LABORATORY 7 - CONNECTIVE TISSUE, continued

OBJECTIVES: (See Laboratory 6)

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

SL 32 (Spleen) Reticular fibers
SL 34 (Adipose tissue) Unilocular fat
SL 12 (Brachial plexus) Adipose tissue
SL 8 Brown fat
SL 35 (Umbilical cord) Embryonic connective tissue
SL 20 (Bronchus) Hyaline cartilage
SL 36A (1/2 Larynx, infant) Hyaline cartilage
SL 36B (Larynx, infant) Hyaline cartilage
SL 184 (Epiglottis) Elastic cartilage
SL 17 (Epiglottis) Elastic cartilage
SL 37 (Meniscus of knee) Fibrous cartilage

POSTED ELECTRON MICROGRAPHS

8 Organelles and inclusions
S-12 Lysosomes
S-23 Plasma cell
Lab 7 Posted EMs

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 5. Connective Tissue
Frames: 274-286
Chapter 7. Supportive Connective Tissue
Frames: 358-372

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., An Atlas of Histology. Copies of this text are on reserve in the HSL.
Fat, pp. 95 - 96
Cartilage, pp. 100 - 104

G. RETICULAR CONNECTIVE TISSUE

SL 32 (J. 5-46; W. 4.3) (Spleen) - A specific stain was used in which unreduced silver was selectively deposited on the fibers and subsequently reduced forming a black deposit outlining the fiber. Scan the slide and find the smallest fine black fibers scattered throughout the slide, these are the reticular fibers. Some of the largest fibers seen in more dense areas may be collagenous fibers that are highly glycosylated and, therefore, also stained by this method. Note - Reticular fibers always remain small and unless special stains are used, they are barely resolvable with the light microscope. Reticular fibers are associated with carbohydrates and therefore stain with both PAS and silver stains (low, med, reticular fibers, blue arrows; reticular fibers at periphery of splenic venous sinusoids, red circles).

H. ADIPOSE TISSUE

1. SL 34 (J. 6-1; W. 4.14, 4.15). (Thymus) Unilocular fat - Find clusters of clear areas surrounded by irregular outlines of tissue. The clear areas (blue arrows) are the regions of the cell where the lipids (fat) have been dissolved from the section by the reagents used in the preparation of the tissue. The thin outlines represent the cell cytoplasm plus the reticular fiber support that, at this level of microscopy, are indistinguishable from one another. In some areas the nuclei of the fat cell may be identified.

2. SL 12 (J. 6-5; W. 4.16, 4.17) - Locate areas where unilocular fat (red circle) cells are visible mixed in with cells having multiple clear areas in the cytoplasm. This is a region of developing adipose tissue. Within these fat cells, several vacuoles (multilocular) of fat will eventually coalesce to form one large vacuole (unilocular). This multilocular stage in development is similar in appearance to brown fat (J. 131-133). Compare this slide with SL 8 that is a section of brown fat where the fat has been retained and stained black.

3. Electron Microscope. Some unique characteristics to note are: brown fat cells have abundant mitochondria; lipid droplets are usually considered to lack a surrounding unit membrane; and, unlike other cells of the c.t., most fat cells have an associated basal lamina (also called an external lamina).

I. EMBRYONIC (MESENCHYMAL) CONNECTIVE TISSUE

SL 35 (Umbilical cord) (W. 4.10) - The appearance of this type of tissue is exemplified by the mucous connective tissue of the umbilical cord. Three rounded structures can be seen which are blood vessels. Connective tissue fills in around the vessel that shows branching, stellate "mesenchymal cells" contained within abundant ground substance (spaces).

II. HARD CONNECTIVE TISSUE

A. HYALINE CARTILAGE (J. 7-2; W. 10.1)

SL 20 (Bronchus) - Within the wall of the large tubular structure locate areas of hyaline cartilage appearing as shown in W. 10.1. Note the homogeneous appearance of the matrix, the variation in size and shape of the lacunae from the surface toward the center. Near the central region of the cartilage plates isogenous groups of cells (blue circles) (J. 7-2) are present. Within the lacunae, nuclei and the shrunken cytoplasm of chondrocytes can be seen. It is evident that after fixation for electron microscopy the cytoplasm of the chondrocyte fills the lacuna (W. 10.2). Identify the perichondrium and note the more peripheral region is fibrous and the area adjacent to the matrix is more cellular (W. 10.1). Compare the mature cartilage on this slide with the immature cartilage in SL 36A (even desks only, is ½ of the larynx i.e. one side) and SL 36B (odd desks, is whole larynx). Note that the immature cartilage has more cells and less extracellular matrix. The lacunae are less distinct, but they have a more uniform size and shape.

B. ELASTIC CARTILAGE

1. SL 184 (J. 7-7; W. 10.4) - There are three sections on this slide.

The C or U shaped piece (arrow) contains a section of elastic cartilage. The elastic fibers are specifically stained (purple) and are contained in the cartilage matrix. The rest of the tissue is relatively unstained, but under high power the general outline of the cartilage can be determined elastic fibers.

2. SL 17 (low, med, oil)- In the center of this piece of tissue areas of elastic cartilage can be found. In this H & E stained preparation abundant fine fibers (red circle; thicker elastic fibers, blue arrows) can be seen in the matrix, which are elastic fibers as demonstrated in SL 184. Compare the appearance of the matrix to that in hyaline cartilage.

C. FIBROUS CARTILAGE

SL 37 (J. 7-8; W. 10.3) fibrous cartilage (fibrocartilage) (med, high) from different locations varies in appearance. On this slide, some regions may lack cartilage. This is a section from the meniscus of the knee. The characteristics observed include small regions of cartilage recognized by the presence of chondrocytes (blue arrows) in lacunae and the absence of a perichondrium. Note other examples in text and atlases.

OBJECTIVES FOR LABORATORY 7: ADIPOSE AND CARTILAGE

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

    - Reticular fibers (using special stains, including PAS)
    - Adipose tissue
        - Unilocular
        - Multilocular
    - Mesenchyme
        - Mesenchymal cells
    - Cartilage
        - Hyaline
            - Lacunae
            - Chondrocytes
                - Isogenous groups
            - Matrix
            - perichondrium
        - Elastic
            - Same as hyaline + elastic fibers
        - Fibrocartilage
            - Same as hyaline + collagen fibers – perichondrium

2. On electron micrographs, identify:

    - Review organelles