EXERCISE 34

Frog Cardiovascular Physiology

Time Allotment: 3 hours. (Allow additional time if students must learn to use equipment.)

Multimedia Resources: See Appendix B for Guide to Multimedia Resource Distributors.
PhysioEx™ 9.0 Exercise 6 (PE: CD-ROM, Website)

Solutions:

Ringer's Solution, Frog
- 6.50 grams sodium chloride
- 0.14 gram potassium chloride
- 0.12 gram calcium chloride
- 0.20 gram sodium bicarbonate

Combine salts in flask and add distilled water to make 1 liter of solution.

Test Solutions:

Atropine Sulfate in Frog Ringer's Solution, 5%
Weigh out 5 grams of atropine sulfate. Add frog Ringer's solution to a final volume of 100 milliliters. **Caution! Atropine sulfate is toxic. Label TOXIC.**

Calcium Chloride in Frog Ringer's Solution, 2%
Weigh out 2 grams of calcium chloride. Add frog Ringer's solution to a final volume of 100 milliliters.

Digitalis in Frog Ringer's Solution, 2%
Weigh out 2 grams of digitoxin. Add frog Ringer's solution to a final volume of 100 milliliters.

Epinephrine in Frog Ringer's Solution, 1%
Weigh out 1 gram of epinephrine (Carolina). Dissolve in 0.5 milliliter of 1 *N* HCl. Add frog Ringer's solution to a final volume of 100 milliliters. **Caution! Epinephrine is toxic. Label TOXIC.**

Histamine in Frog Ringer's Solution, 0.01%
Weigh out 0.01 gram histamine. Add frog Ringer's solution to a final volume of 100 milliliters.

Hydrochloric Acid (HCl) in Frog Ringer's Solution, 0.01 N
Add 0.8 milliliter concentrated HCl to 900 milliliters frog Ringer's solution. Add distilled water to make 1 liter of solution.

Pilocarpine in Frog Ringer's Solution, 2.5%
Weigh out 2.5 grams of pilocarpine chloride. Add frog Ringer's solution to a final volume of 100 milliliters.

Potassium Chloride in Frog Ringer's Solution, 5%
Weigh out 5 grams of potassium chloride. Add frog Ringer's solution to a final volume of 100 milliliters.

Sodium Chloride (NaCl) in Frog Ringer's Solution, 0.7%
Weigh out 0.7 gram of sodium chloride. Add frog Ringer's solution to a final volume of 100 milliliters.

Laboratory Materials

Ordering information is based on a lab size of 24 students, working in groups of 4. A list of supply house addresses appears in Appendix A.

6 frogs
Disposable gloves
Apparatus A or B:
A: physiograph (polygraph), paper, ink, myograph transducer, transducer cables, stimulator output extension cable, electrodes
B: BIOPAC® BSL PRO Software, MP36/35 or MP45 data acquisition unit, PC or Mac computer, BIOPAC® HDW100A tension adjuster (or equivalent). BIOPAC® SS12LA force transducer with S-hook, small hook with thread, and transducer (or ring) stand
Disposable container for organic debris
Dissecting pins
18 bottles of Ringer's solution, frog
Water bath at 32°C and 5°C
12 petri dishes
6 medicine droppers
6 dissecting pans and dissecting kits
6 millimeter rulers
Thread
6 large rubber bands
Box of fine common pins
6 frog boards
Cotton balls
Paper towels
6 compound microscopes, lens paper, lens cleaning solution
6 dropper bottles of each of the following (using frog Ringer's solution as the solvent):
5% atropine sulfate
2% digitalis
1% epinephrine
2.5% pilocarpine
5% potassium chloride (KCl)
2% calcium chloride ($CaCl_2$)
0.7% sodium chloride (NaCl)
0.01% histamine
0.01 *N* HCl

Advance Preparation

1. Order frogs to be delivered close to the date of the lab (see the frog experiment in Exercise 14). Each group will need one double-pithed frog. If time (or student aversion) is a problem, frogs can be pithed just before the lab begins (see Exercise 14). Keep the frogs moist with *frog Ringer's solution*.
2. Set out data acquisition equipment (one per group of four). If the equipment has not been used in an earlier experiment, acquaint students with its set-up and use (see Exercise 14).
   a. BIOPAC®. Set out equipment and materials for the conduction of the BIOPAC® activity, including a computer with BIOPAC® BSL Pro Software installed, tension adjuster, force transducer, and transducer (or ring) stand.
   b. *Physiograph*. For each physiograph, set out paper, ink, transducer stand, myograph transducer, transducer cables, stimulator output extension cable, and electrodes.
3. Put bottles of *frog Ringer's solution* in a water bath set at 32°C and in a refrigerator set at 5°C. Have a supply bottle of room temperature *frog Ringer's solution* available.
4. Each group should be provided with disposable gloves, a dissecting pan and instruments, a 250-milliliter bottle of *frog Ringer's solution*, two petri dishes, a medicine dropper, a millimeter ruler, thread, sturdy rubber bands, several fine common pins, a frog board with a hole in one end (biology supply company), cotton balls, paper towels, and dropper bottles of *test solutions*. Have supply bottles of the *test solutions* available.
5. Have microscopes, lens paper, and lens cleaning solution available.
6. Designate an appropriate disposal area for the frogs.

Comments and Pitfalls

1. Remind students to keep the tissue moist with *frog Ringer's solution* at all times.
2. Be sure the students have correctly located the vagus nerve and have not invented a nerve from connective tissue.
3. Do not overstretch the heart when attaching it to the recording equipment.

4. A Stannius ligature is a simple overhand knot in a loop of thread that can be tightened by pulling on both ends of the thread.
5. See Exercise 14 for additional comments on troubleshooting the recording equipment.

Answers to Pre-Lab Quiz (pp. 513–514)

1. true
2. b, rhythmicity
3. b, three
4. true
5. An extra beat that shows up on the ventricular contraction peak
6. b, digitalis
7. d, vagus
8. vagal escape
9. true
10. c, Histamine

Answers to Activity Questions

Activity 1: Investigating the Automaticity and Rhythmicity of Heart Muscle (p. 514)

4. The heart is contracting rhythmically while the gastrocnemius muscle is not contracting at all.
5. The sinus venosus will continue to beat.
6. Each atrium should continue to beat, as well as the ventricle.
7. The sinus venosus usually displays the most automaticity (contracts at the fastest rate) and the ventricle the least.

Activity 2: Recording Baseline Frog Heart Activity (pp. 516–519)

Preparation of the Frog

4. Yes. The atrium contracts before the ventricle.

Activity 3: Investigating the Refractory Period of Cardiac Muscle Using the Physiograph (p. 519)

3. Extrasystole can be induced during the first part of ventricular relaxation.
4. The heart does not go into tetanus. The heart would be of no value as a pump if it could go into tetanus as a result of rapid repeated stimulation.

Activity 4: Assessing Physical and Chemical Modifiers of Heart Rate (pp. 519–521)

Temperature

5. Cold Ringer's solution slows down the heart rate. Warm Ringer's solution speeds it up.

Chemical Agents: Pilocarpine

Pilocarpine slows the heart. Pilocarpine is an *agonist* of acetylcholine (cholinergic agonist).

Chemical Agents: Atropine Sulfate

The heart rate should increase. Atropine is *antagonistic* to acetylcholine (cholinergic antagonist).

Chemical Agents: Epinephrine

Epinephrine increases heart rate, imitating the sympathetic nervous system.

Chemical Agents: Digitalis

Digitalis slows and steadies heart contraction.

Various Ions

Ca^{2+} increases strength of contraction.

Na^{+} decreases strength and rate of contraction.

K^{+} weakens heart contractions and causes premature beats.

Yes. Students may observe arrhythmia with all three ions.

Vagus Nerve Stimulation

3. Vagal stimulation slows down and eventually stops the heart.

Intrinsic Conduction System Disturbance (Heart Block)

4. A normal AV rhythm should reestablish after removing the block.

Activity 5: Investigating the Effect of Various Factors on the Microcirculation (pp. 521–522)

5. RBCs move through capillaries in single file. They are flexible and they may appear "stacked" and slightly curved as they move through. White blood cells resembling monocytes may be seen.
6. Blood flow in the arterioles is rapid and pulsating, while it is slow and steady in the venules. Movement is very slow in the capillaries. The capillaries are much smaller in diameter than the arterioles.

Temperature

Arterioles respond most noticeably to the temperature change. Cold saline causes a reduction in diameter and warm saline an increase in diameter.

Inflammation

HCl causes vasodilation, increasing capillary blood flow. This is a local response to bring more inflammatory cells to the damaged area.

Histamine

1. Histamine also causes vasodilation and increased blood flow. The response to histamine is similar to the response to HCI.
2. Epinephrine causes vasoconstriction and reduced blood flow.

Name _____

Lab Time/Date _____

EXERCISE 34

REVIEW SHEET

Frog Cardiovascular Physiology

Special Electrical Properties of Cardiac Muscle: Automaticity and Rhythmicity

1. Define the following terms.

automaticity: *Ability to depolarize spontaneously in the absence of external stimulation.*

rhythmicity: *Depolarization/repolarization events occur in a regular and continuous manner.*

2. Discuss the anatomical differences between frog and human hearts. *The frog heart has a single ventricle and two atria. Dorsally there is an expanded area called the sinus venosus. The human heart has two atria and two ventricles. No sinus venosus is present.*

3. Which region of the dissected frog heart had the highest intrinsic rate of contraction? *Sinus venosus*

The greatest automaticity? *Sinus venosus*

The greatest regularity or rhythmicity? *Sinus venosus* How do these properties correlate with the duties of a pacemaker? *The human pacemaker (SA node) has automaticity, rhythmicity, and the highest depolarization rate in the heart.*

Is this region the pacemaker of the frog heart? *Yes*

Which region had the lowest intrinsic rate of contraction? *Ventricle*

Investigating the Refractory Period of Cardiac Muscle

4. Define *extrasystole.* *An extra beat occurring before the time a normal contraction would occur*

5. Respond to the following questions if you used a physiograph. _____

What was the effect of stimulation of the heart during ventricular contraction? *No effect*

During ventricular relaxation (first portion)? *Extrasystole*

During the pause interval? *No effect*

What does this indicate about the refractory period of cardiac muscle? *Much longer than that of skeletal muscle*

Assessing Physical and Chemical Modifiers of Heart Rate

6. Describe the effect of thermal factors on the frog heart.

cold: *Decreased heart rate* heat: *increased heart rate*

7. Once again refer to your recordings. Did the administration of the following produce any changes in force of contraction (shown by peaks of increasing or decreasing height)? If so, explain the mechanism.

epinephrine: *Increases heart rate and force of contraction. Acts on the SA and AV nodes and the myocardium to increase membrane permeability to NA+ and Ca2+.*

pilocarpine: *Decreases heart rate; no effect on force of contraction*

calcium ions: *Increases force of contraction; effects in skeletal muscle, i.e., Ca2+ is the "trigger" for sliding of myofilaments*

8. Excessive amounts of each of the following ions would most likely interfere with normal heart activity. Note the type of changes caused in each case.

K^+: *Heart block; cardiac arrest*

Ca^{2+}: *Increases force of contraction; causes arrythmias*

Na^+: *Decreases force of contraction; causes arrythmias*

9. Respond to the following questions if you used a physiograph. What was the effect of vagal stimulation on heart rate?

Decreased heart rate

Which of the following factors cause the same (or very similar) heart rate–reducing effects: epinephrine, acetylcholine, atropine sulfate, pilocarpine, sympathetic nervous system activity, digitalis, potassium ions?

Acetylcholine, pilocarpine, digitalis, potassium ions

Which of the factors listed above would reverse or antagonize vagal effects? *Epinephrine, atropine sulfate, sympathetic nervous system activity*

10. What is vagal escape? *Return to a normal heart rate after a period of rate depression by the vagus nerve*

Why is vagal escape valuable in maintaining homeostasis? *Continued vagal depression can completely stop the heart and lead to death; vagal escape allows the heart to begin beating again even though the vagus nerve continues to be stimulated.*

11. How does the Stannius ligature used in the laboratory produce heart block? *It physically blocks transmission of impulses from the atria to the ventricle.*

12. Define *partial heart block,* and describe how it was recognized in the laboratory. *Partial heart block occurs when the electrical signal is partially prevented from reaching the ventricles. The 1:1 contraction ratio of atrial to ventricular contractions is replaced by different whole number ratios, e.g., 2:1, 3:1.*

13. Define *total heart block,* and describe how it was recognized in the laboratory. *Total heart block occurs when the electrical signal from the SA node is prevented from reaching the ventricles. Atria and ventricles beat independently of each other, and you can no longer count a whole number ratio of atrial to ventricular contractions.*

14. What do your heart block experiment results indicate about the spread of impulses from the atria to the ventricles?

In normal heart activity, the ventricles are depolarized by the depolarization wave spreading from the atria.

Observing the Microcirculation Under Various Conditions

15. In what way are the red blood cells of the frog different from those of the human? *Frog RBCs are nucleated; human RBCs are anucleate.*

On the basis of this one factor, would you expect their life spans to be longer or shorter? *Longer*

16. The following statements refer to your observation of one or more of the vessel types observed in the microcirculation in the frog's web. Characterize each statement by choosing the best response from the key.

Key: a. arteriole b. venule c. capillary

c 1. smallest vessels observed

a 2. vessel in which blood flow is rapid, pulsating

c 3. vessel in which blood flow is least rapid

c 4. red blood cells pass through these vessels in single file

b 5. blood flow smooth and steady

c 6. most numerous vessels

a 7. vessels that deliver blood to the capillary bed

c 8. vessels that serve the needs of the tissues via exchanges

b 9. vessels that drain the capillary beds

17. Which of the vessel diameters changed most? *Arterioles*

What division of the nervous system controls the vessels? *Autonomic nervous system, sympathetic division*

18. Discuss the effects of the following on blood vessel diameter (state specifically the blood vessels involved) and rate of blood flow. Then explain the importance of the reaction observed to the general well-being of the body.

local application of cold: *Vasoconstriction of arterioles; bypasses the skin capillaries and withdraws blood to deeper body tissues to prevent heat loss to the external environment*

local application of heat: *Vasodilation of arterioles and flushing of capillary bed with blood; increases the local blood supply and allows heat radiation from the skin surface*

inflammation (or application of HCl): *Vasodilation locally bringing in WBCs and more nutrients to help fight the inflammatory stimulus*

histamine: *Same reaction as with inflammation*