Biology 347 General Physiology Lab
Microscopic Examination of Muscle Contraction

Objectives

- Students will be able to identify the microscopic structures associated with striated muscle
- Students will gain exposure using the following techniques:
  - Polarized light microscopy
  - Light microscopy
  - Microscopic measurements
  - Tissue dissection and observation

Introduction

Motility is a fundamental life process. It is carried out by systems of filamentous proteins, including actin, myosin and tubulin. This exercise concentrates on the former two components. Certain cells are specialized to perform almost exclusively in contraction; these are referred to as "muscle," and they function in locomotion of the organism and transport of fluids down the various tubes of the digestive, circulatory, and glandular systems.
In vertebrates there are three distinct types of muscle, differing in function, structure, and distribution.

1. Smooth muscle is composed of sheets of cells, each cell with one nucleus. Contraction is involuntary. This tissue is found in the walls of blood vessels, the digestive tract and the reproductive system.

2. Cardiac muscle, found only in the heart, is composed of uninucleate cells bound together at dense plaques called intercalated disks. This tissue appears to be striated, or striped, owing to the parallel arrangements of the bundles of contractile fibers. It, too, is under involuntary control.

3. Skeletal muscle, under voluntary control, is generally attached to the skeleton. In order to give the maximum strong, directional contraction, individual cells have fused into multinucleated fibers. Prominent striations are visible in the light microscope, due to the extreme regularity of arrangement of the contractile proteins.

We will concentrate on examining the third type, muscle fibers from the rabbit psoas muscle. This muscle, from the small of the back, has long fibers and little connective tissue, making it ideally suited to studies of this kind. Fresh muscle fibers are difficult to use since they deteriorate rapidly. We will use instead fibers that have been treated with glycerol (glycerinated). This treatment makes the membranes permeable, so all the low-molecular-weight material is extracted, but the proteins of the contractile apparatus remain behind. The preparation can be stored in the freezer for long periods. By adding solutions of different compositions to the glycerinated muscle fibers, it is possible to identify the requirements for contraction. Occasionally relaxation can also be demonstrated, but this function is controlled by a regulatory system usually lost after glycerination

Procedure: Examination of Prepared Muscle Slides

I. Skeletal Muscle

Skeletal or striated muscles are attached to the skeleton and are controlled voluntarily. The cells of skeletal muscle are merged together to form one long muscle fiber. The banding patterns of the actin and myosin lend to the striated appearance of the tissue. Nuclei can be seen surrounding the muscle fibers.

Slide- Skeletal Muscle of Primate

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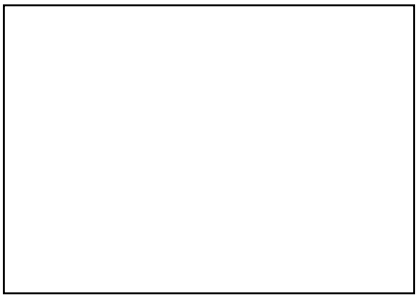
II. Smooth Muscle

Smooth muscle is also referred to as involuntary muscle. The cells are long, spindle shaped cells that contain their own nucleus. They differ from skeletal muscle in appearance as they lack the banding patterns. Smooth muscle is usually arranged into sheets of cells that contract in a rhythmic fashion.

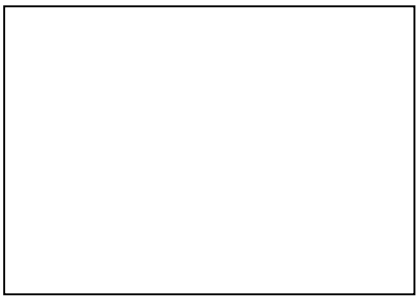
Slide- Smooth Muscle of Primate



III. Cardiac Muscle

Cardiac muscle is similar in appearance to skeletal muscle, as it is striated. However, cardiac muscle differs from skeletal muscle because it is involuntary and the muscle fibers are uninucleated. These muscle cells have a specialized cell junction, called intercalated discs that allow the cells to communicate and contract as a unit.

Slide- Cardiac Muscle of Primate



Procedure: Preparation of Muscle Mounts

1. Take a 2 cm segment of glycerinated muscle and soak it for 30 minutes in ice-cold standard salt solution: 100 mM KCl, 5.0 mM Pipes buffer, 4 mM EDTA, 4 mM MgCl2, pH 7.0
2. Move the fibers and some of the salt solution to a small Petri dish set inside a larger Petri dish of ice. Using two pins or dissecting needles, shred the fibers longitudinally until they are less than 0.2 mm in diameter. You may find it helpful to examine them under a dissecting microscope as you work. The finer the filaments, the better the preparation.
3. Make a wet mount of a portion of the suspension with a glass slide and coverslip. Examine the finest filaments at low and high power and record your observations in as much detail as you are able. Try using both the polarized light microscopes and regular light microscopes. Decide which one works best for you and your ability to resolve the structures. Seal the coverslip to the slide with nail polish and examine with oil immersion. It may be necessary to withdraw some of the fluid with a filter paper wick or with bibulous paper; if so, avoid jarring the coverslip.
4. Relate the structure you see to the model of a sarcomere. Draw the repeating units in the banding pattern and identify them. The long dark transverse bands are A bands. The long light bands are I bands. Across the middle of the A bands, you can distinguish a lighter zone, the H zone, and you may be able to see a narrow, dense line across the middle of the I band, the Z line. The basic unit of contractility is the sarcomere, extending from Z line to Z line.
5. Measure the average sarcomere length using the ocular micrometer; measure the widths of the various bands also. If you have calibrated your micrometer, you can convert these measurements into units of μm.

Procedure: Sarcomere Length Analysis

1. Make a wet mount of another fibril and seal the coverslip to the slide on two parallel sides with nail polish to form a chamber. Locate the fibril under the microscope and test to see if it is attached to the slide, by drawing standard salt solution through the chamber with a wick of filter paper. If it is not displaced, it is attached.
2. While observing the fibril, draw a small drop of solution 1 through the chamber. Now add a drop of ATP. What happens? Measure the band lengths and sarcomere lengths and prepare a drawing as before. Which have changed? Relate the structure to the model of a sarcomere.
3. See if any change results by now drawing solution 2 over the fibril.
4. With a fresh wet mount of fibrils make another chamber. This time add the solutions in the order: standard salt solution, solution 2, ATP, solution 1, more ATP. What can you conclude about the requirements for contraction of skeletal muscle?
5. It is known that one of the contractile proteins, myosin, is soluble in 0.1 M sodium pyrophosphate at pH 6.4. Prepare a fresh wet mount of fibrils. Draw a drop of solution 3 into the chamber. Do you observe any change in the banding pattern?

Table 1: Composition of Solutions

	KCl	Pipes buffer	$MgCl_2$	$CaCl_2$	EDTA	ATP	Na pyro-phosphate	pH
Solution 1:	85 mM	5 mM	5 mM	0.1 mM	--	--	--	7.0
Solution 2:	85 mM	5 mM	5 mM	--	5 mM	--	--	7.0
Solution 3:		5 mM	1 mM	--	--	--	100 mM	6.4
ATP solution	85 mM	5 mM	5 mM	--	--	10 mM	--	7.0

Figure 4: Muscle Mount

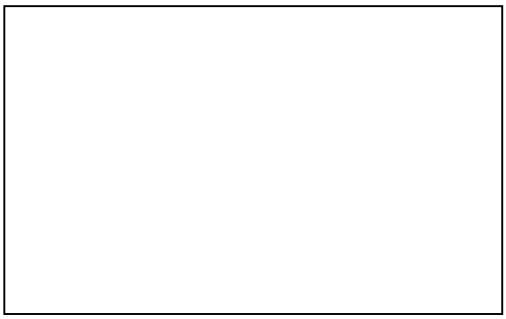


Figure 5: Sarcomere Length- Solution 1 and ATP

Figure 6: Sarcomere Length- Solution 2

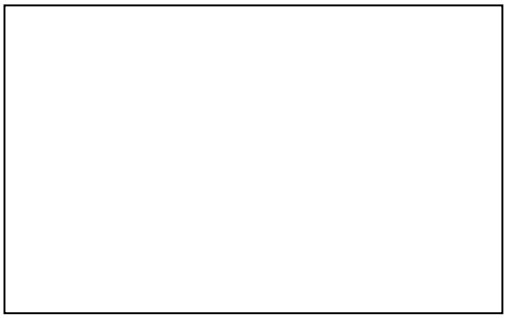


Figure 7: Sarcomere Length- standard salt solution, Solution 2, ATP, Solution 1, more ATP

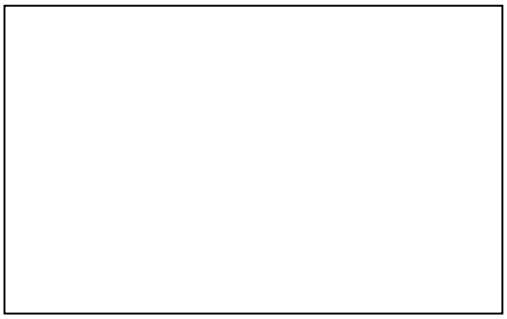


Figure 8: 0.1 M Sodium Pyrophosphate at pH 6.4