The Case of Water Poisoning?
Regulation of Body Fluid Volumes and Electrolytes in Vertebrates

By
Melody Danley, Bonnie Leksrisawat, Ann S. Cooper, Jeff Osborn, and Robin Cooper

PURPOSE AND OBJECTIVES

The purpose of this experiment is to observe the response of a vertebrate renal system to water and salt loading.

Learning Objectives:

1. Students will understand how body fluid volumes are distributed among the intracellular, extracellular, and vascular spaces.
2. Students will understand the mechanisms responsible for the regulation of water reabsorption and excretion in the control of total body water.
3. Students will understand how to measure and calculate renal (kidney) excretion responses to ingestion of fluid and electrolyte loads.

NOTE: Participation in any laboratory where students are used as the test subject is strictly voluntary. No student is required to serve as a test subject for the exercises herein. Students opting not to serve as a test subject will not be penalized. While each volunteer's test results will be recorded onto a group spreadsheet for use by the class, effort is made to maintain privacy: Data collected onto the spreadsheet is never identified by the individual(s) from which it came.

INTRODUCTION

According to the news reports, Ms. Jennifer Strange was 28 years old when she died (AP 2007). She left behind three young children. When she died, she had just returned home after participating in a local radio station's contest. Contestants of the contest had be given the chance to win a Nintendo Wii video game system by drinking as much water as possible during a 3 hour period, without urinating or vomiting. According to the lawsuit filed by Roger Dreyer, the family attorney acting on behalf of the decedent, the jockeys at the contest had mocked about how Jennifer's stomach was so swollen she had looked like she was pregnant (Strange vs. ENTERCOM 2007). Jennifer Strange had complained of having a terrible headache towards the end of the contest. Other contestants around her were vomiting and several had

collapsed onto the ground, clearly ill. The organizers of the contest laughed at the contestants. How much damage could be done by drinking a bunch of water?

For cells to survive, they must maintain the proper osmolarity and balance of ions such as potassium, sodium, and chloride. The freshwater ancestry of vertebrates is striking relation to the relative concentrations of ions in intra- and extracellular environments. The fluid within the vertebrate body contains about one-third the ion concentration as that of standard seawater (Randall 2002). If the osmolarity, or concentration of solutes dissolved in the watery fluids of the body, is not regulated within tolerable ranges, then weakened metabolism, brain damage, muscle rigor, and even death can occur. The osmotic state of the body is heavily influenced by an organism's rate of salt and water intake balanced with its rate of excretion.

The excretory system, also referred to as the renal system in some animal systems, plays a major role in osmoregulation of the internal body fluids. In humans, blood is filtered by the nephra of the kidneys, where excess salts and water are rapidly removed. The volume of fluid and salts removed by the kidneys is tightly controlled by hormonal signaling, the most famous of which is probably the renin-angiotension II negative feedback pathway. Organisms that actively regulate their internal salt and fluid content are referred to as osmoregulators. Humans are a model species of such an animal.

Osmoregulators are generally able to maintain internal salt and fluid levels within a tolerable range, despite changes in their external environment. But, as with most physiological systems, the kidneys' ability to regulate the salt and fluid volume is likely rate limiting. The purpose of today's laboratory exercise is to investigate the osmoregulatory abilities of the human renal system, and to postulate whether or not it could be possible to overwhelm the system with water and salts in such short period of time (a few hours). For the safety of all students, consumption of any materials is strictly voluntary. The volume of fluids and salts will be limited to that which is known to be well within a tolerable range for human.

METHODS

Pre-lab Preparation

Students potentially serving as test subjects should avoid very salty foods (which can cause water retention) and beverages containing caffeine (which can increase urine production rates) for at least 2 hours prior to the start of lab. These types of items can skew the results of some of the tests included in this lab.

1. For the 2 hours proceeding laboratory, note the type and quantity of any food or beverages consumed.

a.

b.

c.

d.

2. Record the most recent time of bladder emptying BEFORE you came to lab. Avoid going to the bathroom within 15 min of the start of lab.

time: _____

Lab Procedures:

A basic outline of the procedure for this lab activity is as follows:

1. Select volunteers – preferably about 1 volunteer per student group
2. Select tests - test 1, 2 and 6 are mandatory.
3. Volunteers collect a urine sample as a control (before water/salt treatments are given).
4. Volunteers consume appropriate treatment materials (water, salt, and/or pretzels) within 10 minutes.
5. Volunteers collect urine samples every 30 minutes for 2 hours, after the control sample was collected. A total of five urine samples will be collected.
6. Analyze the urine for the appropriate parameters immediately after each sample is collected.

As a group (section), determine the tests that would be most appropriate for your study. You must complete tests 1, 2, and 6. You must also select at least one other test from the list, but can elect to perform as many tests as your group determines necessary/useful. In general, the more tests you perform, the more information you will have to work with, and the more thorough your conclusions can be.

Next, a minimum of three volunteers per treatment group should be selected. There are three treatment groups. This will provide three replicates per treatment group for your section. The treatments are as follows:

1. Control – no ingestion of materials
2. Water only group – ingestion of water only within 10 minutes
3. Salt and water group – ingestion of water and salt/salty pretzels within 10 minutes[1]

For treatments 2 and 3, you should consume approximately 12 mL water/kg body weight, up to a 1200 ml maximum.

For treatment group 3, you should consume a total of 2 grams worth of table salt with the cup of pretzels. For most people, it is easiest to eat the salt in its dry form, and then drink the water to wash it down. Do not mix the salt into the water! Trying to consume saltwater will make most people gag.

******* IF YOU HAVE HIGH BLOOD PRESSURE DO NOT CHOOSE THE HIGH SALT GROUP**

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When conducting renal analysis/water balance tests, some tests require only a single sample for testing whereas other tests require samples to be collected every 30 minutes for a minimum of 2 hours. Multiple samples collected over time allow for rates of production and clearance to be determined.

[1] ***If you have a salt restricted diet, have been diagnosed with hypertension, or have any known heart problems, do not volunteer for this group***

For the purpose of this activity, collection of urine samples should be done every 30 minutes for a minimum of 2 hours. **The first urine sample should be collected before the treatments are given to the test subjects.**

Test 1. Volume production rates. This test measures the volume of urine produced through time.

Procedure:

1. Void urine in specimen cup and return sample to lab for testing.
2. Use the graduated cylinders at your workstation to measure the volume of the sample. Record the sample collection time and volume on kidney function results page.
3. Pour the sample back into the specimen collection container if you still have other tests to perform. Otherwise, it can be taken back to the bathroom and flushed down the toilet.
4. Thoroughly rinse the graduated cylinder with distilled water and set aside to dry.
5. To calculate production rates, samples should be collected every 30 minutes for a minimum of 2 hours.

Test 2. Sodium concentration. This test is used to detect the concentration of sodium in the urine. Urine sodium concentrations vary depending on diet, fluid intake levels, and overall osmostic state of the body. When measured over time, this test can be used to observe the ability of the kidneys to respond to different osmotic loads in the body.

Procedure.

1. Prepare two samples for sodium analysis per sample of urine collected.
2. Samples must be diluted before they can be measured by the flame photometer. For each sample, pipette 0.1 mL of urine into a clean microcentrifuge tube.
3. Next, add 1.9 mL of lithium diluent to the tube from the stock bottle. Cap and mix thoroughly. This procedure dilutes the sample by 20X.
4. To measure each sample, uncap the tube, and place the aspiration tubing from the flame photometer into the microcentrifuge tube.
5. Aspirate the sample for at least 5 seconds.
6. Record the sodium concentration from the digital display once the reading becomes stable. Replace the photometer's aspiration tubing back into the beaker of lithium diluent. Repeat procedure for the second sample.
7. The used pipettes and microcentrifuge tubes can be thrown into the trash can.

8. Calculate the actual sodium concentration, from the recordings of the 20X diluted samples. Then calculate the average for the two samples.
9. To observe changes through time, samples should be collected/examined every 30 minutes for a minimum of 2 hours.

Test 3. Chloride concentration. This test is used to detect the concentration of chloride in the urine. Urine chloride concentrations vary depending on diet, fluid intake levels, and overall osmostic state of the body. When measured over time, this test can be used to observe the ability of the kidneys to respond to different osmotic loads in the body.

Procedure.

1. Use a new dropper for each solution described below – do not cross contaminate solutions. Be sure to hold the dropper perfectly upright when dispensing any solutions. Holding a pipette or dropper at an angle can influence the effects of gravity on the volume, and change the volume dispensed.
2. Prepare two samples for chloride analysis, per sample of urine collected.
3. Measure 0.5 mL of fresh urine into a new 13x100 mL test tube.
4. Next, add one drop of the 20% potassium chromate solution[2]. Swirl the solution to mix.
5. Determine the chloride concentration by adding the 2.9% silver nitrate solution, one drop at a time. Swirl thoroughly between drops.
6. Continue to add drops until a permanent color change is observed: usually the solution turns from clear yellow to cloudy grayish-brown.
7. Record the number of drops it took to cause the color change.
8. Calculate the chloride concentration: Each drop of silver nitrite added is equivalent to 61 mg Cl^- per 100 mL of urine.
9. Record the concentration of chloride for each sample. Then calculate the average for the two samples.
10. Pour the solution into the waste bottle labelled “chromate waste.” Chromate is a heavy metal and should not be poured down the drain.
11. Finally, rinse the test tubes thoroughly and set aside to dry.
12. To observe changes through time, samples should be collected/examined every 30 minutes for a minimum of 2 hours.

Test 4. Osmotic concentration.

[2] Potassium chromate is a known human carcinogen. Use of gloves is required when performing this test.

This test is used to detect the concentration of dissolved solutes in the urine. Urine solute concentrations vary depending on diet, fluid intake levels, and overall osmostic state of the body. When measured over time, this test can be used to observe the ability of the kidneys to respond to different osmotic loads in the body.

1. Prepare two samples for osmotic analysis, per sample of urine collected.
2. To prepare each osmotic sample, pipette 50 mL of fresh urine into a microcentrifuge tube.
3. Place the sample into the refrigerator well of the osmometer. Ensure the sample slips completely down into the well. If it does not, there may be ice built up at the bottom of the well which must be removed before the sample can be analyzed.
4. Once the sample tube is seated into the well, gently lower the operating head so that the seed wire and probe enter the tube. The operating head should click down into place.
5. The osmometer will freeze, then melt the sample before displaying the final reading, after approximately 70 seconds.
6. Record the value as mOsm/L in the table below. Repeat the procedure for the second sample. Then calculate the average for the two samples.
7. To observe changes through time, samples should be collected/examined every 30 minutes for a minimum of 2 hours.

Test 5. pH

This test is used to determine the relative concentration of hydrogen ions in the urine. Urine hydrogen ion concentrations vary depending on diet, fluid intake levels, and overall osmotic state of the body. When measured over time, this test can be used to observe the ability of the kidneys to respond to different osmotic loads in the body.

Procedure[3].

1. Determine the pH of a fresh urine sample by dipping a new litmus test strip into the sample.
2. Allow the color on the test strip to develop for 2-3 seconds.
3. Compare the colors on the test strip with those from the color chart on the pH test strip container.
4. Record the urine pH in the chart below.

[3] Note, if you have already measured the pH using Test 1 for a given urine sample, it is not necessary to measure the pH again using test 5.

5. The used test strip should be thrown into the trash can.
6. To observe changes through time, samples should be collected/examined every 30 minutes for a minimum of 2 hours.

Table 1. Results of urine content collected over a 2-hour time interval.

Treatment _____

	Sample	Control, U_0	U_1	U_2	U_3	U_4
	Time (min) **(note the actual times)**	Pre-treatment	30 min	60 min	90 min	120 min
	Volume (mL)					
	Solute content (mOsm/L)					
U_{Cl}	Cl^- content (mg/mL)					
U_{Na}	Na+ content (mg/mL)					
V	Urine Flow Rate (ml/min)					

Calculations

1. Urine Flow Rate (V): Divide the total urine volume (mL) collected by the total time elapsed during the collection period. Note, this is a dynamic value – it changes all the time.
2. Excretion rate of chloride (or sodium): Multiply the urine chloride concentration by the urine flow rate. Note, this is a dynamic value – it changes all the time.

ONE-TIME TESTS

Test 6. 9-point clinical evaluation. This test is used to detect potential underlying medical conditions such as diabetes, liver disease, urinary tract infections, etc. This test only needs to be performed once per volunteer.

Procedure:

1. Dip the test strip into the freshly collected urine sample. Wait one minute.
2. Compare the colors of each test square on the strip with the corresponding color chart on the Chemistrip bottle.
3. Record the results on the Chemstip results page.
4. The used test strip can then be thrown away in the trash can.

Test 7. Microscopic examination of urine sediment.

Microscopic examination can be used to identify the presence of red and white blood cells, bacteria, protein casts, and mineral precipitates (crystals). This test only needs to be performed once per volunteer.

Procedure:

1. Add 2 mL of fresh urine to a microcentrifuge tube. Cap the sample.
2. Centrifuge at a moderate speed for 5 minutes.
3. Discard most of the supernatant taking care not to disturb the sediment at the bottom.
4. Place one drop of the Sedi Stain onto the sediment at the bottom of the tube. Mix by capping the tube and gently flicking it with your finger.
5. Transfer the stained sediment solution onto a clean microscope slide. Place a cover slip over the sample.
6. Exam under 400X magnification for the presence of crystals (10X ocular lens x 40X objective lens) under dimmed, higher contrast light.
7. Exam the sample for the presence of casts, bacteria or human cells using regular, high power light.
8. Record your results onto the table below. Results should be indicated as positive or negative. If positive, indicate the relative number of units observed per field of view. Collect pictures for any positive results.

Table 2. CHEMSTRIP results. This test only needs to be performed one time per volunteer. Note that the order of parameters on the list may be different than the order of parameters as listed on the test strip.

Item Observed	**Typical Results**	**Actual Results**
WBCs/leukocytes	Negative	
Nitrites	Negative	
pH	4.5 - 8.0	
Protein/Albumin	Negative	
Glucose	Negative	
Ketone bodies	Negative	
Urobilinogen	Negative	
Bilirubin	Negative	
Blood/hemoglobin	Negative	

Table 3. Results of the Microscopic examination of urine using Sedi Stain™. This test only needs to be performed one time per volunteer.

Item observed	**Typical results**	**Actual results**
Human red blood cells	Negative	
Human white blood cells	Negative, very few	
Human epithelial cells	Positive	
Bacterial cells	Negative	
Casts	Negative, very few	
Crystals	Negative, very few	

If cells or crystals are observed, identify the type of cell/crystal present, the quantity present, and the magnification under which the cell/crystals were observed. Be sure to obtain pictures using your phone or the instructor's camera.

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