Laboratory Exercise S-1

Skeletal Muscle Contraction

Purpose of the Exercise

To observe and record the responses of an isolated frog muscle to electrical stimulation of varying strength and frequency.

Materials Needed

Recording system (kymograph, Physiograph, etc.)
Stimulator and connecting wires
Live frog
Dissecting tray
Dissecting instruments
Probe for pithing
Heavy thread
Frog Ringer's solution

For Demonstration A—The Kymograph:
Kymograph recording system
Electronic stimulator (or inductorium)
Frog muscle (from pithed frog)
Probe for pithing
Dissecting instruments
Frog Ringer's solution

For Demonstration B—The Physiograph:
Physiograph
Myograph and stand
Frog muscle (from pithed frog)
Probe for pithing
Dissecting instruments
Frog Ringer's solution

For Procedure F—Skeletal Muscle Function: Principles of Summation and Tetanus:
Ph.I.L.S. 4.0

Safety

- Wear disposable gloves when handling the frogs.
- Dispose of gloves and frogs as instructed.
- Wash your hands before leaving the laboratory.

Learning Outcomes

After completing this exercise, you should be able to

1. Choose a recording system and stimulator to record frog muscle responses to electrical stimulation.
2. Determine the threshold level of electrical stimulation in frog muscle.
3. Determine the intensity of stimulation needed for maximal muscle contraction.
4. Record a single muscle twitch and identify its phases.
5. Record the response of a muscle to increasing frequency of stimulation and identify the patterns of tetanic contraction and fatigue.
6. Describe the relationship of calcium release from the sarcoplasmic reticulum to the mechanical events of muscle contraction and relaxation.

Pre-Lab

1. Carefully read the introductory material and examine the entire lab.
2. Be familiar with a muscle twitch, threshold stimulus, summation, and tetanus from lecture or the textbook.

To study the characteristics of certain physiological events such as muscle contractions, it often is necessary to use a recording device, such as a *kymograph* or a *Physiograph.* These devices can provide accurate recordings of various physiological changes.

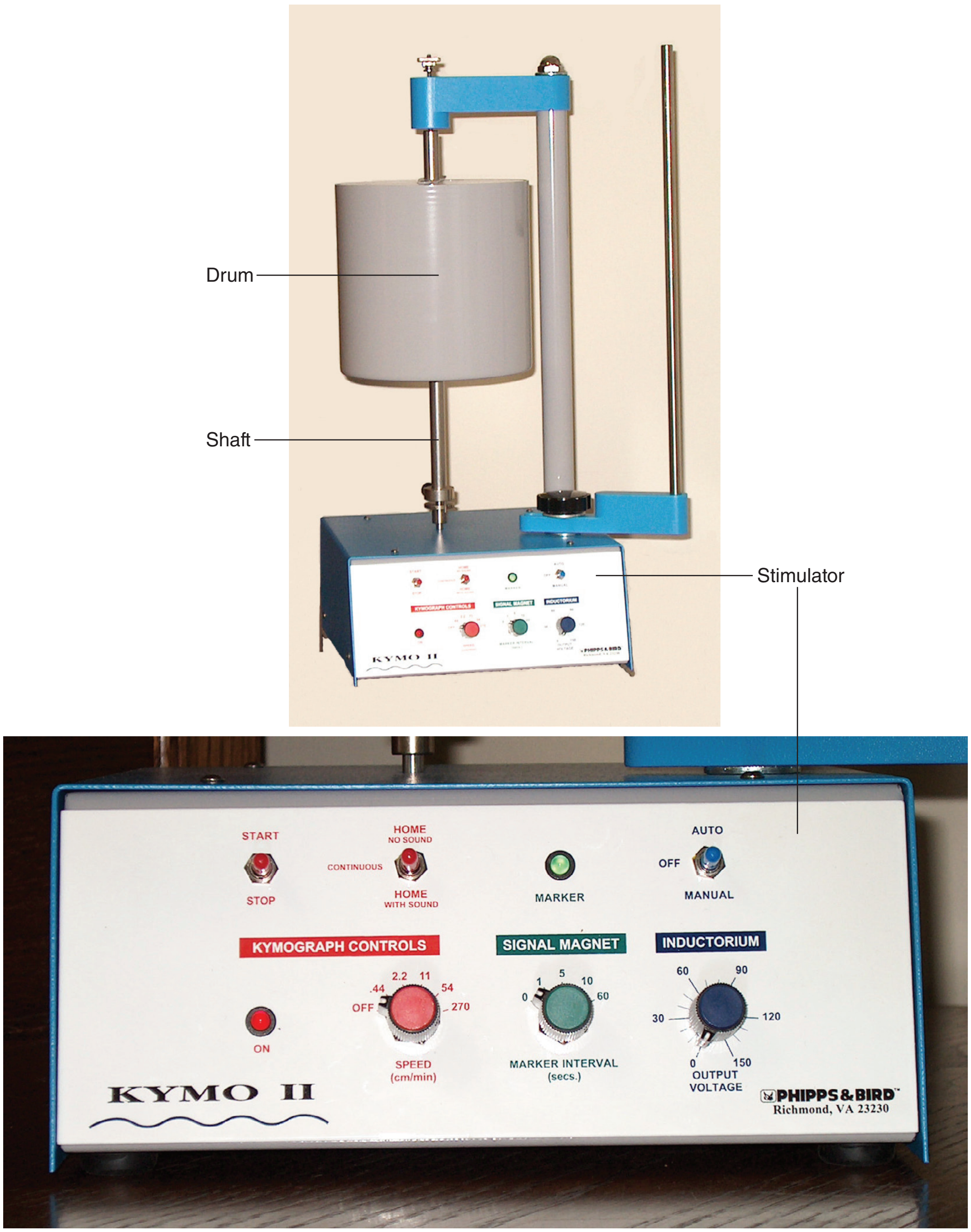
To observe the phenomenon of skeletal muscle contractions, muscles can be removed from anesthetized frogs. These muscles can be attached to recording systems and stimulated by electrical shocks of varying strength, duration, and frequency. Recordings obtained from such procedures can be used to study the basic characteristics of skeletal muscle contractions.

The principles of changing frequency of stimulation to investigate summation and tetanus in skeletal muscle can also be investigated with Ph.I.L.S. 3.0. The relationship of calcium levels in the sarcoplasm to the mechanical events of muscle contraction and relaxation is emphasized.

Demonstration A—The Kymograph

1. Observe the kymograph and, at the same time, study figure S-1.1 to examine the names of its major parts.
2. Note that the kymograph consists of a cylindrical *drum* around which a sheet of paper is wrapped. The drum

FIGURE S-1.1 Kymograph to record frog muscle contractions.



is mounted on a motor-driven *shaft,* and the speed of the motor can be varied. Thus, the drum can be rotated rapidly if rapid physiological events are being recorded or rotated slowly for events that occur more slowly.

A *stylus* that can mark on the paper is attached to a *movable lever,* and the lever, in turn, is connected to an isolated muscle. The origin of the muscle is fixed in position by a *clamp,* and its insertion is hooked to the muscle lever. The muscle also is connected by wires to an *electronic stimulator* (or inductorium). The stimulator can deliver single or multiple electrical shocks to the muscle, and it can be adjusted so that the intensity (voltage), duration (milliseconds), and frequency (stimuli per second) can be varied. Another stylus, on the *signal marker,* records the time each stimulus is given to the muscle. As the muscle responds, the duration and relative length of its contraction are recorded by the stylus on the muscle lever.

3. Watch carefully while the laboratory instructor demonstrates the operation of the kymograph to record a frog muscle contraction.

Demonstration B—The Physiograph

1. Observe the Physiograph and, at the same time, study figures S-1.2 and S-1.3 to examine the names of its major parts.
2. Note that the recording system of the Physiograph includes a transducer, an amplifier, and a recording pen. The *transducer* is a sensing device that can respond to some kind of physiological change by sending an electrical signal to the amplifier. The *amplifier* increases the strength of the electrical signal and relays it to an electric motor that moves the *recording pen.* As the pen moves, a line is drawn on paper.

   To record a frog muscle contraction, a transducer called a *myograph* is used (fig. S-1.3). The origin of the muscle is held in a fixed position, and its insertion is attached to a small lever in the myograph by a thread. The myograph, in turn, is connected to the amplifier by a transducer cable. The muscle also is connected by wires to the electronic stimulator, which is part of the Physiograph. This stimulator can be adjusted to deliver single or multiple electrical shocks to the muscle, and the intensity (voltage), duration (milliseconds), and frequency (stimuli per second) can be varied.

   The speed at which the paper moves under the recording pen can be controlled. A second pen, driven by a timer, marks time units on the paper and indicates when the stimulator is activated. As the muscle responds to stimuli, the recording pen records the duration and relative length of each muscle contraction.
3. Watch carefully while the laboratory instructor operates the Physiograph to record a frog muscle contraction.

FIGURE S-1.2 Physiograph to record frog muscle contractions.

Procedure A—Recording System

1. Set up the recording system and stimulator to record the contractions of a frog muscle according to the directions provided by the laboratory instructor.
2. Obtain a live frog, and prepare its calf muscle (gastrocnemius) as described in Procedure B.

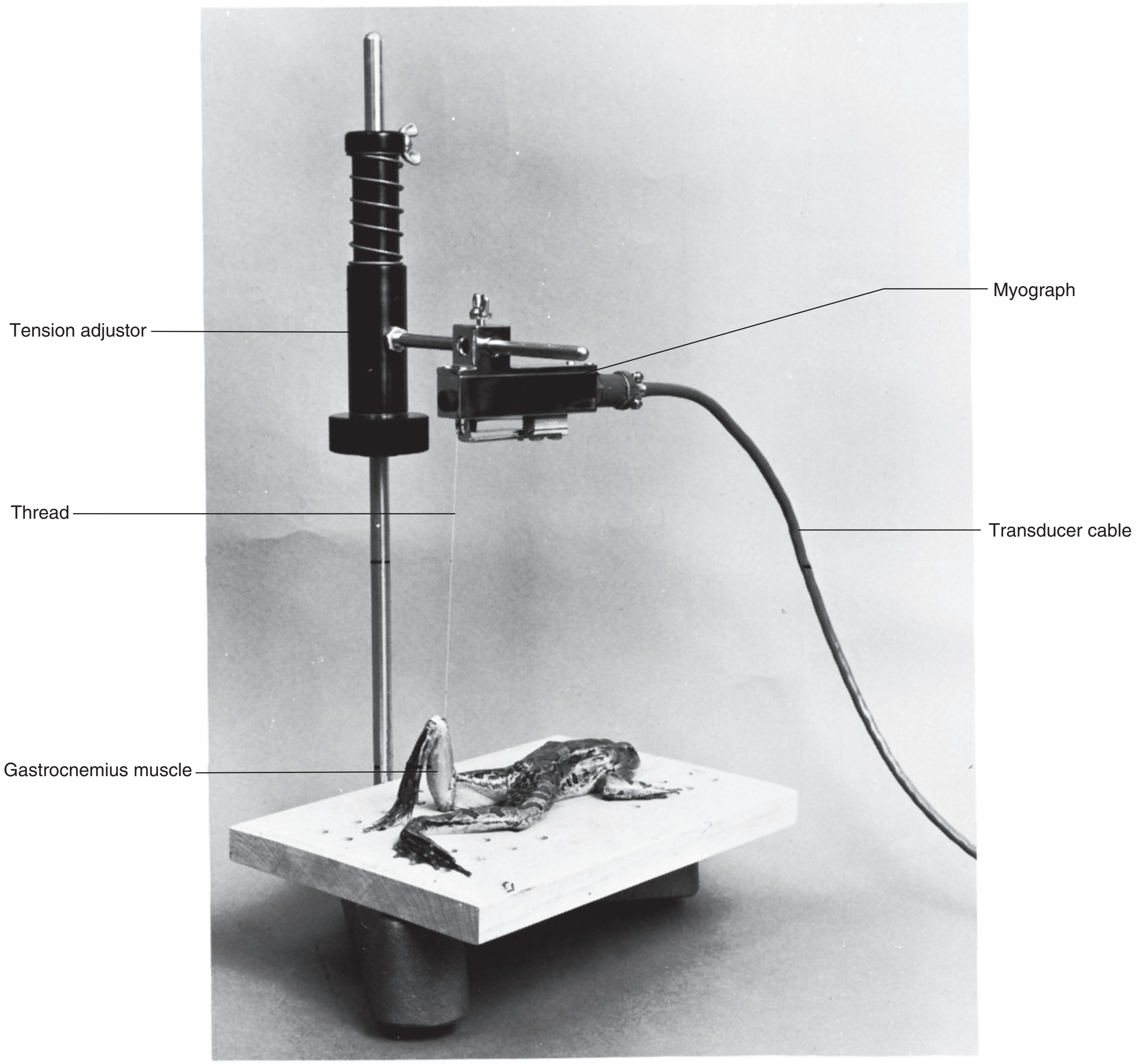
Procedure B—Muscle Preparation

1. Prepare the live frog by pithing so that it will not have sensations or movements when its muscle is removed. To do this, follow these steps:
   a. Hold the frog securely in one hand so that its limbs are extended downward.
   b. Position the frog's head between your thumb and index finger (fig. S-1.4*a*).
   c. Bend the frog's head forward at an angle of about 90° by pressing on its snout with your index finger.
   d. Use a sharp probe to locate the foramen magnum between the occipital condyles in the midline between the frog's tympanic membranes.
   e. Insert the probe through the skin and into the foramen magnum, and then quickly move the probe from side to side to separate the brain from the spinal cord.
   f. Slide the probe forward into the braincase, and continue to move the probe from side to side to destroy the brain.
   g. Remove the probe from the braincase, and insert it into the spinal cord through the same opening in the skin (fig. S-1.4*b*).
   h. Move the probe up and down the spinal cord to destroy it. If the frog has been pithed correctly, its legs will be extended and relaxed. Also, the eyes will not respond when touched with a probe.

Alternative Procedure

An anesthetizing agent, tricaine methane sulfonate, can be used to prepare frogs for this lab. This procedure eliminates the need to pith frogs.

FIGURE S-1.3 Myograph attached to frog muscle.



2. Remove the frog's gastrocnemius muscle by proceeding as follows:
   - **a.** Place the pithed frog in a dissecting tray.
   - **b.** Use scissors to cut through the skin completely around the hindlimb in the thigh.
   - **c.** Pull the skin downward and off the hindlimb.
   - **d.** Locate the gastrocnemius muscle in the calf and the calcaneal tendon (Achilles tendon) at its distal end (fig. S-1.5*a*).
   - **e.** Separate the calcaneal tendon from the underlying tissue, using forceps.
   - **f.** Tie a thread firmly around the tendon (fig. S-1.5*b*).
   - **g.** When the thread is secure, free the distal end of the tendon by cutting it with scissors.
   - **h.** Attach the frog muscle to the recording system in the manner suggested by your laboratory instructor (figs. S-1.1 and S-1.3).
   - **i.** Insert the ends of the stimulator wires into the muscle so that one wire is located on either side of the belly of the muscle.

Keep the frog muscle moist at all times by dripping frog Ringer's solution on it. When the muscle is not being used, cover it with some paper toweling that has been saturated with frog Ringer's solution.

Before you begin operating the recording system and stimulator, have the laboratory instructor inspect your setup.

FIGURE S-1.4 Hold the frog's head between your thumb and index finger to pith (a) its brain and (b) its spinal cord.

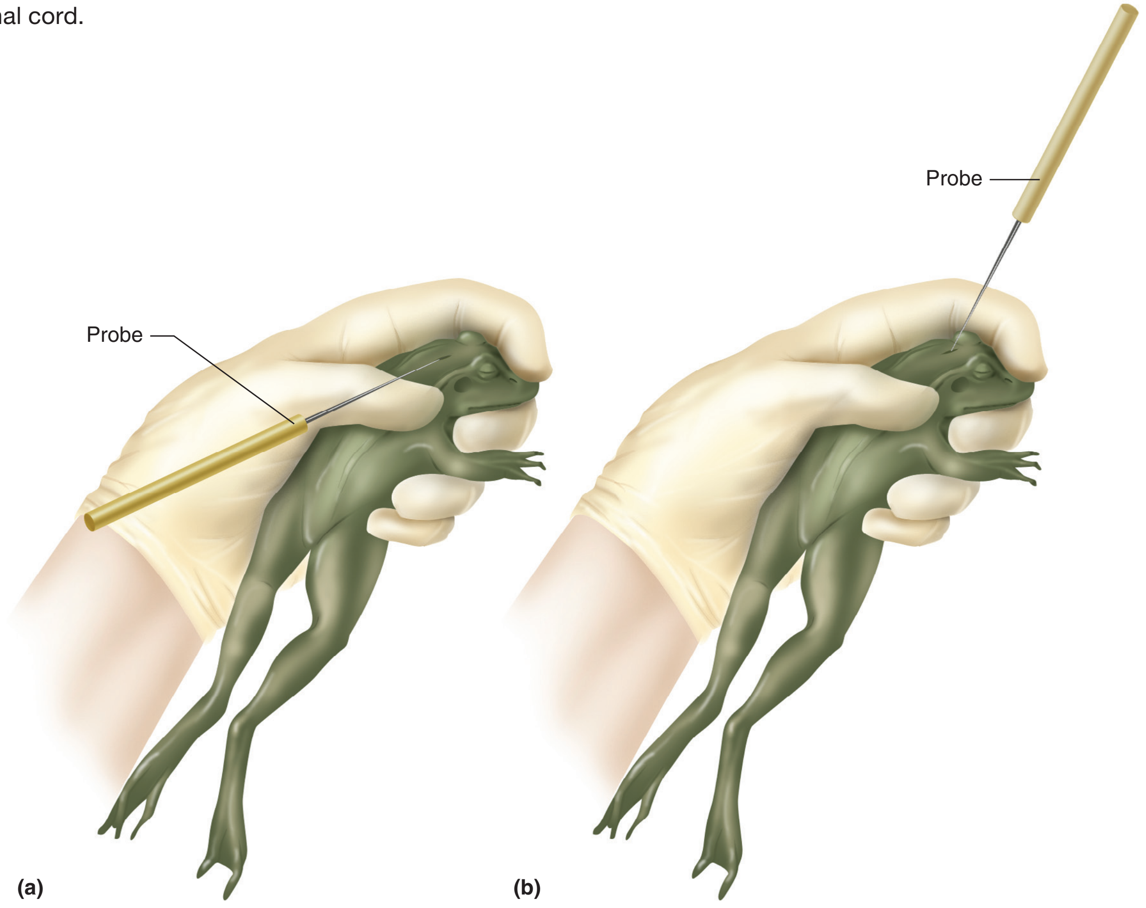
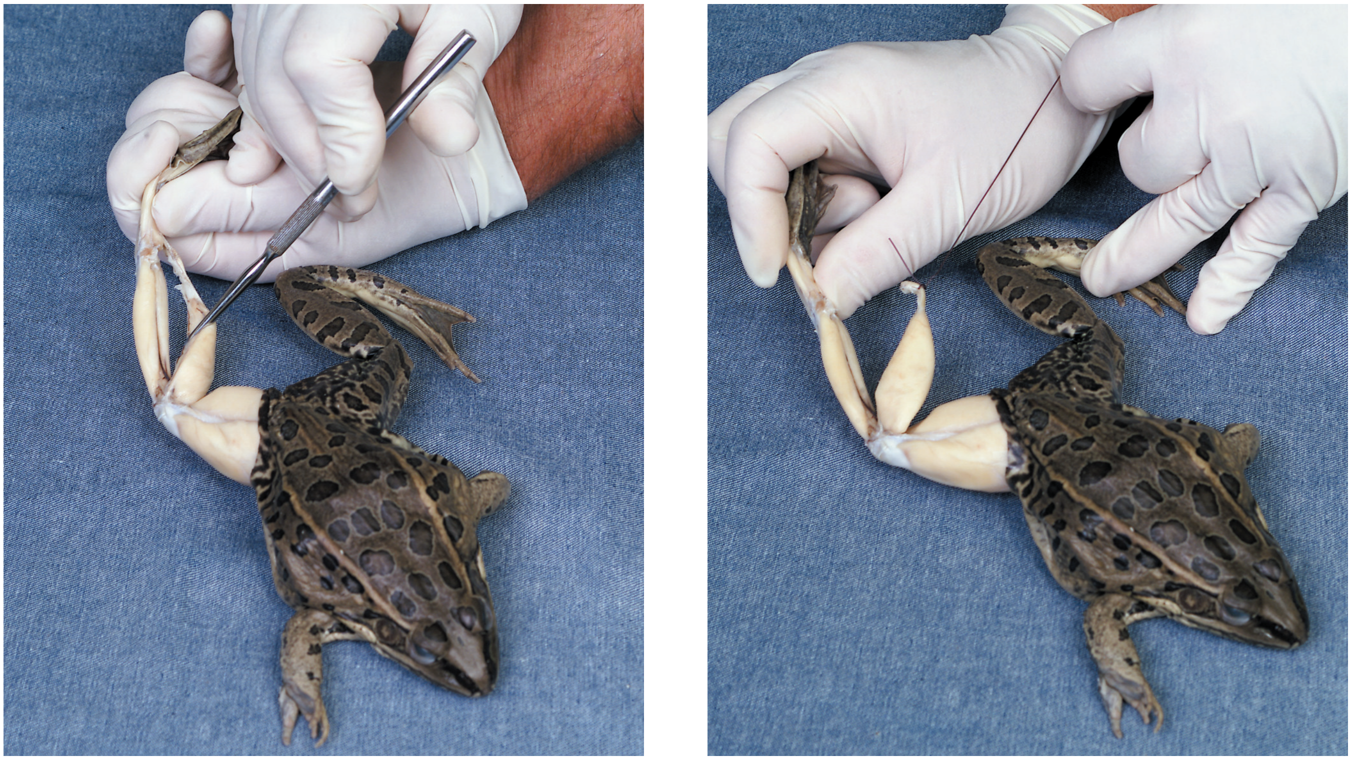


FIGURE S-1.5 (a) Separate the calcaneal (Achilles) tendon from the underlying tissue. (b) Tie a thread around the tendon, and cut its distal attachments.



Procedure C—Threshold Stimulation

1. To determine the threshold or minimal strength of electrical stimulation (voltage) needed to elicit a contraction in the frog muscle, follow these steps:
   a. Set the stimulus duration to a minimum (about 0.1 milliseconds).
   b. Set the voltage to a minimum (about 0.1 volts).
   c. Set the stimulator so that it will administer single stimuli.
2. Administer a single stimulus to the muscle and watch to see if it responds. If no response is observed, increase the voltage to the next higher setting and repeat the procedure until the muscle responds by contracting.
3. After determining the threshold level of stimulation, continue to increase the voltage in increments of 1 or 2 volts until a maximal muscle contraction is obtained.
4. Complete Part A of Laboratory Assessment S-1.

Procedure D—Single Muscle Twitch

1. To record a single muscle twitch, set the voltage for a maximal muscle contraction as determined in Procedure C.
2. Set the paper speed at maximum, and with the paper moving, administer a single electrical stimulus to the frog muscle.
3. Repeat this procedure to obtain several recordings of single muscle twitches.
4. Complete Part B of the laboratory assessment.

Procedure E—Sustained Contraction

1. To record a sustained muscle contraction, follow these steps:
   a. Set the stimulator for continuous stimulation.
   b. Set the voltage for maximal muscle contraction as determined in Procedure C.
   c. Set the frequency of stimulation at a minimum.
   d. Set the paper speed at about 0.05 cm/sec.
   e. With the paper moving, administer electrical stimulation and slowly increase the frequency of stimulation until the muscle sustains a contraction (tetanic contraction or tetanus).
   f. Continue to stimulate the muscle at the frequency that produces sustained contractions until the muscle fatigues and relaxes.
2. Every 15 seconds for the next several minutes, stimulate the muscle to see how long it takes to recover from the fatigue.
3. Complete Part C of the laboratory assessment.

Learning Extension Activity

To demonstrate the staircase effect (treppe), obtain a fresh frog gastrocnemius muscle and attach it to the recording system as before. Set the paper control for slow speed, and set the stimulator voltage to produce a maximal muscle contraction. Stimulate the muscle once each second for several seconds. How do you explain the differences in the lengths of successive muscle contractions?

Procedure F—Ph.I.L.S. Lesson 8 Skeletal Muscle Function: Principles of Summation and Tetanus

1. Open Exercise 8, Skeletal Muscle Function: Principles of Summation and Tetanus.
2. Read the objectives and introduction and take the pre-lab quiz.
3. After completing the pre-lab quiz, read through the wet lab.
4. The lab exercise will open when you have completed the wet lab (fig. S-1.6).

Setup

5. Follow the instructions at the bottom of the screen to turn on the power of the Virtual Computer Screen, the Data Acquisition Unit, and to connect the transducer and electrodes.
6. Set the voltage between 1.6 and 2.0 volts. (This voltage will elicit maximal muscle contraction.)

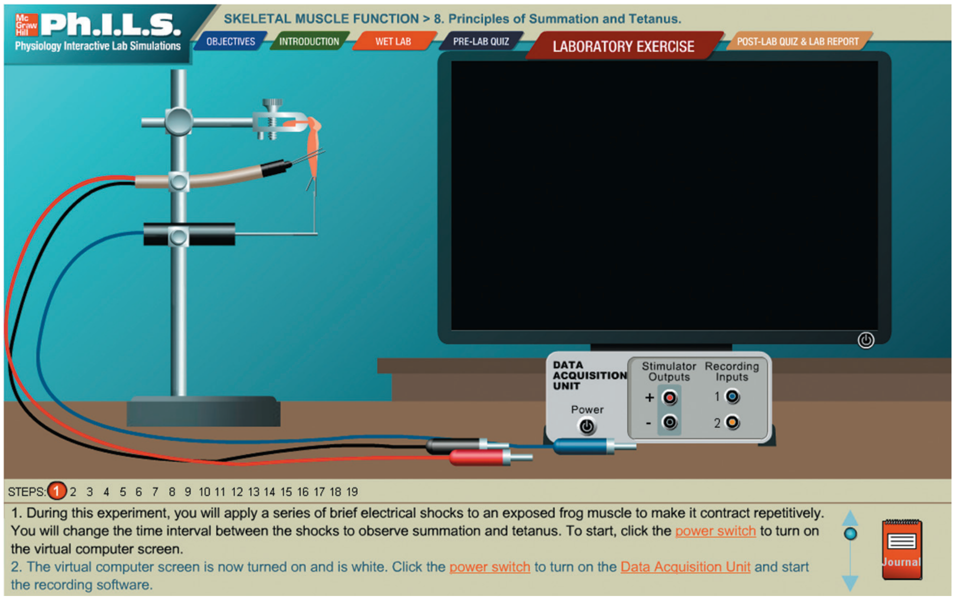
Summation, Incomplete Tetanus, Tetanus

7. Determine summation, incomplete tetanus, and tetanus (See table S-1.1 for description of trace, the red line).
   a. Click Start to initiate a continuous series of electrical shocks.
   b. As the tracing continues to scroll across the screen, increase the frequency (decrease the time interval

TABLE S-1.1 Observing Muscle Response on the Computer Screen

Muscle Response	Observation on Computer Screen
Wave summation	Trace moves off the baseline (lower gray line)
Incomplete tetanus	Trace becomes higher than in a single twitch (above upper gray line)
Complete tetanus	Trace is higher than a single twitch (above upper gray line) and becomes smooth

FIGURE S-1.6 Opening screen for the laboratory exercise on Skeletal Muscle Function: Principles of Summation and Tetanus.



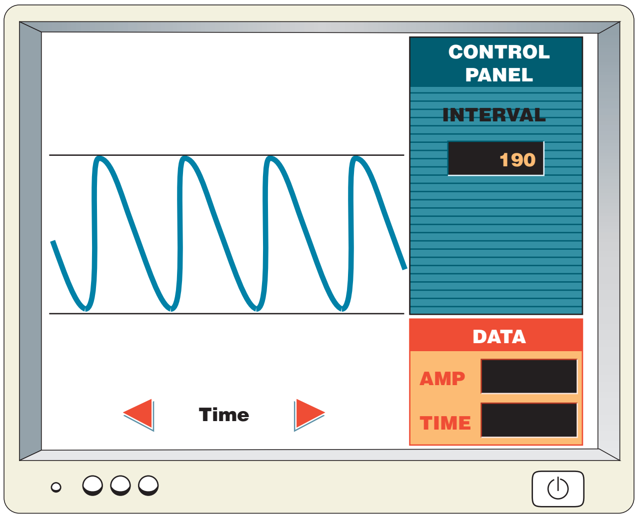
between shocks) by clicking on the Down arrow in the control panel.

c. Click the Journal panel (red rectangle at bottom right of screen) to enter your value into the Journal for summation, incomplete tetanus, and complete tetanus.

Interpreting Results

8. *With the Journal still open on the screen,* answer the questions in Part D of the laboratory assessment. If you accidentally closed the graph, click on the Journal panel (red rectangle at bottom right of screen).
9. After finishing the laboratory exercise you can print the line tracing by clicking on the P in the bottom left of the monitor screen (fig. S-1.7).
10. Complete the post-lab quiz (click open Post-Lab Quiz & Lab Report) by answering the ten questions on the computer screen.
11. Read the conclusion on the computer screen.
12. You may print the Lab Report for Skeletal Muscle Function: Principles of Summation and Tetanus.

FIGURE S-1.7 An example of a line tracing showing the amount of tension produced by successive contractions of an isolated frog gastrocnemius muscle.



NOTES

Laboratory Assessment

S-1

Name ____________________

Date ____________________

Section ____________________

The A corresponds to the indicated outcome(s) found at the beginning of the laboratory exercise.

Skeletal Muscle Contraction

Part A Threshold Stimulation Assessments

Complete the following:

1. What recording system was used for this laboratory exercise? 1 Answers will vary.
2. What was the threshold voltage for stimulation of the frog gastrocnemius muscle? 2 (experimental results)
3. What voltage produced maximal contraction of this muscle? 3 (experimental results)

Critical Thinking Assessment

Do you think other frog muscles would respond in an identical way to these voltages of stimulation?

probably not

Why or why not?

The differences in electrolyte concentrations and various other factors would probably result in slightly different values.

Part B Single Muscle Twitch Assessments

Complete the following:

1. Fasten a recording of two single muscle twitches in the following space. 4

   (attached myogram with labels)

2. On a muscle twitch recording, label the *latent period, period of contraction,* and *period of relaxation,* and indicate the time it took for each of these phases to occur. 4

3. What differences, if any, do you note in the two myograms of a single muscle twitch? How do you explain these differences?

   Answers will vary.

Part C Sustained Contraction Assessments

Complete the following:

1. Fasten a recording of a sustained contraction in the following space. 5

   (attached myogram with labels)

2. On the sustained contraction recording, indicate when the muscle twitches began to combine (summate), and label the period of tetanic contraction and the period of fatigue.

3. At what frequency of stimulation did tetanic contraction occur? (experimental results)

4. How long did it take for the tetanic muscle to fatigue? (experimental results)

5. Is the length of muscle contraction at the beginning of tetanic contraction the same as or different from the length of the single muscle contractions before tetanic contraction occurred? different How do you explain this?

   There is a greater concentration during tetanic contraction from recruitment or more motor units.

6. How long did it take for the fatigued muscle to become responsive again? (experimental results)

Part D Ph.I.L.S. Lesson 8, Skeletal Muscle Function: Principles of Summation and Tetanus Assessments

1. At what frequency did muscle twitches begin to combine (summate)? ~200 ms Demonstrate incomplete tetanus? ~170 ms Demonstrate complete tetanus? ~70 ms

2. Calcium is released from the sarcoplasmic reticulum through voltage-gated calcium channels to initiate muscle contraction. When the muscle is no longer stimulated, calcium pumps move the calcium from the sarcoplasm back into the sarcoplasmic reticulum. Complete the following:

   a. During muscle *contraction,* is the net movement of calcium into or out of the sarcoplasmic reticulum?

   The net movement is out of the sarcoplasmic reticulum.

   b. During muscle *relaxation,* is the net movement of calcium into or out of the sarcoplasmic reticulum?

   The net movement is into the sarcoplasmic reticulum.

   c. During incomplete tetanus, the amount of tension produced by the muscle is greater than during a single twitch. What does this tell you about the relative amount of calcium in the muscle sarcoplasm during incomplete tetanus?

   The Ca^{2+} flowing out of the sarcoplasmic reticulum is greater than the amount of Ca^{2+} pumped into the sarcoplasmic reticulum.

   d. Describe the net movement of calcium (into or out of the sarcoplasmic reticulum) during complete tetanus.

   The Ca^{2+} levels remain high enough in sarcoplasm for sustained contraction.

   e. Explain why the rate of muscle relaxation would be slower after tetanus than after a single twitch.

   More time is required to pump the additional Ca^{2+} back into the sarcoplasmic reticulum.

Critical Thinking Assessment

The toxins released from the bacterium that causes tetanus bind permanently to the acetylcholine receptors of the skeletal muscle, resulting in continuous stimulation of the muscle. Explain the result to the skeletal muscle of the body.

Normally, acetylcholine (the neurotransmitter that binds to the receptors of the muscle fiber to stimulate an action potential down the sarcolemma) is destroyed by acetylcholinesterase. This allows for only a brief stimulation of the muscle fiber. When the toxin produced from the bacteria that causes tetanus binds with the acetylcholine receptor, it does so permanently. The result is continuous stimulation of the muscle with continuous release of calcium from the sarcoplasmic reticulum and sustained contraction (tetanus).

NOTES