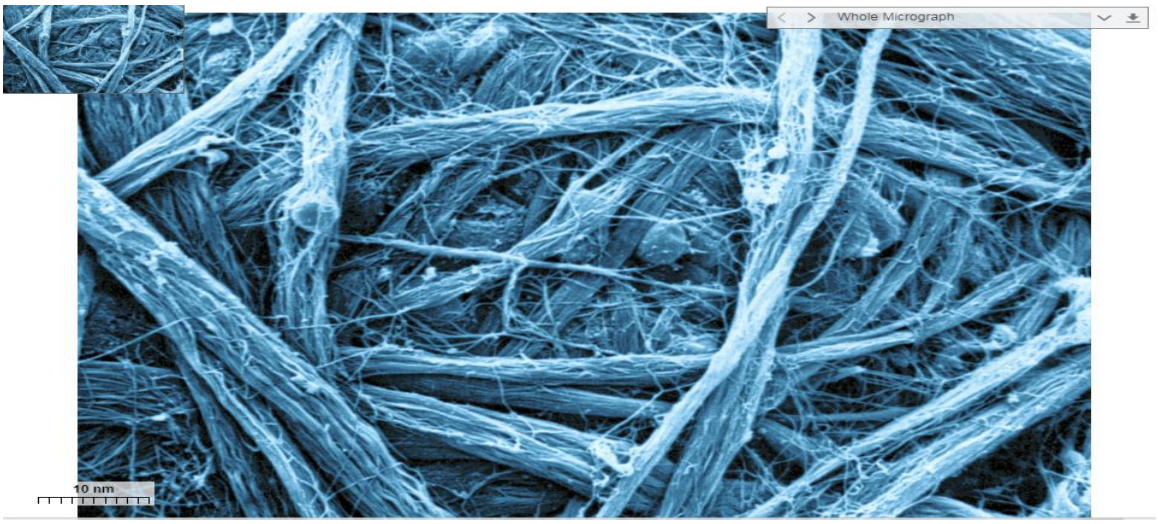
Dense irregular connective tissue is found prominently in the dermis of the skin and the lamina propria of the gastrointestinal tract.

The dermis is the layer of skin between the epidermis (stratified squamous keratinized epithelium) and the subcutaneous tissue. The dermis provides tensile strength and elasticity to skin.

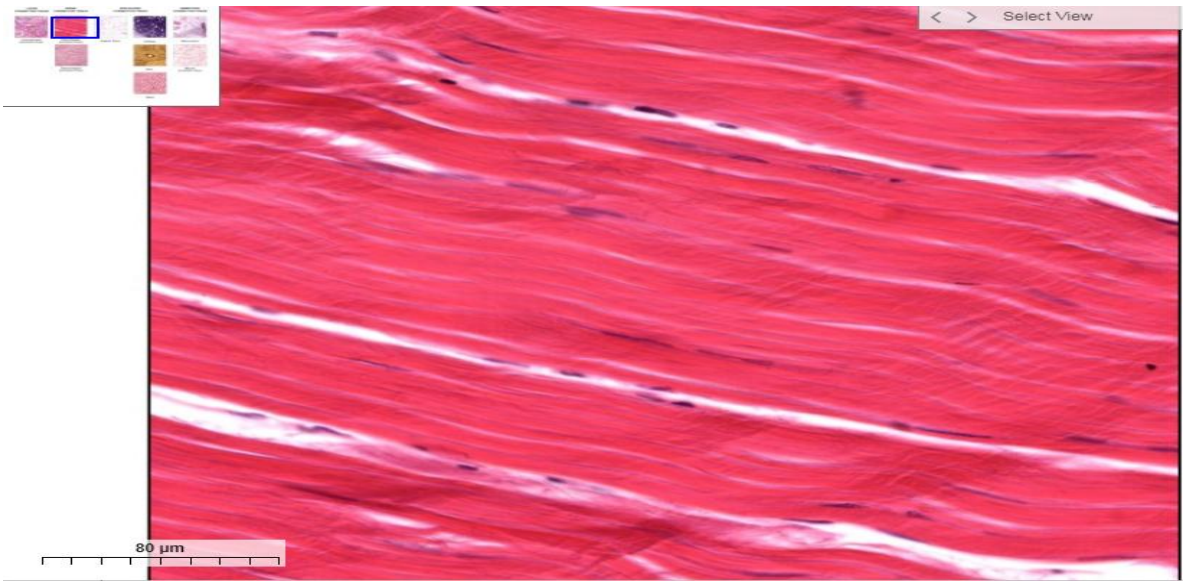
Fibroblasts are the most common cell type.

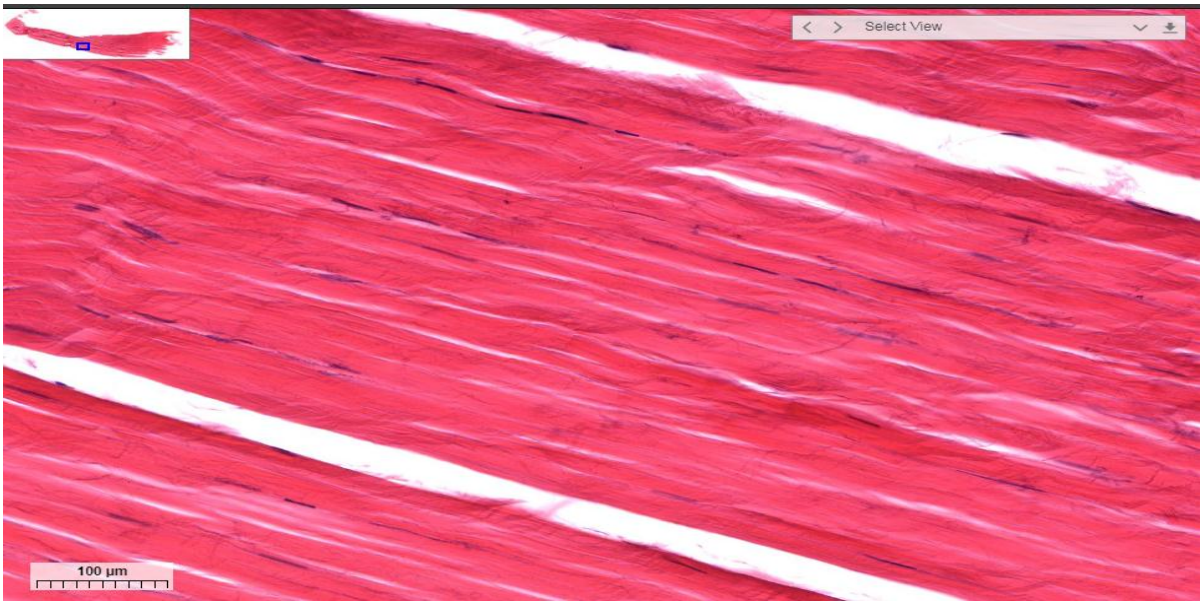
By scanning electron microscopy, the interwoven collagen fibers are easily seen. See EM 283 Dense Irregular Connective Tissue.





The excretory ducts of the mammary glands are called lactiferous ducts. They are lined by a quite nice stratified columnar epithelium. If you are working with a section of non-lactating breast look for the lactiferous ducts in the connective tissue.



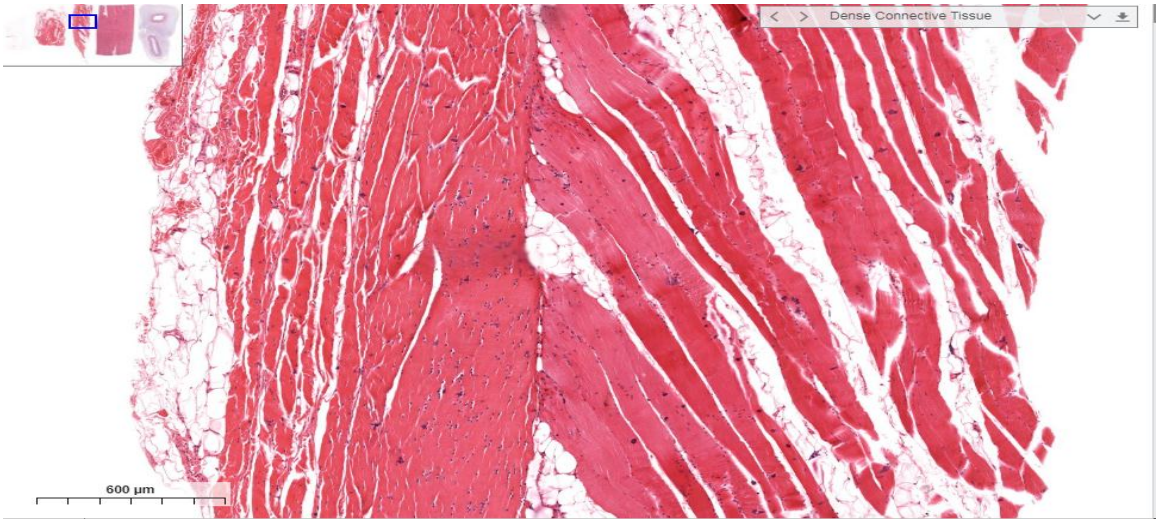


Dense Regular Connective Tissue

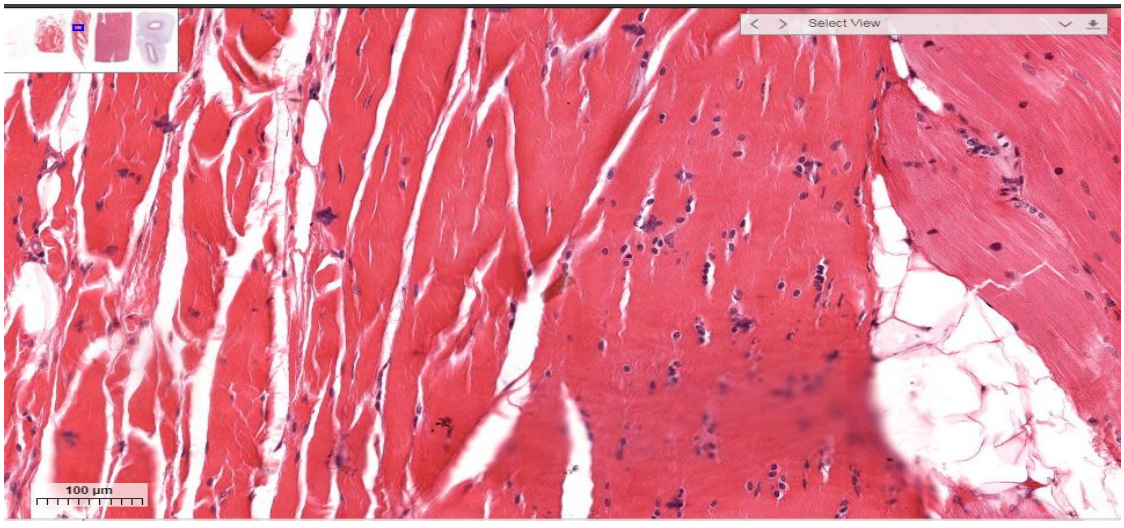
Dense regular connective tissue contains densely packed collagen fibers arranged in parallel bundles.

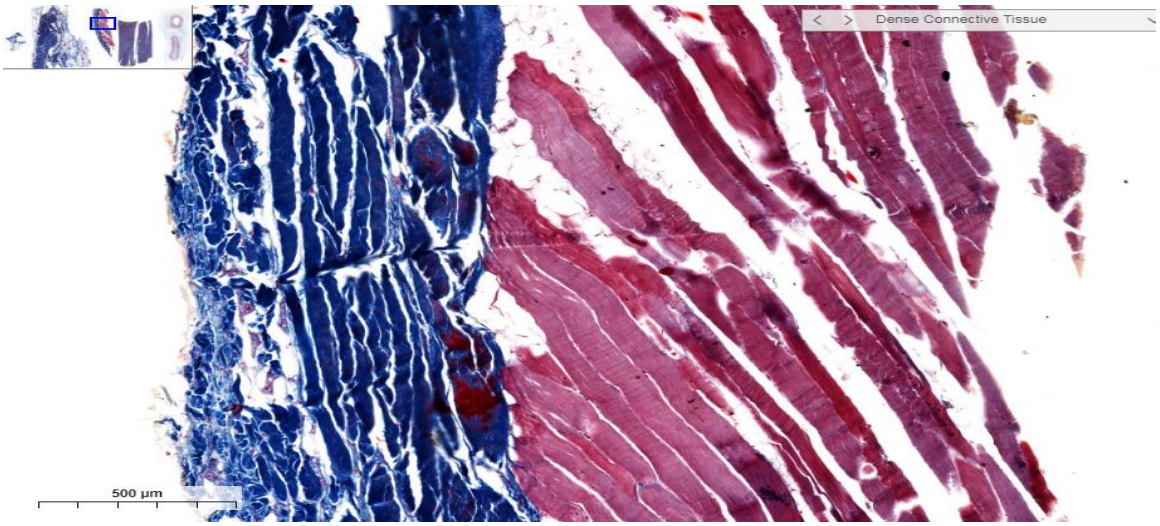
It is found in tendons (joins muscles to bone or cartilage) and ligaments (bone to bone). The primary function of the collagen fibers is to provide tensile strength to tissues.

The fibroblasts that produced the fibers are relatively inactive in adults. They reside in close proximity to the collagen fibers and often only their flattened nuclei are visible. Their sparse cytoplasm is not visible largely because it blends in with the collagen fibers.



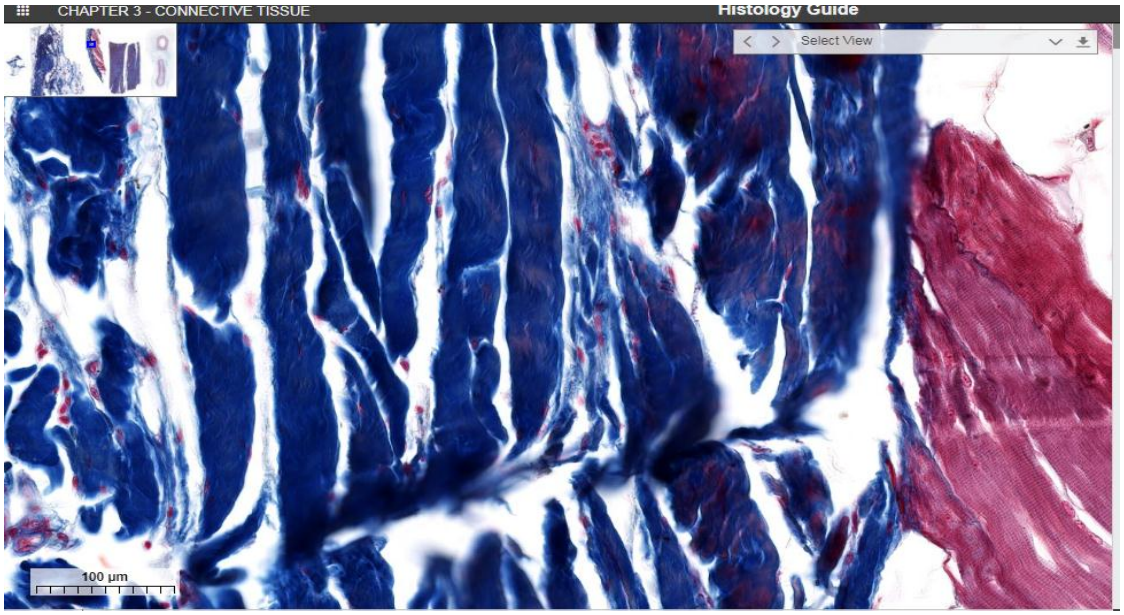
Dense regular connective tissue with both collagen and elastic fibers. These are difficult to distinguish from each other with H&E. (Verhoeff stain can be used to identify elastic fibers.)

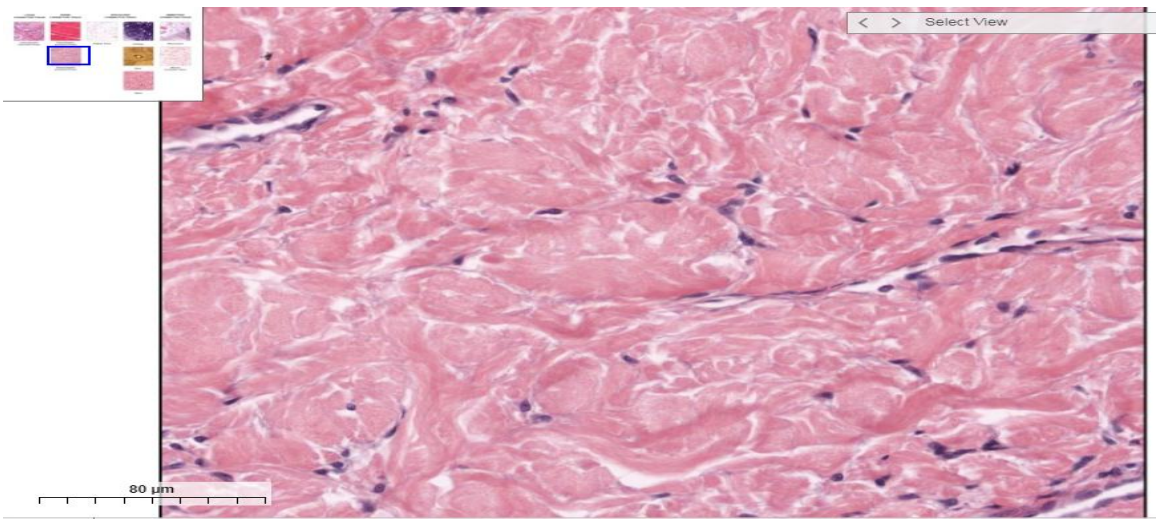




Azan

- **Dense Connective Tissue** - fascia lata is an example of dense connective tissue. The fascia lata is connective tissue that encloses thigh muscles. Collagen fibers are located to the left are stained blue and muscle fibers to the right are stained red.





Reticular connective tissue

Reticular connective tissue consists of reticular cells and the network of reticular fibres formed by them. Most connective tissues contain reticular fibres, but only in reticular connective tissue are they the dominant fibre type. In a number of tissues and organs, reticular connective tissue forms the structural framework in which the cells of the organ are suspended. The open meshwork of fine fibres is particularly useful in tissues and organs in which diffusion and / or cell movements are functionally important, for example, in the liver, lymph nodes and the spleen.

Adipose tissue

Adipose tissue is essentially loose connective tissue containing large numbers of adipocytes. There are two types of adipose tissue, which derive their names from the colour of the tissue (white or brown) and/or the number of lipid droplets found in the adipocytes.

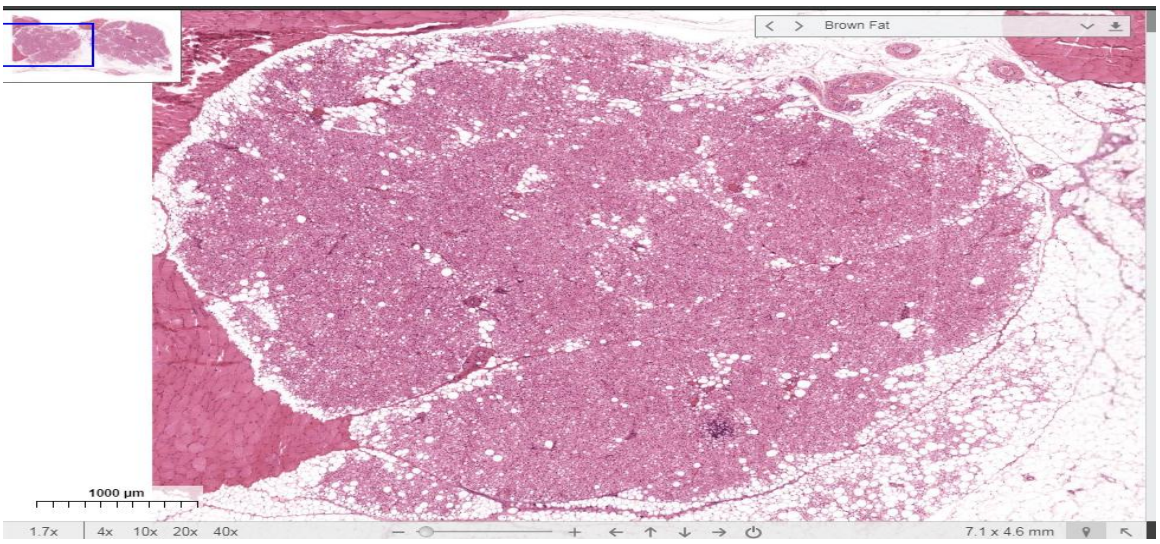
- Adipocytes of white, unilocular adipose tissue contain one large lipid droplet.
- Adipocytes of brown, multilocular adipose tissue contain many lipid droplets.

The storage/mobilisation of lipids does require quite some metabolic activity of the tissue. Consequently, adipose tissue has a rich supply of capillaries. Lipid storage/mobilisation is under nervous (sympathetic) and hormonal (insulin) control.

White adipose tissue does not only function in the storage of lipids. For example, in the palms of the hands, on the plantar surface (sole) of the feet and in the gluteal region (buttocks) it has a structural, cushioning function. In these regions, accumulations of adipocytes are surrounded by strong connective tissue fibres. Also, the distribution of white adipose tissue is different in males and females and is part of the secondary sexual characteristics.

Brown adipose tissue occurs mainly during development and may account for 2-5% of the body weight in a newborn. In adult individuals most of the brown fat has further differentiated into white fat. Adipocytes in brown fat contain heaps of mitochondria. The cytochromes found in the mitochondria give the tissue its characteristic colour. A special feature of these mitochondria is the decoupling of the oxidation of fatty acids from the generation of ATP. Instead, these cells generate heat.

The location of the brown fat reflects its heat-generating function. It is located in the axilla (armpits), between the shoulder blades, in the region of the neck and along large blood vessels. The heat generated by the brown fat warms the blood which supplies nearby organs or which re-enters the trunk from the limbs.



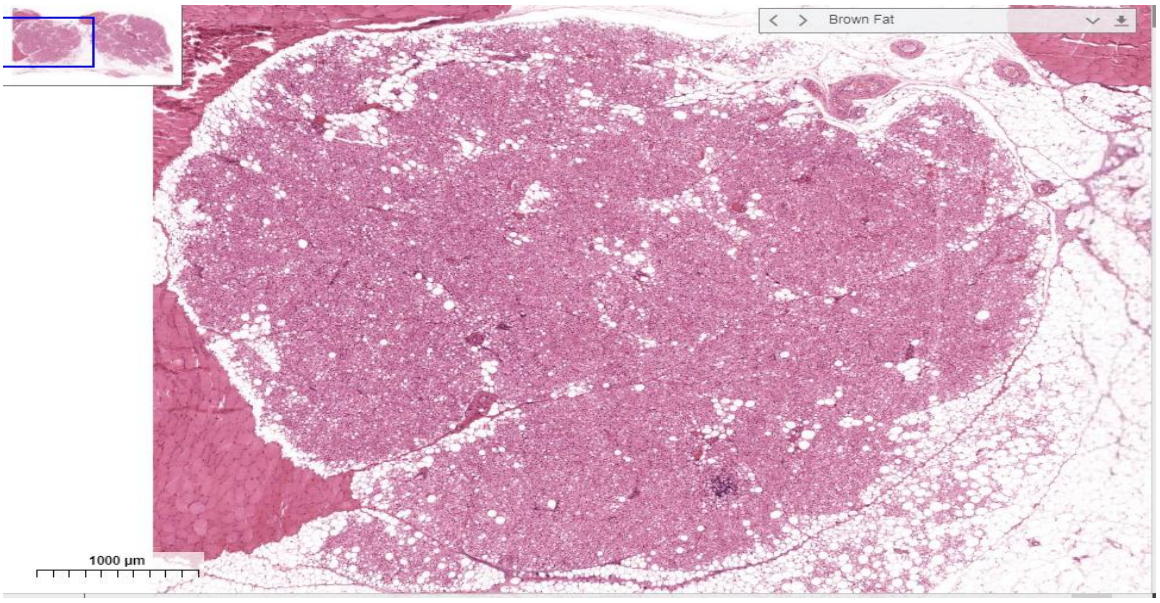
Brown Adipocytes

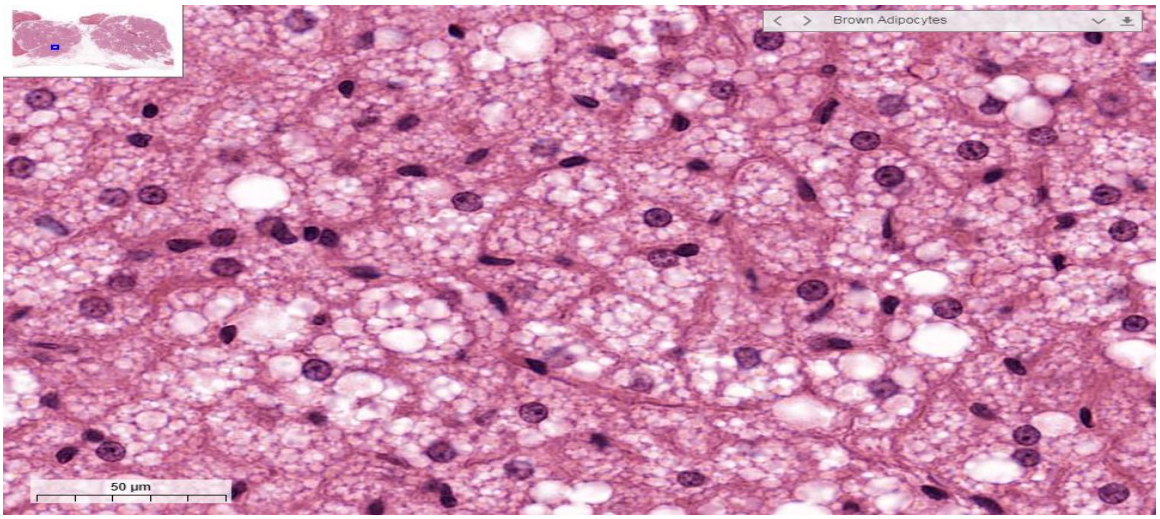
Brown fat is specialized for the generation of heat (thermogenesis). In adult humans, it is found in small amounts in the upper chest and neck.

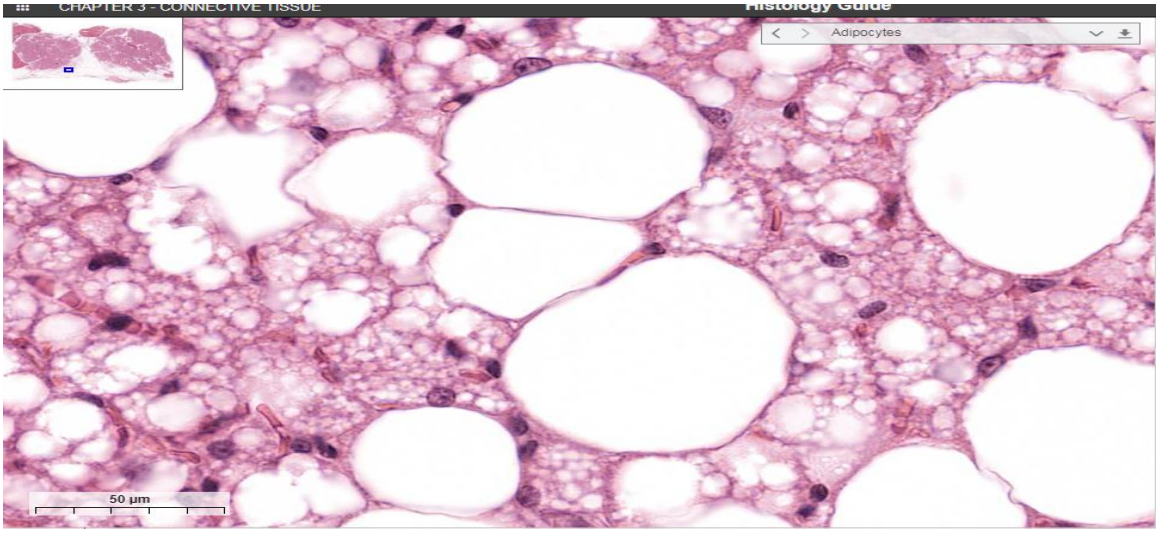
Brown adipocytes are smaller cells with a centrally located nucleus and many small lipid droplets (multilocular). The abundance of mitochondria accounts for the brown color characteristic of its gross appearance.

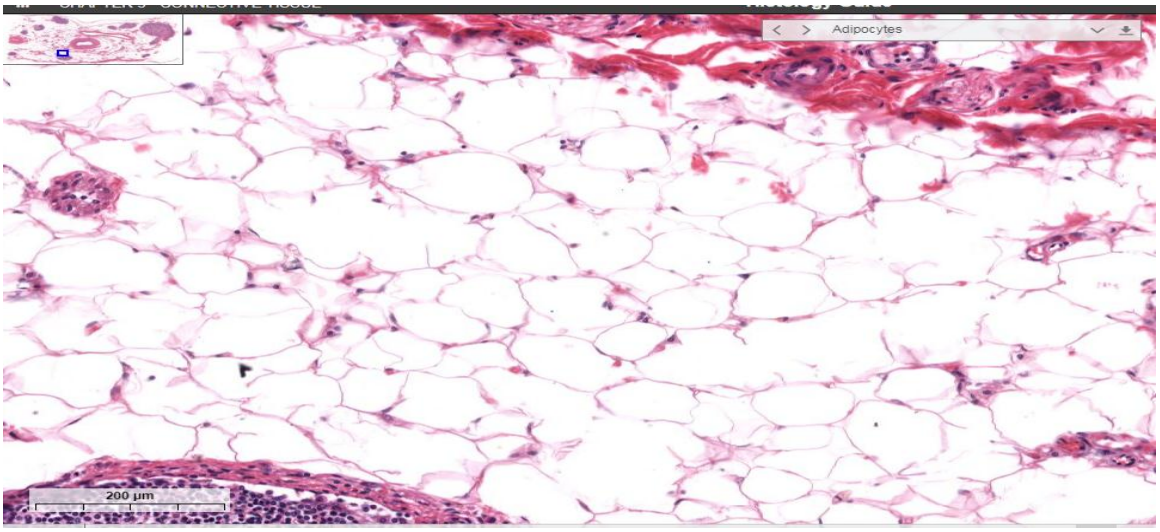
Brown adipocytes uniquely express uncoupling protein-1 (UCP-1) in their mitochondria. It uncouples oxidation of fatty acids by allowing an influx of protons into the matrix of mitochondria. This energy is dissipated as heat.

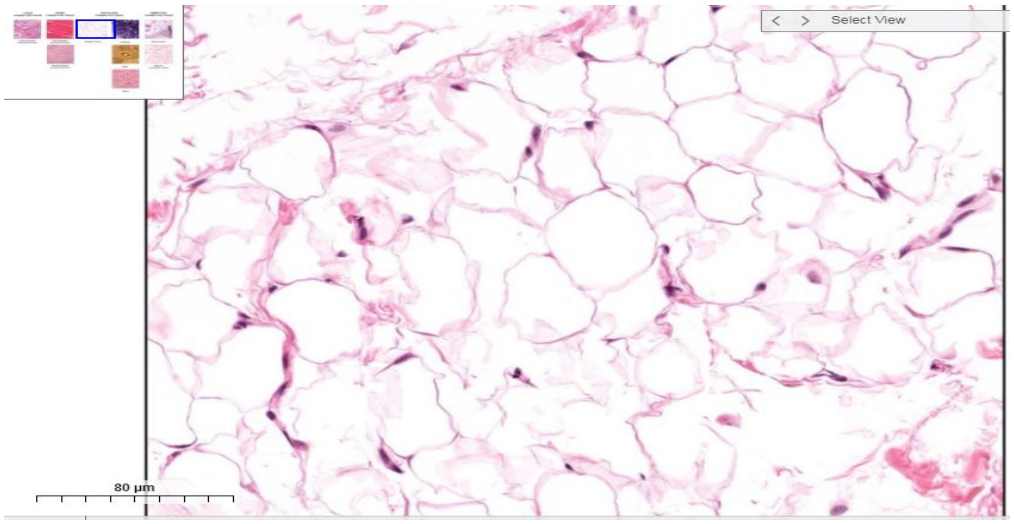
The differences between the much larger, unilocular white adipocytes and the smaller, multilocular brown adipocytes and can be easily seen where they are mixed together.

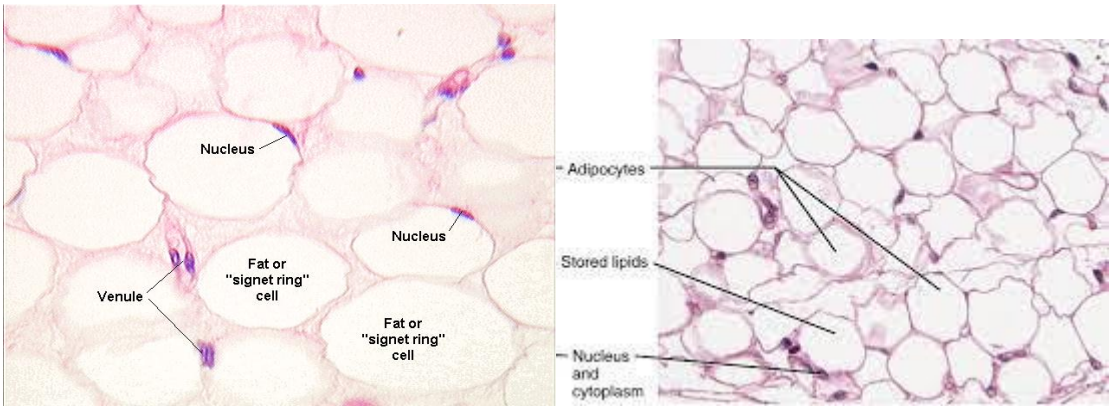



Brown Adipocytes
50 µm


Adipocytes
50 µm

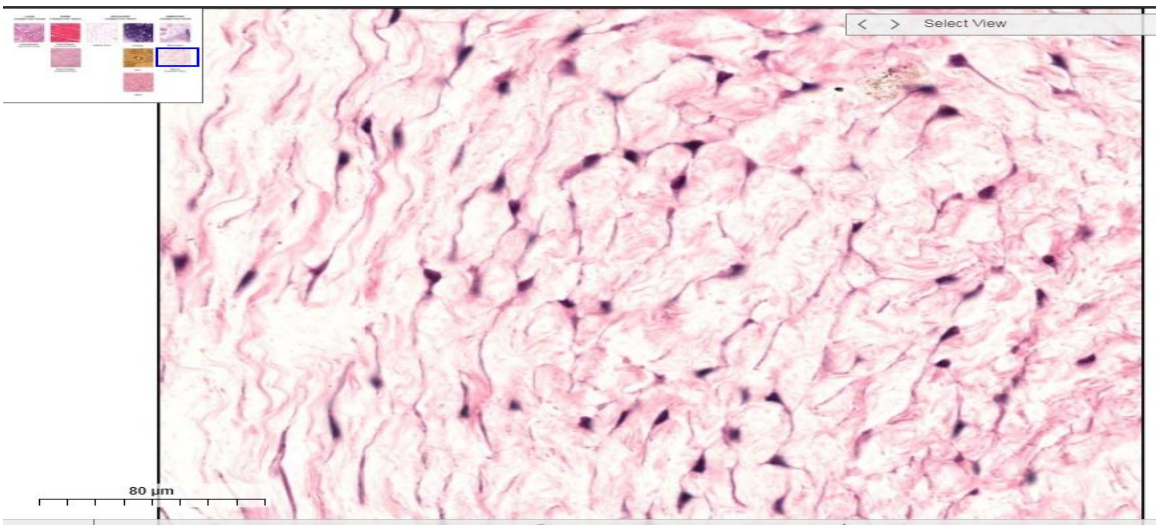

Adipocytes
200 µm





Mucous connective tissue

Mucous connective tissue is quite common in the foetus, particularly beneath the skin. It is also characteristic for the umbilical cord, where it is also called Wharton's jelly. It contains many cells, delicate collagen fibres and plenty of ground substance. Mucous connective tissue can be stained by the same dyes that are used to show mucin - hence its name. In adult individuals, mucous connective tissue is only found in the dental pulp.



Suitable Slides

sections of umbilical cord, some early foetal organs or tooth (pulp) - H&E or Alcian blue & van Gieson section usable for "intramembranous ossification" during foetal development will contain areas of mucous connective tissue.Umbilical Cord, human - H&E Within the umbilical cord you will be able to identify three large vessels and their walls. Mucous connective tissue fills the space between the vessels and the simple squamous epithelium lining the surface of the umbilical cord. Note the very fine appearance of the collagen fibres and the lack of apparent specialisations in this type of connective tissue. The number of cells and appearance of the collagen fibres vary depending on the precise location of the tissue. In some locations, mucous connective tissues will contain a large number of cells and only a few, very delicate collagen fibres. Examples are dental pulp and the mucous connective tissue which is found between the developing tubuli and glomeruli of the foetal kidney. A small drawing should be sufficient to capture the appearance of the tissue.