

LABORATORY 6 - CONNECTIVE TISSUE (first of three laboratory sessions)

Throughout the course, you will see connective tissue on virtually every slide, since connective tissue occurs under all epithelia. In order to acquire an initial understanding of the appearance and variety of connective tissues, you will first look at several types of soft connective tissue in the light microscope and in electron micrographs. During the second laboratory session you will study a few of the more specialized connective tissues and the three types of cartilage. The structure of bone is rather complicated and we will consider its development, mature structure and maintenance on a third day.

OBJECTIVES: LIGHT MICROSCOPY - Recognize types of soft and hard connective tissues including the cell types, fibers and matrix that each contains. Understand how the structure of connective tissues and their components are related to function.
ELECTRON MICROSCOPY - In soft and hard connective tissues recognize the cell types and fibers and know their characteristics.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

[SL 1](#) (Spread) Loose connective tissue (areolar)
[SL 91](#) (Salivary gland) Loose to dense irregular connective tissue
[SL 25](#) (Skin) Dense irregular connective tissue
[SL 24](#) (Bronchus) Loose to dense irregular C.T.
[SL 118](#) (Esophagus - stomach) - Mast cells in C.T.
[SL 27](#) (Tendon) Dense regular C.T.
[SL 184](#) (Large artery) Elastic C.T. - resorcin stained
[SL 31](#) (Large elastic artery) Elastic C.T.

ELECTRON MICROGRAPHS

EM 15 Elastin and collagen
EM 21-4 Fibroblasts

POSTED ELECTRON MICROGRAPHS

#10 Connective tissue fibers
#14 Basal lamina
#15 Mast cell
#29 Cytochemical method
[Lab 6 Posted EMs](#); [Lab 6 Posted EMs with some yellow labels](#)

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 5. Connective Tissue
Frames: 236-274

EXTRA CREDIT CASE ON BLACKBOARD: MARFANS SYNDROME

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., [An Atlas of Histology](#)
Copies of this text are on reserve
in the HSL.
Connective tissue pp. 82 – 94

I. SOFT CONNECTIVE TISSUE

A. The prototype for the connective tissues is LOOSE c.t. or AREOLAR c.t. (also may be referred to as loose fibro-elastic connective tissue)

1. [SL 1 \(med\)](#) - (J. 5-2, 5-24; W. 4.6a) The tissue in this slide has not been sectioned, rather it is a spread of connective tissue in which a piece of c.t., usually from under the skin, is placed on a slide and torn apart in such a manner that individual elements can be identified when the connective tissue is fixed and stained. Identify the following:
 - a) Collagen fibers - stained pink, size variable
 - b) Elastic fibers - stained purple to black, thin fibers, often long, occasionally coiled
 - c) Reticular fibers - cannot be identified without special staining
 - d) Fibroblasts - oval blue nuclei, usually no cytoplasm visible (cytoplasm minimal and acidophilic when evident)

[\(collagen fibers, red arrows; elastic fibers, blue arrows; fibroblasts, green arrows\)](#)

Note most of the large clear "spaces" are artifacts of the preparation, however, the tissue is "loosely" organized with spaces between the various elements. This space is occupied by the extracellular matrix consisting of fibers and the amorphous material called "ground substance" which does not stain specifically in this preparation. In life, it is through the ground substance that many metabolically active agents "flow".

2. [SL 91 \(scan\)](#) (Salivary gland) (W. 4.12) Histological section showing [loose connective tissue](#) and [dense irregular connective tissue \(loose c.t., red circle; dense irregular c.t., blue circle\)](#). This slide is a section of salivary gland and most of the tissue is glandular epithelium. Regions of connective tissue separate regions of the tissue into compartments. Scan over the slide and note that the irregular c.t. varies in appearance, the fibers are more loosely arranged (more space per unit area) in some regions and blend with more densely arranged tissue in other regions. The most loosely arranged regions would be designated as loose c.t. (areolar), while the regions that are most dense are designated as dense irregular c.t. A typical example of a large area of dense irregular c.t. is seen on the next slide ([SL 25](#)) for comparison.

B. DENSE IRREGULAR CONNECTIVE TISSUE

1. [SL 25 \(Skin\)](#) (W. 4.12) - An excellent example of [dense irregular connective tissue](#) is located in this section ([blue rectangle](#)). Beneath the epithelium is a large area of dense irregular c.t. In this region note the collagenous fibers and large bundles of fibers irregularly arranged with minimal amounts of "space" (ground substance) between them. Observe fibroblast nuclei sparsely scattered throughout. Elastic fibers are less prominent in this tissue and are difficult to identify. Also look at [SL 26](#) where the tissue has been stained with a different stain.
2. [SL 24 \(Bronchus\)](#) Review the appearance of irregular c.t. on this slide noting areas of loose (areolar) and more densely arranged c.t. ([scan](#), [low](#), [high](#), [loose c.t., red circle; dense c.t., blue circle](#)).

C. SPECIALIZED CELLS OF CONNECTIVE TISSUE

1. [SL 118](#) - Esophagus-stomach transition stained for MAST CELLS ([scan](#),). All of the tissues on this slide are stained light blue. Scan the slide under [low power](#) and find the small cells, that appear to stain "purple", scattered throughout the c.t. ([red circle](#)). Under [high power \(red circles\)](#) observe that the cytoplasm contains "purple" staining granules with blue staining nuclei present in some cells. These are the mast cells.

It is important to realize that a single stain (Azure A) was used on this section that resulted in the nuclei of cells staining blue whereas, in contrast, the mast cell granules stained reddish purple. This shift in the staining color is referred to as metachromasia, the granules stain metachromatically. It should be noted that mast cells vary in shape, size, etc. and in the human they are smaller than those in rat mast cells that often appear in textbooks. Text – J. 101-103 (J. 5-10; W. 4.18). Mast cells in routine H & E preparations usually cannot be delineated.

- D. ELECTRON MICROSCOPE – Observe EM 15-1 (elastic) and 2 (collagen) fibers. Note EM 21-4 two active fibroblasts with RER, collagen EM 21-3 for more detail see J. 5-26 to 5-30; W. 4.2, 4.9). Does a fibroblast have an external lamina? Review formation of collagen in text (J. pp106-112, Fig. 5-21).
- E. DENSE REGULAR CONNECTIVE TISSUE
1. SL 27 (J. 5-44 to 5-45; W. 10.31, 10.32) - Tendon and skeletal muscle. As you scan the slide you will find that there are two different areas of tissues, similar to those seen in W. 10.31, 10.32 (low). The tendon is usually the more compact region that is stained lighter (region of tendon in blue rectangle). Frequently, the thin fibroblast nuclei (blue arrows) are arranged in rows between wide areas of longitudinally oriented bundles of collagenous fibers. In places the bundles may appear wavy or shredded; however, the tight parallel arrangement is the normal appearance.
- F. ELASTIC CONNECTIVE TISSUE
- Elastic fibers may occur as either dense bands or fine fibers, but they are difficult to distinguish in routinely stained preparations. However, they can be stained specifically by a large variety of techniques.
1. SL 184 (scan)- A specific procedure was used, resorcin - basic fuchsin which stains elastic fibers "purple" (W. 8.9). There are 3 sections on this slide. Observe the middle, tubular structure (large artery) in which numerous "purple" staining elastic fibers or lamellae make up the wall (high). The dense purple regions going across several fibers are folds (artifact, between pairs of blue arrows) in the section of tissue. Also, locate small elastic fibers scattered throughout the tissues surrounding the artery.
 2. SL 31 (high) - Large artery stained with H & E. Compare the appearance with that in SL 184. By lowering the condenser on your microscope, the elastic lamellae (between pairs of blue arrows) will appear as shiny pink (refractile) fibers that are somewhat wavy.

OBJECTIVES FOR LABORATORY 6: CONNECTIVE TISSUE I

1. Using the light microscope or digital slides, identify:

Components of connective tissue

Collagen fibers

Elastic fibers (may need special stains)

Reticular fibers (seen with special stains only)

Fibroblasts

Mast cells (seen in special stain stains only - metachromasia)

Ground substance

Types of connective tissue (covered in this lab)

Loose (areolar)

Dense irregular

Dense regular (e.g. tendon)

Elastic tissue

2. On electron micrographs, identify:

Collagen fibers

Elastic fibers

Fibroblast

Basal lamina