

Dissection and Contraction of Frog Heart

Rob MacLeod and Alex Brownell (aabrownell@yahoo.com)

March 4, 2006

1 Purpose and Background

1.1 Purpose:

To examine the anatomy and basic contractile physiology of the frog heart. This lab provides background information for the subsequent study of the physiological response of the frog heart to external stimuli, which we will cover in the next lab session.

1.2 Background

1.2.1 Frog anatomy/physiology

There are a number of excellent web sites you can peruse to find background information relevant to this lab. We will cover some basics in class, but please at least go through the virtual dissection site at curry.edschool.virginia.edu/go/frog/Frog1/menu.html.

The full list of web sites to visit include:

- curry.edschool.virginia.edu/go/frog/Frog1/: a simpler site that describes dissection and provides useful images.
- curry.edschool.virginia.edu/go/frog/Frog2/: a more elaborate version of the previous site with interaction and animations.
- biog-101-104.bio.cornell.edu/Biog101_104/tutorials/frog.html: a frog morphology & physiology tutorial.

1.2.2 Matlab

We assume you have a working knowledge of MATLAB. If this is not the case, please let me know and the TA for the lab, Alex Brownell (aabrownell@yahoo.com), will give a tutorial on Matlab. For more information, you can go to the web sites listed at www.cvrti.utah.edu/~macleod/software/matlab-info.html and consult any of the enormous number of books written about Matlab.

2 Procedure

2.1 Materials

The equipment required consists of:

- Digital camera to take photos of the frog during dissection
- Dissection pan with 4 needles
- Dissection kit you used in the anatomy experiment.
- Two bioamplifiers
- Force transducer
- 2 magnetic clamp stands
- Bipolar electrode
- Oscilloscope
- Computer with acquisition program (C:\\bioen\\CB8ChScope)
- 20 ml vials for drug samples
- Plastic eye-droppers
- suture needle with thread attached
- Batteries for the force transducer
- medium sized vial containing Ringer's solution, composed of:
 - NaCl: 200 ml (stock 4M),
 - KCl: 20 ml (stock 1M),
 - MgCl₂: 20 ml (stock 1M),
 - CaCl₂: 4 ml (stock 1 M),
 - NaOH: 25.8 ml (stock 1 M)
 - D-Glucose: 1.8 g,
 - HEPES: 11.44 ml (stock 1 M),
 - pH: 7.4,
 - De-ionized water: to make 2 L,
 - Total Volume: 2 L.

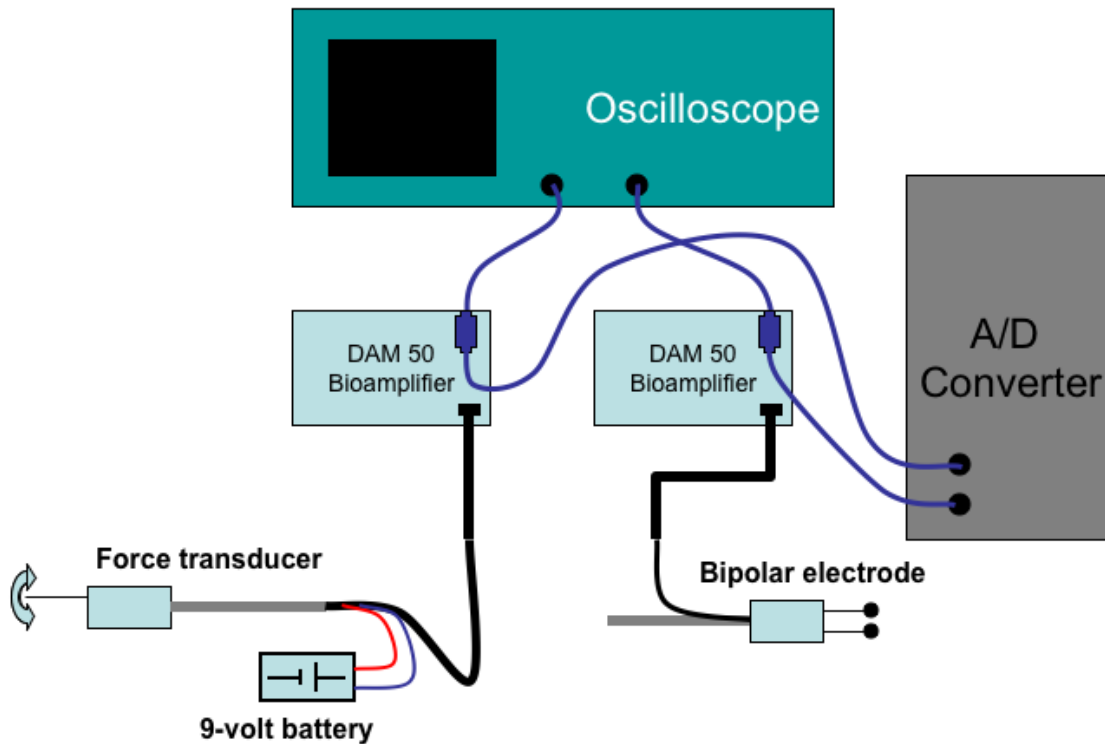


Figure 1: Circuit diagram for the recording of contraction and electrograms from the frog heart.

2.2 Preparing the force transducer and circuit

Please carry out the following steps (Note **Do not start the frog dissection until you have completed all the setup steps!**):

1. Setting up the measurement circuit according to Figure 1:
 - (a) Connect the battery to the pressure transducer and hook up the wires from it to the input of one of the bioamplifiers.
 - (b) Place a T-connector on the output of the bioamplifier and then connect one end to the input of the oscilloscope and the other to the inputs for the computer A/D converter. Use channel one for both the oscilloscope and A/D converter.
 - (c) Adjust the settings on the bioamplifier to get a clean signal on the oscilloscope in which you can see the response to **gentle** bending of the force transducer. Start with the following settings on the bioamplifier:
 - DC coupling

- Low filter at lowest frequency setting
- High filter at low to moderate frequency
- Gain at or near maximum

On the oscilloscope, try the following settings (make sure all settings are in calibrated mode, *i.e.*, latched into fixed settings):

- DC coupling
 - 1 Volt/div
 - ≈ 0.5 s/div
- (d) Launch the acquisition program (C:\bioen\CB8ChanScop) computers for acquiring the signals. Then select sampling parameters from the program (sampling rate of 100–200 is adequate) and run it to make sure it acquires signal.

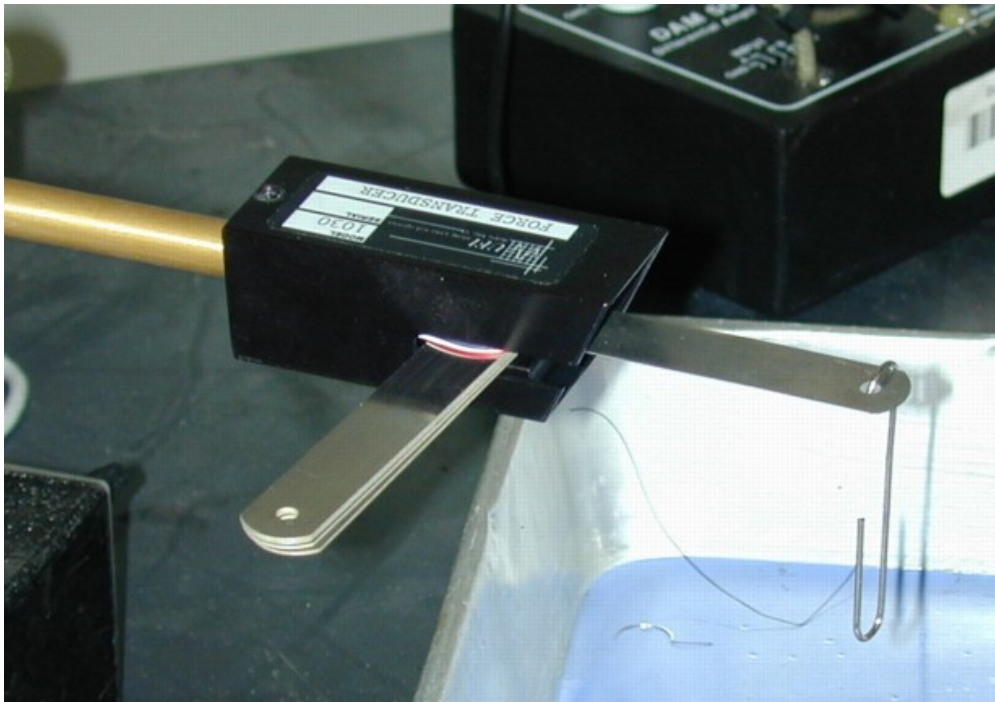


Figure 2: Calibration of the force transducer.

2. Calibration of the force transducer:

- Mount the transducer in the magnetic stand and make sure only the thinnest blade is aligned with the transducer handle (all the other blades should be perpendicular to the handle), as shown in Figure 2. Rotate the transducer so the flat side of the blade is parallel (horizontal) to the table; deflections in the up and down directions should cause the signal on the oscilloscope to change.
- Use a small Phillips screwdriver to remove the screw and slide the cover out of the way. Put the cover and the screw carefully aside for later re-attachment.

- (c) Use a small straight screwdriver to adjust the zero of the transducer. This can be a little tedious as many turns of the screw may be necessary before the zero level shifts, then it may shift quite suddenly. Watch the oscilloscope display the whole time you are turning the adjustment screw and when in the working range, tune it so that there is no or little offset from zero. You may have to repeat this adjustment during the experiment.
- (d) Hang a series of the large (0.75 g) and small (0.35 g) paper clips from the transducer blade and note the total weight and the associated deflection of the signal on the oscilloscope; the resulting table of values will be the basis for calibration of the transducer.
- (e) When you are done, rotate the blade of the transducer back into the vertical orientation and, if necessary, re-zero it using the small screwdriver. You should now be ready to perform the measurements of the contracting heart.

2.3 Frog Preparation

Once you have everything set up and the force transducer calibrated, you can move on to the frog preparation as follows:

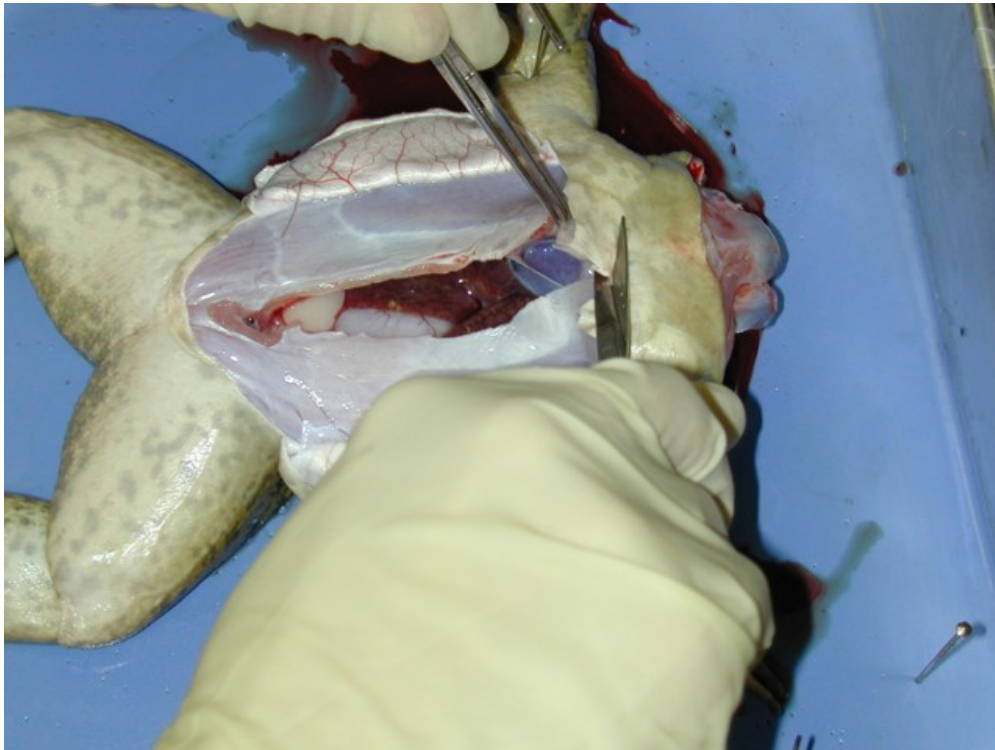


Figure 3: Dissection of the frog showing the open skin flaps, the removal of the sternum and, underneath, the exposed heart inside the pericardial sack.

1. Obtain a pithed frog from the lab TA/Instructor and fix the frog on its back using the big needles in the pan. Open the thorax of the frog with a central incision and two flaps, which

is also shown nicely through a series of images in the web site curry.edschool.virginia.edu/go/frog/Frog1/menu.html. Go to the point of the Layer One section and focus on the heart. The point here is not to perform a detailed dissection but to make you familiar with the general anatomy and comfortable with the preparation of the animal. **Do not cut or remove any organs other than the skin and some of the ribs covering the heart.**

2. To expose the heart, make sure to remove the lower and middle sections of the rib cage as they will interfere with the transducer you will use to measure contraction. The heart of the bullfrog is quite large and red and should be slowly beating. Figure 3 shows the process of removing the ribs and sternum and the exposed heart below. If the frog is still cold, the rate may be very slow so run some Ringer's solution over the heart to help it warm up. Observe the atria and single ventricle of the heart and note the sequence of contraction of each.
3. Once the heart is open, regularly apply a few drops of Ringer's solution to keep it moist.
4. If you have a camera available, take photos of the thorax and mark organs on them. If you do not have a camera, find images from a classmate and label them for your lab report.
5. Attaching transducer to the frog (See Figure 4):
 - (a) Very carefully, cut open and remove the pericardium from the heart so you can see it fully exposed.
 - (b) Using the curved needle and suture provided, run the needle through the lower part of the ventricle, about 5 mm from the apex of the heart, and tie a loop with the suture thread. Then clip off the needle and discard it carefully. Run the other end of the suture through the hole in the transducer blade and tie a knot there as well. Make sure there is at least 30 cm of suture available between the heart and the force transducer.
 - (c) Place the transducer at the end of the pan, elevated about 20 cm above the table surface with the blade oriented perpendicular to the table. The thread from the frog heart to the transducer should be quite flat (horizontal) so that you apply tension to the long axis of the heart. See Figure 4 for reference.
 - (d) Apply a ground wire between the metal dissection tray and the large metal plate on which you are working. This can reduce the electrical noise levels substantially when we start to perform electrocardiographic measurements.
 - (e) Now apply enough tension to the thread that you start to see a signal on the oscilloscope that reflects the contraction of the heart. Sensitivity of the 'scope should be in the range of 1–5 V/div. Adjust locations and tension so as to generate as clean a signal as possible, ideally one that reveals the separate components of atrial and ventricular contractions. Make sure the tension of the thread is just enough to pull the thread taut and lift the heart slightly, but not that it yanks the heart from the animal. Check also that there is no obstruction from the side of the pan or any other object. Place the pan and the stand well away from the edge of the lab bench and always be careful not to accidentally touch the post or the thread so as not to change the orientation or lose the reference signals, which will be important later in the experiment.

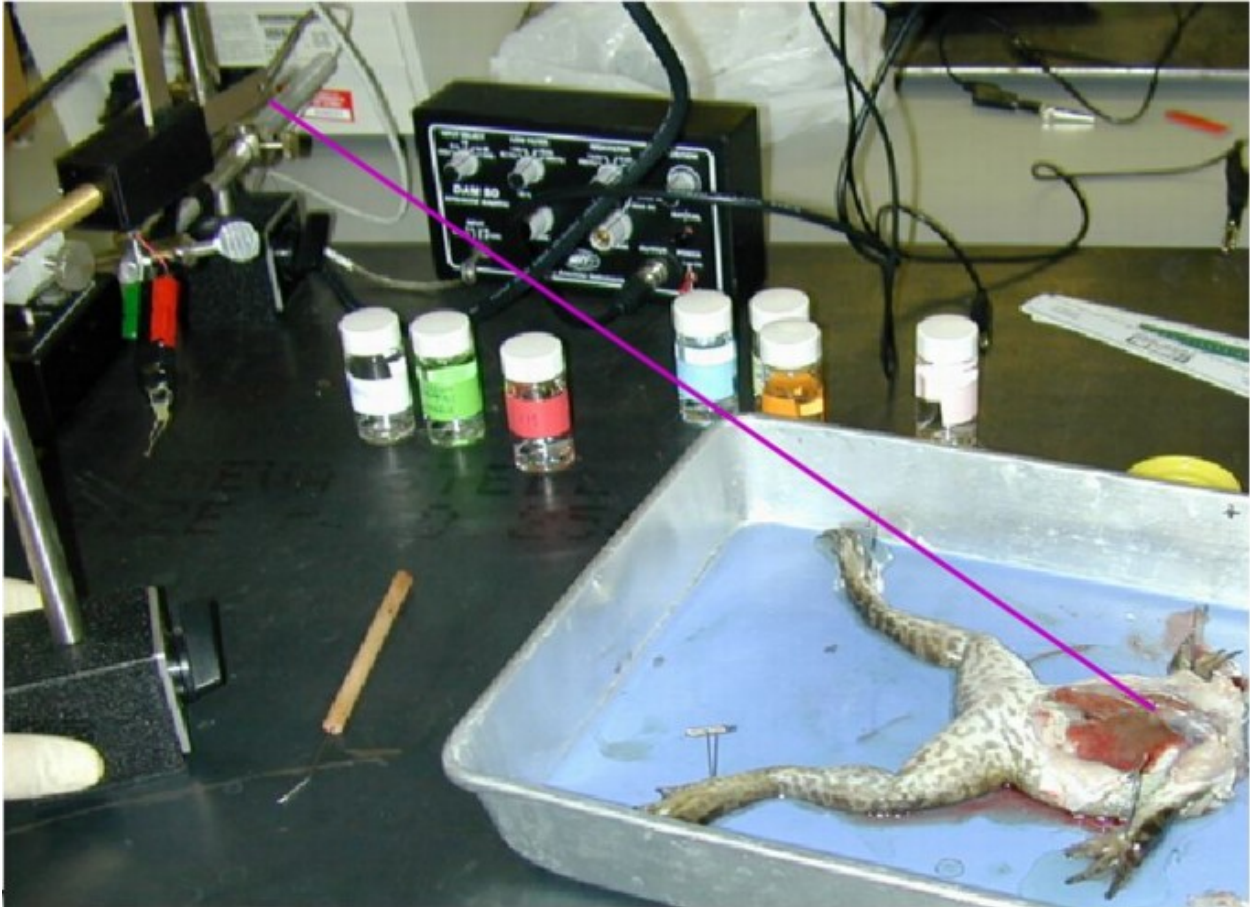


Figure 4: Photo of the complete frog preparation include the thread connecting the heart to the transducer.

2.4 ECG and and Data acquisition

Now, to visualize and acquire the electrical signals, carry out the following steps. We will refer to the “ECG” or electrocardiogram to mean the electrical signal acquired from the surface of the animal and the “electrogram” to be the signal acquired directly from the surface of the heart.

1. Arrange a second bioamplifier with the output going to the second channel of the oscilloscope and the channel 2 of the A/D converter.
2. Try the following settings on the bioamplifier:
 - AC coupling
 - A-B mode
 - Low filter at lowest frequency setting
 - High filter at low to moderate frequency
 - Gain at or near maximum

First for the limb-lead ECG:

1. Attach three wires from input of the bioamplifier to the needles that attach the frog to the dissection pan. The green lead is the reference and the red and black feed the signal to the bioamplifier. Monitor the output of the bioamplifier on the oscilloscope and adjust so as to get a clear signal.
2. Try different arrangements of the wires and see which gives the nicest and largest ECG signal, one that includes both atrial and ventricular activity.
3. Record the ECG together with the contraction signal and save on the computer.



Figure 5: Exposed heart with applied bipolar electrodes. The electrodes should touch the exposed heart lightly.

Now for the electrogram, the signal one can record directly from the heart surface, as follows (see Figure 5):

1. Take a bipolar electrode holder, attach it to a magnetic stand that can lift up and down, and place the electrodes in contact with the heart surface.
2. Connect the wire from the electrode to a bioamplifier (use the same one as previously for the ECG). Connect the reference lead to one of the pins that hold down the feet of the frog. Adjust the electrode location so as to get a clean signal of both atrial and ventricular “electrograms”.
3. Record the electrogram together with the contraction signal on the computer.

2.5 Experimental protocol

There are a set of interventions that you should carry out to see how the heart responds to external stimuli, both mechanical and chemical.

2.6 Response to stretch

Now try and replicate the effect of the Frank Starling mechanism with the preparation by measuring contraction and progressively stretching the heart to simulate the effect of increased venous return.

1. Arrange the pan and transducer so that there is just enough tension for the thread to clear the edge of the pan (adjust the height of the transducer if necessary) and for you to get a contraction signal. Take this as the baseline value and measure the applied force and the amplitude of the contractions. Take a sample of data with the computer.
2. Move the pan a few millimeters further away from the transducer so that it increases tension on the heart slightly. Then once again measure applied force and contractile force amplitude and take a sample of the signals on the computer.
3. Repeat this process in **8–10** small steps until the heart looks dangerously stretched, at each step recording applied force and contractile response of the heart on the computer. Check with the TA or instructor if in doubt about how far to stretch the heart.
4. For the report, construct a plot of twitch contraction versus pre-tension and explain which mechanism(s) explains the results. The pre-tension is the base tension, which is visible in the force transducer before (and after) the time-varying signal (twitch) from the heartbeat. For the graph, you will need to make measurements of the base level of the contraction signal as the independent (x axis) variable. The dependent variable (y axis) you can then determine from the peak contraction for each heartbeat by subtracting peak force values from the pre-tension value. The resulting plot of contraction versus pre-tension should look something like the Frank-Starling curves from the text (or class).
5. **Note: make sure to apply the calibration curve to all the data you acquire from the lab so that units are in grams or newtons.**

2.7 Final dissection

Now dissect the heart from the animal and see if you can recognize at least gross anatomical features like the chambers and major vessels.

1. Remove the heart and attempt to open it up and see structure.
2. Remove lungs and attempt to inflate them, then slice them open and observe structure.

3 Post processing

After the lab you will have a lot of data from the tension and electrical recordings from the frog and should now process, visualize, and analyze these data for the report. MATLAB is probably the best tool for much of this and is one tool that can do all of it.

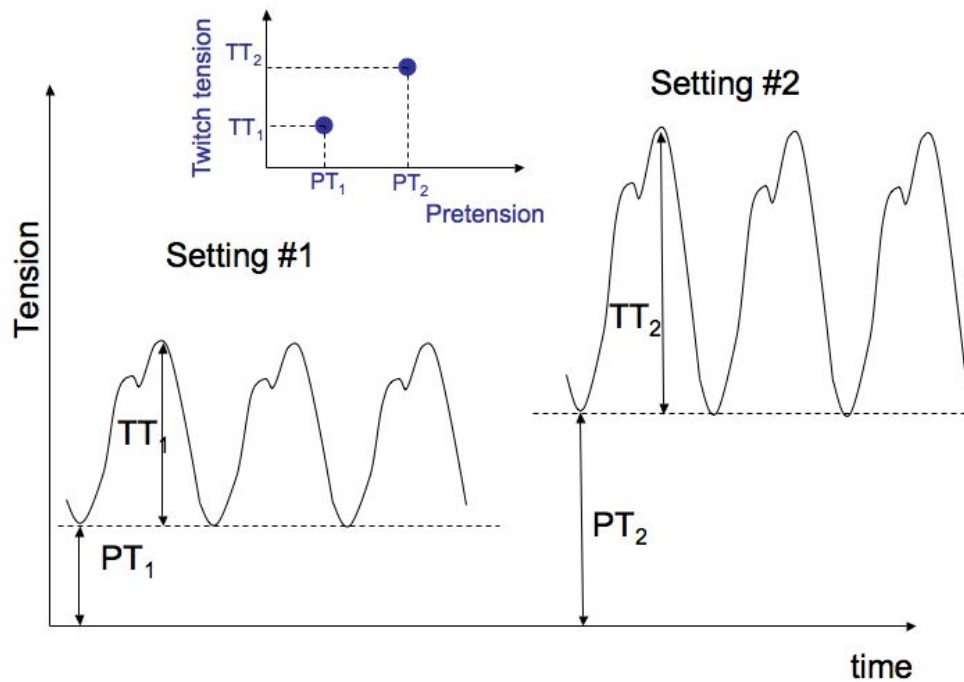


Figure 6: Example of the response to pre-tension. The lower axes show two measurements of resting or pre-tension and the resulting twitch tension during contraction. The inset graph shows the resulting points in a twitch tension versus pretension curve.

3.1 Response to stretch

Figure 6 illustrates one facet of the signal processing and parameter extraction you should perform. The two time signals show contraction under two different levels of pretension and the resulting contraction tension. Note how the parameters from the time signals then come together in a plot of twitch tension versus pretension—one form of the Frank-Starling curve. You should construct a curve like this from your own data.

4 Lab Report

Prepare just one lab report for this and the next lab together in which you document your findings. For this section, include brief background and methods sections and concentrate on showing the following:

- Labeled images of the thorax.
- Plots from the computer acquisition of contraction signal—please use MATLAB to create these.

- Calibration curve for the transducer, which you should apply to all the measured signals so that all plots are expressed in units of mass (or force).
- Plot of contraction as a function of pre-tension (Frank-Starling curve).

Wherever relevant, please attempt to describe and to interpret and discuss your results. Did the results match your expectations? What are the possible mechanisms for the response of the heart to contraction? Compare atrial and ventricular contraction as you observed it in the frog heart.

The tips for homework assignments provided at www.cvrtri.utah.edu/~macleod/bioen/be6900/homework/homework-tips.html also apply to lab reports.