BE172 Spring 2018

Week II: Mechanical Properties of Skeletal Muscle

I. The Length-Tension Relationship

Introduction

This purpose of this experiment is to determine the passive and active length-tension relationships in the frog semitendinosis muscle. Twitch and tetanic properties will be studied, and measurements made in a viable, excised muscle.

It is the function of muscle cells to generate force and shorten, and in doing so, perform mechanical work. There are two basic properties of skeletal muscle which make this possible: (l) excitability, or the ability to respond to an electrical stimulus, and (2) contractility, or the ability of muscle cells to forcefully shorten.

When a muscle fiber is stimulated by its motor neuron, the 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 which releases calcium that 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), (3) the stress-strain characteristics of the elastic elements of muscle, and (4) the force generating capability of the contractile component at constant sarcomere length (the active force curve).

In the following exercises, you will experimentally determine some of these relations and will use the information to understand how structural properties at the sarcomere level lead to functional responses at the whole cell level.

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 the so-called 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 (60-100 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 (3.65 mm), the active tension produced quickly falls to zero. Between sarcomere lengths 3.65 and 2.2 mm, 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 which have been the subject of investigation by other workers in the field. In muscle physiology, a great majority of the data published has come from experiments on the semitendinosis 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.

Equipment

- Stimulator
- Oscilloscope
- Muscle chamber
- Isometric force transducer
- Weights/weight tray
- Pole/clamp setup
- Syringe w/tube for emptying/filling chamber
- Computer data acquisition system

In this first muscle-mechanics lab, we will make the majority of measurements directly on the oscilloscope. At the end of the experiment, use the computer to acquire a signal for graphing purposes of a representative contraction. Use the Labview acquisition program to acquire a single tetanic contraction to include in your write up as an example plot. Labview instructions (including downloading and using the VI) are on the Course Web Page.

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: semitendinosis muscle

Prelab Questions

• Define excitability and contractility in skeletal muscle.

• Why does increasing the stimulus frequency start to fuse together contractions?

• In the experimental system for this lab, how do we change the length of the muscle specimen?

• How long does a typical skeletal muscle twitch last?

• Describe the relationship between change in muscle length and change in sarcomere length in a muscle. Give a factor that could alter this relationship.

Experimental Procedure

Isometric Force Setup

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

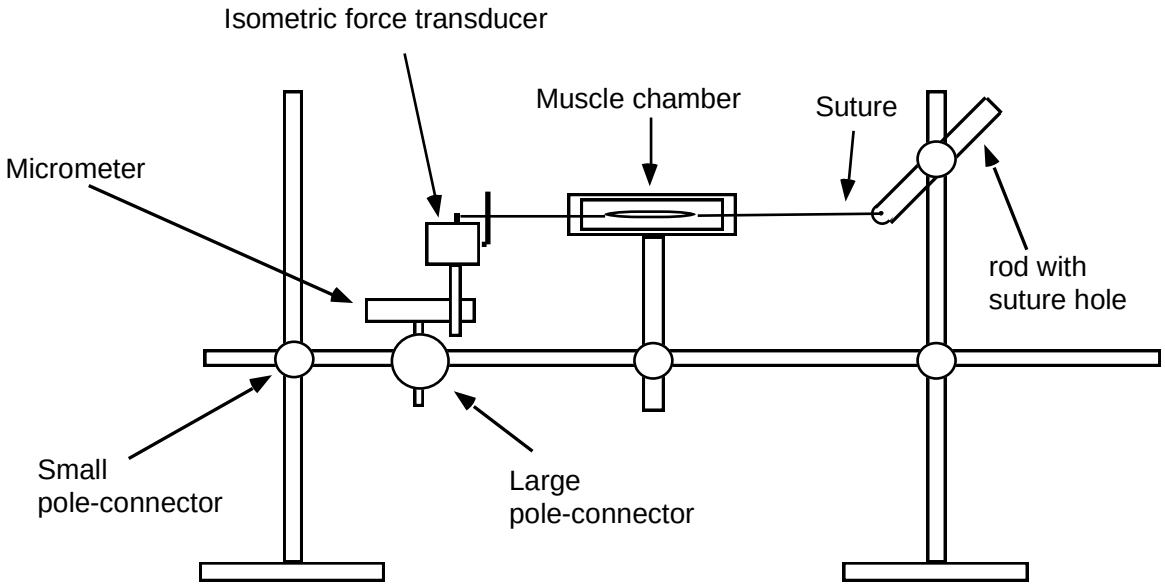

Figure 1: Experimental setup for isolated skeletal muscle

Calibration of the Force Transducer

Finish the setup by connecting the stimulation and data acquisition equipment as shown in Figure 2. **Do not tie a tight knot on the transducer, make a secure "loop" with the suture to place around the transducer.** 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-rod in place of the rod with the suture hole, and place 2 different weights in the weight pan to generate a linear calibration curve (3 points, 0 plus 2 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. Also use the tip/BNC adaptor wire (red/black 6" wires) for the output of the stimulator, and plug it into the side holes of the output posts.

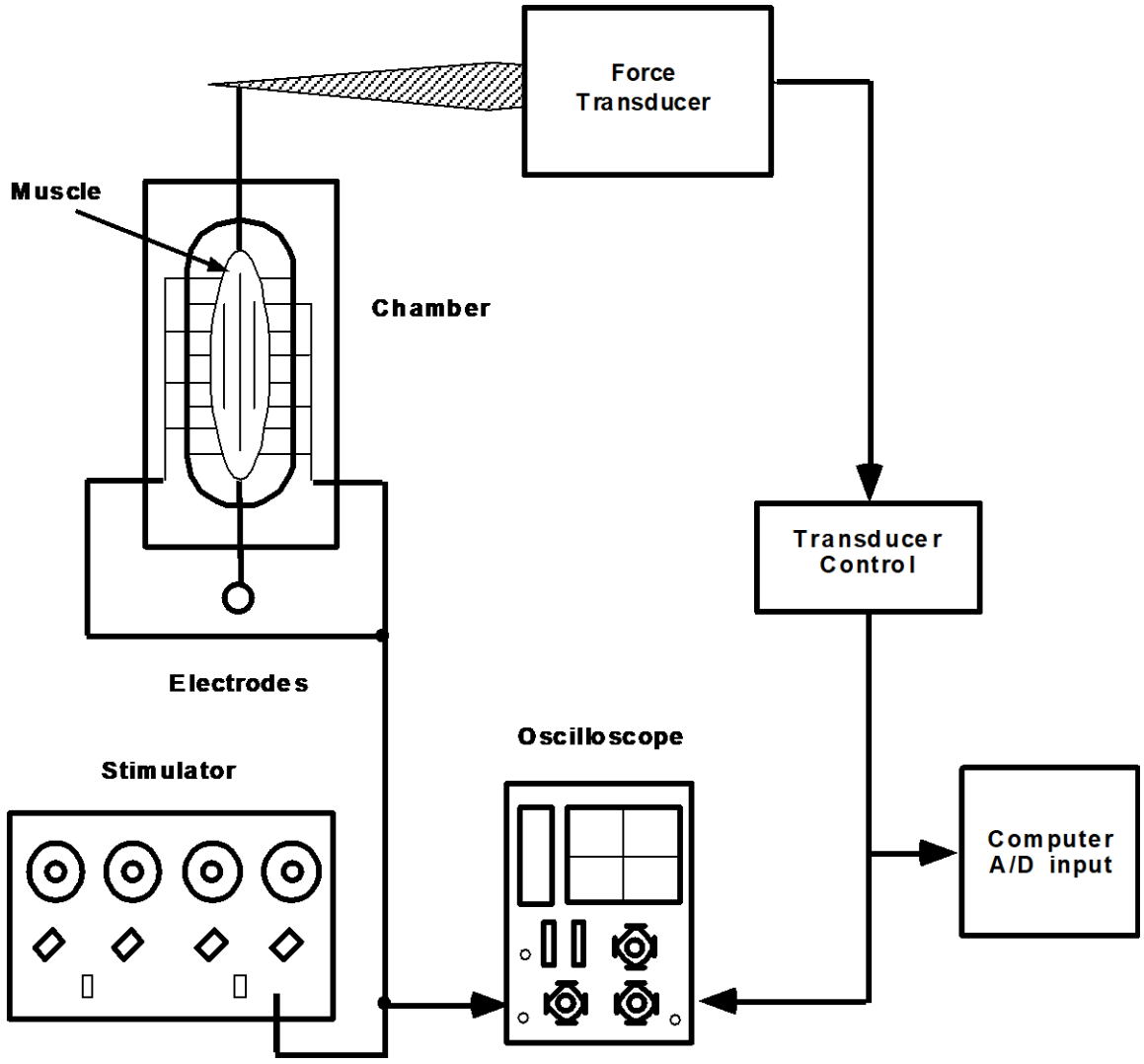

Figure 2: Block diagram for isometric recording

Dissection of the frog semitendinosis muscle of the frog (*Grass frogs or Bull frogs*)

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 trousers. Place the frog on its back, and remove the thin muscle layer above the semitendinosis muscle starting at the knee end.

3. Place a length of suture around the tendon of insertion (at the proximal tibiofibula) of the semitendinosis muscle (see Figure 3). Remove the ventral head of the semitendinosis. Tie another suture on the distal end of the muscle around the 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 (<u>in situ</u> length) of the dorsal head of the between the 2 suture knots.

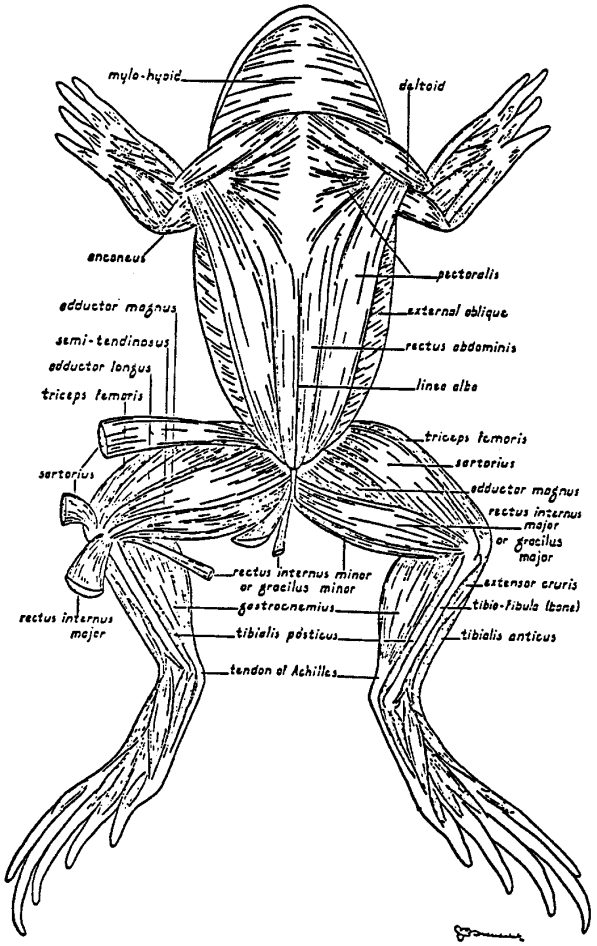

Figure 3: Bullfrog (Rana catesbeiana) muscles - Ventral view.

4. Cut the tendon distal to the knot and carefully dissect the muscle anteriorly by applying a *slight* tension to the string as you cut the surrounding connective tissue. *Keep the muscle wet with Ringer's solution at all times.*

5. Prepare the dorsal head of the muscle with sutures from the other leg in a similar fashion. Keep this muscle bathed in Ringer's as a backup in case of disaster!

6. Use the laser diffraction setup to find sarcomere length at a known muscle length. First use the calibration grid to find h, the distance from the ruler to the slide. This can be found with

$$n \cdot \lambda = d \cdot \sin(\tan^{-1}(x_n/h))$$

where n is the order of the diffraction pattern, λ is the wavelength of the laser (632 nm for a He-Ne laser), d is the calibration grid spacing, and x_n is the distance measured on the ruler from the vertical (center) to the diffraction band. Once you have determined h, use the same equation, this time with the muscle diffraction, to determine the sarcomere spacing d. You may assume sarcomere length varies linearly with muscle length.

The Twitch

1. After the isometric tension transducer has been calibrated, place the excised muscle in the muscle chamber by looping one thread around the tension transducer and securing the opposite thread through the hole in the chamber at attaching it through the hole in the rod. **Do not tie a tight knot on the transducer, make a secure "loop".** The end connected to the rod can be a knot, and needs to be secure so that a true isometric twitch is produced.

2. 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.

3. Using a pulse duration of 0.5 msec, commence stimulating the muscle with single pulses in order to determine the threshold voltage. Start at 0.5-1.0 volt amplitude, and increase the output up to a maximum of 10V. For the stimulation to reach the muscle, the fluid must be drained from the chamber so that the electrodes are not shorted. Visually watch the muscle for a contraction, each time increasing the voltage until a twitch is seen. Record the threshold voltage from the simulator. It is OK to do many twitches in short time.

4. 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 is less than 5g. 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; or use normal trigger and set the level. Auto trigger will not work for this part. 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 stimulator voltage setting at this level for the duration of the lab.

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 maximum.

2. The muscle will show a fused tetanus at about 50Hz or higher. 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, 10Hz, 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 (about 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 tetanus/twitch peak force ratio.

Passive and Active Tension

1. Leave the stimulation frequency at 50Hz. Produce another tetanic contraction at L_o, and from this curve measure the passive and active tensions (in grams). Remember that the total tension is passive plus active. This will be the first point on your length-tension curve.

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 at each muscle length. 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 may continue to increase as the length gets 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. At this point, 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.

5. Construct 3 length-tension curves: passive, active and total. Use grams for the units of force and sarcomere length (μm) for length. Plot them all on the same graph. Compare your curves to published data, noting differences and possible limitations of your study.

Write-up notes

Introduction:

Introduce the write-up with background concerning active force generation in skeletal muscle, and how sarcomeres and their length relate to muscle force generation. Relate these known properties to what you will measure in the lab.

Methods:

Briefly give an overview of the system for measuring force and controlling length. Briefly describe the tissue, and the protocol you will be using (steps used to get data). Mention the sarcomere length measurement equipment.

Results:

Sarcomere length information (sarcomere length/muscle length ratio) from laser diffraction.

Twitch threshold and twitch parameters in #4 in "The Twitch" section above.

Peak tension during a tetanic contraction as a function of frequency. Twitch/tetanus ratio. Compare your twitch and tetanic peak values to known values as described in the pre-lab question above.

Active, passive and total length-tension curves for tetanic contractions as described in "Passive and Active Tension" above.

Picture of an example tetanic contraction recorded with Labview.

Discussion:

A brief explanation of how the laser diffraction system works for measuring sarcomere length.

Differences between a twitch and tetanic contraction, experimentally and theoretically. Include comment on "waiting period" between experimental tetanic contractions.

Explain the shape of your active and passive length tension curves, in terms of sarcomere length.

Comparison of your length tension curves to some published data for skeletal muscle, make sure to quote your reference.

Limitations of this experimental setup.

References

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