1724 Lab: Frog Skeletal Muscle Physiology
(Marieb Exercise 16A)

I. Introduction.
Read the introductory material in your lab manual Marieb Ex 16A: Skeletal Muscle Physiology – Frog Experimentation.

II. Preparation of the Frog
You will not be preparing the frog as described in the lab manual; instead follow the dissection procedure below:

After the frog has been double pithed you are ready to remove a muscle for testing. One of the largest and easiest muscles to obtain is the gastrocnemius of the lower leg. Before beginning the procedure rinse the frog briefly in the sink.

Place the frog on an uncontaminated dissecting pan. Be sure that neither the pan nor the dissecting instruments have been contaminated with preservatives such as formaldehyde. **The instructor will provide you with pans and tools reserved specifically for the dissection of fresh materials.**

It is not necessary to tie down a double pithed frog. As the muscle is dissected assign one of your coworkers the task of frequently flooding the surgical area with frog ringers using a small beaker and dropper. The muscle must not dry out while dissecting or it will be useless.

With forceps, lift the skin from one thigh and cut the skin completely around the leg using scissors. Pull the cut end back and peel the skin off the leg. Be careful not to touch the outer surface of the skin to the muscle tissues or to touch your contaminated hands to the muscle as poisons on the skin may damage some of the muscle cells. With a blunt probe or the blunt end of the forceps separate the body of the gastrocnemius from the underlying bone. The distal end of the muscle is attached to the Achilles tendon – a silvery white to yellow strand of fibrous tissue. Loosen a portion of the Achilles tendon from the bone then insert a piece of thread about 10 inches long around the tendon and tie it securely. Cut the tendon distal to this knot. The proximal end of the gastrocnemius is attached to the femur. **Do not remove it from the bone.** Carefully remove all other muscles from the upper leg but leave the gastrocnemius attached. Then cut the lower leg bone at or slightly distal to the "knee" joint with strong scissors or bone cutters (DO NOT USE A SCALPEL). Cut the proximal end of the femur leaving at least one half inch of bone attached to the muscle. You should now have a preparation resembling that in the figure. With the proximal end of the gastrocnemius attached to the femur and the distal end attached to a portion of thread. This is the "muscle – bone preparation" you will use for your muscle physiology experiments. continue to periodically flood the tissue preparation with saline now ant throughout the duration of the experiment.

III. iWorx Frog Skeletal Muscle Physiology Equipment Setup

1. Make sure computer and iWorx interface (black box) are unplugged)
2. Attach the cable of the Displacement Transducer to the bottom channel 3 input plug
3. Insert the "stimulator cable" plug into channel 4 of the iWorx box
    (which is attached to the stimulator section of the iWorx interface)
4. Plug the computer power cord into the power outlet.
5. Plug the iWorx power cord into the outlet
6. Turn on the iWorx interface
7. Turn on the computer
8. After the computer has booted up, click on the iWorx icon
9. Use cursor to select "settings" → choose "load group" in the drop down menu that appears
10. Use cursor to select "AK204" in the box that appears and choose "load" in the same box
11. Use cursor to select "settings" again → choose "Muscle #2"

Your equipment is now ready for the experiment

Review basic instructions for using and interpreting data from the iWorx software

IV. Standardize the movement of the transducer

1. When the transducer is pulled by the contracting muscle, the amplitude of the contraction will be given as "volts" in the window to the upper right of channel 3 (muscle) (ignore any – signs).
2. To convert this reading to actual millimeters of muscle contraction
    a. click start
    b. pull the transducer rod as high as it will go and the release it
    c. click stop
    d. now use a mm ruler to measure the actual distance that the rod moved (this is usually about 30 mm)
    e. click "autoscale" on the channel 3 if needed
    e. click the 2-cursor icon and measure the height of the deflection on channel 3 by placing the first blue line on the peak of the deflection and the second blue line on the baseline after the deflection has returned to its original position.
    f. read and record the Volts reading above and to the right of channel 3 (ignore negative signs)
    g. this "voltage" reading is equivalent to the actual mm of movement measured by the ruler
    h. divide the mm of movement by the "voltage" to get a **conversion factor**
3. whenever you measure amplitude of a contraction in "voltage" you can now convert it to millimeters of contraction by multiplying this value by your **conversion factor** determined above

V. Set up the frog muscle preparation as follows:

1. compress the femur in the femur clamp
2. adjust the clamps so that the thread is vertical and the displacement transducer rod rests just on the upper stop with no slack in the thread; try to make the thread vertical
3. position the stimulator electrodes so that they lie against the muscle about midway between the knee and the tendon; the two electrodes should not touch one another, but both must be in contact with the muscle
4. place two nickels (10g) in the weight pan
5. do not add any more frog ringers for the remainder of the experiments

VI. Exercises in Frog Muscle Physiology

1. Set up the stimulator (select <preferences> in Edit menu) as follows:

    Pulse Width   =   10 ms
    Delay   =   50 ms
    Amplitude   =   0 Volts
    Frequency   =   0.5 Hz
    # of Pulses   =   1

2. Type "0 v" in the 'marks box', click 'Start' to begin recording and press 'enter'. The stimulus and a record of the muscles response (if any) will be displayed in channel #4 (shock) and channel #3 (muscle), respectively .
3. Quickly click 'stop'.

Data Analysis:

4. click the <2 cursor> icon (fig 2-14) so that two blue vertical lines appear over the recording window.
5. Adjust the display time if needed and move the mouse to place the cursor on the left line, click the mouse and drag it to the peak of the first response wave (if there is one). Drag the second cursor, to the baseline after the response. (see fig 4-8)
6. The value in the channel three title area, above and to the right of the channel three window is the value for the amplitude of contraction in volts at the stimulus voltage used. Record this value in the table on your data sheet
7. Convert this amplitude 'voltage' to millimeters of contraction by multiplying it by the conversion factor determined in part IV and record this in the table on your data sheet as well.

8. Open the stimulator dialog box and change the stimulus amplitude to 0.25 v
9. Repeat steps 2 through 7, this time typing .25 v in the 'marks' box before starting

10. Repeat the above procedure increasing the voltage in 0.25 volt increments until you are stimulating the muscle with 5 volts
11. Once you have completed your table you should be able to complete the observations below:

Determining the Threshold Stimulus

12. The threshold stimulus is the minimum stimulus needed to get any kind of a response from the frog muscle.
13. Record the threshold stimulus on your data sheet

Observing Graded Muscle Response to Increased Stimulus Intensity

14. Use a piece of graph paper to graph the stimulus voltage on the x-axis and the amplitude of contraction in millimeters on the y-axis.
15. Draw a "best fit" line for your data points (It is best NOT to generate a graph by computer for this part of the exercise, since computers don't know how to draw "best fit" lines, they can only connect the dots).
16. Note the range of stimulus voltages in which you produced a graded response by the muscle. What is occurring in the muscle organ at this point?
17. The amount of contraction should peak and remain relatively constant at some stimulus voltage, this is your **maximal contraction** and **maximal stimulus voltage**
    When the curve levels off you have reached maximal stimulus voltage. Estimate the maximal stimulus voltage from your graph and record it on your data sheet. Why can't the muscle contract any more since you are still increasing the stimulus after this point?
18. Attach this graph to your data sheet.

Timing the Muscle Twitch
1. set the stimulus voltage at the value you determined was the maximal stimulus (usually 3-4 volts)
2. Scroll to one of the contractions at or near maximal contraction
3. Type "twitch" in the marks area; click 'start' and quickly press 'enter' to mark the record and produce a single twitch
4. Click stop
5. If necessary, right click after recording to invert trace.
6. If necessary adjust the display time as needed by clicking the <half display time> icon one or more times to facilitate your analysis

Data Analysis:
7. Scroll to the beginning of the section of data you want to investigate; click AutoScale if necessary.
8. Click the 2 cursor icon (Fig 2-14) so that two blue vertical lines appear over the recording window and measure:
    a. the amplitude of the twitch (Fig. 4-8) and convert it to mm as before
    b. The duration of the latent period: Place one cursor line at the stimulus mark (in window 4) and the other on window 3 just before a contraction begins (L in Fig. 4-9) and read T2-T1 in upper left corner of screen. this is the duration of the latent period in seconds.
    c. Use the double cursors to measure the duration of the period of contraction (C in Fig 4-9) in the same way
    d. Use the double cursors to also measure the duration of the period of relaxation (R in Fig 4-9)
9. Convert these values to milliseconds and record them on your data sheet.
10. Print a copy of this tracing for your group.

Inducing Treppe, or the Staircase Phenomenon
skip

Observing Graded Muscle Response to Increased Stimulus Frequency
1. open the stimulator dialog box and change the number of pulses to 250, make sure the stimulus is still set to the maximal stimulus voltage as determined earlier
2. Type 0.5 Hz in the marks box, click 'Start' and press 'Enter' on the keyboard to mark your record.
3. Click 'stop'
4. Increase the frequency to 1, 5, 10 and then 20 Hz and repeat steps 2 and 3. Notice that the first few contractions increase in intensity even though the stimulus is the same. Notice also that at a certain frequency the muscle does not have sufficient time to fully relax so that the response does not return to baseline, this is *summation*(Fig 4-13).

5. Record the frequency at which summation first appears on your data sheet
6. Print a group copy of the tracing on your screen at this frequency
7. As you continue to increase the frequency of stimulation, notice that at some frequency there is no relaxation at all between each stimulus, this is *tetanus*. (Fig 4-14)

Inducing Muscle Fatigue
Our program will not allow you to stimulate the muscle long enough to induce fatigue

Determining the Effect of Load on Skeletal Muscle
1. Set the stimulus voltage to produce maximal stimulus as determined previously (usually between 3 to 5 volts)
2. Reset stimulator settings to those used in part VI on the previous page
3. With 2 nickels in the hanger type "10 g" on the keyboard
4. Click 'Start' and press 'Enter' to mark the record and produce a single twitch.
5. Click Stop.
6. Add two more nickels (10 g) to the hanger for a total of 20 g
7. Repeat the run as above (#3 & 4)
8. Continue increasing the weight in the hanger in 10 g (two nickel) increments until there is no discernable contraction of the muscle

Data Analysis:
9. Adjust the display time and autoscale as needed
10. Scroll to the beginning of this section of data or use the 'Marks' icon to find each of the above runs
11. click the 2cursor icon (Fig 4-7) so that two blue vertical lines appear over the recording window and
12. for each twitch at each weight, measure the *amplitude* of the twitch both as a voltage measurement and after converting it to millimeters using your conversion factor and record on your data sheet
13. Calculate the "work done" for each weight that produced a measurable contraction of the muscle as:

work done (g-mm) = weight of load (g) X amplitude of contraction (mm)

13. record this value in the table on your data sheet
14. Make **two graphs** on a sheet of graph paper. On the first graph plot the load on the x-axis and the amplitude of contraction in mm on the y axis. On the second graph (or you can combine them) plot load on the x-axis and work done on the y axis.

VII. Completing your report
When you complete the lab you should turn in:
     Your completed data sheet
     a graph of the graded muscle response and maximal stimulus
     a graph of load vs work done

     your group should turn in a printout of a muscle twitch with the phases of a twitch and the duration of each handwritten in
     a printout showing summation
     a printout showing tetanus

Cleanup and Disposal

Wrap frog and muscle preparation in paper towels or plastic bag provided and discard in trash can.
Clean all dissecting equipment with soap and water, blot dry with paper towels and return to tray
Disconnect the femur clamp from your station, rinse with DI water, and replace on the ring stand at your station
Wet a paper towel with DI water and wipe down the transducer, stimulator probes and clamps, blot dry with a paper towel
Turn off the iWorx station.
Remember to spray your work area with disinfectant before leaving the labroom.

Exercises in
Frog Skeletal Muscle Physiology
Bio 2402 Data Sheet

Conversion factor to convert "voltage" to mm of displacement:_____

Record the values in the table below:

stimulus voltage																
contraction (volts)																
contraction (mm)																

stimulus voltage																
contraction (volts)																
contraction (mm)																

Determining the Threshold Stimulus

At what stimulus voltage did a measurable contraction first occur?_____

Do all motor units have the same stimulus threshold? Explain

Observing Graded Muscle Response to Increased Stimulus Intensity

Attach graph of degree of contraction by stimulus voltage to this data sheet

How much did the muscle contract at maximal contraction?_____

What was the stimulus voltage at maximal contraction?_____

What was happening to the muscle cells within the muscle as the stimulus voltage was increased up to maximal stimulus in terms of recruitment of motor units?

What happened to the cells within the muscle once maximal contraction was reached? Explain why the strength of the contraction does not increase once the maximal stimulus is reached.

Timing the Muscle Twitch

Stimulus voltage used :_____   Amplitude of contraction (mm):_____

Durations of:

      Latent Period (ms):   _____

      Period of Contraction (ms):   _____

      Period of Relaxation (ms):   _____

Label the tracing of a twitch with each phase and the duration of each phase.

How do these values compare with the time intervals given in lecture or text? Explain.

Inducing Treppe, or the Staircase Phenomenon

skip

Observing Graded Muscle Response to Increased Stimulus Frequency

1. What was the frequency that produced summation: _____

2. What was the frequency that produced tetanus: _____

Inducing Muscle Fatigue

You did not demonstrate this on your muscle preparation but explain what is happening in the muscle
    cells during fatigue.

Determining the Effect of Load on Skeletal Muscle

Load (g)	Amplitude of Contraction (volts)	Amplitude of Contraction (mm)	Work Done (gmm)
10 g			
20 g			
30 g			
40 g			
50 g			
60 g			
70 g			
80 g			

Did the amplitude of the contraction increase or decrease with increasing load? Explain.

Did the work done increase or decrease with increasing load? Explain.

Explain your results in terms of energy required, and the interplay between isotonic and isometric contractions.