Laboratory Manual in Animal Histology (Biology 134)

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EXERCISE 3: THE CONNECTIVE TISSUE

Introduction

Among the four types of animal tissues, the connective tissue is distinctly different in many ways. The cells are derived from embryonic cells called mesenchyme which are pluripotent, that is, they can give rise to several cell types in adult. Also, there is a large amount of extracellular molecules (ECM) associated with the cells. This ECM is responsible for the wide variety of connective tissue and thus, its morphology and functions.

Objectives

At the end of the exercise, the student should be able to:

1. identify the different types of tissues based on certain criteria; and,
2. predict the functions of the different types of connective tissue based on their histological features.

Classification of Connective Tissue

The main basis for classifying the connective tissue is the amount and kind of cells and fibers that make up the tissue.

A. **General Connective Tissue** – based on the preponderance of the cells relative to the fibers that make up the tissue.

1. **Loose Connective Tissue** - also called the ***areolar connective tissue.*** It has greater number of cells than fibers. The most prominent cells are:
    a. **Fibroblasts** – long thin cells with short cytoplasmic processes that are invisible with H & E prepared slides. The nucleus is visible and appears oval and lightly stained located at the center of the cell.
    b. **Mast cells** - large oval cells with numerous granules in the cytoplasm that usually obliterate the nucleus. They may appear bluish purple and thus, exhibit metachromasia.
    c. **Plasma cells** - small round cells that have a clear region on the apical cytoplasm. Focus on the nucleus under HPO and observe the arrangement of the chromatin granules. ***What does it indicate?***
    d. **Adipose cells** - large cells that are round or polygonal with a "signet-ring" appearance. The circular "empty" part is the cytoplasm which has lost its fats during histological preparation of the tissue. The thin densely-stained nucleus is at the periphery of the cell.

The cells are interspersed among the extracellular molecules visible as fibers :

a. **Collagen fibers** – white, thick bands between the cells. They may appear straight or wavy in form.
b. **Elastic fibers** – dark, thin bands between the cells. Straight but disposed randomly among the other fibers and cells.
c. **Reticular fibers** - threadlike structures that form a network among the fibers and cells.

2. **Modified loose CT**- based on the predominant components, either cells or fibers.
   a. **Adipose tissue-** aggregate of adipose cells that appear polygonal with large fat vacuoles.
   b. **Mucous tissue** – small,l stellate mesenchymal cells are embedded in a viscous and light intercellular substance. This constitutes the so-called Wharton's jelly of the umbilical cord.
   c. **Reticular tissue** - the cells are surrounded by a meshwork of reticular fibers that are stained black by silver impregnation. The cell types vary according to the organ. Found in the stroma of the bone marrow, liver and lymph nodes.

3. **Dense Connective Tissue** – the fibers are closely packed with few cells in between them. On the basis of the orientation of the fibers, two types are known:
   a. **Regular** - the fibers are oriented parallel to one another. The nuclei of the fibroblasts appear elongated and dark between the fibers. Found in tendons and ligaments.
   b. **Irregular** - the fibers are randomly disposed among the cells. Study the dermis of the skin and note the orientation of the fibers and the cells in them. **Of what significance is the arrangement of the fibers?**

B. **Specialized Connective Tissue –** exhibits different consistency of the intercellular substances, thus, serve as the basis for its classification.
   1. **Cartilage** – flexible supporting structure in the adult. The three types are hyaline, elastic and fibrocartilages which vary according to the ratio of cells to intercellular substances.

1.a. Hyaline Cartilage - a transverse section would show the following parts:

- **Perichondrium** - a dense connective tissue capsule composed of thin layer of cells, the chondroblasts. This stained eosinophilic and clearly show the basophilic oval nuclei of the cells.
- **Chondrocytes** - large cells with round nuclei and pale cytoplasm. They are usually seen in pairs or singly inside the cavities called lacunae. The paired or group of cells are called isogenous chondrocytes. ***Why?***

- **Matrix** –an amorphous ground substance where the chondrocytes are embedded. Note that the territorial matrix is which is immediately surrounding the lacunae is stained more intensely (violet in color) compared to the interterritorial matrix. ***What accounts for this difference in staining affinity?***

1.b. Elastic Cartilage – almost identical to hyaline cartilage but for the abundance of elastic fibers between the chondrocytes. When stained with resorcin, the fibers appear black.

1.c. Fibrocartilage – are easily identified by the linear organization of chondrocytes separated by bundles of collagen fibers. The chondrocytes are smaller, enclosed within the oval or round lacunae. **Is there a perichondrium?**

2. **Bone –** highly rigid mineralized tissue that protects vital organs and leverage for locomotion. Based on the organization of the ECM , there are two types known.
   2.a. Compact Bone- exhibits a solid organization of ECM forming a concentric mass.
   - **Haversian Canal System** – large round bodies that are repeatedly seen in the entire bone. They are the structural and functional units of the bone. They are also called osteons. Each of these are composed of the same structures as follows:
   - **Haversian Canal** – central round space that is actually a branch of blood vessel.
   - **Concentric lamellae** - about 5-8 circumferential layers of matrix surrounding each Haversian canal.
   - **Lacunae** - thin, dense elliptical structures in each lamella. In ground section, they appear empty, but *in vivo*, they contain the osteocytes.
   - **Canaliculi** – appear as long thin lines radiating from the lacunae.
   - **Cement Line** – a thin dense line surrounding the osteon.
   - **Volkmann's Canal** - seen as tubular space bisecting the lamellae and may unite with the haversian canal.
   - **Interstitial Lamellae** - remnants of the remodeled bone. They are small, triangular lamellae between adjacent osteons.
   - **Periosteum** – dense connective tissue that cover the outer surface of the bone. It contains blood vessels and nerves.
   - **Sharpey's Fibers** - bundles of collagen fibers from the outer layer that enter the bone.

   2.b. Spongy Bone- has a porous or spongy appearance owing to the

presence of bars. This is located between the compact bones.
Identify the following structures:

- **Trabeculae** – bars or plates of bones that anastomose with one another.
- **Matrix** – the ground substance of developing bone seen as the component of the trabeculae. Being immature, it is mostly unorganized but some may show incipient concentric lamellae with lacunae.
- **Lacunae** – small and ovoid cavities that contain the bone cells called osteocytes.
- **Endosteum** - inner surfaces of the bone lined with cuboidal cells called osteoblasts.
- **Howship's Lacunae** – shallow depression containing giant multinucleated cells called Osteoclasts. These are usually found on or near the corner of the trabeculae.
- **Osteoid** – newly-formed bone matrix which is still uncalcified. It appears as a light line between the layer of osteoblasts and developing bone.

Osteogenesis - the development of bone occurs in two ways:

a. **Intramembranous ossification** – formation of bone from a membrane bone.
b. **Endochondral ossification** – bone is formed from a cartilage model. The latter is responsible for the increase in the length of the long bone, hence, the growth of man. The sequence of events in the transformation of a cartilage into a bone is best observed in the longitudinal section of a long bone like femur. From top to bottom, there are five zones in endochondral ossification, namely:

- **Resting Zone** - the chondrocytes are flat, small and fusiform which are far apart separated by the matrix.
- **Proliferative Zone** – chondrocytes are clustered into column-like array of 3 – 10 cells.
- **Hypertrophic Zone** - chondrocytes appear much larger and irregular in shape inside their lacunae. They are closely packed with very little intercellular substance.
- **Calcification Zone** – the chondrocytes have little nuclei and highly irregular in shape. They are dying or dead due to mineralization of the matrix.
- **Ossification Zone** – the matrix is calcified or mineralized and the cells within the lacunae are now called osteocytes.

3. **Blood-** unique among the connective tissue since the intercellular substance is fluid with cells suspended in it. Important in the transport of

oxygen and other substances throughout the body. There are three main types of cells, each with different specific functions.

3.a. Red Blood Cells/Erythrocytes- tiny biconcave discs without nuclei. They are about 7.5 µm in diameter and often seen as file of coins (Rouleux formation) or clumped together in smeared preparation. ***Why?*** OIO must be used if they are to be studied in detail. Since there are 5.5 M/mm^3 RBC in men, they are the most predominant cells in a drop of blood smear preparation.

3.b. White Blood Cells/Leukocytes – larger but fewer cells than RBC. They are variable in morphology and classified into two main types:

3.b.1. Granulocytes - cells that have cytoplasmic granules when viewed under LM. Based on their staining affinity and nuclear morphology, they are further subdivided into three types:

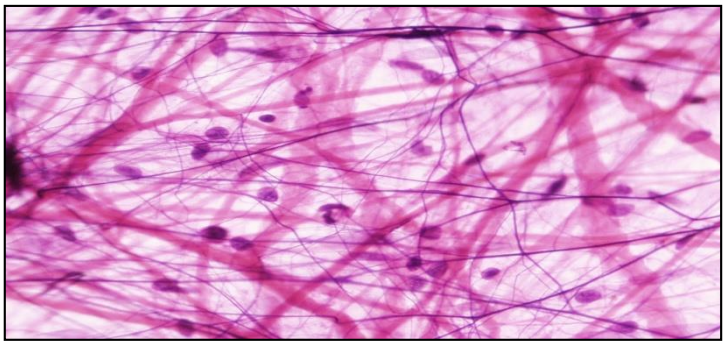
- **Neutrophils -** most numerous among the WBC with varied nuclear morphology. The single nucleus has 3-5 lobes joined by thin chromatin granules.
- **Eosinophils** – their cytoplasmic granules have affinity to eosin dyes, hence, the name. A large bilobes nucleus can be observed in them.
- **Basophils** – least in number among the WBC, hence, seldom observed. Their nuclei have ill-defined lobes and usually obscured from view by many granules.

3.b.2. Agranulocytes –cells that appeared to be devoid of cytoplasmic granules when viewed under the light microscope but contain certain granules under the electron microscope.

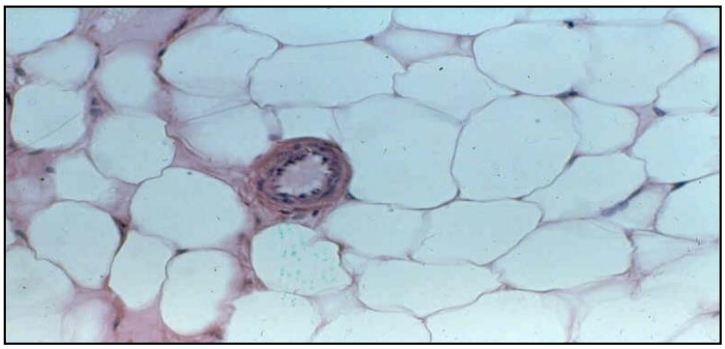
- **Lymphocytes** – cells that range in size from 6-15 µm in diameter and are thus, classified into small, medium and large lymphocytes. They all have a large round nuclei surrounded by a thin rim of cytoplasm.
- **Monocytes** - the largest among the WBC with a diameter of 12-20 µm. The large nucleus is often kidney-shaped or deeply indented. The cytoplasm is thin on one side and thicker in the opposite side.

3.c. Platelets/Thrombocytes – a unique type of cells by virtue of its being fragments rather than a true cell. They are enucleated and often seen as clumps of cells among the other blood cells. They are derived from giant multinucleated cells called megakaryocytes in the bone marrow.

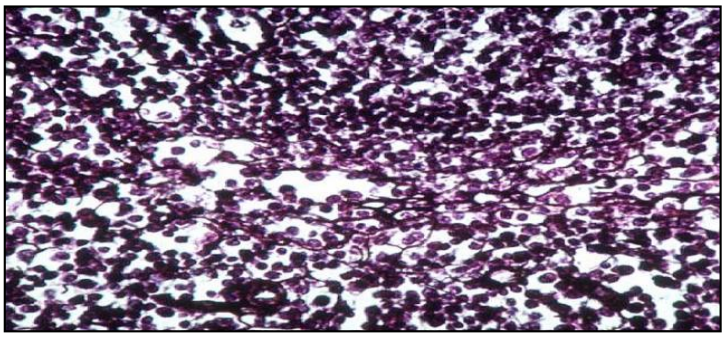
Illustrations



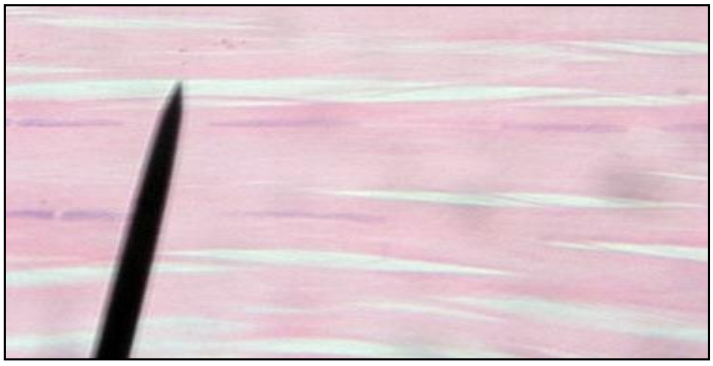
Areolar connective tissue



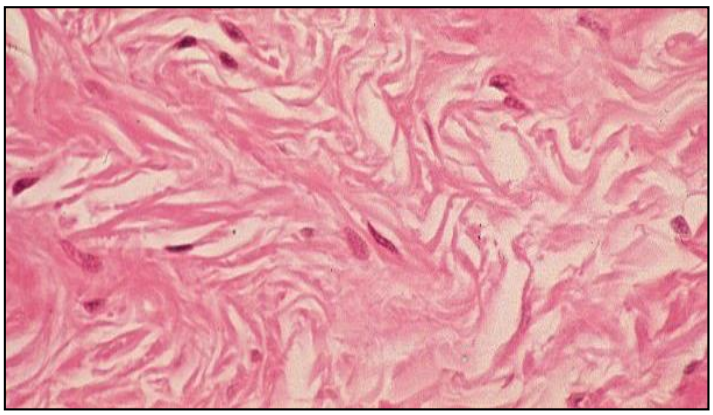
Adipose connective tissue



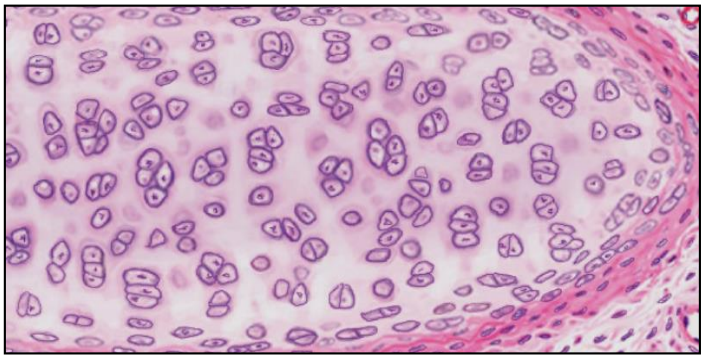
Reticular Connective tissu



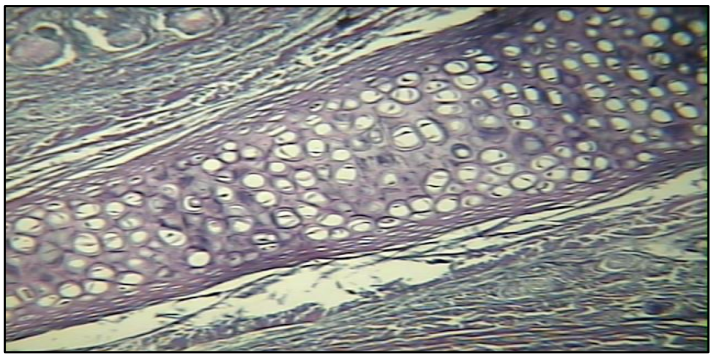
Dense regular connective tissue



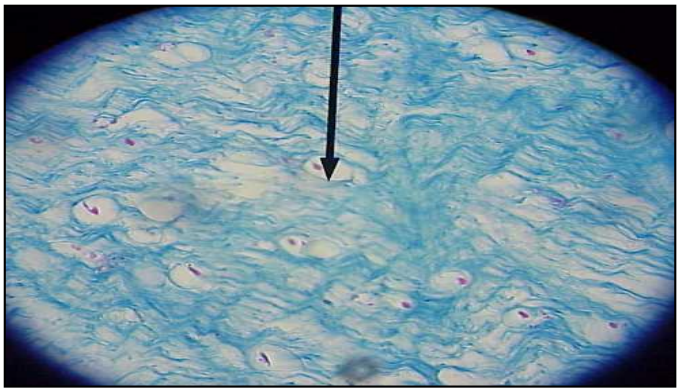
Dense irregular connective tissue



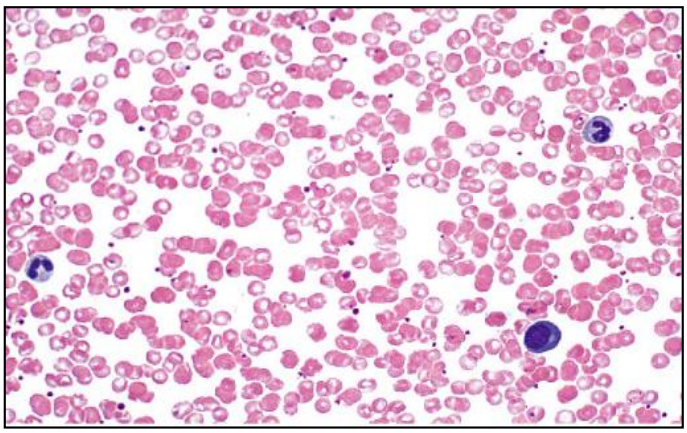
Hyaline cartilage



Elastic cartilage



Fibrocartilage



Human Blood smear

Compact Bone

Guide Questions

1. Tabulate the differences between collagen, elastic and reticular fibers based on the following criteria.

Criteria	Collagen	Elastic	Reticular
Abundance			
Thickness			
LM Appearance			
Staining affinity			
Mechanical Property			
Molecular structure			
Location			
Primary Function			

2. What is meant by metachromasia? Give examples of cells exhibiting this characteristic.

_____
_____
_____

3. Differentiate the following based on their histological appearance and functions.

A.Hyaline and elastic cartilage

_____
_____
_____

B. Spongy and compact bone

_____
_____
_____

C. Neutrophil and lymphocyte

_____
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