SKELETAL MUSCLE PROPERTIES

Background :

In this experiment, you will investigate the physiological properties of skeletal muscle from the isolated amphibian gastrocnemius. You will examine skeletal muscle functions such as the single twitch, multiple motor unit summation, the relationship between muscle length and tension generated, wave summation and tetanus, and muscle fatigue.

The physiology of skeletal muscle was not fully understood until the early 20th century. Some of the earliest experiments on muscle physiology were performed between 1661 and 1665 by Jan Swammerdam, who demonstrated that an isolated frog muscle could be made to contract when the sciatic nerve was irritated with a metal object. Later, Luigi Galvani (1737–1798) demonstrated that frog muscle responded to electrical currents (Fulton and Wilson, 1966).

The basic unit of a muscle is the muscle cell, or myofiber, and whole muscles are made up of bundles of these myofibers. A single muscle fiber has a very regular structure, and is composed of contractile units called myofibrils. Each myofibril consists of an orderly repeated arrangement of the contractile proteins actin and myosin (sarcomere), which slide past each other in the presence of Ca^{2+} and ATP.

A motor unit is a motor neuron and all the myofibers it innervates. Greater numbers of motor neurons associated with a muscle give finer control over that muscle. Motor neurons release the neurotransmitter acetylcholine from their synaptic bulbs onto the muscle. This junction between a nerve and a muscle is called the neuromuscular junction. The release of acetylcholine at the motor end plate depolarizes the muscle tissue and in turn leads to the release of intracellular calcium from the sarcoplasmic reticulum, a variant of smooth endoplasmic reticulum. This release of intracellular calcium sets in motion the biochemical events that allow actin and myosin to interact and slide past each other, a process ultimately driven by ATP hydrolysis.

Skeletal muscle is similar to nerve tissue in that it responds to a stimulus in an all-or-none fashion. This response is called a twitch. Depending on the intensity and frequency of stimulation, greater numbers of fibers are activated. By increasing the number of active muscle fibers, the muscle is able to increase the force it generates. Muscle contractions are graded in size by wave summation, which depends on stimulus frequency, or by recruitment of more motor units. Thus, muscles with large cross-sectional areas are able to generate larger forces than those with small cross-sectional areas.

In this experiment, you will examine the basic principles of skeletal muscle physiology, including the all-or-none response, the effect of stimulus intensity and frequency on contraction force and the phenomenon of muscle fatigue. These experiments illustrate the collective understanding of muscle physiology gained from over 400 years of

research (adapted from AD Instruments Lab Protocols, for complete document see
http://www.powerlab-teaching.com/experiments/TE04a_Frog_Muscle.html).

Experimental apparatus and tissue preparation :

Dissection. Double pith a 3-4" Northern Grass Frog (Rana pipiens). Cut the skin all the way around the abdomen, and peel away the skin over the legs by pulling down with a pair of tissue-gripping forceps. Sever one leg by cutting through the femur. Remove the muscles of the upper leg and trim the femur to about one inch. Identify the gastrocnemius and **measure its resting length**. Now separate the gastrocnemius muscle from other muscles of the calf, and cut through the Achilles tendon above the ankle leaving most of the tendon attached to the muscle preparation. Next, cut the tibiofibula at the knee, along with the other calf muscles. You should be left with the gastrocnemius and the Achilles tendon at the distal end, and an inch of femur attached to the proximal end of the muscle. Place the muscle in a bath of room temperature Ringer's solution at pH 7.2-7.4. (For a diagrammatic representation see **Appendix I: Dissection**)

The muscle will contract in vitro when stimulated by an adequate electrical stimulus, just as it would in vivo when a motor unit is stimulated by a motor neuron.

Grass Stimulator. The SD9 stimulators are electronic devices that supply a known stimulus to the preparation. We can set the intensity (voltage), duration (time in msec), and frequency (number of stimulation pulses/sec) of stimulation with the dials on the front panel of the instrument. Note that the switch below each dial is a multiplier that multiplies the dial value by 0.01 to 10X.

Transducer. The device on your ring stand is a semi-isometric strain gage transducer (UFI Inc. Model 1030) which converts the mechanical tension produced by muscle contraction into an electronic signal that the recording system can detect. Other kinds of transducers exist for pressure, temperature, muscle shortening, fluid flow, sound, and light--each of these forms of energy can be converted by a transducer to an electrical signal.

Bridge amplifier and PowerLab. The PowerLab units contain 2 to 4 banks of analog-to-digital conversion circuits which will record electrical signals from our preparation. The bridge amplifier is a Wheatstone bridge circuit which collects data from the strain gauge transducer and provides a signal to PowerLab proportional to tension produced by contracting muscle. In some of the experimental stations the transducer may be connected directly to the PowerLab unit (the amplification is built into the PowerLab unit)

PC. At your table is a desktop computer that can be used to compose graphs, analyze experimental results, work with text, and record experimental data. You will record data using Chart 4.0 PowerLab software. This software simulates a pen-and-paper chart recorder.

# Getting Started:

1. Starting up and working with PowerLab.

a. Make sure the PowerLab unit in your station is turned on -- to turn it on use the power switch on the back of the unit.

b. Log onto the PC.

c. Launch Chart 3.6.3., an application that simulates a pen-and-paper chart recorder.

d. The screen should look similar to **Figure 1**. The window shown in **Figure 1** looks rather complex, but the software is very user friendly. We will introduce you to a few key features and you will gradually pick up other details as you work with the program.
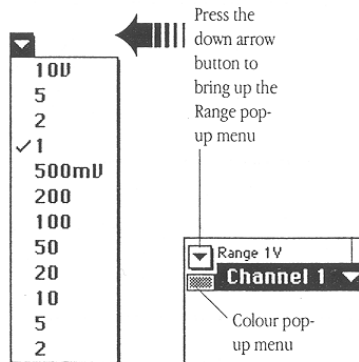
Figure 1
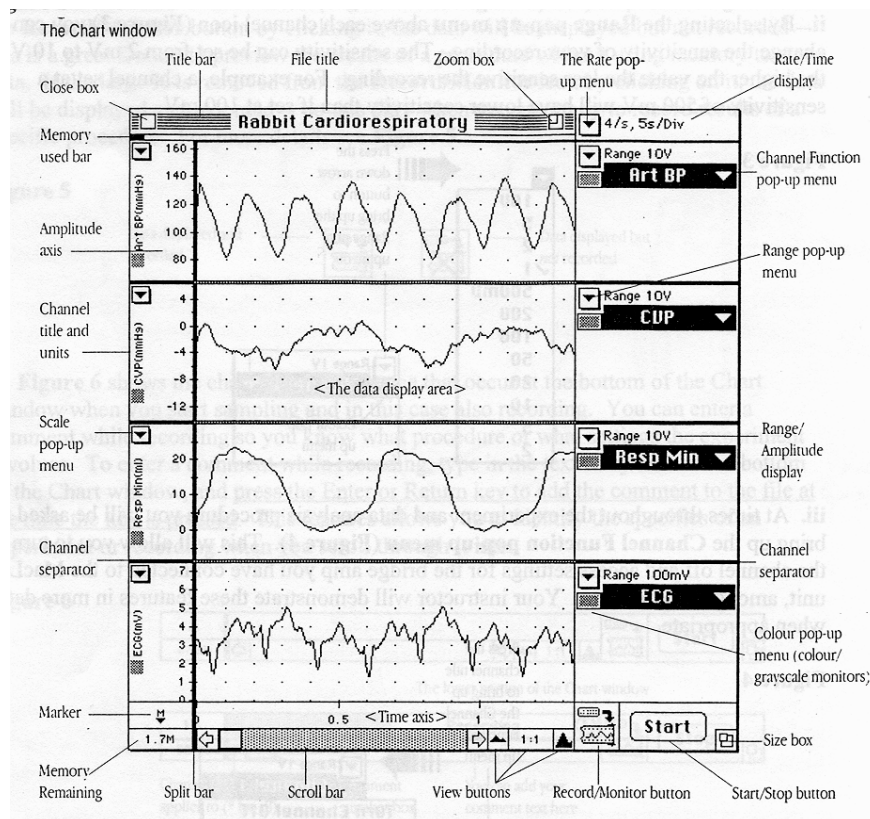
i. By selecting the **Rate pop-up menu** you can choose the sampling rate – the number of data samples that are stored per unit time, and the time taken by each sampling division to scroll past. For details see **Figure 2** below. By altering the value in this pop-up menu you are doing the same as controlling paper speed in an old-time pen-and-paper recorder.

Figure 2
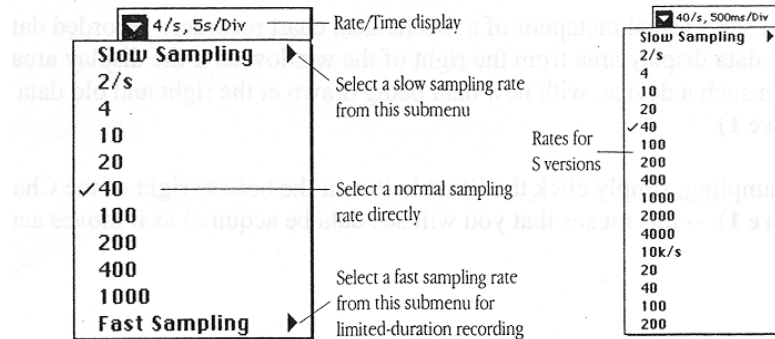
ii. By selecting the **Range pop-up menu** above each channel icon (**Figure 3**) you can change the sensitivity of your recording. The sensitivity can be set from 2 mV to 10 V -- the higher the value the less sensitive the recording. For example, a channel set at a sensitivity of 500 mV will have lower sensitivity than if set at 100 mV.

Figure 3

iii. At times throughout the experiment and data analysis procedure you will be asked to bring up the **Channel Function pop-up menu** (**Figure 4**). This will allow you to turn the channel off and access settings for the bridge amp you have connected to the PowerLab unit, among other features. Your instructor will demonstrate these features in more detail when appropriate.
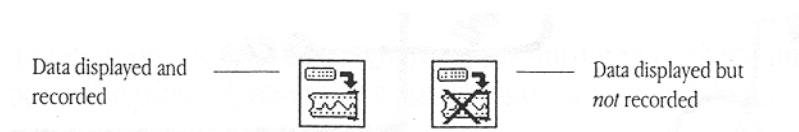
Figure 4
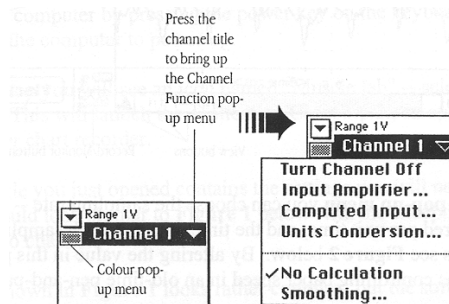
iv. Chart uses a visual metaphor of a mechanical chart recorder: recorded data scroll across the data display area from the right of the window as if the display area were a roll of paper in such a device, with new data being drawn at the right and old data moving left (see **Figure 1**).
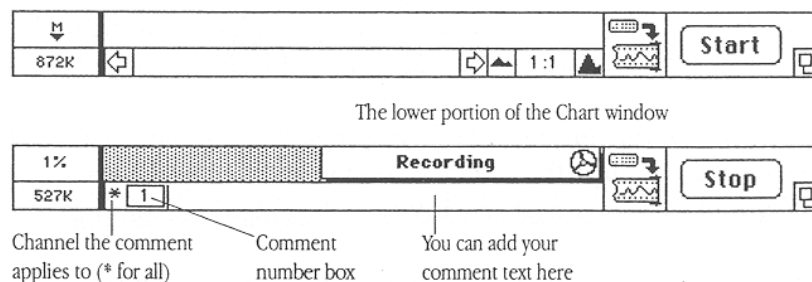
To start sampling, simply click the **Start** button at the bottom right of the Chart window (**see Figure 1**). -- this means that you will see data be acquired as it moves across the screen. Whether the data is recorded or not depends on the status of the **Record/Monitor** icon to the left of the **Start button** (**Figure 1** and **Figure 5**). If you place a large X over the **Record/Monitor** button by clicking it, the data will be displayed but not recorded -- this is a great feature to preview the results of a procedure without using memory to store data. If the large X is removed from the **Record/Monitor** icon by clicking on it, the data will be displayed and recorded -- do this only when you are ready to record results of a specific procedure. For more details see **Figure 5**.

Figure 5

Data displayed and recorded — Data displayed but *not* recorded

v. Figure 6 shows the changes in appearance that occur at the bottom of the Chart window when you start sampling and in this case also recording. You can enter a comment while recording so you know what procedure or what settings the experiment involves. To enter a comment while recording, type in the text entry area at the bottom of the Chart window, and **press the Enter or Return key to add the comment to the file at the time the key is pressed**. This features allows you to identify the specifics of an experiment or recording when you scroll through it later.

Figure 6

The lower portion of the Chart window

Channel the comment applies to (* for all)   Comment number box   You can add your comment text here

To look at several comments at once, locate a comment in a file, or delete or edit comments, choose Comments from the Windows menu to bring up the Comments window. The comments are listed in a scrolling field in the window in the order that they appear left to right across a file, with the comment numbers in boxes.

Try out some of the above features of CHART -- don't hesitate to try anything! <u>When you're done exploring, check with an instructor to see that your PowerLab is ready to record.</u>

2. Secure the muscle to the apparatus (Figure 7)

Figure 7 (autographed at request)
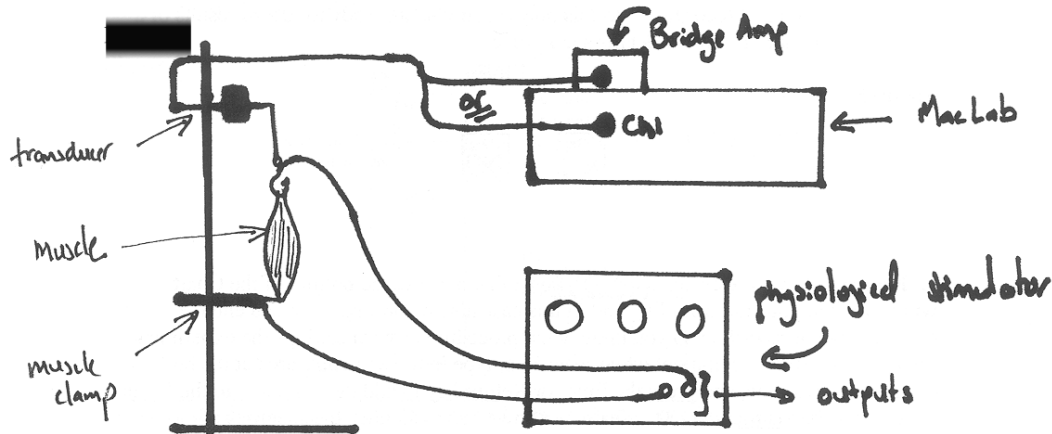
a. Remove the muscle from its Ringer's bath. Attach the muscle to the femur clamp <u>with the bone attached parallel to the clamp and at right angles to the muscle</u>.

b. At the top end of the muscle, attach the preparation to the isometric transducer using the fishhook thrust through the Achilles' tendon and tied to the transducer lever. <u>Use two- three leaves of the transducer for best sensitivity and isometric recording</u>. Stretch the muscle between the femur clamp and the transducer so that the muscle length is close to resting muscle length you measured prior to the dissection procedure.

c. Attach wires from the <u>stimulator output</u> to the femur clamp (via a screw terminal if possible) and to the fishhook at the top (via a soldered connection). Polarity (which one is + or -) doesn't matter.

d. Once you've got the muscle in place move the clamps apart so as to exert a slight tension on the resting muscle.

e. <u>Liberally douse the muscle with Ringer's saline solution now and throughout the procedure. Place a beaker underneath to catch the excess. It is extremely important that you keep the muscle wet throughout the procedure.</u>

f. In some units the transducer's output cable plugs into the bridge amplifier which in turn plugs into PowerLab Channel 1 by a BNC shielded cable. In other units the transducer's output cable plugs directly into PowerLab Channel 1.

2. Calibration.

During the experiments, <u>after the muscle is set in place,</u> and between individual procedures, you will calibrate the transducer/PowerLab apparatus by placing a weight (10 or 20 grams) on the end of the transducer lever and recording the displacement caused by that weight. Record at a moderate speed of 1 - 5 s/div, use **Rate/Time pop-up menu** to set this. You will use this calibration to compute the tension developed in your muscle under various conditions.

<u>Repeat the calibration procedure at frequent intervals during your work, and record the new calibration on the PowerLab record as a comment or in your notebook for reference</u>.

During experimentation, adjust sensitivity (use **Range pop-up menu**) as needed (except during an experimental procedure) to yield a good-sized contraction record.

<u>Always record the settings (especially sensitivity) in your written notes and/or as a comment in CHART</u>.

Liberally douse the muscle with Ringer's saline solution now and throughout the procedure. Place a beaker underneath to catch the excess.

Experimental procedures :

1. Threshold and recruitment of fibers. For the first experiment, we will find the lowest stimulus voltage needed to start a muscle action potential and cause contraction.

a. The sensitivity should be set relatively low.

b. Make sure that the stimulator wires are properly attached to the muscle prep, and the stimulator is set in the following fashion:

Duration: 30 msec
Intensity: 0.1 V
Frequency: single pulse

c. Stimulate the muscle with single pulses using the mode switch on the stimulator.

d. Record at a slow chart speed and <u>note in the Comments the voltage applied for each stimulus</u>.

e. Gradually increase the stimulus intensity in 0.1 V or 0.2 V increments, giving a single pulse at each voltage -- keep increasing the voltage until you get a response. That stimulus intensity is the threshold for the most sensitive fibers in your muscle.

f. Continue increasing the stimulus intensity (now in 0.5 -1.0 V increments) until a maximal contraction is obtained.

g. Now, with CHART running at a slow/moderate speed, <u>quickly</u> repeat this stepwise progression (0.5 - 1.0 V increments) from threshold to high stimulus intensities to obtain a <u>clean, short record</u> of the effects of recruitment of muscle fibers. Record comments that include the stimulus voltage used.

h. <u>Save your data</u> by click-dragging from the File menu down to SAVE or SAVE AS. Give your file a new name that you'll recognize and remember. Save to the M-drive.

i. Investigate the effects of applying a train of repeated subliminal or subthreshold stimuli. Do successive stimuli add together to cause a contraction?

j. Does duration of the stimulus alter the threshold? Adjust duration to various values and record the voltage needed at each duration to elicit a certain level of response.

2. The muscle twitch. The individual muscle contractions seen with a single stimulation are called <u>twitch</u> contractions. A <u>maximal twitch</u> involves all the muscle's fibers contracting at once and was observed above at the point when further increases in stimulus voltage didn't cause any further increase in tension.

During this procedure you will record a single maximal twitch at very high speed in Channel 1, also recording in Channel 2 the exact time when you applied the stimulus.

a. Calibrate your prep as described above.

b. Click Channel 2 **Range pop-up menu** and turn Channel 2 on.

c. Use a black probe or dual-banana plug cable provided by your instructor to connect the stimulator output to PowerLab Input 2. **NOTE:** Do not exceed 10 V stimulus voltages when connected to PowerLab or you'll blow the A/D board, a very expensive mistake (has been done before…).

d. Set up Channel 2 to record on 10 V range. The size of the stimulus wave is irrelevant; all we need is a mark time for the stimulation. Check with the Input amplifier in the **Channel 2 pop-up menu** to see that the stimulator pulse is displayed.

e. Record several individual twitches at <u>high speed</u>, about 100 - 200 msec/division -- use a maximal stimulus intensity and keep frequency and stimulus duration set as in **1**.

Before you start, check that the sampling rate is maximal (400 samples/div) so that you'll be able to precisely measure time in your recordings. You should see the muscle twitch in Channel 1 and the stimulus wave on Channel 2.

f. Don't forget to bathe the muscle periodically with Ringer's saline solution to rinse away waste products and keep the prep moist.

g. <u>Save your data again</u> by choosing SAVE from the File Menu.

h. Disconnect the stimulator from PowerLab Channel 2 and turn Channel 2 off using the **Channel 2 pop-up menu**.

3. Twitch fusion and tetany.

a. Calibrate your prep as described above.

b. Use a stimulus voltage which will produce a maximal contraction to investigate the effects of applying a series of repeated stimuli to the muscle.

c. Set the stimulator frequency to 1/sec, then 2/sec, then 3/sec, 5/sec, 7/sec, 10/sec, 15/sec, 20/sec. Stimulus duration stays as above in **1** and **2**.

d. Apply a train of repeated stimuli by briefly switching the stimulator **Mode switch** to **Repeat** or **Multiple**. Use only a short period of stimulation, about 5 sec, and be consistent in applying the train of stimuli. Allow the muscle to rest for about 30 sec. between stimulations.

e. Record all information in your notebook--stimulus frequency, voltage, recording speed, sensitivity -- also insert appropriate Comments during the recording.

The muscle twitches you observe will fuse together, and at high frequency stimulation will reach a condition of tetanic, or constant contraction. If the recording goes off scale you'll have to reduce the sensitivity, recalibrate, and repeat the experiment for all stimulation frequencies.

f. Save your data again by choosing SAVE from the File Menu.

4. Length-tension curve. You will examine the effects of changing the initial length of the muscle on the tension generated by stimulation with a maximal stimulus.

a. Calibrate your prep as described above.

b. Move the transducer up or down and measure the distance between clamps ("muscle length") with a ruler or calipers.

c. At each length stimulate the muscle with a single pulse using a maximal stimulus to elicit the maximum muscle tension. Use only a short range of lengths in your experiment -- perhaps $\pm$ **0.1 – 1.0 cm** total. Collect 8-10 data points. For each contraction, save the length value in a comment. **Make sure you recalibrate every time you change muscle length.**

d. Save your data again by choosing SAVE from the File Menu.

5. Pre-loading. Examine the effects of pre-loading on muscle contraction.

a. Invert the apparatus by switching the positions of the femur clamp and transducer.
Stretch the muscle to the resting muscle length in the frog.

b. Hang a pan for weights from the bottom of the muscle, below the transducer, start the
recording at **low paper speed -- record continuously throughout this procedure**.
Gradually increase the load by successively adding 10 to 400 g to the pan (work by
increments of 10 or 20 g). Each time you add weight, let the baseline stabilize, insert an
appropriate comment with weight, and stimulate muscle with a supermaximal stimulus to
produce a twitch. Add the next weight increment and repeat procedure.

6. Fatigue. When you have completed all the above experiments [not before!], look at
the effects of a continuous, high frequency stimulation on muscle tension.

a. Calibrate your prep as described above.

b. Reduce the sampling rate and recording time base so you can record over a longer
period of time.

c. Apply a tetanizing pulse (20-50 pulses/sec) and watch the development of fatigue in
the muscle. With **low paper speed**, record for about 3 minutes, then turn off the
stimulator and allow the muscle to relax completely before you stop the recording.

d. During the stimulation apply several drops of Ringer's after tension has declined
significantly. Does tension increase after a Ringer's wash?

e. Save your data again by choosing SAVE from the File Menu.

Clean up

You have completed your experimental procedures. Before leaving do the following:

1. Remove the muscle from the apparatus and dispose of it in an animal waste bag.

2. Clean all instruments and equipment.

3. Close the Chart program, shutdown the Mac, and turn off the power in the back of the
PowerLab unit.

4. Data analysis will be done next cycle.

<u>Analysis</u> :

Before starting analysis covert your data to grams as necessary by using calibration waves you collected along the procedure and the Units Conversion feature of Chart 4.0.

1. Threshold and recruitment of fibers.

Examine your data recording from Part 1.

a. Place the **marker (M)** on the baseline of the waveform in the Force channel.

b. Using the mouse, place the **waveform cursor** at the top of the contraction peak. Record the contraction amplitude in Table 1.

c. From your data, determine the minimum voltage required to elicit a maximal contraction. This is the maximum excitation voltage, a maximal stimulus. Determine your value for a supramaximal stimulus by multiplying this voltage by 1.5. Record these values in Table 2.

2. The muscle twitch.

a. Go to a recording of a high speed twitch from Part 2. Highlight both channels so that you include the full twitch. Zoom into this window and using the **marker (M)** and the waveform cursor, measure the duration of the latent period, contraction period, and relaxation period. Record these values in Table 3.

b. Either copy or print the zoom window. Identify the latent period, contraction period, relaxation period.

3. Twitch fusion and tetany.

a. Examine your data from Part 3. There will be eight blocks of recorded data.

b. For each data block, determine the maximum contraction force using the marker and waveform cursor.

c. Record your results in Table 4 of your Data Notebook.

4. Length-tension curve.

a. Examine your data from part 4.

b. For each initial muscle length, measure force produced by muscle contraction – use **marker (M)** and waveform cursor. Don't forget to recalibrate after each trial.

c. Record your data in Table 5

5. Pre-loading.

a. Examine your data from Part 5 – go to beginning of the low speed recording.

b. Recalibrate your data based upon the displacement produced by your first 10 or 20 load.

c. Return the **marker (M)** to baseline, previous to addition of any load.

d. Using the **waveform cursor**, measure the displacement produced by addition of weight. This is the preload force.

e. Next, determine the raw twitch force with the **waveform cursor** by placing the cursor at the peak of the contraction produced at this pre-load.. Enter your results in Table 6.

f. repeat steps d and e for the other data blocks

g. Calculate the net twitch force by subtracting the preload (baseline) value from the raw twitch force value.

6. Muscle fatigue

a. Examine your data from Part 6.

b. Place the **marker (M)** on the waveform immediately prior to stimulation.

c. Use the waveform cursor to determine maximum contraction force. Record this value and the time of maximal stimulation in Table 7 of your Data Notebook.

d. Determine the contraction force at t= 15, 30, 60, 90, 120, 150, and 180 seconds after stimulation.

f. Record your results in Table 6 of your Data Notebook.