



# The Osmoregulatory Function of the Kidney

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**Grade Level:**

High School

## **The Osmoregulatory Function of the Kidney Amphiuma's kidney tubules/Volume regulation**

### **Introduction:**

This is a wonderful inquiry lab for both Biology and Physiology students. It requires advance preparation. It can be used in many different contexts; general osmoregulation, volume regulatory function of cells, cell membrane transport, introduction to kidney tubules' function, filtration, secretion, and reabsorption. Possibilities are many. Use your knowledge and references to come up with other ideas.

### **Purpose:**

To study the osmoregulatory function of kidney tubules of a species of *Amphiuma*

### **Objectives:**

Students will be able to:

- understand the concept of osmosis, diffusion, osmoregulation done by cells, and the notion of concentration gradient of different ions.
- observe the change in the appearance of the kidney under the dissecting microscope over a short period of time.
- observe the colored (dye) indicator moved from outside to inside the lumen of the proximal tubules of the kidney (secretion).
- observe the outline of the cells around the lumen.
- observe the volume regulation of the cells surrounding the lumen of the tubules.
- design a model of a kidney according to the need of a hypothetical organism to osmoregulate.
- make the actual model of the kidney that shows filtration, secretion, and reabsorption (Advance for extra credit or can be required for students who have desire for A+).

### **Materials:**

- **Amphiuma** (*Amphiuma Tridactylum*) can be ordered from:  
**Atchafalaya Biological (Danny Kramer)**  
Phone: 1-504-446-5929  
OR  
**Carolina Biological** Phone: 1-800-334-5551 Ext.: 5310  
For those of you who have access to Flounder of any type this is an ultimate choice of fish for its kidney tissue can be obtained easier and the result of the experiment would be clear.
- **Aeration apparatus:**  
For bubbling the kidney tissue obtained from the Amphiuma (**Figure 1**)  
plastic graduated cylinder

stopper with two holes

two pieces of 1/16 3/16 5/16 plastic tubes TYGON® brand

bubbling stone

syringe tip

petri dish

- **Dissection tools**

picture or drawing of the internal organ of the Amphiuma (**Figure 2 & 3**)

dissecting scope

scalpel

forceps (watchmen tweezers) two pairs

scissors

dropper (both glass and plastic)

- **Chemicals** (Listed in the order of use.):

Sigma Chemical Co. (1-800-325-3010)

Anesthetic

3- Amino Benzoic Acid Ethyl Ester (Comes in powder form, two tablespoons for a large bucket of water can be used for both fish and Amphiuma; can be ordered from SIGMA 98 CATALOG; CALL #A-5040 5g @ \$10.85; 25g/@ \$43.0

Ringer solution

Isotonic, Hypotonic, and Hypertonic Table 1 & 2 show the recipes for Amphiuma and fish appropriate Ringer

Collagenase

For dissolving the tissues surrounding the kidney; this is a brown powder kept in 0° C; Can be ordered from SIGMA; CALL # C-5138: 1g @ \$154.50; 100mg @ \$24.55 (Liquid form); can be dissolved in the Ringer solution 1mg/1mL

Dye or Indicator

Phenol red (Phenol Sulfo Naphthalein) Recent studies show the Chloro form of this dye works better. This information is for the phenol red. This is a dark rusty red solution; can be ordered from SIGMA 98 CATALOG; CALL # P-0290; 0.5/ IN D- PBS @ \$5.88.

### **Making your solutions:**

- You need to have a stock of the Ringer for students' use. Usually the chemistry department can help you find and make your solutions. Asking students to help with this process is strongly suggested.
- 1 L Flask
  - seven Glass Beakers
  - stock of 1 Mole NaCl, KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, Hepes, NaOH, and Glucose

- formulas to find the concentration of each you need (See Table 3)
- pH meter
- osmolality meter
- pipette with different tips for precise measurements

You can freeze your ringer solution, and thaw them out the day of the lab in hot water. Make sure you pH your solution every time you freeze/thaw.

- Glass ware:
  - Beakers 10ml, 20ml, 25ml, 50ml, 100ml, 150ml,
  - 3X20ml for Ringers
- Other equipment:
  - Pipette, manual or digital
    - Tips for pipetting: you can request a copy of instruction for pipetting from the same company you purchase your pipettes from cuvettes or plastic vials
  - Filter paper or Kim-wipe (brand name for soft wipe paper found in professional laboratories)
  - Air Supply (If not available, small aquarium pump would be sufficient)

### **Procedure:**

Teacher preparation: It is recommended for you to do the whole lab once before the lab date. This will help you trouble-shoot for your own settings.

Study: Find online information. Check sites such as *PubMed* for current information about this subject. An example is attached. You can use this or more recent one.

**References:**

Prepare references for students' use, such as library books, books or articles about Amphiuma, Salt-water fish and how they live under their environmental conditions. The more references you make available to the students, you will have an easier time when it comes to the actual lab.

**Ordering:****Months in advance**

- Make sure you order everything you need way in advance. The Amphiuma are available in certain months of the year. Check with your source to find out what is the best time to order. Plan your lab according to the date Amphiumas are more available. (Check the Material section)

**Days in advance**

- Make all the solution you need and label. Keep in the freezer. You can thaw them out the day of the lab, usually a hot water bath.
- Assemble the Apparatus in **Figure 1 and Figure 2.**

**Handling the Amphiuma:**

- **Habitat:** Use a large tank with lockable cover. Cover the bottom with large smooth rocks. Temperature should be slightly cooler than the room temperature ~ 20-22°C. A fish tank with aeration mechanism works well if you have one available on site.
- **How to feed:** Gold fish or pieces of fish are good food.
- **Anesthetizing:** About one hour before the lab, add two tablespoons of the powdered anesthetic to a bucket of cold water. Label and cover.
  - You can keep this bucket with active anesthetic for a long time. If securely covered it can be kept for about a month. Make sure you keep it covered, labeled and in a locked place at all times. **This is particularly important as a safety measure. Make sure the students don't have access to this bucket.**
  - When you are ready to anesthetize the Amphiuma (Eel), capture one and leave in the bucket for at least ½ an hour, keeping the cover tight. Eels can bite, so use safety or gardening gloves. The Amphiuma shows an unfamiliar behavior when in the bucket; it becomes hyperactive before it becomes anesthetized.

**Dissection:**

- The apparatus should be ready.
- Dissection should follow immediately after anesthetic.
- Open up the gorp (Amphiuma) mid-sagittally through out the body. (**Figure 1 and Figure 2**)
- Obtain both kidneys. Leave in petri dish with the isotonic (IR) Ringer solution. Start bubbling this is to keep your tissue alive. Divide both kidneys into enough

pieces, so that each group of students will have one. Give each group a piece of kidney tissue in a petri dish with IR Ringer.

**Photocopy:**

Make enough copies of the student procedure sheet, Student work-sheet #1, Student work-sheet #2, Figures and Tables and the *PubMed* Query sheet

Students: Use your judgment about the students. You may divide them in to groups as you see fit. If you teach small groups like are in AP Biology, you can have them work in pairs. Depending on the students you have, you may want to have everything ready at their stations or to have them obtain their materials and needed solutions. Depending on the number of dissecting scopes you have available you may want your students to work in pairs or groups of four.

**Procedure for students**

Each student obtains a piece of kidney in a petridish with IR ringer enough, to slightly cover the tissue

- Obtain Collagenase.
- Cover the specimen with “kim-wipe”<sup>®</sup> or thin filter paper.
- Add three to five ml of the Collagenase; add as needed until the surrounding tissues are easy to tease away (at least 15 minutes) under the dissecting microscope.
- Under the dissection Microscope, use tweezers to tease away the surrounding tissues. Be very, very gentle!! The objective is to tease away other tissues and keep the tubules.
- Remove the specimen; leave in a fresh IR ringer enough to cover.
- Observe under microscope the tubules appear as bunch of spaghetti noodles in a plastic bag try to tease away the plastic bag, isolating the tubules for further study of the tubules.
- As soon as you see the tubules, using the glass dropper gently add one drop of the phenol red dye close to one of the tubules.
- Observe closely; the dye will move in to the lumen of the tubules (secretion).
- Put your specimen in to a fresh Hypotonic (RVD) ringer, wait about 10 minutes, and observe again. The cells of the lumen are swelling, pushing the dye out of the lumen; therefore, the red string inside the lumen appears thinner.
- Put your specimen in to a fresh Hypertonic (RVI) ringer, wait about 10 minutes, and observe again. The cells of the lumen are shrinking the lumen is getting wider the dye inside will appear lighter and wider

## Pre-Operation Day 1

### Student Worksheet

Name: \_\_\_\_\_

Period: \_\_\_\_\_

Date: \_\_\_\_\_

Answer these questions using any of the reference books, computer data collections and or any other reference material your teacher requires:

1. What is a liquid?
2. What is a solution?
3. What is a concentration?
4. What is a gradient?
5. What is osmosis? What are isotonic, hypotonic, and hypertonic solutions? Draw a picture to illustrate each.
6. What is diffusion? Draw a picture to illustrate.
7. When substances move across the cell membrane, what components do they have to pass through?
8. What materials can pass most easily across the cell membrane?

9. What does ATP stand for? What is the function of this molecule?

10. How do cells use this molecule?

11. Is there any question you want to ask about the movement of material across the cell membrane? On a separate sheet of paper, list all your questions for homework. Due tomorrow.

## Lab Activity: Volume Regulation of Cells

### Students' Procedure sheet

Student Names:

Student#1 \_\_\_\_\_

Student#2 \_\_\_\_\_

Student#3 \_\_\_\_\_

Student#4 \_\_\_\_\_

Group# \_\_\_\_\_

Date: \_\_\_\_\_

#### Materials:

beakers 10ml , 20 ml, 25ml, 50 ml, 100ml, 150ml,  
five Petri dishes  
tweezers  
ringer solutions (3 different): IR, RVD, and RVI  
collagenase solution  
Phenol Red dye solution  
goggles

#### Procedure:

##### **Pay close attention to the Lab Safety rules!**

Before you start you need to have paper and pencil ready to make sketches of all the observations. You can go back later and complete your drawing, coloring, and labeling them.

1. Groups of 3-4 people are suggested. Your teacher will tell you how you should be grouped
2. Obtain all your solutions and goggles
  - a. Ringer solutions in the 25 ml beaker
  - b. Collagenase in the plastic vial
  - c. Dye in the plastic vial
3. Obtain the sample specimen from the teacher in a petridish filled with IR Ringer.
4. Look at the sample under the dissecting microscope
5. Cover the sample with filter paper/kim-wipe®
6. Add three to five drops of the collagenase solution to the filter paper/kim-wipe®.
7. Leave for 15-20 minutes.
8. Observe under the dissecting-scope try to gently tease the tissues with the watchmen tweezers.
9. If hard to tease, add more collagenase and leave until the tissues are dissolved.
10. Find structure that resembles the spaghetti noodles wrapped in plastic bag  
Congratulations!! These are the tubules!!
11. Very gently tease the “plastic bag” around the tubules, isolating the tubules.
12. Remove the tissue; leave it in fresh Ringer solution.

13. Under the scope, using the glass dropper add a drop of the dye solution close to one of the tubules.
14. Observe closely the dye will be moving into the tubules (secretion).
15. When movement is completed, remove the specimen. Leave it in the hypotonic Ringer solution.
16. Observe closely and carefully. The cells around the lumen of the tubules will be swelled, squeezing the dye out of the lumen, leaving a narrow trace of the dye in the lumen.
17. When movement is completed, remove the specimen and leave in the hypertonic solution. Observe closely and carefully. The cells around the lumen will shrink to their original size or even less, causing the lumen to become wider. Therefore, the trace of dye will become lighter.
18. **Carefully clean up your station. Listen carefully to the directions given by the teacher.**

## Post Operation Day 2

### Student worksheet #3

#### Student Names

Student#1 \_\_\_\_\_

Student#2 \_\_\_\_\_

Student#3 \_\_\_\_\_

Student#4 \_\_\_\_\_

**When done with this assignment, collect all members' work, staple this sheet on top and hand in.**

#### Part I: Drawing

Each student is required to draw on a separate sheet of drawing paper.

All drawings should be titled, labeled, and colored.

Drawing #1: The apparatus used by the teacher and its function.

Drawing #2: The Amphiumas internal organs.

Drawing #3: The kidney and write the description of the kidney

Drawing #4: The kidney tubules under the dissecting microscope showing the lumen and the cells surrounding it

Drawing #5: The appearance of the cells around the lumen in the Hypotonic solution, showing the location and the amount of dye inside (thick or thin).

Drawing#6: The appearance of the cells around the lumen in the Hypertonic solution.

#### Part II: Explanation

On a separate sheet of writing paper, explain the following inquiries, basing your answer on the recent knowledge you have gained about the movement across the cell membrane.

Use scientific terminology.

Inquiry#1: Explain what happened through out your lab activity.

Inquiry#2: Explain the events in the right sequence.

Inquiry#3: Find new research done on this same topic. On the Internet, you can check *PubMed*. Look for recent abstracts searching for the same topic. You will be able to find wonderful information in *American Journal of Physiology-Cell*. Don't forget to cite your research. Use the correct format for the bibliography.

Inquiry#4: Using your imagination and previous knowledge, what other issues may be related to this topic? This is a very broad inquiry.

**Lab Activity**  
**Student's Work-Sheet #4**  
**Cooperative worksheet**  
**All student Names:**

**Names:** \_\_\_\_\_  
\_\_\_\_\_

**Question #1: Why did the dye move into the tubules?**

**Question #2: Why did the cells swell up when you added the hypotonic solution?**

**Question#3: Why did the cells shrink, when you added the hypertonic solution?**

**Question#4: Why did the dye leave the lumen of the tubules?**

**Question#5: What is homeostasis? What are some structures organisms use to maintain their homeostasis?**

**References:**

1. Dr. Peter M. Cala. Ph.D., Department of Physiology, Professor at University of California Davis
2. Mr. Rob Rigor, Laboratory Manager, Cala lab, University of California - Davis
3. Dr. Zhenpheng Zhuang, Ph.D., Post Doc at University of California - Davis
4. Tanner; GA Ph.D., Excretion of Phenol Red by Necturus kidney tubules  
*American Journal of Physiology* 1970, May; 236(5):F442-7

### Table 3.

Formula for the ringer solution:

Each of the ion solutions should have an Alpha# associated to the stock.

The best is to use a computer spreadsheet application program. Make a template for calculating the concentration. When you create your template, all you need to do is to punch in some numbers, the computer will do the rest for you.

**Alpha =**

**Example:** 1M NaCl \_\_\_\_ 1000mM X 2 osmolytes = 2000 mOsm

Osmometer: Linear range ~ 250 mOsm

Dilute 1:8 in H<sub>2</sub>O

Theory should be 125 mM NaCl

Should be 250 mOsm

Measure it -Osmometer experimental; Value (Milosm Expt)

Alpha =

After you find the concentration of each of the ions using this formula, add components bring to 95% total final volume with dd H<sub>2</sub>O.

Check the pH to 7.6 +/- .02

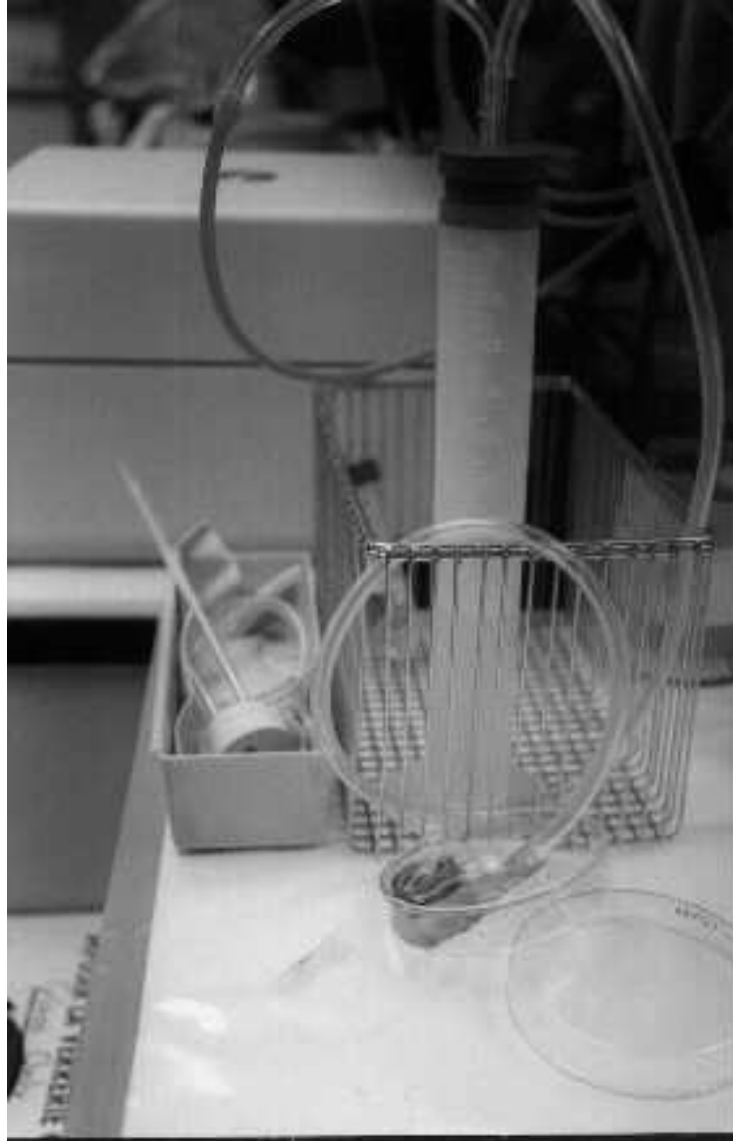
Bring to final volume

Check osmolality should be 297 to 303

**Ion Solutions: 1 Mole of each of NaCl, KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, Hepes, NaOH, and Glucose**

**RVD Solution** (Regulatory Volume Decrease) 0.55 127 mOsm **Hypotonic**

**RVI Solution** (Regulatory Volume Increase) 1.60 367 mOsm **Hypertonic**



**Figure #1. Aeration Apparatus**

## **Figure #2 Internal organs of the Amphiuma**

KIDNEYS are the dark structures located close to the tail



