## Laboratory Exercise

## **Factors Affecting the Cardiac Cycle**

#### Purpose of the Exercise

To review the mechanism by which the heartbeat is regulated, to observe the action of a frog heart, and to investigate the effects of various factors on the frog heartbeat.

### 🕌 Materials Needed

Physiological recording apparatus such as a kymograph or Physiograph Live frog Dissecting tray Dissecting instruments Dissecting pins Frog Ringer's solution in plastic squeeze bottle Thread Small hook Medicine dropper Thermometer Ice Hot plate Calcium chloride, 2% solution Potassium chloride, 5% solution Ph.I.L.S. 4.0 For Learning Extension Activity: Epinephrine, 1:10,000 solution Acetylcholine, 1:10,000 solution

### Safety

Caffeine, 0.2% solution

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

#### **Learning Outcomes**

#### After completing this exercise, you should be able to

- Distinguish the atrial and ventricular contractions and determine the heart rate from a recording of a frog heartbeat.
- 2 Assess the effects of various factors on the action of a frog heart.
- Measure the amplitude of a heart contraction (indication of stroke volume).
- Apply the concepts of thermal and chemical effects to the human heart.

#### Pre-Lab

- **1.** Carefully read the introductory material and examine the entire lab.
- **2.** Be familiar with the autonomic nervous system, the cardiac cycle, and the cardiac conduction system from lecture or the textbook.

Although the cardiac cycle is controlled by the SA node serving as the pacemaker, the rate of heart action can be altered by various other factors. These factors include parasympathetic and sympathetic nerve impulses that originate in the cardiac center of the medulla oblongata, changes in body temperature, and concentrations of certain ions.

#### Procedure A—Live Frog Exercise

#### **General Suggestion**

Try to become familiar with the content and organization of this lab before you pith a frog. If you work quickly, one pithed frog should last for all of the experimental steps.

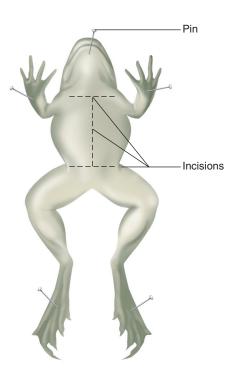
- **1.** Observe the normal action of a frog heart. To do this, follow these steps:
  - **a.** Obtain a live frog, and pith it according to the directions in Procedure B of Laboratory Exercise S-1.
  - **b.** Place the frog in a dissecting tray with its ventral surface up, and pin its jaw and limbs to the tray with dissecting pins (fig. S-3.1).
  - **c.** Use scissors to make a midline incision through the skin from the pelvis to the jaw.
  - **d.** Cut the skin laterally on each side in the pelvic and pectoral regions, and pin the resulting flaps of skin to the tray (fig. S-3.1).

#### **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.

- e. Remove the exposed pectoral muscles and the sternum, being careful not to injure the underlying organs.
- **f.** Note the beating heart surrounded by the thin-walled pericardium. Use forceps to lift the pericardium upward, and carefully slit it open with scissors, thus exposing the heart.
- **g.** Flood the heart with frog Ringer's solution, and keep it moist throughout this exercise.
- **h.** Note that the frog heart has only three chambers two atria and a ventricle. Watch the heart carefully as it beats, and note the sequence of chamber movements during a cardiac cycle.
- **2.** Tie a piece of thread about 45 cm long to a small metal hook, and insert the hook into the tip (apex) of the ventricle without penetrating the chamber (fig. S-3.2). The laboratory instructor will demonstrate how to connect the thread to a physiological recording apparatus so that you can record the frog heart movements. The thread should be adjusted so that there is no slack in it, but at the same time, it should not be so taut that it pulls the heart out of its normal position (fig. S-3.3).

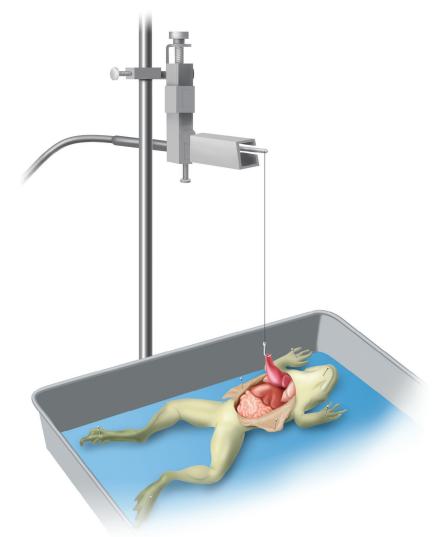
**FIGURE S-3.1** Pin the frog to the dissecting tray and make incisions through the skin as indicated.



**FIGURE S-3.2** Attach a hook and thread to the tip of the ventricle.



FIGURE S-3.3 Attach the thread from the heart to the recording apparatus so that there is no slack in the thread.



- **3.** Record the movements of the frog heart for 2–3 minutes. Identify on the recording the smaller atrial contraction waves and the larger ventricular contraction waves. Also, determine the heart rate (beats per minute) for each minute of recording, and calculate the average rate. Enter the results in Part A of Laboratory Assessment S-3.
- **4.** Test the effect of temperature change on the frog's heart rate. To do this, follow these steps:
  - **a.** Remove as much as possible of the Ringer's solution from around the heart, using a medicine dropper.
  - **b.** Flood the heart with fresh Ringer's solution that has been cooled in an ice water bath to about  $10^{\circ}C$  (50°F).
  - **c.** Record the heart movements, and determine the heart rate as before.
  - **d.** Remove the cool liquid from around the heart, and replace it with room-temperature Ringer's solution.
  - **e.** After the heart is beating at its normal rate again, flood it with Ringer's solution that has been heated on a hot plate to about 35°C (95°F).

- **f.** Record the heart movements, and determine the heart rate as before.
- **g.** Enter the results in Part A of the laboratory assessment.
- 5. Complete Part A of the laboratory assessment.
- **6.** Test the effect of an increased concentration of calcium ions on the frog heart. If the frog heart from the previous experiment is still beating, replace the fluid around it with room-temperature Ringer's solution, and wait until its rate is normal. Otherwise, prepare a fresh specimen, and determine its normal rate as before. To perform the test, follow these steps:
  - **a.** Flood the frog heart with 2% calcium chloride. (This solution of calcium chloride will allow ionization to occur, providing Ca<sup>++</sup>.)
  - **b.** Record the heartbeat for about 5 minutes, and note any change in rate.
  - **c.** Flood the heart with fresh Ringer's solution until heart rate returns to normal.

#### Learning Extension Activity

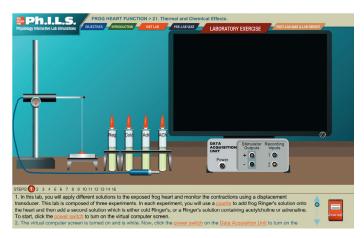
Plan an experiment to test the effect of an additional factor on the action of a frog heart. For example, you might test the effect of epinephrine, acetylcholine, caffeine, or some other available substance. If the laboratory instructor approves your plan, perform the experiment and record the heart movements. What do you conclude from the results of your experiment?

- **7.** Test the effect of an increased concentration of potassium ions on the frog heart. To do this, follow these steps:
  - **a.** Flood the heart with 5% potassium chloride. (This solution of potassium chloride will allow ionization to occur, providing  $K^+$ .)
  - **b.** Record the heartbeat for about 5 minutes, and note any change in rate.
- 8. Complete Part B of the laboratory assessment.

#### Procedure B: Ph.I.L.S. Lesson 21 Frog Heart Function: Thermal and Chemical Effects

- **1.** Open Exercise 21, Frog Heart Function: Thermal and Chemical Effects.
- **2.** Read the objectives and introduction and take the prelab 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-3.4).

**FIGURE S-3.4** Opening screen for the laboratory exercise on Frog Heart Function: Thermal and Chemical Effects.



#### 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 to the Data Acquisition Unit.

#### Cold Saline

- 6. Addition of Regular Saline (Ringer's) and Cold Saline
  - **a.** Click the Start button and follow the instructions on screen to stretch the heart. A blue line (when stimulus applied) will be displayed on the monitor and will continue to scroll across the screen until you push Stop. As it scrolls, you must click the Up arrow above the clamp to increase the heart response. Proceed to the next step as it scrolls.
  - **b.** Follow instructions on screen. Add a drop of "regular saline solution"; wait 3 heart cycles and add cold saline solution; wait 4 heart cycles and click Stop.
  - **c.** Scroll back to the graphic recording after regular Ringer's solution was applied.
  - **d.** Measure the amplitude:
    - i. Position the crosshairs (using the mouse) at the top of the wave and click. If it is not in the correct location, reposition by dragging it to a new position. Now position the crosshairs at the bottom of the deflection and click. Amplitude (amp) is the force of contraction of the heart (and is an indication of stroke volume, the amount of blood pumped per beat).
    - ii. Click the Journal panel (red rectangle at bottom right of screen) to enter your value into the Journal. A table with amplitude, interval, frequency, and cardiac output will be displayed. (Amplitude data will appear in the table.)
    - **iii.** Close the Journal window by clicking on the X in the right-hand corner.
  - e. Measure the interval of time for one cycle:
    - i. Position the crosshairs (using the mouse) at the top of a wave and click. Now position the crosshairs at the top of the *next* wave and click. The result will display in the (yellow) data panel.
    - **ii.** Click the Journal panel. (The remainder of the table for regular Ringer's solution will be complete.)

*Note:* The interval of time was used to calculate heart rate by dividing 60 (seconds) by the interval of time. For example: 60/2.0 = 30.0. (This is done by the computer and is shown in the Journal.)

Calculate the heart rate (frequency) for your results:

 $\frac{60}{[]} = [$ (interval of time)

] (frequency or bpm)

iii. Cardiac output was calculated by multiplying the amplitude (indication of stroke volume)  $\times$ frequency (heart rate). For example 33.1  $\times$  1.2  $\times$  40. (This is done by the computer and is shown in the Journal.)

Calculate the cardiac output for your results: \_\_\_\_\_ (amplitude) × \_\_\_\_\_ (frequency) = \_\_\_\_\_ cardiac output

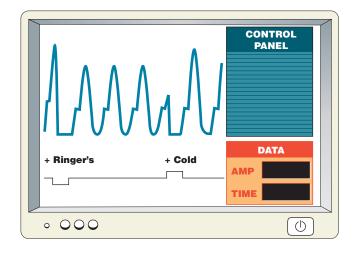
- f. Close the Journal.
- **g.** After scrolling to the section labeled "cold," repeat steps d and e to obtain results for cold Ringer's solution.
- **h.** After finishing this section you can print the line tracing for regular Ringer's solution and cold Ringer's solution by clicking on the P in the bottom left of the monitor screen (fig. S-3.5).
- **i.** Close the Journal window by clicking on the X in the right-hand corner.

#### Adrenaline

**7.** Addition of Regular Saline (Ringer's) and Adrenaline (*Epinephrine*): Repeat number 6 (Ph.I.L.S. steps 6–8), except add adrenaline in place of cold saline. (Do not need to stretch heart.)

#### Acetylcholine

**8.** Addition of Regular Saline (Ringer's) and Acetylcholine: Repeat number 6 (Ph.I.L.S. steps 6–8), except add acetylcholine in place of cold saline. (Do not need to stretch heart.) **FIGURE S-3.5** An example of a recording of contractions (upper trace) from an exposed frog heart. The response in normal Ringer's solution is shown to the left of the upward deflection, and the response after the addition of cold saline is shown to the right.



#### Interpreting Results

- **9.** With the Journal still open on the screen, answer the questions in Part C of the laboratory assessment. If you accidentally closed the graph, click on the Journal panel (red rectangle at bottom right of screen).
- **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 Frog Heart Function: Thermal and Chemical Effects.

## NOTES


Laboratory Assessment



Name	
Date	

Section \_

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

# **Factors Affecting the Cardiac Cycle**

#### Part A Assessments

1. Describe the actions of the frog heart chambers during a cardiac cycle. <u>1</u>

The atria contract as a unit followed by the contraction of the ventricle (the ventricle is single in frogs).

2. Attach a short segment of the normal frog heart recording in the following space. Label the atrial and ventricular waves of one cardiac cycle. Enter the average heart rate beneath the recording.

(experimental results)

3. Temperature effect results: 🙎

Temperature	Heart Rate
10°C (50°F)	(experimental results)
Room temperature	
35°C (95°F)	

4. Summarize the effect of temperature on the frog's heart action that was demonstrated by this experiment. 🙎 \_\_\_\_\_

The SA node activity at room temperature is slower than the human resting heart rate. Lower

temperatures slow the rate; warmer temperatures increase the rate.

#### Part B Assessments

Complete the following:

**1.** Describe the effect of an increased calcium ion (Ca<sup>++</sup>) concentration on the frog's heart rate. 2 The force

(amplitude) of the contractions normally increases as calcium ions help initiate muscle contractions.

2. Describe the effect of an increased potassium ion  $(K^+)$  concentration on the frog's heart rate. 2 Normally the

rate and force of contractions decrease by altering the usual polarized state of cardiac muscle cells.

Excessive potassium ions may result in cardiac arrest.

Critical Thinking Assessment
In testing the effects of different ions on heart action, why were chlorides used in each case?
This will allow the ionization to occur providing K <sup>+</sup> and Ca <sup>++</sup> when in a solution of water.

#### Part C Ph.I.L.S. Lesson 21, Frog Heart Function: Thermal and Chemical Effects Assessments

1. Complete the table for temperature effects on amplitude, frequency, and cardiac output. A (sample data)

Heart Variable	<b>Regular Ringer's Solution</b>	Cold Ringer's Solution
Amplitude	1.5 mm	1.7 mm
Frequency	34.2 bpm	25.5 bpm
Cardiac output	52	44

- 2. As the temperature decreases, the frequency (heart rate) <u>decreases</u> (increases or decreases) and cardiac output <u>decreases</u> (increases or decreases). As a result, temperature is <u>directly</u> (directly or inversely) related to heart rate and cardiac output.
- 3. If you added warm Ringer's solution, what prediction would you make about heart rate and cardiac output? 🙎 \_\_\_\_\_

#### Both heart rate and cardiac output would increase.

4. Complete the table for the effects of adrenaline (epinephrine) on amplitude, frequency, and cardiac output. 🕂 🔉 (sample data)

Heart Variable	<b>Regular Ringer's Solution</b>	Adrenaline (Epinephrine)
Amplitude	1.6 mm	2 mm
Frequency	34.4 bpm	49.5 bpm
Cardiac output	56	99

5. With the addition of adrenaline (epinephrine) to the heart, amplitude <u>increases</u> (increases or decreases), frequency <u>increases</u> (increases or decreases), and cardiac output <u>increases</u> (increases or decreases).

6. Complete the table for the effects of acetylcholine on amplitude, frequency, and cardiac output: A 3 (sample data)

Heart Variable	<b>Regular Ringer's Solution</b>	Acetylcholine
Amplitude	1.5 mm	1.4 mm
Frequency	38.9 bpm	27.1 bpm
Cardiac output	59	38

7. With the addition of acetylcholine to the heart, frequency <u>decreases</u> (increases or decreases) and cardiac output <u>decreases</u> (increases or decreases). Is there a significant change in amplitude?
<u>no</u>

#### Critical Thinking Assessment 🔼

Body Temperature and Cardiac Output: A patient enters the hospital with hypothermia (low body temperature); you would predict what change (increase or decrease) in heart rate? <u>decrease</u> cardiac output? <u>decrease</u> A child has the flu and is "running a fever"; you would predict what change in heart rate? <u>increase</u> cardiac output? <u>increase</u> cardiac output?

Autonomic Nervous System and Cardiac Output: When you are excited, exercising, or experiencing an emergency, the sympathetic nervous system will be activated. If stimulation of the sympathetic nervous system releases adrenaline (epinephrine), what effect would this have on the amount of blood pumped from the heart per stroke (stroke volume)? increase heart rate? increase cardiac output? increase When you are resting, the parasympathetic nervous system releases acetylcholine, what effect does this have on heart rate? decrease cardiac output? decrease Is there a significant change in stroke volume? no

#### **Photo Credits**

#### Laboratory Exercise S-1

Figure S-1.1(top)–(bottom): © Phipps & Bird, Inc. Used with permission; S-1.2: © Narco Bio-Systems Narco Trace® Narco Bio-Systems is a division of International Biomedical Inc.; S-1.3: © The McGraw-Hill Companies, Inc./ Richard Pflanzer; S-1.5a–b: © The McGraw-Hill Companies, Inc./J. Womack Photography.

#### Laboratory Exercise S-2

Figure S-2.1: © The McGraw-Hill Companies, Inc./J. Womack Photography.

#### Laboratory Exercise S-3

Figure S-3.2: © The McGraw-Hill Companies, Inc./J. Womack Photography.