Urine Composition and Fluid Balance

Objective:

To explain in words the composition of urine, and the control of fluid and electrolyte balance following water and/or salt consumption, at the level of 85% proficiency for each student.

In order to achieve this objective, you will need to be able to:

1. Measure the composition of urine, including specific gravity, osmolarity, sodium chloride, protein, glucose, ketones, hemoglobin, bilirubin, and bile salts.
2. Measure urine volume and composition in response to various water and salt intake.

Materials:

Group Supplies:

5 - paper cups for urine collection
1 test tube rack
2 - 4 test tubes
20% potassium chromate solution (in dropper bottle)
2.9% silver nitrate (in dropper bottle)
Labstix Test Strip (leave in stock bottle until you are ready to use it)
2 – 500 mL bottled water (for each of 4 groups)
1 - package of pretzels weighed for 1 g Na (use pretzels with a high sodium content) or salt packet with 1 g Na (for each of 4 groups

Lab Supplies

Biohazard disposal bag

Methods:

Students should work in groups of four. A student in each group should be the subject. Initially, each student voids, emptying the bladder as completely as possible. The urine is saved, labeled, and used for baseline information, including the measurement of urine composition (A and B below). The same student in each group will participate in one of three conditions of water and salt ingestion and thereafter collect urine samples at 30 minute intervals (C below).

A. Baseline Urine Sample

For the baseline urine sample determine urine volume, urine formation rate, appearance, sodium concentration, and osmolarity. Record the baseline values in Table 1a and in Table 2: In addition, complete a urinalysis using Labstix® or other urine test strip (see next page).

1. **Urine Volume:** It is necessary to determine the total volume voided. This is conveniently done using a calibrated specimen container. Keep 50 mL of urine for Labstix® measurement.

2. **Urine Formation Rate:** Now divide your urine volume by the number of minutes since you last voided. This is your urine formation rate (UFR) in milliliters per minute. Normal urine formation rate is about 1 mL/min.

3. **Appearance:** Normal urine is light straw-amber color due to the pigment urochrome, which is the end product of hemoglobin breakdown.

4. **Sodium Chloride Concentration:** The normal amount of sodium chloride in urine for a 24-hour period is 15 grams. Using the following procedure, determine the sodium chloride concentration of your specimen:

   a. Measure 10 drops of urine into a test tube using a standard medicine dropper. Add 1 drop of 20% potassium chromate solution to the urine.

   b. Add 2.9% silver Nitrate solution 1 drop at a time using the dropper in the bottle. Vigorously swirl the test tube after each drop of silver nitrate added.

   c. Count the drops of silver nitrate solution required to turn the solution from a bright yellow to an orange brown, color.

   d. Each drop of 2.9% silver nitrate required to produce the color change represents approximately 1 g/L of NaCI.

5. **Osmolarity and Osmolality.** These are measurements of the number of osmoles (Osm) in a solution and usually refer to the concentration of ions (and/or other dissolved particles). For example, one mole of NaCl dissolved in water produces two osmoles of ions (one osmole of Na^+ ions and one osmole of Cl^- ions). **Osmolarity** is osmoles per liter of solution (Osm/L). **Osmolality** is osmoles per kilogram of solvent (Osm/kg).

In human plasma, osmolarity is about 0.290 Osm/L (often expressed as 290 mOsm/L.) In urine the osmolality can range from about 100 to about 1200 mOsm/kg.

a. The molar mass (weight of mole in grams) of NaCl is 58.44 g/mol. Use the concentration of NaCl, determined in 4d above, to calculate osmolarity.

b. For example, if the concentration of NaCl is 8.5 g/L the osmolarity will be

Expressed as Osm/L:

$$\frac{8.5 \text{ g/L of NaCI}}{58.44 \text{ g/mol}} = 0.145 \text{ mol/L x 2 ions/molecule} = 0.290 \text{ Osm/L}$$

Expressed as mOsm/L:

$$\frac{0.290 \text{ Osm/L x 1000 mOsm}}{1 \text{ Osm}} = 290 \text{ mOsm/L}$$

6. **Urinalysis using Labstix or other Test Strips:** Follow the instructions on the bottle containing the Test Strips. Dip the test strip into the urine sample and match the color of the strip with the chart provided on the bottle. Be sure to observe the proper amount of time required for the color to develop. Record data in Table 1b for each of the following tests:

a. **Glucose:** Glucose is absent in normal urine. The excretion of readily detectable amounts of glucose is known as glucosuria. Glucosuria may be benign or pathological. Renal diabetes is a benign condition where the kidney threshold for glucose is reduced but blood glucose is normal. Diabetes mellitus is a pathological condition where blood glucose shows a marked elevation and urine volume is greatly increased with a glucose content from 3 to 10% or more.

b. **Bilirubin:** Normal urine is free of bilirubin. Bilirubin is formed from decomposition of hemoglobin by the reticuloendothelial system and then bound to protein. It is removed from the plasma by the liver and bound with glucuronic acid. In this form it is secreted in the bile. Plasma and hence urinary levels of bilirubin increase when there is a biliary obstruction, hepatitis, or liver cirrhosis.

c. **Ketones:** Ketones include acetoacetic acid, B-hydroxybutyric acid, and acetone. Such substances are present in urine of individuals who are using body fat as their primary energy source as would occur during starvation, prolonged fasting, or untreated Diabetes Mellitus. Normal urine may contain variable amounts of total ketones (mostly acetone) to the extent of about 20-mg per day. Pathological values for ketones range from 0.02 to 6 grams per day.

d. **Specific Gravity:** Specific gravity (SG) less than 1.008 is dilute and greater than 1.020 is concentrated. Decreased SG is seen in excessive fluid intake, renal failure, pyelonephritis, and central and nephrogenic diabetes insipidus. Increased SG is seen in conditions causing dehydration, glycosuria, renal artery stenosis, heart failure (secondary to decreased blood flow to the kidneys), inappropriate antidiuretic hormone secretion and proteinuria.

e. **Blood:** When blood appears in the urine it may be classified as hematuria or hemoglobinuria. Hematuria consists of red blood cells and pigments in the urine, where hemaglobinuria involves the pigment only present in the urine. Hematuria is caused by lesions in the kidney or urinary tract. Hemoglobinuria results from hemolysis of red blood cells and liberation of hemoglobin. Causes of hemoglobinuria include malaria, typhoid, yellow fever, hepatitis, hemolytic jaundice, transfusion reactions, and bums that cover a considerable amount of the body.

f. **pH:** Normal pH ranges from 4.8 to 8.0 but usually urine has a pH value 5.5 - 6.5. High acidity may be due to acidosis, fever, or a high protein diet. Low acidity values may be the result of bladder retention, cystitis, or anemia.

g. **Protein:** Normal urine is free of protein although traces of serum albumin and globulin may be present. When proteinuria (protein in the urine) occurs the major type of protein is albumin. The most common cause of proteinuria is renal disease, including toxemia of pregnancy, Albuminuria may also be indicative of ischemic kidneys, congestive heart failure, fever, anemia and liver disease.

h. **Urobilinogen:** A small amount of urobilinogen is normally found in urine, but significant amounts suggest that further assessment for haemolytic and hepatocellular disease is indicated. Urobilinogen levels can be increased in conditions associated with elevated nitrite levels (eg, UTIs).

i. **Nitrite:** This test relies on the breakdown of urinary nitrates to nitrites, which are not found in normal urine. Many Gram-negative and some Gram-positive bacteria are capable of producing this reaction and a positive test suggests their presence in significant numbers (ie more than 10,000 per ml). A negative result does not rule out a UTI.

j. **Leukocytes**: This relies on the reaction of leukocyte esterase produced by neutrophils and a positive result suggests pyuria associated with UTI.

B. Fluid Balance following Water and/or Salt Ingestion:

The student(s) in each group that provided baseline data will participate in one of three conditions of water and salt ingestion and thereafter collect urine samples at 30 minute intervals

The student(s) in each group will be assigned one of three conditions of water and salt ingestion:

1. Drink 1000 mL of water as rapidly as comfortable.
2. Drink 1000 mL of water and eat pretzels (weighed to contain 1 g of Na) as rapidly as comfortable.
3. Eat pretzels (weighed to contain 1 g of Na) as rapidly as comfortable.

Each participant is expected to consume their water and/or salt as rapidly as comfortable. No other fluids or food will be ingested during the experimental period.

At 30 minute intervals for 2 hours after consuming the water and/or pretzels, each subject will empty their bladder completely to determine volume, and save a small quantity to examine appearance and measure NaCl concentration. Record these values in Table 2:

1. **Urine Volume:** Instructions are the same as A1, except only a small quantity needs to be saved for further analysis.
2. **Urine Formation Rate:** Instructions are the same as A2
3. **Appearance:** Instructions are the same as A3
4. **Sodium Chloride Concentration:** Instructions are the same as A4
5. **Osmolarity:** Instructions are the same as A5

Results:

Table 1a. Baseline Urine Sample.

Urine Volume Produced (mL)	Urine Formation Rate (mL/min)	Appearance	NaCl conc.	Osmolarity (mOsm/L)

Table 1b. Composition of Baseline Urine Sample (Labstix 10).

Leukocytes	Nitrite	Urobilinogen	Protein	pH
Blood	Specific Gravity	Ketones	Bilirubin	Glucose (mg/dL)

Table 2. Effects of Water & Salt Ingestion on Renal Fluid and Electrolyte Excretion

Solution Ingested	Time (min)	Urine Volume Produced (mL)		Urine Rate (mL/min)		Appearance		NaCl concentration (g/L)		Osmolarity (mOsm/L)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
1000 mL H_2O	0										
	30										
	60										
	90										
	120										
1 gm Na Pretzels	0										
	30										
	60										
	90										
	120										
1000 mL H_2O and 1 gm Na Pretzels	0										
	30										
	60										
	90										
	120										

Discussion:

A.

1. What is the significance of urinalysis information?
2. Explain the relation between specific gravity and osmolarity (or osmolality).
3. Explain the relation between osmolarity (or osmolality).and sodium chloride concentration.

B.

4. According to your group data, how does NaCl and/or water intake influence urine production?
5. Speculate on the function of vasopressin (ADH)? Propose a homeostatic system that controls water excretion (diuresis) or water retention.
6. Speculate on the function of angiotensin II / aldosterone? Propose a homeostatic system that controls sodium excretion (natriuresis) or sodium retention.