Bio 236 Lab: **Osmoregulation and Renal Function (updated 10/30/23)**

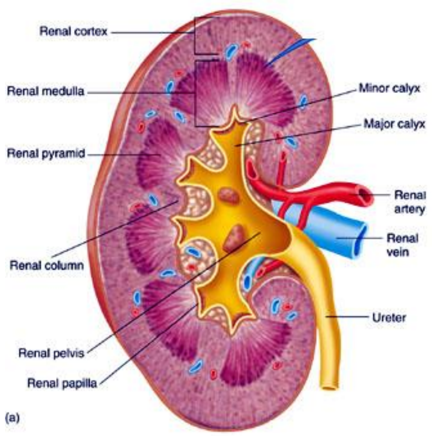


Fig. 1: Kidney Anatomy

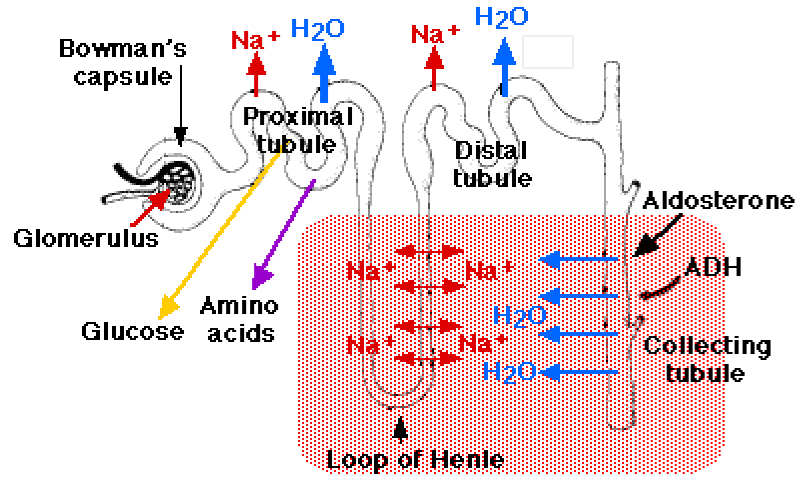


Fig. 2: Renal Nephron

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 (or pyramids). Within each renal lobe are millions of **nephrons** that are the functional units of filtration (Fig. 1). Arterial blood entering the kidney, from the afferent arteriole, enters the **glomerulus** (a capillary network), and then passes out of pores into the **Bowman's capsule** as "**filtrate**" (the acellular fluid that will be filtered by the renal nephron) into the renal tubules. The cellular component of blood (WBCs, RBCs, and large molecules) 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 then passes through 3 different tubules (see below), 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 automatically 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 would be lost 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 peritubullar capillaries for excretion from the body as urine include ions (potassium K+ and bicarbonate HCO3-), uric acid, creatine, and pharmaceutical agents (e.g. some antibiotics and diuretics).

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 **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.

The substances reabsorbed automatically from the PCT and Loop of Henle are not influences by hormones such as ADH or aldosterone. Only after passing the Loop of Henle will hormones affect reabsorption of salt and water.

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 presence or absence of aldosterone. 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, produced by the adrenal cortex, which stimulates arteriole vasoconstriction and to increase salt (Na+ and Cl-) reabsorption within the DCT. Where salt goes water follows by osmosis. Thus, the more salt that is reabsorbed the 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 (supraoptic nucleus) will produce ADH 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 Procedure:

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

1. Urine Volume

2. Urine Specific Gravity (S.G.)

The solutions we will ingest will vary in salt content. Divide evenly into groups, and drink one solution.

Solutions: **1. 350 ml of tap water (salt free)**

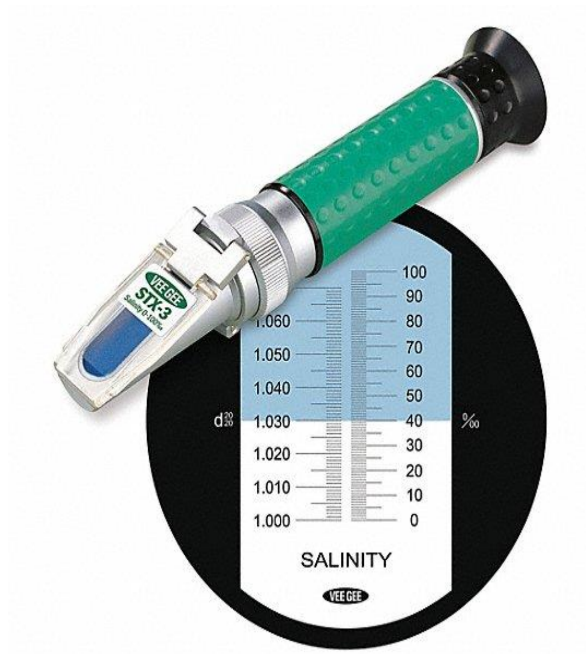
2. 75 ml of 5% saline

Urine collection and volume measurement:

Get a plastic urine cup and a plastic pipette, and go to the bathroom to collect your urine sample. Completely void your bladder (pee all that you can), record the volume of urine excreted by looking at the ml gradations on the beaker. Use the plastic pipette to draw up 1 – 2 ml of urine. Pour the rest of the urine in the toilet, use water from the sink to rinse the cup, and pour the rinse water in the toilet before flushing. MAKE SURE YOU KEEP THE BATHROOM CLEAN! If the volume of urine is too small to be measured by the urine cup (it can only measure 50 ml or higher), use a graduated cylinder provided in the lab to measure urine volume. 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.

Specific gravity measurement:

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 blue glass 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 likely have a specific gravity likely between 1.000 and about 1.030. See Figure 3 for refractometer scale. As the refractometer is a veterinary refractometer, read from the scale in the dog column, as humans concentration urine similar to canines.



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

	Time (min)		
	0	**30**	**60**
Water group urine volume			
Water group urine S.G.			
5% Saline group urine volume			
5% Saline group urine S.G.			