Length-Tension Properties Of Skeletal Muscle

Introduction

This purpose of this experiment is to determine the passive and active length-tension relationships in the frog semitendinosus muscle. Twitch and tetanic properties will be studied. It is the function of muscle cells to produce tension. In doing so they may shorten, remain at the same length, or even lengthen. There are two basic properties of skeletal muscle that make this possible: (l) excitability and (2) contractility.

When a muscle fiber is stimulated by its motor neuron, chemicals released at the motor end plate region of the fiber spread a wave of excitation, which is propagated along the muscle membrane. This electrical signal is then transmitted to an internal membrane system, which is composed of a transverse tubular system (or T-system) and elements of the sarcoplasmic reticulum (or SR). It is this latter structure that releases calcium and initiates the chemical breakdown of ATP and the other processes associated with muscle contraction. The contractile behavior of muscle then becomes the direct manifestation of these electrical, chemical, and mechanical changes taking place on the molecular level. For this reason, muscle physiologists have used parameters associated with contractility to elucidate and quantify many of the basic mechanisms of muscle contraction.

The dynamic aspects of contractility are experimentally described in terms of (l) the relation between the degree of filament overlap and the isometric force generated (the length-tension curve), (2) the relation between the capacity to bear a load and the speed of shortening (the force-velocity curve), and (3) the stress-strain characteristics of the elastic elements of muscle.

In the following exercise, you will determine experimentally some of these relations and will use the information to construct a mechanical model for muscle contraction that will enable you to describe and predict the muscle's behavior functionally.

Background

Two types of muscle contraction have been defined. If a muscle is required to lift a load and in doing so is allowed to shorten, the muscle is said to be contracting isotonically (isotonic = constant tension). On the other hand, when a muscle is stimulated while both of its ends are rigidly fixed so that no shortening can occur, the muscle will contract isometrically (isometric = constant length).

The tension *vs*. time characteristic of an isometrically contracting muscle in response to a single impulse produces a muscle twitch. If a second stimulus is delivered to the muscle before the first response completely decays, the phenomenon of summation occurs. When many stimuli are applied with a sufficiently high frequency (40-100 pulses per second in this experiment), a fused tetanus ensues, the height of which can be 3-4 times greater than that of a single isometric twitch.

According to the widely-accepted sliding filament theory of muscle contraction (Huxley and Hanson 1954; Huxley and Niedergerke 1954), the ability of a muscle to contract depends upon the amount of cross-bridge interaction between thick and thin filaments i.e., on the degree of filament overlap. It follows then that there should be a relation between the length at which the muscle is held when stimulated and the maximum twitch tension it is able to develop.

Gordon, Huxley and Julian demonstrated in 1966 that when a frog muscle fiber is stimulated after being stretched to a length where its sarcomeres are greater than the sum of the thick and thin filament lengths (3.65μm), the active tension produced quickly falls to zero. Between sarcomere lengths 3.65 and 2.2μm, where the number of crossbridges increases linearly with decreasing length, the active tension produced was found to increase with a linear fashion. The length-tension relation has been the subject of much investigation (Ramsey and Strett 1940; Edman 1966; Podolsky 1964) and you should be familiar with this aspect of contractility.

Experimental Model:

Investigators often choose experimental systems that have been the subject of investigation by other workers in the field. In muscle physiology, a great majority of the data published have come from experiments on the semitendinosus muscle for several technical reasons. It is a very thin muscle (1-2 mm thick) possessing fibers arranged in a parallel fashion with fibers running almost the entire length of the muscle. As a result, electrical stimulation of all of the fibers will be synchronous and diffusion of Ringer's solution and O_2 will be complete.

Stimulation:

It is a common practice in laboratory courses to stimulate muscles via their motor nerves or by direct application of artificial stimulation from pulse generators. Artificial stimulation is massive and insures that all of the fibers of the muscle are activated synchronously. In addition, nerve and muscle propagation times as well as transmitter release times are abolished.

Equipment
• Stimulator (Grass SD-9)
• Oscilloscope (HP 54600B)
• Muscle chamber
• Isometric force transducer (Harvard Apparatus #52-9529)
• Weights/weight tray
• Pole/clamp setup
• Syringe w/tube for emptying/filling chamber

In this first lab, we will make the majority of measurements directly on the oscilloscope. At the end of the experiment, it will be possible to use the computer to acquire a signal for plotting purposes. Use the LabVIEW program to acquire a tetanic contraction to including in your write up as an example plot (optional).

Surgical Equipment/Supplies
• Frog Ringer's Solution (FRS: 110 mM NaCl, 2.5 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.0 mM D-glucose, 5 mM HEPES)

• Squirt bottle
• Dissection instruments
• Dissection board
• Suture

Tissue

• Frog: semitendinosus muscle

Prelab Questions:

• Explain how calcium is involved in striated muscle contraction.
• Explain the structural basis for the shape of the skeletal muscle length-tension curve.
• How can one determine the sarcomere length in an isolated muscle? Does it matter how long the muscle is when sarcomere length is measured?
• What is the time-to-peak tension of a single twitch last in a frog semitendinosus muscle?
• A "good" muscle preparation will last for several hours with the muscle developing the same amount of force with each contraction. Give 2 reasons why the force of contraction might change over this time.

Experimental Procedure

Isometric Force Setup

Assemble the components of the setup as shown in Figure 1. Try to avoid friction between the suture and the apparatus by lining up the force transducer and the mounting rod with the holes in the muscle chamber and the connecting rod.
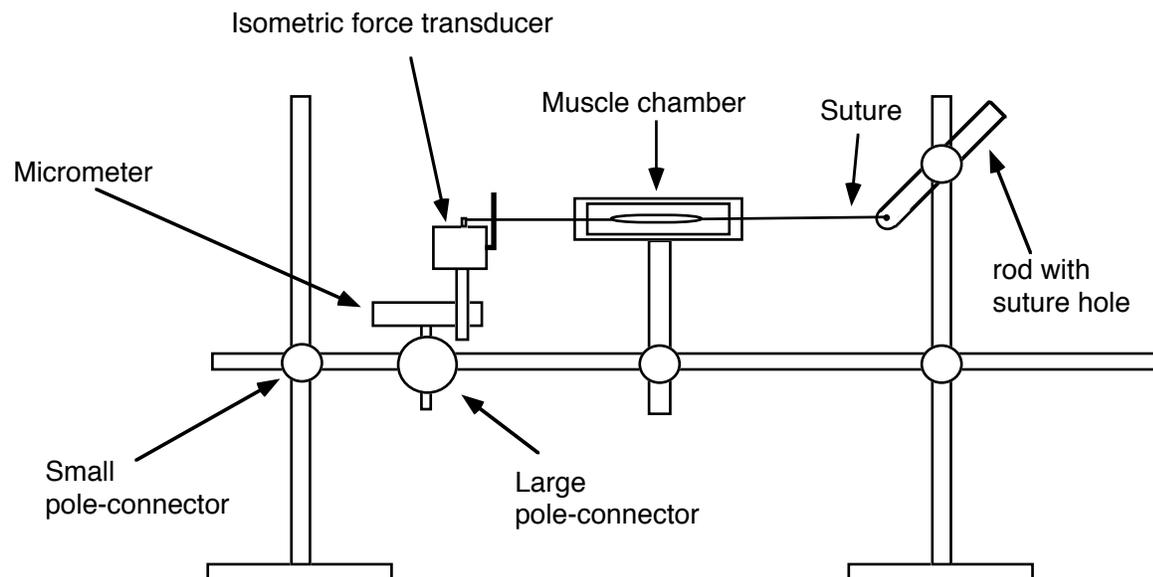

Figure 1: **Experimental setup for mechanical testing of isolated skeletal muscle**

Calibration of the Force Transducer

Finish the setup by connecting the stimulation and data acquisition equipment as shown in Figure 2. Balance and calibrate the force transducer output using the oscilloscope: first adjust the balance when the load is zero so that the transducer output is zero, then calibrate the transducer with known weight to obtain a "calibration factor." To do this you can temporarily attach the pulley in place of the rod with the suture hole, and place different weights in the weight pan to generate a linear calibration curve (5 points, 0 plus 4 weights). You should obtain a single calibration factor in grams/volt. After calibration, prepare the system for a muscle by lining up the original rod with the end hole with the chamber and transducer. Finally, make sure that the oscilloscope is "triggering" off of the synchronization pulse that comes from the stimulator.
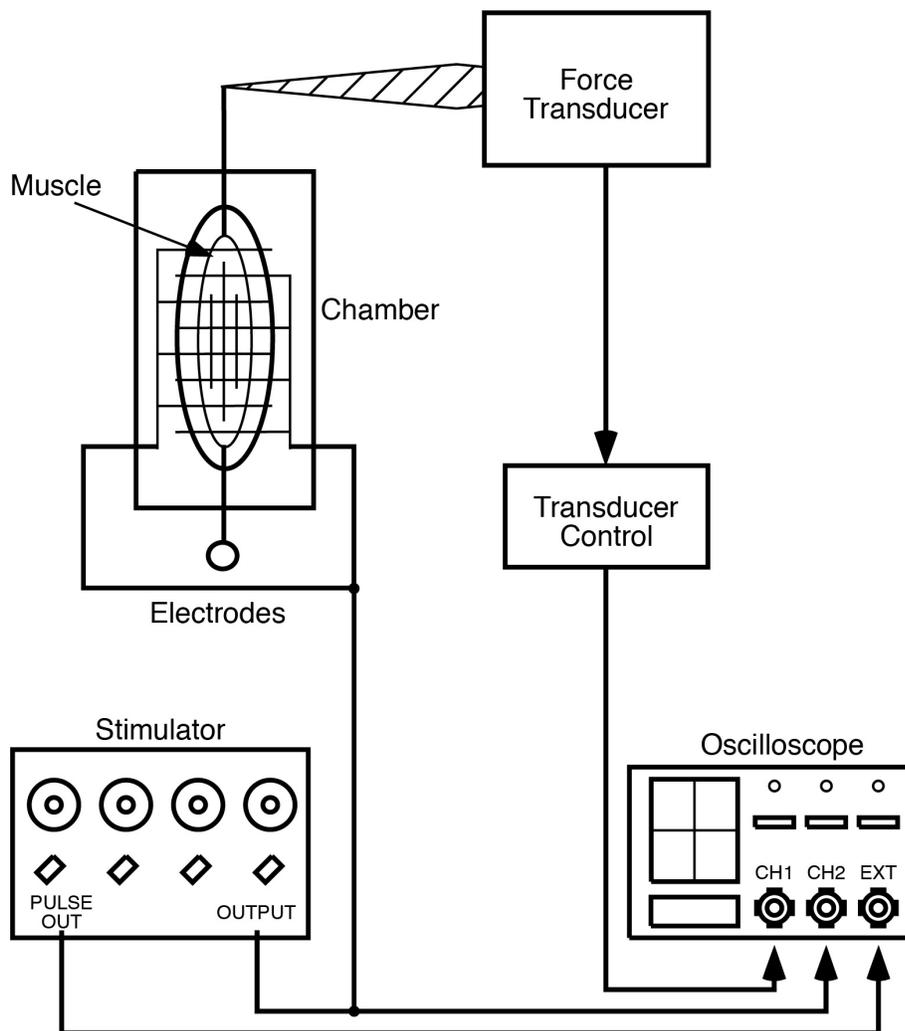
Figure 2: **Block diagram for isometric recording from a frog skeletal muscle.**

Dissection of the frog semitendinosus muscle of the frog (*Rana pipiens*)

1. Sacrifice the animal by double pithing. Insert the probe into the cranium and destroy the brain as judged by the absence of a corneal reflex. Then reverse the direction of the pith into the spinal column until the entire length of the spinal cord has been disrupted and rigidity ensues.

2. Remove the skin of the hind limbs by cutting around the pelvic region and pulling the skin from the legs as if you were removing a pair of pants. Place the frog on its back, and remove the thin muscle layer above the semitendinosus muscle starting at the knee end where the semitendinosus insertion tendon is clearly visible.

3. Place a length of suture around the insertion tendon (at the proximal tibiofibula) of the semitendinosus muscle (see Figure 3). Remove the ventral head of the semitendinosus which is not appropriate for this experiment. Tie another suture on the proximal end of the muscle around the origin tendon. Position the thigh at right angles to the trunk and place the lower leg at a right angle to the thigh. With a ruler, measure the reference length (in situ length) of the dorsal head between the 2 suture knots. Cut the insertion tendon distal to the knot and carefully dissect the muscle anteriorly by applying a gentle tension to the suture. Cut the proximal end proximal to the knot.

4. Using the apparatus supplied in the laboratory, transilluminate the muscle with the HeNe laser and measure the spacing between the +2 and the -2 diffraction orders. You will use these to calculated sarcomere length as described in the lecture. Make sure to measure the knot-to-knot spacing of the muscle that corresponds to this sarcomere length.

5. After the isometric tension transducer has been calibrated, place the excised muscle in the muscle chamber by fixing one thread to the tension transducer and securing the opposite thread through the hole in the chamber at attaching it through the hole in the rod. This end needs to be secure so that a true isometric twitch is produced.

6. Adjust the length of the muscle with the calibrated micrometer so that the muscle is held at its reference length. Record the length reading on the micrometer. Keep the muscle chamber full of Ringer's solution at all times when not simulating the muscle. Use a syringe and tube to fill or empty the chamber when needed.

The Twitch

7. Using a pulse duration of 0.5 ms, begin stimulating the muscle with single pulses in order to determine the threshold voltage. Start at 0.1 volts amplitude, and increase the output by 0.1V (0-1 volt range) or 1.0V (1-10V range). For the stimulation to reach the muscle, the fluid must be drained from the chamber so that the electrodes are not shorted. Visually observe the muscle for a contraction, each time increasing the voltage until a twitch is seen. Record the threshold voltage from the simulator.

8. After you have found the threshold voltage, increase the voltage and produce twitches until the twitch magnitude is 5-10 grams. The maximum voltage to use is 10V, even if the twitch

magnitude is less than 5 grams. Record one twitch from the muscle at this voltage using the storage functions on the oscilloscope. You can set the scope for a single trigger, then follow with the single pulse from the stimulator. From this twitch recording, determine the peak contraction time (from the beginning of the twitch to the peak), the contraction force in grams, and 1/2 relaxation time (time from peak to 1/2 of this value). Leave the voltage setting at this level for the duration of the lab.
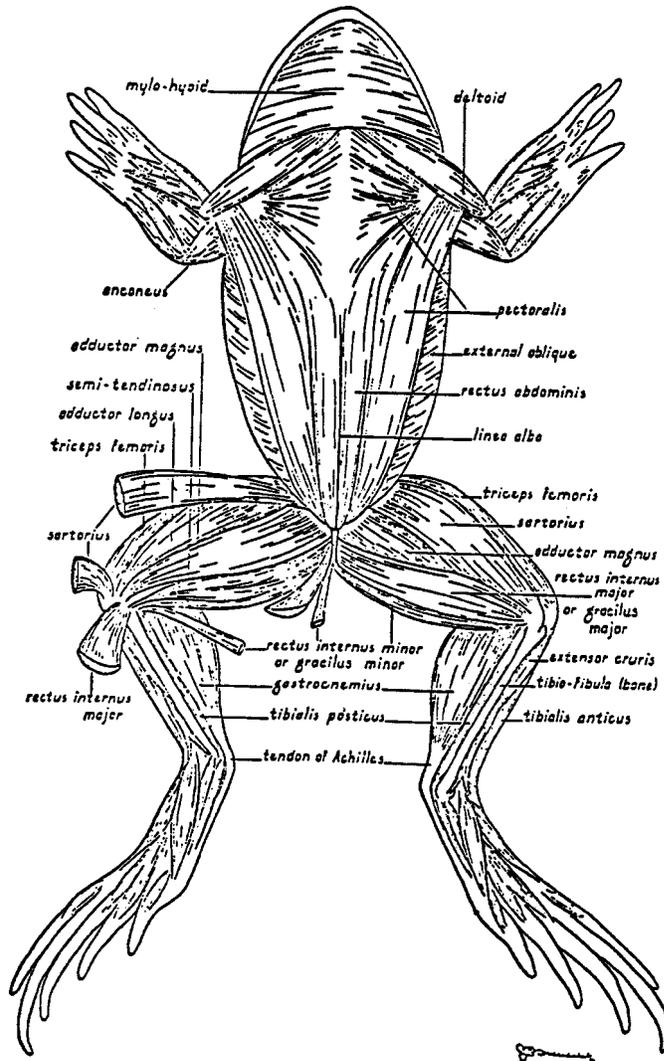

Figure 3: Bullfrog (Rana catesbeiana) muscles —Ventral view.

The Tetanus

1. To produce a tetanic contraction, deliver a train of pulses to the muscle using the "repeat" function on the stimulator. Remember that this pulse train will drain the ATP supply from the muscle, so only hold the repeat button for 1-2 seconds.

2. The muscle will show a fused tetanus at 50Hz or lower. The peak tetanic force should increase with increasing frequency. Record the peak tension value (grams) from the oscilloscope during a train of pulses at 5Hz, 10 Hz, 25Hz and 50Hz. In order to watch the peak contraction in real time, use the oscilloscope with a time base of about 500ms. For each tetanic contraction, hold the repeat button only until the peak twitch height has reach a plateau (~1-2 seconds). Remember to wait 2 minutes before repeating a tetanic pulse train.

3. Determine the fused tetanus force (at 50 Hz) in grams at this length, L_o, and calculate the twitch-tetanus ratio.

Passive and Active Tension

1. Leave the stimulation frequency at 50Hz. Produce another tetanic contraction at L_o, and determine the passive and active tensions (in grams). Remember that the total tension is passive plus active.

2. Increase the passive length of the muscle in 1.0 mm steps and repeat the contraction acquisition sequence above, recording both active and passive tension. Remember to wait 2 minutes between steps to allow for recovery from fatigue. Continue this process until the *active* force has decreased substantially (to about 10% of the L_o value) near the end of the muscle length-tension curve. Note that total tension will continue to increase as the length gets longer and longer.

3. Reset the length to L_o and check for peak isometric force to make sure the muscle is viable. You should note any effects of fatigue in your write-up. Repeat step 2, this time *decreasing* the passive length in 1-mm increments. This will form the ascending limb of the length-tension curve. Continue until almost no active force is produced.

4. (OPTIONAL) At this point you may use the LabVIEW data acquisition system to record one or more tetanic contractions on the computer. If you enter a calibration factor in the program, your results will be in units of force. You can plot the digital output file and include the plot in your report as an example of a tetanic contraction. There is a note on the Web page on direct screen images from LabVIEW for including in your report.

5. Construct a passive "length-tension" curve for the muscle; use grams for the units of force and both muscle fiber length (mm) and sarcomere length (μm) for length.

6. Construct an active "length-tension" curve for the muscle in the same absolute units as above. Compare your curves to published data, noting differences and possible limitations of your study.

Literature References

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