LABORATORY 9 - MUSCLE

This lab has an associated homework assignment that is posted on If you have not done this assignment already, it would Blackboard. make this lab more efficient if you did so.

OBJECTIVES: LIGHT MICROSCOPY: Recognize three types of muscle as individual cells, bundles or sheets in any orientation. Know cell types, their structure and connective tissue coverings. Complete chart at end of this section.

> ELECTRON MICROSCOPY: Recognize three types of muscle cells, their characteristics and ultrastructural features. Relate structure to function. Recognize structures including the following:

Smooth muscle: central nucleus, microfilaments, dense plaques (or dense bodies) caveolae, external lamina.

Skeletal and cardiac muscle: muscle fibers, fibrils and filaments, bands and their significance, distribution of membranous structures, position of nucleus. Cardiac muscle: intercalated discs and their subcomponents and function.

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

Smo	ooth Mus	<u>scle</u>					
SL	57	(Bladder)	fiber arrangement				
SL	<u>53</u>	(Colon)	sheets of muscle				
<u>SL</u>	<u>138</u>	(Uterus)	bundles of muscle				
<u>SL</u>	<u>155</u>	(Nipple)	isolated bundles of muscle in connective tissue				
Skeletal Muscle							
<u>SL</u>	<u>86</u>	Thin plastic section (longitudinal). Bands most evident.					
SL	27	Skeletal muscle and dense regular connective tissue					
<u>SL</u>	61	(Diaphragm) cross section					
<u>SL</u>	<u>15A</u>	(Esophagus) smooth and skeletal muscle					
SL	60	(Skeletal Muscle) cross section					

Cardiac Muscle

88 Cross section and some longitudinal sections

64 Bencosme's stain

ELECTRON MICROGRAPHS

Smooth muscle J. 10-32; W. 6.19 Skeletal muscle EM 23; J. 10-10, 10-12, 10-15, 10-16, 10-17,; W. 6.9 to 6.13 Cardiac muscle J. 10-24 to 10-28; W. 6.25 to 6.26

POSTED ELECTRON MICROGRAPHS

- #2 Mitochondria
- #17 Muscle
- #18 Muscle

Lab 9 EMs; Lab 9 EMs with some yellow labels

HISTOLOGY IMAGE REVIEW - available on computers in HSL

Chapter 2. Muscle Tissue Frames: 18-72

SUPPLEMENTARY ELECTRON MICROGRAPHS in Rhodin Atlas Muscle pp. 128 – 141

MUSCLE

As you analyze your slides, note especially the nuclear location within the cells (is it central or peripheral) and whether there is one nucleus or many nuclei per cell. Also observe the varieties and organization of striations and the nature of the cytoplasm and organelles and then complete the chart at the end of this section.

Initially, it may be difficult for you to distinguish the different types of muscle from dense connective tissues and from peripheral nerves in both longitudinal and transverse sections of tissues. As you study other tissues, add them to the chart. Suggest functional significance for the different types of organization found in muscle.

- I. <u>SMOOTH MUSCLE</u> (J. pp. 207-213)
 - A. <u>Smooth muscle</u> (J. 10-29 to 10-33; W. 6.15 to 6.18)

<u>SL 57</u> (bladder) - Muscle fibers and arrangement. Note the network of fine reticular fibers around the muscle fibers, and other connective tissue. These extracellular elements constitute the "framework" of this variety of muscle (med, high, near areas indicated by red arrows, high (a)).

- B. Smooth muscle in "<u>sheets</u>" <u>SL 53</u> (colon) - At the periphery of this section two layers of smooth muscle are evident. The outermost layer is composed of smooth muscle cut in cross section, adjacent to this layer is a wider layer of longitudinally sectioned smooth muscle (<u>low</u>, <u>high</u>, cross sections in red circle, long. section in green circle <u>high (a)</u>).
- C. Smooth muscle in <u>interlacing bundles</u> (W. 6.15, 19.21) <u>SL 138</u> (<u>uterus</u>) - Compact "bundles" of muscle cut in various planes comprise the bulk of this organ. The muscle bundles are separated by minimal amounts of connective tissue.
- D. Smooth muscle in small groups of fibers or thin layers
 - <u>SL 155</u> (nipple) In this section small bundles of smooth muscle are scattered in the dense irregular connective tissue. The muscle bundles have a variety of orientations. This slide provides a good comparison between smooth muscle and dense irregular connective tissue (<u>low</u>, <u>high</u>, muscle bundles enclosed by green lines, <u>high (a)</u>).
 - 2. Electron microscope (J. 10-32; W. 6.19).
 - Question: What kinds of functional significance can you attribute to each of the patterns of smooth muscle observed in A, B, C and D?
- **II.** <u>SKELETAL MUSCLE</u> (J. pp. 191- 205).
 - A. Skeletal muscle in <u>longitudinal sections</u> (J. 10-1, 10-2, 10-5 to 10-9; W. 6.3).
 - 1. <u>SL 86</u> Skeletal muscle, thin section (monkey). In this section the A, H, I and Z bands are visible. Also, observe the following: shape of fibers, sarcolemma, frequency and position of nuclei (<u>high</u>, <u>oil</u>, red vertical lines indicate width of a fiber or cell <u>high</u> (a)).
 - 2. <u>SL 27</u> Skeletal muscle and dense regular connective tissue (ligament). The two types of tissue can be distinguished by their densities of staining and distribution of nuclei. In routine histology preparations cross-banding of muscle may be indistinct or absent. In the muscle on this slide, individual muscle fibers are evident and myofibrils may be observed in both longitudinal and cross sections due to the shrinkage of the tissue (low, med, high). At both low (a) and high (a) mag. dense regular c.t. is enclosed by a blue line.

- 3. <u>SL 61</u> (W. 6.2, 6.3, 6.5) Diaphragm <u>skeletal muscle</u> in cross and longitudinal sections.
- 4. Electron microscope. Study <u>EM 23</u> (J. 10-10, 10-12, 10-15 to 10-18; W. 6.9 to 6.13) compare longitudinal and cross sections from the text or atlas with the longitudinal section EM image 23, and note the morphological changes that occur during muscle contraction. Also, find <u>T tubules</u> and the <u>terminal cisternae</u> of the sarcoplasmic reticulum (see W. 6.12). T tubules are a number of invaginations of the plasma membrane (see J. 10-15) that allow the muscle's depolarization to reach the deeper portions of the cell, which has a wide diameter. Depolarization of the T tubules influences adjacent terminal cisternae to release calcium. In human skeletal muscle, each T tubule is flanked by terminal cisterna, forming a <u>triad</u> located near the junction of the A and I bands. The arrangement in human cardiac muscle is usually a <u>diad</u> consisting of only one terminal cisternae associated with a T tubule (see W. 6.25). Other species have different arrangements of these structures.
- B. Comparison of smooth and skeletal muscle

<u>SL 15A</u> (esophagus) – The wide band of muscle on the side of the section opposite to the epithelium (indicated by arrow in diagram and between arrows in micrograph to right) is a mixture of smooth and skeletal muscle in both longitudinal and cross section. This region provides an excellent opportunity to compare the two types of muscle (<u>scan</u>, <u>low</u>, <u>med</u>).



- C. Skeletal <u>muscles as organs</u> <u>SL 60</u> - A muscle in cross section. Distinguish the various connective tissue sheaths and the location of blood vessels. Review the way in which skeletal muscle may attach to cartilage or bone (<u>low</u>, <u>high</u>).
- III. <u>CARDIAC MUSCLE</u>. (J. pp. 206-210).
 - A. <u>SL 88</u> (J. 10-22, 10-23; W. 6.21, 6.22). Most of the muscle appears in <u>cross</u> or oblique sections. However, the few outermost fibers are cut <u>longitudinally</u> and demonstrate intercalated discs here seen as faint lines within <u>blue circles</u>. The intercalated discs may be difficult to identify, but they can be located on most slides.
 - B. <u>SL 64</u> (Bencosme's stain). Note contrast of muscle (red) and connective tissue (yellow), intercalated discs (easily seen on this slide), and muscle in various planes of section. Compare the structure of cardiac muscle seen in longitudinal and cross section with the other two types. Cardiac muscle fibers branch yielding irregular outlines in cross section (med, high 1, high 2). Intercalated discs are indicated (red arrows)
 - C. Electron microscope (J. 10-24 to 10-28; W. 6.25, 6.26). The structure of intercalated discs is illustrated and described in figure, J. 10-26.

OBJECTIVES FOR LABORATORY 9: MUSCLE

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

Muscle type (in all planes of section) Skeletal Myofibers (muscle fibers, muscle cells) Myofibrils Fascicle Endomysium Perimysium Epimysium Cardiac Intercalated disks Smooth

Bands seen in skeletal and cardiac muscle A band H band I band Z line M line Sarcomere

2. On electron micrographs, identify:

Muscle types Skeletal Myofibers (muscle fibers, muscle cells) **Myofibrils Myofilaments** Thick filaments Thin filaments Sarcolemma External lamina Sarcoplasmic reticulum Triad Terminal cisternae of sarcoplasmic reticulum (x2) Transverse (T) tubule (x1) Bands noted above for light microscopy Glycogen and mitochondria Cardiac Structures noted above for skeletal muscle Diad instead of triad (only one terminal cisternae) Intercalated disks Gap junction Smooth Myofilaments (mostly actin filaments) Dense bodies (cytoplasmic densities) Caveolae (pinocytotic vesicles) External lamina Gap junctions

REVIEW

- 1. Compare a contracted sarcomere with a relaxed sarcomere. How is the pattern of bands changed?
- 2. Compare an intercalated disc with the components of a junctional complex in epithelium.
- 3. In skeletal muscle and smooth muscle where is the intermediate filament desmin located?
- 4. What is the structure in muscle that is comparable to the basal lamina?
- 5. For review purposes from your visual picture, complete the chart on the following page. Check completeness and accuracy in your text or atlas.

THE DIAGNOSTIC CHARACTERISTICS OF SELECTED HISTOLOGICAL SPECIMENS

	<u>_</u>	ELECTRON MICROSCOPE		
<u>TISSUE</u> <u>NAME</u>	Position of visible nuclei in relation to fibrous material? (Central, peripheral, other)	Striations in longitudinal sections. (Longitudinal Transverse, or both?)	Appearance of fibrous material in cross section. (Homogeneous, grainy, of uniform diameter, organization, etc.)	Suggest at least one distinctive feature seen at this level of organization
<u>Dense Regular</u> <u>Connective Tissue</u>	Outside of fibers (Fibroblasts)			
Smooth Muscle				
Cardiac Muscle		Both L. and T.		
Skeletal Muscle				