

## PCB 4023 – Cell Biology

### Lab 5: Muscle

Name: \_\_\_\_\_

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**N.B.** Since this document is in “pdf” format, the URLs (web addresses) cannot be linked. To use them, simply highlight and copy the address and paste it into the address box of your browser.

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#### Preparation assignment (to be completed before lab (1/25/02):

- 1) Kerr (1999) Chapter 5. Muscle (pp. 81-106).

#### Web resources:

**N.B.** Again, you may wish to examine the modules pertaining to muscle at the University of Florida College of Medicine histology tutorial. For muscle there is a review module and an on-line quiz. As before, these modules may contain information that is not covered in this course and you will not be held responsible for any information presented there. However, you may wish to consider using them as additional preparation and/or review. The URL is as follows:

<http://www.medinfo.ufl.edu/year1/histo/index.html>

Know of any other web sites pertaining to muscle that you have found helpful or interesting? E-mail me the link at [condon@fiu.edu](mailto:condon@fiu.edu). and let me know what and why you found it informative and/or interesting.

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## MUSCLE

You will recall that there are four basic types of tissue and the vast majority of cell types can be classified as one of these four:

- 1) epithelia - continuous layers of cells with little intercellular space which line surfaces and form glands
- 2) connective tissue - cells embedded in intercellular substances (extra-cellular matrix); the matrix is typically abundant and the cells less so.
- 3) muscles - specialized contractile cells with electrically excitable membranes
- 4) nervous tissue - (1) cells specialized for conducting and transmitting electrochemically mediated information (i.e., neurons) and (2) the cells which support those engaging in this activity (e.g., neuroglia or glia).

Today we will examine muscle. Like the neurons of the nervous tissue, muscle cells have membranes capable of generating electrical charges. In contrast to neurons, however, they use these charges to control the activity of internal contractile (tension generating) proteins, rather than for communication with other cells. Muscles, along with glands, are the major effectors of the nervous system and there is an intimate relationship between these two tissue.

Muscles serve a variety of functions. (1) They supply force for movement, both internally and externally. Internally, they act upon viscera to affect their activity, e.g., peristalsis, sphincters, constrictors (vessels and air passages), etc.. Externally, with the skeletal system, muscles provide the force to effect

behavior. (2) Muscles supply non-skeletal support to much of the body (e.g., organs of the abdomen and perineum, pectoral girdle, etc.). (3) Muscles are also important in the endothermic generation of heat, both by means of non-shivering thermogenesis (the waste product of cellular respiration) and shivering thermogenesis. In some species of deep-diving fish (e.g., swordfish, marlins, spearfish, etc.), some cranial muscles lose their contractile proteins and hypertrophy their membrane to become thermogenic organs. (4) As noted above, in order to contract, muscle cells must depolarize their membrane by producing a weak electrical signal of very low voltage. Many species of fish (500+) have modified some of their muscles to produce electric organs, blocks of specialized muscles that produce high levels of voltage rather than mechanical force.

figure: WL9-1

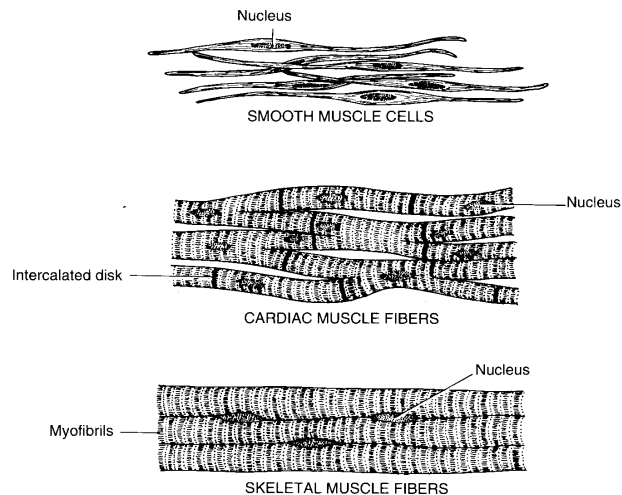
There are three types of muscle cells or fibers present in craniates (vertebrates): smooth, cardiac, and skeletal. All are specialized for contraction and all work on the same principle. ATP-powered sliding filaments. Like neurons, all possess electrically excitable membranes, and almost all can generate action potentials (tonic skeletal muscle fibers cannot

Smooth muscle is found in the viscera of the digestive, respiratory, excretory and reproductive systems, blood vessels, hair follicles, etc. It occurs in both sheets (e.g., muscular walls of blood vessels and organs) and as isolated cells (e.g., myoepithelial cells).

Smooth muscle is under involuntary control of the autonomic nervous system and keeps air, food, waste and blood moving. Histologically, smooth muscle fibers are elongated, spindle-shaped cells with a single, centrally located nucleus. Since the contractile proteins are not systematically arrayed, they lack striations. Physiologically, smooth muscle fibers produce slow and sustained levels of tension and are resistant to fatigue. The fibers are electrically coupled (gap junctions) to one another resulting in the spread of action potential across adjacent fibers. Thus, in contrast to skeletal muscle, each smooth muscle fiber is not individually innervated. Contraction (= tension generation) can either be myogenic (self-initiated, often in response to stretch) or neurogenic.

Cardiac muscle fibers are found in the heart and in the proximal regions of the great arteries (aortae, pulmonary trunk, etc.). These are single, branching cells (cardiocytes) arranged in a meshwork which can resist high pressure without tearing. Their sarcoplasm (cytoplasm) appears striated due to the orderly arrangement of contractile proteins into units known as sarcomeres. Cardiac muscle fibers are joined to one another by intercalated discs. These specialized intercellular junctions provide strong mechanical adhesion and are also the site of electrical coupling (gap junctions). Thus, as in smooth muscle, it is not necessary that every cardiac muscle fiber be individually innervated. Physiologically, cardiac muscle fibers produce rapid and forceful levels of tension, and, thankfully, are resistant to fatigue. The rate of the heart beat is under control of autonomic nervous system but the heart beat itself is intrinsic (myogenic), being initiated by pacemaker cells in sinoatrial node.

Skeletal muscles fibers are not true cells but syncytiums containing multiple nuclei and developing from the fusion of many cells. This fusion permits the fibers to grow very large and are easily visible to the naked eye. Skeletal muscle fibers, like cardiac fibers and for the same reason, are striated in appearance. In contrast to cardiac and smooth fibers, skeletal fibers are not electrically coupled (at least when mature) but are individually innervated and contraction (tension generation) is neurogenic. These muscles are under voluntary control of the somatic nervous system. As pointed out by that great muscle biology, Harlan K. Sanders, III, not all skeletal muscle fibers are the same, that is, there are varying types of skeletal muscles fibers which differ in their speed of contraction and/or ability to resist fatigue.



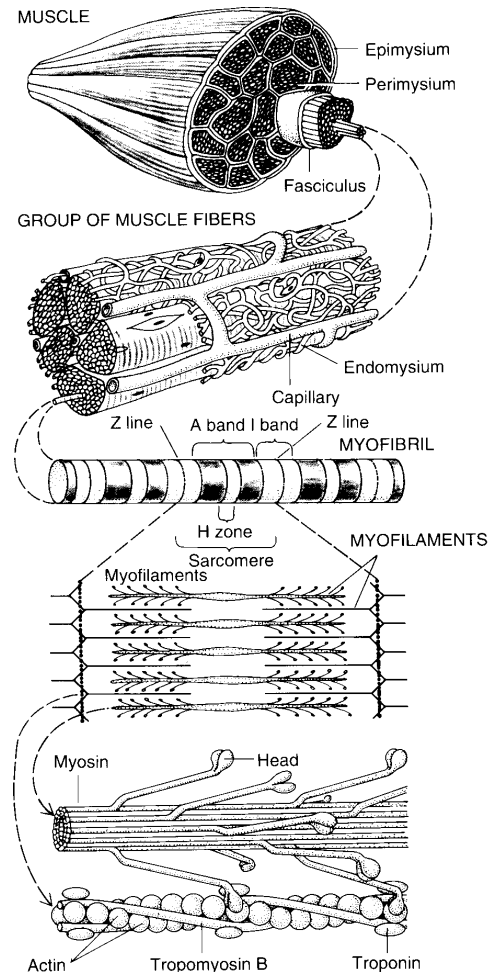
Unfortunately, like the term “bone”, “skeletal muscle” can refer either to a tissue (skeletal muscle fibers) or an organ (a skeletal muscle such as soleus, masseter, etc.). A skeletal muscle [organ] typically consists of (1) skeletal muscle fibers, forming the gaster or belly, (2) three connective tissue coats (endo, peri- and epi-myseium); (3) tendon, (4) nerves (both afferent (sensory) and efferent (motor)), and (5) vessels. The latter are particularly abundant, each muscle fiber being surround by a capillary bed. Muscle fibers are metabolically active and require a constant supply of oxygen and nutrients for cellular respiration to produce ATP.

figure: W&L9-2

The structure of a skeletal muscle [organ] is illustrated in the accompanying figure. Three layers of irregular dense connective tissue envelop each skeletal muscle: (1) The endomysium (Gr., within + muscle) surrounds each muscle fiber; (2) the perimysium (Gr., around + muscle) surrounds groups of muscle fibers into fasciculae or fascicles (L., dim of bundle); and (3) epimysium (Gr., upon + muscle) surrounds entire muscle and is forms the deep fascia of gross dissection.

Tendons attach the organ to the periostium of bone or dermis of the skin and are comprised of regular dense connective tissue. The myotendinous

As noted previously, each muscle is comprised of one or more fascicles, and each fascicle is comprised of 2 or more muscle fibers. Each muscle fibers is comprised of hundreds to thousands of myofibrils, cylindrical bundles of contractile proteins which span length of cell and are typically 1-2 microns diameter. The force output of a muscle is determined by the number of myofibrils. Each myofibrils is comprised of serially (longitudinally) arranged sarcomeres. Sarcomeres, as the name suggests, are the fundamental units of contraction (tension generation) and are comprised of near-dizzying array of proteins (actin, myosin, tropomyosin, etc.). A single sarcomere extends from Z-line to Z-line. Attaching to and extending from each Z line are thin filaments (primarily actin). In the center, between the opposing sets of actin filaments are the thick filaments (myosin). It is the alternating arrangement of thick and thin filaments that gives skeletal (and cardiac) fibers their striated appearance in light microscopy. Just remember, that the I- and A-bands seen under the light microscope are produced by the sarcomeric arrangement but are not-synonymous with them, i.e., the former are optical units whereas the latter are functional units and the two do not correspond in a one-to-one fashion.




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### Lab Assignment: Muscle

Work through the following sections using your atlas as a guide. Make sure to answer the questions (marked by “?”) at the end of the lab; these will be evaluated when you turn in your handout next week. A list of structures which will form the basis for next week’s quiz is given at the end of the handout.

To learn how to identify the structures, write down criteria which will assist you in your identification (e.g., simple squamous epithelium: single layer, flat cells with flattened nuclei). Your text is

a good source for such material as well as your own observations. Some students find it helpful to make rough sketches of the structure to assist in their learning.

**N.B.** Due to [unprogrammed] slide death, your slide box may not contain the required slide. If this is the case, notify an instructor and they will provide a replacement or suggest an alternative. If you end up borrowing a slide from one of your colleagues', please don't forget to return it to them.

## I. Smooth Muscle

muscle types (isolated): 93W3513, HD 5-1  
small intestine: 93W4526, HK 7-24  
colon: 93W4540  
uterus: 93W7314, H 8485 (best), H8486

Although smooth muscle cells can occur in isolation or in small cluster (e.g., myoepithelial cells, erector pili, etc.) it is most easily observed where it forms muscle walls, as in the intestines and the uterus. In the gut smooth muscle forms the thinner muscularis mucosae of the mucosa and the thicker, more prominent muscularis externa, the latter typically containing both circular and longitudinal sheets. Look for areas of clear transverse and longitudinal sections. Smooth muscle forms almost all of the uterine wall.

When sectioned longitudinally (i.e., along their long axis) smooth muscle cells have a slender, fusiform shape with a single central nucleus oriented in the direction of the fibers. Close examination will reveal these contractile cells are embedded within a thin connective tissue with associated fibroblasts. Make observations as to the position of the nucleus, nucleocytoplasmic ratio, presence or absence of striations, wrinkling of nuclei in longitudinal section, and difference in the diameter of muscle fibers.

Look for large arteries in the intestine and uterus. You will note that the tunica media is comprised predominantly of smooth muscle fibers arranged in a circumferential fashion.

**N.B.** Collagenous -tissue and smooth muscle are often confused. In connective tissue there is an interweaving arrangement of fibers and the nuclei are situated between the fibers. In smooth muscle the fibers appear as parallel bands and adjacent fibers tend to overlap; the nuclei are within the fibers.

Identify:           smooth muscle fibers  
                      nucleus

## II. Skeletal muscle

muscle types (isolated): 93W3513; HD 5-1  
tail: H800 (best); 93W3321  
tongue: H2690 (best; extras available); HD 2-212

Examine the skeletal muscle in the comparative slide. In cross-sections notice that the fibers are tightly packed with myofibrils, the nuclei are peripheral and the individual fibers are surrounded with a loose connective tissue, the endomysium. Also in cross section, note that the individual fibers are gathered into groups called fascicles which are surrounded by a perimysium; the entire muscle is encased by the epimysium which forms the deep fascia of gross dissection.

In longitudinal section observe that the fibers are multi-nucleated (all peripheral) and that the cross striations are very obvious. Examine these striations and determine the A and I bands. Note that under LM it is not possible to distinguish satellite cells from myonuclei.

In the tail, the muscles will appear predominately in cross-section. Make sure you can distinguish this tissue (and its "mysiums") from adjacent tendons (denser regular connective tissue).

In the tongue, you will see that the muscle fibers are interlaced in many different planes. This arrangement of the muscle allows for movement of the tongue in many directions.

In cross-sectioned muscle, look for muscle spindles. These sensory organs will appear as a bundle of small-diameter muscle fibers (intrafusals) within a connective tissue capsule or spindle.

Again, look for large arteries in the tail and tongue and note that the tunica media is comprised predominantly of smooth muscle fibers.

Identify:           skeletal muscle  
                      nucleus  
                      endomysium  
                      perimysium  
                      epimysium  
                      I bands  
                      A bands  
                      muscle spindles (possibly)

### III.     Cardiac Muscle

muscle types (isolated): 93W3513; HD 5-1

Observe cardiac tissue in the comparative slide. Because the fibers course in various directions you should look for areas with good transverse or longitudinal cuts. Look for the position of the nuclei, the nucleo-cytoplasmic ratio, presence or absence of striations, intercalated discs and branching of the fibers.

**N.B.** Cardiac and skeletal muscle can be confused. Cardiac muscle fibers are significantly shorter and thinner; contain a single, central nucleus; possess faint cross-striations, possess intercalated disks; and the fibers exhibit a branching, intertwined pattern. Skeletal muscle fibers are significantly wider and longer, contain multiple, peripheral nuclei, have prominent cross-striations, and have a parallel, non-intertwining arrangement.

Identify:           cardiac muscle  
                      nucleus  
                      intercalated disk

### IV.     Demonstration slides:

#### A.     Myotendinous junctions: HD 3-12 (DEMONSTRATION)

No skeletal muscle fiber inserts directly to its attachment (e.g., bone, skin) but does so through a connective tissue intermediary. When attaching to bone, this connective tissue is called a tendon and typically consists of dense regular connective tissue. The muscle – tendon interface, or myotendinous junction, consists of interdigitating skeletal muscle fibers and extensions of the tendon. This interdigitation is difficult to see in this demonstration slide but is nicely illustrated in your text figure 5.9c.

Identify:           myotendinous junction

#### B.     Purkinje fibers: 93W3533 (DEMONSTRATION)

The conduction system of the heart is comprised of modified cardiac myofibers. With normal staining (e.g., H&E), most of the cells of this system are difficult to distinguish from normal cardiac fibers. However, the terminal fibers of the system, the Purkinje fibers, are morphologically distinct and are found in the walls of the ventricles just deep to the endocardium (i.e., in the subendothelial layer). [Ask Dr. Kos about the embryological origin of Purkinje fibers. Also, ask Dr. Kos how not to pronounce "Purkinje".] The Purkinje myocytes are large cells which appear puffy, pale and contain a greater amount of cytoplasm, and fewer fibrils, than the usual cardiac fiber.

Identify: Purkinje fibers

#### V. Questions (Due Week 6)

- ? Which two muscle types appear striated when sectioned longitudinally? What is the basis for this striation? In which of the two are the striations more prominent?
- ? Which two muscle types have a single, centrally located nucleus?
- ? Which two muscle types are electronically coupled?
- ? Which two muscle types are innervated by the autonomic nervous system?
- ? What is the special name given to the cytoplasm of muscle fibers? The plasma membrane? The endoplasmic reticulum? The contractile units? What does the Greek root "sarco-" mean? What is a "sarcophagus", literally and figuratively?
- ? What is the name given to the stem cells found between the sarcolemma and basal lamina of a skeletal muscle fiber? What is their role in muscle injury?
- ? What is the difference between a syncytium and plasmodium (small case 'p')? [Hint: Consult a good medical dictionary.]
- ? Distinguish the epimysium from the perimysium from the endomysium. What type of tissue forms these structures? What type of cells produces them?
- ? Distinguish anatomically between the contents of skeletal muscle the organ versus skeletal muscle the tissue.
- ? Distinguish between a syncytium and plasmodium. [You may have to consult a biological dictionary.] Skeletal muscle fibers are an example of which?
- ? What is a (skeletal muscle) satellite cell? Where is it found? What is its function?
- ? Describe anatomically the following structures of a sarcomere?  
Z – lines

A –bands  
I – bands

Which of the above is not distinguishable in light microscopy? What does the A and I refer to?  
What type of filament(s) (thin/action, thick/myosin) are found in the A band?  
What type of filament(s) (thin/action, thick/myosin) are found in the I band?

- ? Define a motor unit.
- ? What part of the heart , endocardium, myocardium or [visceral] pericardium is formed by cardiac muscle?
- ? What two functions do intercalated disks serve?
- ? Why is it important that the cardiac fibers of the heart be electrically linked?
- ? Who was Purkinje?

VI. Quiz: Be prepared to identify the following structures on next week's quiz:

smooth muscle

- smooth muscle fiber
- nucleus

skeletal muscle

- skeletal muscle fiber
- nucleus
- endomysium
- perimysium
- epimysium
- I bands
- A bands
- muscle spindles (possibly)
- myotendinous junction

cardiac muscle

- cardiac muscle fiber
- nucleus
- intercalated disk
- Purkinje fiber