

Bio 236 Lab:

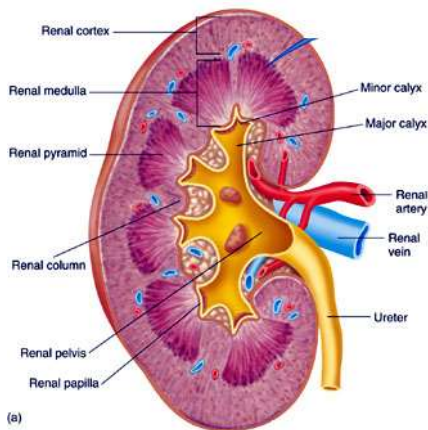


Fig. 1: Kidney Anatomy

The kidneys are paired structures that lie within the posterior abdominal cavity close to the spine. Each kidney contains an outer **renal cortex** and **inner renal medulla**, and approximately 8 – 12 renal lobes. Within each renal lobe are millions of **nephrons** that are the functional units of filtration (Fig. 1). Blood entering the kidney enters the **glomerulus** (a capillary network), delivered by the **afferent arteriole**, and then passes out of pores in the **Bowman's capsule** as "**filtrate**" (the acellular fluid that will be filtered by the renal nephron) into the renal tubules. The acellular component of blood that is not filterable (cannot pass through the Bowman's capsule pores) is removed via the **efferent arteriole** and delivered back into the circulatory system by entering the **peritubular capillaries** surrounding the nephron. The filtrate passes through 3 different tubules, during which salt and water is reabsorbed, before passing into a **collecting duct**. At the collecting duct additional substances might be reabsorbed from the filtrate, depending on your body's state of hydration and salt balance, and the remaining filtrate (now considered urine) is excreted into the kidney's ureters, which then transports urine to the bladder and urethra for exit from the body.

The nephrons of our kidneys can filter our entire blood volume (~5.5 L) every 40 minutes. That equals about 7.7 L of filtration per hour. However, 99% of the filtrate is reabsorbed back into the bloodstream and the remaining 1% of filtrate might be reabsorbed or secreted (as urine) depending on endocrine regulation and our blood osmolarity, blood pressure, and state of hydration. If not for reabsorption the majority of this fluid we would lose large volumes of body fluid to urine. The minimum urine output needed to remove toxic wastes from the bloodstream (**obligatory water loss**) is merely 0.4 L per day. However, normal **average urine output** is about 30 – 40 ml / hr or about 0.7 – 2 L of urine per day. A urine output greater than this is called **polyuria** and a lower urine output is called **oliguria**.

3 Renal Tubules of the Nephron:

1. The **proximal convoluted tubule (PCT)** is where approximately 65% of substances within the filtrate is reabsorbed from the tubule and taken up by the peritubular capillaries. These substances include active transport of sodium ions (Na^+) out of the tubule into the interstitial space of the renal medulla, which precedes the passive following of chloride ions (Cl^-) into the interstitial space. Once salt (NaCl) leaves the tubule water will follow salt (by osmosis) out of the tubule and into the interstitial space where it will be taken up by the "vasa recta" (capillary vessel) and reclaimed to the circulatory system. Additional substances removed from the filtrate within the PCT include glucose and small amino acids. Substances that tend to be "secreted" (added) to the filtrate from the peritubular capillaries for excretion from the body as urine include ions (potassium K^+ and bicarbonate HCO_3^-), uric acid, creatine, and pharmaceutical agents (e.g. some antibiotics and diuretics).

Osmoregulation and Renal Function

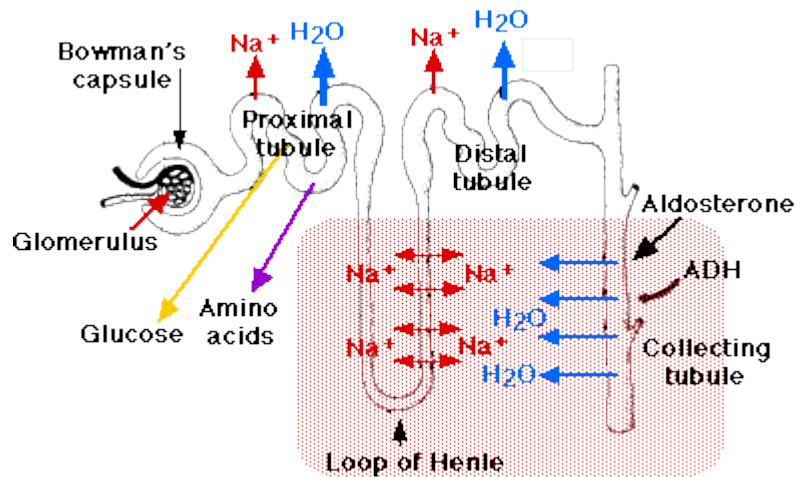


Fig. 2: Renal Nephron

2. The Loop of Henle. In the loop of Henle there are 2 subdivisions: the **descending loop** (which leaves the proximal convoluted tubule and descends into the medullary space) and the **ascending loop**, which rises up from the medulla to meet with the distal convoluted tubule. The **descending loop of Henle** is permeable to allow water to leave the tubule (by osmosis) and enter the interstitial space but it is NOT permeable to salt. Thus salt stays within the descending loop and passes into the ascending loop. *[By the time filtrate has completed its journey through the descending loop approximately 85% of the filtrate fluid volume has left the tubules and been taken up by the vasa recta blood vessel for reincorporation into the body's circulatory system. This 85% volume is always reabsorbed from the filtrate, regardless of your state of hydration or blood osmolarity. The last 15 percent of the total fluid volume of the filtrate is reabsorbed or retained through the collecting duct based on interactions of the duct with endocrine hormones.]*

The **ascending loop of Henle** is impermeable to water (will not allow water to leave the tubule) but IS permeable to salt. Thus, Na⁺ can leave the ascending tubule (through active transport), and Cl⁻ then follows Na⁺ into the interstitial space. Thus, water remains within the ascending tubule while salt is removed from the tubule. As a result of salt exiting the ascending tubule the interstitial space in the medulla becomes salty (hypertonic) relative to the more dilute contents of the tubule. This gradient will become important for the next step of filtration occurring in the collecting duct.

3. The distal convoluted tubule (DCT) receives the approximately 15% volume remaining of the initial filtrate and it is permeable to salt. Whether filtrate is reabsorbed or retained within the tubule for excretion depends on your body's state of hydration or salt balance and is regulated by the endocrine system. Reabsorption of salt (Na⁺ and Cl⁻) in the DCT is regulated by the **renal renin-angiotensin-aldosterone system** and your body's state of hydration and blood osmolarity. If your blood volume and blood pressure is too low the cells of the macula densa sense this and signal the juxtaglomerular apparatus (cells near the afferent arteriole of the nephron) to secrete renin. **Renin** stimulates the liver to convert **angiotensinogen** into **angiotensin 1**. Angiotensin 1 is converted (by ACE enzymes in the lungs) into **angiotensin 2**. Angiotensin 2 stimulates the adrenal cortex to secrete aldosterone. **Aldosterone** is a mineralcorticoid hormone that stimulates arteriole vasoconstriction and to increase salt (Na⁺ and Cl⁻) reabsorption within the DCT. Where salt goes water follows by osmosis. Thus, more water is also reabsorbed into the bloodstream. The net result is that your urine output decreases (you pee a smaller volume of more concentrated urine) and your blood volume, blood pressure, and osmolarity increases. If your blood volume and blood pressure is too high renin secretion is inhibited, leading to less angiotensin 2 and aldosterone production. The net result is that less salt and water is reabsorbed by the DCT, urine output increases (you pee a higher volume of more dilute urine), and your blood volume, pressure, and osmolarity decreases.

The **collecting duct**: The collecting duct can allow some water reabsorption in response to endocrine regulation by **antidiuretic hormone (ADH)**. The first part of the collecting duct is sensitive to stimulation by ADH but the lower regions are not. Your hypothalamus has a "set point" for blood osmolarity at 280-290 mOsm. If your blood osmolarity is above this set point the hypothalamic nuclei (paraventricular nucleus and supraoptic nucleus) will produce ADH (antidiuretic hormone) and release it from the posterior pituitary. ADH will increase the permeability of the collecting duct to water, allowing water to leave the duct and be reclaimed by the bloodstream. The net effect is that more water is returned to your bloodstream, urine output is decreased (you pee a smaller volume of more concentrated urine), your blood volume and blood pressure will rise, and your blood osmolarity will decrease. If your blood osmolarity is lower than the hypothalamic set point the hypothalamus will decrease secretion of ADH, causing decreased water permeability of the collecting duct. The net result is less water is reclaimed by the bloodstream, urine output is increased (you pee a larger volume of more dilute urine), your blood volume and blood pressure decreases, and your blood osmolarity increases. The collecting duct receives the remaining "waste" filtrate and delivers it to the minor calyx, then major calyx of the kidney. Urine then exits the kidney through the ureter.

Osmoregulation Experiment:

In this lab we will investigate the osmoregulatory effects of ingestion of different fluids. After ingestion we will collect urine at 0 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes. Data collected will be:

1. Urine Volume
2. Specific Gravity

The solutions we will ingest will vary in pH and osmolarity. Divide evenly into groups, ensuring that more than one student drinks each solution.

- Solutions:
1. 500 ml of tap water.
 2. 500 ml of isotonic saline (0.9% NaCl) or equivalent.
 3. 75 ml of 5% saline.
 4. 500 ml of unsweet tea.

Note: if ingesting the solutions makes you feel sick STOP DRINKING.

If you cannot void a sample, try to wait until the next 30 minute data collection period. If you have to urinate between data collection times, save the sample and add it to the next collection.

Record all data in the space provided and add it to the class data sheet.

Procedure:

1. Immediately after consuming the prescribed solution take a urine collection container to the bathroom and collect a full specimen of urine for your time 0 collection. It is the aim to completely empty your bladder. Record the volume of urine by looking at the specimen cup. Use a disposable pipette to draw up a sample of urine. Empty the urine from the cup into the toilet, rinse the cup with water from the sink, and dump rinse water into toilet, and flush. Please make sure and be extremely clean during this step! The bathroom should be clean and tidy when you are done. Take the specimen cup with the pipette of urine back to the lab for analysis.
2. As soon as you return to lab, write down the volume of urine you excreted (before you forget!)
3. Measure and record the specific gravity of your sample using a refractometer. Your instructor will have shown you how to do this. Make sure and clean the refractometer after use!
4. Repeat steps 1-3 again at 30 and 60 minutes.
8. Record your individual data in Table 1, and then into the class summary excel spreadsheet your instructor has prepared.

Specific gravity

Specific gravity is a measure of the solute concentration of a solution. It is measured by using the urine refractometer. Use a plastic pipette to add 1 drop of urine to the platform of the refractometer. Lower the plastic lid and hold the refractometer up to a bright light source to read the specific gravity (s.g.) scale, and record the value. Pure water has a specific gravity of 1.000. Your urine sample will have a specific gravity between 1.000 and about 1.050.

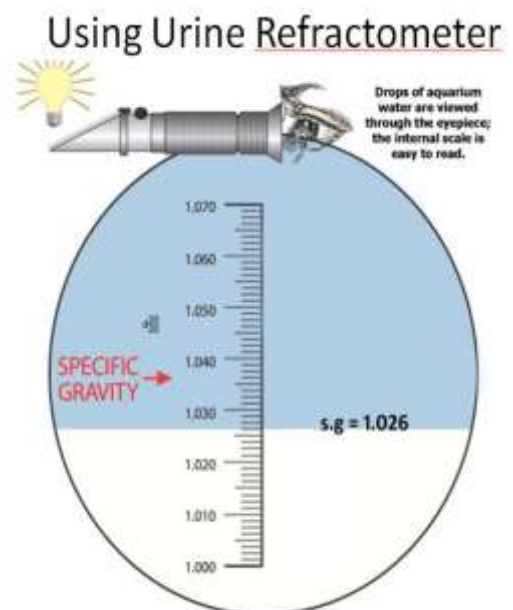


Table 1	Urine Volume (ml)			Urine Specific Gravity (s.g. mOsm)		
	0 min	30 min	60 min	0 min	30 min	60 min
Water						
Unsweet Tea						
0.9% Saline						
5% Saline						

Osmoregulation Questions:

Name _____

1. For water, saline solutions, and tea, at what time interval(s) was there the greatest change in both urine total volume and urine output rate?

Water _____

0.9% (physiologic) saline _____

5% saline _____

Tea _____

2. What was the difference in urine output rate between water, saline solutions, and tea?

3. What would be the physiological explanation for why urine volume and output rate might increase after drinking water?

4. What would be the physiological explanation for why urine volume and output rate might decrease after drinking 5% saline?

5. Which tubule within the nephron allows for absorption of glucose, small amino acids, uric acid, and creatine, along with Na^+ , Cl^- , K^+ , and water? _____

6. Which part of Henle's loop is permeable to:

A) water but not salt (NaCl)? _____

B) salt but not water? _____

7. How is aldosterone produced? _____

8. What is the effect of aldosterone on the distal convoluted tubule? _____

9. What is the effect of Antidiuretic hormone (ADH)? _____

10. What sequence of events would occur if your hypothalamus senses that your blood osmolarity rises above 290 mOsm? _____

11. Blood for filtration enters the Bowman's capsule through which blood vessel? _____

12. What has been your favorite lab from this semester? _____
Why? _____

13. What has been your least favorite lab from this semester? _____
Why? _____