LABORATORY 3

CONNECTIVE TISSUE PROPER & ADIPOSE TISSUE

OBJECTIVES:

At the end of this lab, you should:

1. be familiar with the three major constituents of connective tissues:
   - cells
   - extracellular fibers
   - ground substance

2. recognize by light microscopy (on the videodisks and if possible also on glass slides) the major cell types found in connective tissue:
   - fibroblasts
   - macrophages
   - mast cells
   - adipocytes
   - lymphocytes
   - plasma cells
   - eosinophils
   - neutrophils
   - basophils

3. recognize by electron microscopy the following cell types:
   - fibroblasts
   - macrophages
   - mast cells
   - adipocytes
   - lymphocytes
   - plasma cells
   - eosinophils

4. recognize by light and electron microscopy the three major types of extracellular fibers found in connective tissue:
   - collagen fibers
   - elastic fibers
   - reticular fibers

5. understand the classification of connective tissue and identify by light microscopy the following types of connective tissue:
   - loose (areolar) connective tissue
   - dense irregular connective tissue
   - dense regular connective tissue
   - reticular connective tissue
   - mucous connective tissue
   - adipose tissue

6. be able to give examples of locations where each of these types of connective tissue is normally found

7. distinguish between brown fat and white fat
Connective tissues (CTs) perform many functions. Almost all epithelia are supported by a CT layer. CTs are the site of most inflammatory responses and many immune responses. They connect muscle to bone as tendons, and bone to bone as ligaments. They fill the spaces between organs, form the capsules and sheaths that surround organs, and provide internal support for the cells comprising most organs. CT forms the stroma or supporting elements of most organs. (The other tissues that carry out the unique functions of the organ are referred to as the parenchyma of the organ).

Connective tissue is one of the four basic tissue types. It can be distinguished from the others (epithelia, muscle and nerve) because:

1. CT cells are usually widely separated from one another by an extensive extracellular matrix that they have produced, rather than being in close proximity to one another.
2. most CTs contain a greater variety of differentiated cell types than found in other tissues (i.e., CTs usually have a highly mixed cell population).

There are 2 major groups of CT cells. One is the fibroblast family, which includes ordinary fibroblasts, reticular CT cells, adipocytes, osteoblasts in bone, chondroblasts in cartilage, etc.). In most CTs it is the members of the fibroblast family that produce most of the components of the extracellular matrix. Cells of the fibroblast family differentiate from the mesenchymal cells of embryonic CT.

The second group of CT cells is the bone marrow-derived cells, which arise from stem cells in the bone marrow and then migrate into CT to function there. These cells include monocytes (and macrophages derived from them), lymphocytes (and plasma cells derived from them), neutrophils, eosinophils, basophils and mast cells.

Another way of classifying CT cells is to divide them into resident cells (also called “fixed” cells) and wandering cells (also called “immigrant”, “transient”, or “elicited” cells). Resident cells are those that are usually found in relatively constant numbers in a particular CT (fibroblasts, adipocytes, mast cells, mesenchymal cells, macrophages), while wandering cells can migrate into a CT in large numbers in response to specific stimuli (lymphocytes, plasma cells, neutrophils, eosinophils, basophils, monocytes).

The extracellular matrix includes fibers and ground substance. There are 3 main fiber types: collagen fibers, reticular fibers and elastic fibers. The ground substance includes all the non-fibrous components of the matrix such as glycosaminoglycans (GAGs), proteoglycans, and various adhesive glycoproteins (e.g., fibronectin).

Combining the various cell types, fiber types and ground substance components in different proportions can produce CTs with very different properties and functions. In this lab we will study the following CT types:

**CT proper:**
- Loose (areolar) CT
- Loose cellular CT (the lamina propria of the gut)
- Dense regular CT
- Dense irregular CT

**Specialized CTs:**
- Reticular CT
- Adipose tissue
- Mucous CT

Other specialized CTs such as cartilage, bone, blood and bone marrow will be topics of separate labs.
I. CONNECTIVE TISSUE PROPER

A. Loose (Areolar) Connective Tissue
   Slide 20 (HU Box): Mammary Gland, Inactive, or Slide 34A or B: Mesentery
   Loose CT is characterized by relatively small numbers of fibers, a large amount of ground substance and a greater variety of cell types than other kinds of CT. The fibroblast is the most common cell type, but leukocytes, adipocytes, mast cells and macrophages are also present. The fibers tend to be thin and randomly oriented, and are mainly collagen fibers. Loose CTs bind other tissues together, and surround blood vessels, nerves, hair follicles, etc. The gel-like nature of the ground substance aids diffusion through the tissue. In these slides look for the loose CT around blood or lymphatic vessels (Slide 34A or B) and within the lobules of a gland (Slide 20 HU).

   Slide 62 (HU Box): Ileum, or Slide 64: Jejunum
   A loose CT layer called the lamina propria is part of the wall of many hollow organs (e.g., GI tract). It lies just beneath the epithelium that lines the lumenal surface of these organs. The epithelium and lamina propria are part of a layer called the mucosa. Lamina propria is a true loose CT. However, throughout most of the GI tract it is so highly cellular that it is almost impossible to find the fibers amid the large number of cells. The lamina propria in such locations is sometimes given a special designation and is called loose cellular connective tissue. Confirm for yourself on these slides the large number of cells in the lamina propria. Many of these cells are leukocytes, especially lymphocytes.

B. Dense Regular Connective Tissue
   Slide 97 (HU Box): Tendon, c.s. & l.s. or Slide 2: Tendon, l.s., and Slide 3: Tendon, c.s.
   A dense regular connective tissue is one in which there are many thick, closely packed fibers which are regularly (i.e., non-randomly) arranged. The fiber type is usually predominantly collagen fibers. There are fewer cells than in loose CT and almost all are fibroblasts. Note the different appearance of the fibroblast nucleus when cut in cross section (round appearance) vs. longitudinal section (elongated or oval appearance). Also notice that in a tendon the collagen fibers are all arranged in the same direction (parallel to the long axis of the tendon), thus providing excellent resistance to stretching forces that are consistently applied in the same direction. In a few dense regular CTs the arrangement of fibers is more complex. For example, the cornea resembles plywood in that the collagen fibers within each individual layer of corneal stroma are all oriented parallel to one another, but neighboring layers are arranged so that the fibers are at an angle to one another. Despite these differences in fiber orientation, both cornea and tendon are classified as dense regular CT because the direction of their collagen fibers is predictable (non-random).

C. Dense Irregular Connective Tissue
   Slide 54 (HU Box): Skin, Palm or Sole, or Slide 41: Plantar Skin, (Aldehyde Fuchsins-Masson Trichrome)
In a dense irregular CT: (1) there are more collagen fibers than cells or ground substance; (2) the majority of cells are fibroblasts; and (3) the fibers are not arranged in any regular or predictable orientation. Dense irregular CT is well suited to resisting strong forces that may be applied from any direction. It is found in locations such as the dermis of the skin.

The dermis is the CT layer immediately beneath the epithelium. It has 2 indistinctly separated layers, a narrow papillary layer and a wider reticular layer, each composed of a different type of connective tissue. Consult an atlas to locate these layers. The papillary layer contains loose CT, while the reticular layer contains dense irregular connective tissue.

Slide 41 has been stained with aldehyde fuchsin & Masson trichrome. Masson trichrome stains collagen aqua (greenish blue). In some versions of this slide some of the larger collagen fiber bundles are red. This represents a staining artifact indicating that, in one of the steps of the staining procedure, the sections were not rinsed long enough to remove the red stain from all the collagen fibers. Note that although collagen fibers predominate, there are other fiber types present in both layers of the dermis. Find the elastic fibers, which are stained purple by the aldehyde fuchsin stain. Reticular fibers are also present but are not identifiable with this stain.

II. ELASTIC & RETICULAR FIBERS

A. Elastic Fibers
   Slide 46 (HU Box): Aorta, Human, Elastic Stain, or Slide 29C: Aorta (Aldehyde Fuchsin)
   and Slide 72 (HU Box): Lung, Elastic Stain, or Slide 48B: Lung, (Elastic Fibers)

   Identify the elastic fibers in the aorta and lung. Elastic fibers stain very poorly with H&E, and therefore usually require specialized stains (e.g., aldehyde fuchsin, orcein) to be visualized clearly. We have already seen in the dermis of the skin and again in slide 29C that aldehyde fuchsin stains elastic fibers purple. Other elastic stains cause them to stain brown, black, red, yellow or orange. Therefore you must know the stain used, or be familiar with the normal abundance and location of elastic fibers in different organs in order to identify them confidently. In elastic arteries such as the aorta the elastic tissue forms elastic sheets (elastic lamellae) rather than individual fibers. These sheets are concentrically arranged in the smooth muscle layer of the vessel called the tunica media (see Ross, Fig. 13.17a, p.419).

   In the lung, the elastic fibers are less obvious. They are present in the walls of blood vessels and large airways, but also in the walls of the alveoli where gas exchange occurs. Excessive destruction of these elastic fibers seems to be involved in some forms of emphysema.

   In most CTs elastic material is a minor component of the matrix. However in the tunica media of elastic arteries and in some elastic ligaments (ligamenta flava of the vertebral column, ligamentum nuchae of the neck, vocal cord of the larynx) it is so abundant that these tissues are sometimes referred to as elastic connective tissues.

B. Reticular Fibers
   Slide 51 (HU Box): Spleen or Lymph Node (depending on your box) - Silver, (Reticular Fibers)
Identify the reticular fibers. Reticular fibers tend to form a meshwork or “reticulum” of fibers rather than thick fiber bundles. They provide a delicate supporting network for many soft organs such as the liver, the spleen, adipose tissue, lymph nodes, and many endocrine organs. They are also found underlying the basal lamina of epithelia. What is the chemical composition of reticular fibers? (Answer: They are composed of collagen type III).

In ordinary H&E preparations it is usually impossible to distinguish reticular fibers from other types of collagen fibers. However, after appropriate pretreatment of the sections, certain silver stains will stain reticular fibers black. Note that some of our slides have also been counterstained with a red dye that stains cell nuclei and cytoplasm in order to show the location of the cells relative to the fibers.

Reticular fibers are also PAS positive because they are highly glycosylated. Other types of collagen are less heavily glycosylated and hence are PAS negative. Since elastic fibers and other types of collagen fibers do not stain with silver stains or with the PAS technique, either of these procedures can be used to unequivocally identify reticular fibers.

III. SPECIALIZED CONNECTIVE TISSUES

A. Adipose Tissue
   Slide 44 (HU Box): Cardiac Muscle, or
   Slide 36: Left Ventricle Wall, or
   Slide 36B: Right Ventricle Wall

   The two types of adipose tissue are: unilocular (white) fat, which is quite common, and multilocular (brown) fat, which is rare in adults but more easily found in newborns. Unilocular (white) fat should be abundant in the epicardium on these slides. White fat can be distinguished from brown fat because each mature cell of white fat contains a single large lipid droplet in its cytoplasm while each brown fat cell contains multiple lipid droplets. White fat is therefore said to be unilocular, while brown fat is multilocular. Identify the characteristically flattened nucleus of the white adipocyte, located at the periphery of the cell in the thin rim of cytoplasm surrounding the lipid droplet. When large numbers of white adipocytes abut one another they tend to appear somewhat deformed rather than perfectly round, forming a pattern that often resembles chicken wire.

   Slide 79 (HU Box): Adrenal, Human, or
   Slide 47B: Primary Bronchus, Newborn, or
   Slide 71B: Pancreas, Newborn

   A patient search in the loose connective tissue beyond the adrenal capsule on slide 79 should reward you with some examples of brown fat. Scattered lobules of brown fat may also be present around the bronchi of the newborn in slide 47B or near the pancreas in 71B. (If you have no luck, try looking in the subcutaneous tissue of slide 30 HU (chondroid tissue). Also, about half of the 48 HU slides (fetal heart) have some brown fat around the great vessels). Observe the multiple lipid droplets within each brown fat cell, and the rounded nucleus, which is usually centrally placed in the cell rather than pushed to the side. Also notice the large number of capillaries between adipocytes (brown fat is characteristically more highly vascularized than white) and the almost glandular appearance of brown fat.
In all of these slides of brown and white fat, the lipid has been extracted by the solvents used to prepare the tissues, so the fat droplets within the adipocytes appear empty and unstained. If special fixatives had been used or the specimen had been frozen without fixation, the lipid would still be present and could be stained with appropriate stains (oil red O, osmium tetroxide, Sudan black, etc.)

B. Mucous Connective Tissue

Slide 34 (HU Box): Umbilical Cord, Human

Mucous connective tissue is a type of embryonic CT characterized by a large amount of ground substance rich in hyaluronic acid, very few fibers, and a population of undifferentiated cells. It is found primarily in the umbilical cord.

IV. CONNECTIVE TISSUE CELL TYPES

Many of these cell types are difficult to identify with your microscope unless you use the oil immersion lens. Even then it may be difficult. For good examples of each of these cell types consult the videodisk.

USE OF THE OIL IMMERSION OBJECTIVE:

The oil immersion objective is the 100X objective. It is labeled “OIL” on the side of the barrel or has a narrow black band around the barrel near its tip. To use it, proceed as follows:

1. Without using oil, study the section at low magnification to locate a connective tissue area for further study.
2. Continue your study with progressively stronger objectives, through 40X.
3. When you have finished observing the slide at 40X, swing that objective out of place and, observing from the side of the microscope, slowly swing the oil objective into place to be sure that it clears the coverslip.
4. Still observing from the side, raise the stage until the objective is almost touching the coverslip.
5. Then swing the oil objective out of position so that no objective is lined up with the ocular tube. Place a drop of immersion oil on the slide.
6. Carefully swing the oil immersion objective lens back into position, and slowly focus by LOWERING the stage with the fine focus control knob. DO NOT FOCUS BY RAISING THE STAGE SINCE IT IS EASY TO MISS FOCUS IF YOU GO TOO FAST, AND GRIND THE OBJECTIVE INTO THE COVERSIP, DESTROYING BOTH.
7. Move around the slide as much as you wish. However, if you move very far, the image may become blurry since the oil has become spread out in too thin a layer over the surface of the slide. This can be remedied by adding another drop of oil in the same manner as before.
8. When you have completed your observations with oil immersion, clean the slide and objective lens by first wiping them well with lens paper (do not use paper towels, Kleenex or any other type of paper since these may scratch the surfaces).
9. When you have finished studying the slide and have switched back to using a non-oil objective, you may find that the image appears blurry. This usually means that oil has somehow gotten onto the non-oil objective lens. Clean the lens as described in step #8.
A. Fibroblasts
   Slide 34A or 34B: Mesentery
   Identify the fibroblasts in the loose CT. They have an elongated, flattened, oval nucleus. Sometimes you can see the long, thin, pale-staining cytoplasmic processes of these elongated or fusiform cells, but often only the nucleus is visible. Fibroblasts synthesize virtually all the type I collagen in the body (except in the muscle layer of the wall of blood vessels, where smooth muscle cells perform this function). In connective tissues, fibroblasts produce not only the type I collagen, but also most of the other components of the extracellular matrix as well.

B. Macrophages
   Slide 67 (HU Box): Liver, Stellate Reticuloendothelial Cells, India Ink, or Slide 1A: Liver, Trypan Blue Safranin
   The macrophage is one of the two major phagocytic cell types in humans (the other being the neutrophil). In the preparation of these slides, the macrophages of the living animal were exposed to particulate dyes (either India ink or trypan blue) and allowed to phagocytize the dye particles prior to fixation of the tissue. The dye particles within the cytoplasmic phagosomes act as a marker for the macrophages.
   The macrophages in the liver are given a special name. They are called Kupffer cells. Along with ordinary endothelial cells (which are poorly phagocytic and hence don't take up the dye markers), the Kupffer cells line the small blood vessels of the liver called sinusoids. See if you can identify some of these dye-containing cells lining the lumen of the sinusoids.
   These preparations do not preserve the details of macrophage structure very well. To study these, see the videodisk images of Kupffer cells and other macrophages. Note that most macrophages usually have an irregular nucleus (more lumpy looking rather than perfectly round or oval in outline) and a pale-staining cytoplasm. However the most reliable way to identify a macrophage by LM is to find one that contains visible phagocytized debris in its cytoplasm.

C. Mast Cells
   Slide 87 (HU Box): Knee, Rat, Methylene Blue and Slide 67: Large Intestine
   Search the CT of the intestinal submucosa (Slide 67) or around the knee joint (Slide 87) to find mast cells. They are usually round to oval cells that have numerous large cytoplasmic granules surrounding a round to oval nucleus that is centrally placed in the cell. The granules are usually pale-staining in H&E, but with methylene blue they may stain either dark blue or reddish-purple (metachromatic staining) depending on the vagaries of the tissue and the batch of stain. In most versions of Slide 87 the granules appear dark blue. Again, you should consult the videodisk to find high magnification images that demonstrate the detail of mast cell morphology.
   When activated by appropriate stimuli, mast cells secrete histamine and certain leukotrienes that increase the permeability of small blood vessels, especially postcapillary venules. In CTs mast cells are often located near small blood vessels. See if you can confirm this relationship on Slide 67.
Basophils are leukocytes that leave the blood vessels to carry out their functions in the surrounding CTs. Both cells produce similar secretory products and have similar functions in regulating the permeability of post-capillary venules. They can be distinguished morphologically since the mast cell nucleus is round to oval and fairly easy to see within the cell, while the basophil nucleus is lobulated and often obscured by the cytoplasmic granules.

D. Lymphocytes

Slides 9 (HU Box) or 10 (HU Box): Tonsils, or Slide 22: Tonsils

The lymphoid nodules that make up the tonsils are composed mainly of lymphocytes of different sizes. Small lymphocytes have a heterochromatic nucleus that is round, slightly flattened on one side, or kidney bean-shaped. There is so little cytoplasm that it is often difficult to see it. The cytoplasm tends to be basophilic. When lymphocytes become activated during an immune reaction, they enlarge, their nucleus becomes more euchromatic, and the relative amount of cytoplasm increases. Large lymphocytes can be found in the pale staining center (called the germinal center) found in secondary lymphoid nodules (see Ross, Figs. 14.13 & 14.15a, pp. 458 & 460). Small lymphocytes are found around the edge of a secondary lymphoid nodule (the cap or mantle region), between nodules, and also infiltrating into the epithelium covering the tonsil.

E. Plasma Cells

Slide 60 (HU Box): Duodenum, c.s., or Slide 61A: Fundic Stomach, Bouins, or Slide 62: Stomach, Pyloric, Dog, Sec. or Slide 67A: Colon, Bouins, or Slide 95A: Mammary Gland

Search in the lamina propria beneath the epithelium of the GI tract or in the intralobular CT of the mammary gland to find plasma cells and lymphocytes. Plasma cells have an eccentric nucleus (eccentric = not in the center) with a characteristic pattern of heterochromatin clumps arranged around the periphery of the nucleus in a clockface or cartwheel arrangement. The cytoplasm of plasma cells is basophilic (a distinctive bluish-gray or lilac color) except for a pale area near the nucleus (called the cytocentrum), which contains the Golgi and centrioles. There are no large secretory granules.

F. Eosinophils

Slide 66: Appendix

Look in the lamina propria for eosinophils, which can be recognized by their lobed nucleus (usually bi-lobed) and numerous large secretory granules that cause the cytoplasm to be highly eosinophilic (See Wheater, Fig. 4.21, p. 81).

NOTE: Lymphocytes, eosinophils and basophils are three of the five types of white blood cells or leukocytes (neutrophils, eosinophils, basophils, lymphocytes & monocytes). All leukocytes can leave the blood vessels and enter surrounding CTs. Indeed, their major functions are carried out in the CT. Monocytes rapidly
differentiate into macrophages and so are rarely seen in the CTs. We will study their light microscope morphology when we study blood. Neutrophils and basophils are always present in CTs in small numbers, but increase greatly in number during inflammatory or allergic responses. Consult the videodisk and your atlas for light microscope images of neutrophils and basophils.

V. ELECTRON MICROSCOPY (RHODIN)
A. CONNECTIVE TISSUE FIBERS
1. Collagen fibers: Make sure you can distinguish between a collagen fiber and a collagen fibril. For most types of collagen the mature fibrils have a regular cross-banded pattern (Figs. 7-5 & 7-6). These fibrils then aggregate to form fibers that are seen in cross section and longitudinal section in Fig. 7-4. Realize that each of the small dots within a cross-sectioned fiber in this figure is a collagen fibril.

2. Elastic Fibers: Next distinguish between collagen fibrils and elastic fibers. Elastic fibers have two components: a central core of elastin and numerous microfibrils composed of fibrillin. The elastin core appears rather amorphous (literally amorphous means without shape; used to describe structures which lack distinguishing features or substructure). The microfibrils surround the elastin core, and smaller numbers may be embedded within it. Fig. 7-11 shows a single elastic fiber in cross section. Compare it with the fibrils of the collagen fiber shown at the same magnification in Fig. 7-10. Elastic fibers are usually larger in diameter than collagen fibrils, but smaller in diameter than collagen fibers. Next compare collagen and elastic fibers sectioned more longitudinally (Rhodin, Fig. 7-9). Notice again the amorphous core of the elastic fiber.

3. Reticular Fibers (Fig. 7-16): A reticular fiber is composed of reticular fibrils made of collagen type III. Reticular fibrils tend to be thinner in diameter than collagen fibrils (compare Figs. 7-10 & 7-16) and to form thinner fibers. They usually form interlacing meshworks rather than fiber bundles. In the lymphoid organs and bone marrow, they are produced by a fibroblast-like cell called the reticular connective tissue cell or reticular cell (Fig. 18-14) which is unusual in that the cytoplasmic processes of the reticular cell often remain wrapped around the reticular fibers (Fig. 18-15), to form a supporting network that actually consists of reticular fibers and reticular cells adhering to them. In most other organs, reticular fibers are produced by fibroblasts. In the tunica media of blood vessels they are made by smooth muscle cells.

B. CONNECTIVE TISSUE CELL TYPES
1. Fibroblasts are elongated fusiform (spindle-shaped or cigar-shaped) cells. When the cell is actively synthesizing and secreting proteins, the rough endoplasmic reticulum becomes quite prominent (Figs. 7-22 & 7-37), and the cisternae are often distended and irregular rather than uniformly narrow. When the cells are less active in protein synthesis, they have sparse amounts of cytoplasm with little RER or Golgi (Fig. 7-21). Some authors reserve the term fibrocyte for this less active form of the fibroblast. Note that even active fibroblasts do not contain large secretory granules since they secrete their products constitutively and do not need to concentrate them for intracellular storage.

2. Mast cells contain numerous large, electron-dense cytoplasmic granules and have a centrally placed and relatively round nucleus (Fig. 7-24). What substance is stored in the mast cell granules and then released from the activated cell to
increase the permeability of postcapillary venules? (Answer: Histamine). After activation the cell also begins to secrete other mediators such as certain leukotrienes that have a similar action but are not pre-synthesized and are not stored in cytoplasmic granules. Fig. 25-16 illustrates again that mast cells are often found near the small blood vessels (postcapillary venules and capillaries) that they affect.

3. Macrophages develop from monocytes that have left the blood vessels. Macrophages (Fig. 7-26) have the characteristics you would expect from highly phagocytic cells: a well-developed Golgi complex (where the lysosomes are formed), numerous primary lysosomes, secondary lysosomes, residual bodies, phagosomes, and an active plasmalemma, i.e., one with many projections of the surface membrane that are engaged in the formation of phagosomes (these are labeled "microvilli" in this micrograph).

4. Mature adipocytes in white fat are unilocular, with the nucleus compressed peripherally in the thin rim of cytoplasm surrounding the large lipid droplet (Fig. 7-39). Observe that when lipid is preserved it appears dark by electron microscopy (Fig. 7-39), and when it has been extracted during fixation, the lipid droplets are light-staining (Figs. 7-40 & 7-41). Notice (Figs. 7-40 to 7-42) that during their development the adipocytes of white fat pass through a multilocular stage (i.e., each cell has numerous lipid droplets which later coalesce into one droplet). Brown fat, in contrast, is always multilocular.

5. Lymphocytes are small cells when not activated in an immune response (Fig. 17-23). A small lymphocyte has a heterochromatic nucleus and very little cytoplasm. The cytoplasm is basophilic due to the presence of free polysomes, but other organelles are few in number. After a lymphocyte has been activated in an immune response it enlarges (Fig. 17-24) the nucleus becomes more euchromatic, the relative amount of cytoplasm increases, and the various cytoplasmic organelles become more abundant.

6. Plasma cells (Fig. 7-29) develop from B lymphocytes during an immune response. They have clumps of heterochromatin margined along the inner surface of the nuclear membrane, abundant rough endoplasmic reticulum (which accounts for the basophilia of the cytoplasm by light microscopy) and a well-developed Golgi. The cartwheel or clockface pattern of heterochromatin is not always as apparent in electron micrographs as in light micrographs. What protein is being synthesized on the RER of a plasma cell? (Answer: Immunoglobulins, otherwise known as antibodies).

7. Eosinophils (Fig. 5-12) are another type of leukocyte. They have a lobed nucleus (usually bi-lobed), and numerous large cytoplasmic granules. We have seen that by light microscopy they can be distinguished from mast cells by the fact that the mast cell nucleus tends to be round or oval, and mast cell granules are highly basophilic while those of the eosinophil are eosinophilic (hence the name of the cell). By electron microscopy the two cell types can be distinguished by their nuclear morphology and the fact that eosinophil granules tend to contain a crystalloid (Fig. 5-13) while those of mast cells do not. The crystalloid contents include a protein called major basic protein, which has anti-parasitic activity.
NOTE: The lymphocyte and eosinophil are two of the five types of white blood cells or leukocytes (neutrophils, eosinophils, basophils, lymphocytes & monocytes). All leukocytes can leave the blood vessels and enter surrounding CTs. Indeed, their major functions are carried out in the CT. The detailed ultrastructure of the other leukocytes will be considered when we study blood.

C. CONNECTIVE TISSUE TYPES

1. Mesenchyme (Figs. 7-1 & 7-2): Mesenchyme is an embryonic form of CT. Note that the extracellular matrix consists of abundant ground substance and relatively few fibers. The fibers that are present tend to be very thin reticular fibers. The mesenchymal cells have multiple long, thin cytoplasmic processes and they make up a homogeneous population (i.e., virtually all the cells have the same undifferentiated appearance). The cells somewhat resemble fibroblasts, but if they were mature fibroblasts you would expect to see more fibers in the matrix and at least a few differentiated cells of other types (lymphocytes, mast cells, macrophages, etc.).

2. Loose cellular connective tissue (Fig. 7-31): Characterized by large numbers of cells, many different cell types and relatively few thin fibers.

3. Loose connective tissue vs. dense irregular connective tissue (Fig. 7-32): Compare the number of cells per unit area and the thickness of the collagen fibers in the upper portion (loose CT) vs. the lower portion (dense CT) of this micrograph.
LABORATORY 3 CHECKLIST
CONNECTIVE TISSUE PROPER & ADIPOSE TISSUE

**LIGHT MICROSCOPY**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Example Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>fibroblast</td>
<td>Kupffer cell</td>
</tr>
<tr>
<td>collagen fibers</td>
<td>mast cell</td>
</tr>
<tr>
<td>lamina propria</td>
<td>small lymphocyte</td>
</tr>
<tr>
<td>tendon</td>
<td>large lymphocyte (in germinal center)</td>
</tr>
<tr>
<td>papillary layer of the dermis</td>
<td>plasma cell</td>
</tr>
<tr>
<td>reticular layer of the dermis</td>
<td>eosinophil</td>
</tr>
<tr>
<td>elastic fibers</td>
<td>loose connective tissue</td>
</tr>
<tr>
<td>elastic lamellae</td>
<td>loose cellular connective tissue</td>
</tr>
<tr>
<td>reticular fibers</td>
<td>dense irregular connective tissue</td>
</tr>
<tr>
<td>white fat</td>
<td>dense regular connective tissue</td>
</tr>
<tr>
<td>brown fat</td>
<td>reticular connective tissue</td>
</tr>
<tr>
<td>macrophage</td>
<td>mucous connective tissue</td>
</tr>
</tbody>
</table>

**ELECTRON MICROGRAPHS**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Example Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>collagen fibril</td>
<td>macrophage</td>
</tr>
<tr>
<td>collagen fiber</td>
<td>white adipocyte</td>
</tr>
<tr>
<td>elastic fiber</td>
<td>small lymphocyte</td>
</tr>
<tr>
<td>reticular fiber</td>
<td>plasma cell</td>
</tr>
<tr>
<td>fibroblast</td>
<td>eosinophil</td>
</tr>
<tr>
<td>mast cell</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** These checklists include MOST of the structures that you should be able to identify. Exams may include structures not on these lists.
FOCUS QUESTIONS
LAB 3: CONNECTIVE TISSUE PROPER & ADIPOSE TISSUE

See whether you can answer the following questions. The correct answers are posted on the course website (http://neurobio.drexelmed.edu/education/ifm/microanatomy) under “Lab Focus Questions”.

1. What is the major identifying characteristic that allows you to classify a tissue as a connective tissue?
2. What is the major identifying characteristic that allows you to classify a tissue as an epithelium?
3. What are the major functions of connective tissue?
4. Which type of connective tissue (loose, dense irregular or dense regular) would be best suited for resisting strong forces that are consistently applied in the same direction?
5. What cell type is responsible for the synthesis of collagen in connective tissues? In the muscle layer of the wall of blood vessels?
6. Under what circumstances does the number of “wandering” or “immigrant” cells in a CT increase dramatically?
7. What color do most trichromes stain collagen? What color do most stain smooth muscle?
8. What tissue component is stained by aldehyde fuchsin?
9. What stain is most commonly used to demonstrate reticular fibers?
10. Reticular fibers are PAS positive. What color would they be after PAS staining? What information do you gain from the fact that they are PAS positive whereas other types of collagen fibers are not?
11. What does the term “reticular” tell you about the arrangement of reticular fibers in a tissue?
12. Is there a membrane surrounding each individual lipid droplet in the cytoplasm of brown or white fat cells?
13. Can lipid droplets stain black in an electron micrograph? Can they stain gray or white?
14. Why don’t plasma cells have large secretory granules in their cytoplasm?
15. Protein secreting cells such as plasma cells often have a pale cytoplasmic area called the centrosome (cytocentrum). What organelle is characteristically found in the centrosome and how is it related to protein synthesis and secretion?