KIDNEY FUNCTION: URINE ANALYSIS AND REGULATION OF OSMOLARITY

INTRODUCTION

Kidney structure and function:
When studying any organ system, it is important to recognize the difference between the job of that organ and the mechanism used to accomplish that job. The job of the kidney is to regulate the volume and composition of the body’s fluid compartments (in particular the extracellular fluid, ECF). The mechanism used to accomplish this job is a filtration/reabsorption system, where plasma is first nonselectively filtered at the glomerulus, and then substances needed by the body are selective reabsorbed by the tubules, leaving unwanted substances to form urine. The advantages of this system are 1) a very high capacity to get rid of things by monitoring the levels of substances in the plasma on a moment by moment basis and 2) the ability to eliminate a very broad range of substances without requiring special mechanisms for each substance. The filtration/reabsorption mechanism is able to maintain the composition of body fluids within very narrow limits in spite of wide variation in activity and nourishment. It is important to emphasize to students that the kidneys are relative small organs, representing only 0.5% of the body’s mass. Yet, they receive 25% of the blood flow leaving the heart! Even the much larger brain receives less blood flow than the kidneys. This high rate of blood flow supports the high rates of plasma filtration (Figure 1) and is the basis of the kidney’s rapid response to changes in ECF volume and composition. The energy required to filter the plasma is provided by the beating heat. The process of glomerular filtration can be likened to a cloth sack filled with wet paste being squeezing so the liquid and other small particles ooze out, while larger substances (in the case of blood, the cells and plasma proteins) are retained. About 20% of the plasma flowing to the glomeruli is filtered to form what is referred to as glomerular filtrate.

The functional unit of the kidney is the nephron (Figure 2). The renal filtration locus is formed by the glomerular capillary and Bowman’s capsule, an expansion of the nephron that envelopes the glomerular capillary. It is important to emphasize that the filtration barrier (formed by the wall of the glomerular capillary and the wall of Bowman’s capsule) is a very porous and non-selective structure. About the only things in blood that are not filtered by the glomerulus are blood cells and plasma proteins. All the smaller components of blood (water, salt, minerals, sugars, fats, amino acids, hormones, etc.) are freely filtered. This means that, except for protein, the composition of plasma and glomerular filtrate are essentially identical. The remainder of the nephron, the renal tubule, represents the kidney’s reabsorption locus. (Secretion also occurs in the renal tubules, but the emphasis for beginning students should be on the much higher rates of reabsorption.) Tubular reabsorption recovers all of the sugars, amino acids, and other nutrients in the glomerular filtrate. Substances like salt and
water that are present in the body in variable quantities are reabsorbed on an as-needed basis but, in general more than 95% of filtered salt and water is reabsorbed. Compare the volume of glomerular filtration (180 l/day) to the volume of urine formed (1-4 l/day). Other materials, specifically metabolic by products and potentially toxic materials are not reabsorbed by the kidney and left to be eliminated in the urine. The result of tubular reabsorption is a dramatic reduction in the volume of tubular fluid and changes in the composition of the tubular fluid as the final urine is formed. It is important to emphasize that some things present in the glomerular filtrate are absent from normal urine (e.g., glucose); other things present in the glomerular filtrate in low concentration become concentrated as water is reabsorbed from the tubule (e.g. urea).

Five Distinct Functional Regions of the Nephron

Bowman’s capsule forms part of the filtration barrier and collects the glomerular filtrate. The proximal tubule reabsorbs the filtered nutrients and more than 90% of the filtered salt and water. The ability of the proximal tubule to completely reabsorb specific substances depends on their concentration in the glomerular filtrate and ultimately in the plasma. The absorption capacity is appropriate for normal concentrations, but if concentrations are too high tubular reabsorption will be incomplete and the substance will "spill over" into the urine and be excreted. This is why glucose appears in the urine of diabetics. The function of the proximal tubule is the most diverse and perhaps complex but it is enough for the beginning student to appreciate its central role in the reabsorption of nutrients.

The loop of Henle is required to form an osmotically concentrated urine, and it is unique to birds and mammals. Understanding the mechanism for forming concentrated urine is one of the most conceptually difficult topics in biology. It is important to stress that animals living in air are always losing water to the environment by evaporation. Because water is often in limited supply it is necessary to conserve water where possible and forming a concentrated urine allows organisms living in air to get rid of metabolic waste products with a minimal amount of water loss. Because birds and mammals have high metabolic rates (compared to amphibians and reptiles) this is a much more serious problem for them, hence the fact that only birds and mammals form concentrated urine. The specific function of the loop of Henle is to use metabolic energy to pump salt out of the tubular fluid creating a space inside the kidney that has a very high osmotic concentration. This hyperosmotic compartment will be used by the kidney to make a concentrated urine as needed. Note that the fluid in the tubule is not concentrated as it flows out of the loop of Henle (Figure 2). Urine concentration occurs in the collecting duct.

The distal tubule is primarily responsible for regulating the concentration of Na+, K+, and H+ in the blood. Students who have had an introduction to nerve and muscle function will appreciate that the concentrations of Na+ and K+ in the ECF are critical to formation of membrane potentials and the propagation of action potentials. As a result, the concentrations of these ions are closely regulated. The hydrogen ion is the most ubiquitous reactant in the body and the pH of ECF
is also tightly regulated. The collecting duct is where the final concentration of urine is determined. If there is a shortage of water in the body the kidney will excrete a concentrated urine. It does this by making the walls of the collecting duct permeable to water so that water in the tubular fluid will be absorbed into the compartment made concentrated by the loop of Henle (i.e., the interstitium of the medulla). This is an example of the movement of water down and osmotic gradient. The mechanisms for shunting this water out of the kidney and conserving the concentration gradient are the job of the vasa recta and probably beyond the scope of what high school students can understand, but suffice it to say such mechanisms exist. The conservation of water is called anti-diuresis and it is regulated by the antidiuretic hormone (ADH) which makes the wall of the collecting duct permeable to water. If there is an excess of water in the body the kidney will excrete a dilute urine. It does this by making the wall of the collecting duct impermeable to water so that as the tubular fluid flows through it no additional water is reabsorbed. Note than the fluid entering the collecting duct is dilute (Figure 2). This condition is called diuresis and is determined by the absence of the hormone ADH. Two topics of interest are the effects of caffeinated and alcoholic beverages on urine volume and composition. While both beverages are typically more dilute than body fluids, their consumption results in dehydration. Caffeine stimulates water excretion by a number of mechanisms and ethanol inhibits the release of ADH. In each case there is loss of fluid in excess of intake and the result is dehydration. Individuals who consume significant amounts of coffee or alcohol must take care to consume additional water. In conclusion, the specific sequential action of the different tubular segments determines the final volume and composition of urine. Many disease states can be diagnosed by the absence or presence of substances in the urine. The tests conducted in this lab are typical for routine urinalysis, but it is possible to use urinalysis to test for many specific conditions using the same principle (i.e., there are things that should be in the urine and things that should not be).

Other Uses for Urinalysis
Pregnancy testing: The kidney normally scavenges complex biomolecules such as peptide hormones, which can then be reused or recycled. During pregnancy, however, the placenta produces very high levels of several hormones that spill over into the urine. One in particular, human chorionic gonadotropin (HGC) is diagnostic of pregnancy because its only produced by the placenta, while other hormones that also increase during pregnancy might be increased by factors other than pregnancy. There are currently a number of over-the-counter early pregnancy tests on the market that utilize the presence of HCG in urine to indicate a pregnancy.

Drug testing: Because virtually all drugs whether licit or illicit are small enough to be filtered by the kidney and most are substances the kidney has no means to reabsorb, urine can be tested to detect the presence of many drugs. This can be quite sophisticated as in the case of testing athletes for synthetic versions of
naturally occurring compounds in the human body (e.g., anabolic steroids) or relatively straightforward as in the case of detecting the presence of drugs like marijuana. It might be interesting for students to contemplate why urine testing for alcohol is not done. Ethanol is certainly small and filterable and it is present in urine after one has been drinking. But ethanol per se is not illegal, only if the blood concentration is very high. Because substances in the urine can be concentrated passively if the body is trying to save water, it is fairly difficult (although not impossible) to back calculate blood concentrations accurately from urine concentrations. For illegal drugs their presence, not concentration, is the issue and passive concentration actually makes them easier to detect in urine than in blood. There are other, simpler methods for determining blood alcohol levels.

Urinalysis
Color, volume, and specific gravity (osmotic concentration) of urine vary tremendously depending on the body’s need for salt and water. Normally, as volume of urine goes down specific gravity goes up and vice versa. The color of urine is due to pigments in the diet or is derived from metabolism. Concentrated urine will have a darker color and stronger smell as chromogens and volatile wastes are concentrated. For students who take B-vitamin supplements, an interesting observation is to see how quickly their urine turns bright yellow following ingestion of vitamin B. Many people also metabolize a chemical in asparagus to a highly odoriferous and volatile compound that can be smelled in urine literally within minutes of eating asparagus. Glucose is not normally present in the urine because reabsorption in the proximal tubule is complete. Its presence typically indicates that plasma levels are well above normal, as in diabetes. Historically diabetes was diagnosed by tasting the urine for the characteristic sweetness imparted by the sugar present. Students considering careers in medicine will appreciate the development of glucose test strips currently used to make this measurement! Glucose in urine attracts water by osmosis increasing the volume of urine formed. Diabetics urinate frequently and are excessively thirsty as a result. The loss of water dehydrates the body causing a response to conserve water by concentrating urine. Ketones are by-products of the metabolism of fat. Ketosis and ketonuria are characteristic of starvation, various metabolic disorders, and diabetes. For diabetics the production of ketones results from the inability to properly regulate insulin, which in addition to its role in the metabolism of glucose also plays a key role in the metabolism of fats. Protein is not normally present in urine. The glomerulus should not allow filtration of plasma protein. In the early stages of glomerular disease the filtration barrier can become leaky to protein. This generally increases glomerular filtration and can also lead to increased urine volume. Renal disease and damage to the glomerular filtration barrier can result from a bewildering array of conditions. Untreated high blood pressure is probably the most significant and rapidly increasing cause of chronic renal failure. Diabetes and a variety of autoimmune syndromes also result in glomerular damage. Protein in urine might also result from bacterial infection. Bilirubin and urobilinogen are breakdown products of
Bilirubin is not normally found in urine. Normal levels for urobilinogen are more controversial and not considered in this lab. Red blood cells in the body, live for about 120 days after which time they are broken down and the hemoglobin they contain is recycled in a complex process that results in formation of bilirubin. Bilirubin is formed in the liver and excreted with the bile into the intestine. The presence of bilirubin in the urine indicates blood levels that are too high, either because excessive numbers of red blood cells are being broken down (hemolytic anemia - infrequently observed) or because the liver is failing to properly metabolize the hemoglobin (hepatitis, cirrhosis, or bile blockage due to gall stones). Either situation can result in anemia as valuable blood protein and iron are lost. Nitrite is a product of the bacterial reduction of nitrate and indicates the presence of a bacterial infection. Nitrate is produced in the body through the metabolism of food. Normally there is no nitrite in urine (normal urine is sterile). Infections in the kidney do not normally produce pain during urination. Painful urination is typical of lower urinary tract (urethra and bladder) infections. These infections usually travel up the urinary tract. The amount of nitrite formed depends on many things including: the type of bacteria (whether or not it has reductase), how long the urine is stored in the bladder, the presence of nitrate in the diet. Lower urinary tract infections can travel up into the kidney if left untreated. They may also be sexually transmitted. Antibiotics are used to treat infections. Bacterial culture (identification) is important to guide proper antibiotic therapy. Blood, often referred to as occult blood (meaning mysterious origin) indicates bleeding somewhere in the urinary system and is an abnormal finding. Sediment and crystals are solids found in urine. Common sediments include dead epithelial cells sloughed from the urinary tract lining, bacteria and white blood cells suggestive of infection, crystals of certain minerals that precipitate out of solution. The most common stone-forming minerals are calcium and oxalate that can lead to the formation of kidney stones that can obstruct tubules, causing significant damage and pain. Twelve percent of males form stones, with those between the ages of 30-50 years at greatest risk for stone disease. Oxalate is common in nuts and dark green leafy vegetables. It is the reason rhubarb leaves are considered toxic. When pets or children drink antifreeze, oxalate is formed from the ethylene glycol in antifreeze by metabolism in the liver. In both cases, kidney failure due to massive tubular damage is the cause of death. To avoid kidney stones, at risk individuals are urged to keep urine volumes high, and oxalate levels and urine pH low. This reduces the concentration and increases solubility of calcium oxalate in tubular fluid.

Use of Dip-stick Technology for Chemical Analysis (Multistix)

In the clinical setting it is desirable to be able to obtain quick and highly reproducible analyses – often performed by individuals with limited background and technical expertise. There are a variety of methods used (for example, automated chemical analyzers that can perform a variety of tests on small samples with a high degree of accuracy). These analyzers are expensive, limiting their use to hospital or contract lab settings. The desirability of tests that the
consumer can take home and perform themselves for routine monitoring of blood sugar, by diabetics, and recently urine tests for pregnancy has provided a tremendous economic incentive to develop these tests. Most dip stick methods utilize enzyme-based colorimetric reactions. The principles are quite basic and well known. The methods for quality control during production are trade secrets! The difficulty is getting the different reagents to coexist on the paper strip without reacting together during manufacture. The basic technique is to introduce the different reagents in immiscible phases. In the example described here the paper is impregnated with the enzymes in an aqueous solution, while the chromogenic reagent is added in an organic phase. The point is made that oil and water don’t mix! The reaction is used to test for the presence of glucose involves the oxidation of glucose using the enzyme glucose oxidase (G.O.) to form peroxide (H2O2). The peroxide is activated by another enzyme, peroxidase, forming a reactive oxygen compound that reacts with the indicator substance tetramethyl benzidine (TMB) resulting in the formation of a blue color.

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\text{G.O.} \quad \text{peroxidase}
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glucose \quad \rightarrow \quad \text{gluconic acid} + \text{H}_2\text{O}_2 \quad \rightarrow \quad \text{reactive oxygen}
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\text{TMB (brown)} \quad \rightarrow \quad \text{TMB (blue)}
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By this reaction the quantity of glucose present is estimated by the amount of blue colored TMB that is produced. The enzymes are not consumed in the reaction so all of the glucose absorbed to the dipstick is converted to gluconic acid and H2O2 on a mole for mole basis. Because the TMB is activated by the reactive oxygen (formed when the peroxide is broken down by the peroxidase) on a mole for mole basis, it follows that the amount of blue color is determined by the amount of glucose present. The highest concentration of glucose that can be detected is determined by the amount of TMB present. The amount of glucose that is consumed in the reaction is related to the concentration of glucose in solution by the volume of solution that absorbs to the area of the dipstick containing the chemicals. This can be expressed by the equation: concentration = quantity/volume.

Background for this lab:

It is the job of the kidney to regulate the volume and composition of body fluids. Body fluids include the extracellular fluid (ECF, the blood plasma) and the intracellular fluid (ICF, inside body cells). Regulating these fluids requires that the kidney conserves (keeps) or eliminates (discards) materials in just the right amounts to balance intakes and non-renal losses to maintain body fluid homeostasis.

The mechanism of kidney function is a 2-step process. First, the blood is filtered through the glomerulus, forming glomerular filtrate. Second, the filtered fluid is conditioned in the tubule through reabsorption of some chemicals and secretion.
of others. The final product is urine, a solution that normally contains water, salt, urea and other substances the body needs to eliminate. The kidney responds to body demands and conditions by changing how much of each material is conserved and how much eliminated, so the volume and composition of urine are quite variable, while the volume and composition of the ECF remains constant. The problem in maintaining constancy in body fluids is that changes caused by eating, drinking, body demands, and activity are very irregular and unpredictable. Even so, the chemical composition of the blood and other body fluids must remain very constant for the body to function normally. The kidneys have the major responsibility for maintaining body fluid homeostasis no matter what the individual eats, drinks, or does. The solution to the problem is to rapidly turn over the body fluids by glomerular filtration and tubular reabsorption, continually filtering and monitoring the plasma for changes that require correction. The adult kidney filters 170 liters of blood plasma per day. An average adult has a plasma volume of about 3.5 liters, meaning that the kidney completely filters the plasma about 48 times per day, or twice per hour! Obviously, all of the material filtered by the kidney cannot be excreted, so the second step in normal kidney function is to selectively recover the parts of the filtrate that are necessary and useful to the body. During the process of selective reabsorption, some substances (like glucose) are completely recovered from the urine and other substances (like urea) remain to be eliminated.

The composition of normal urine is highly variable because our diet and level of activity are highly variable. However, abnormal urine may contain substances that are never present in normal urine. Sometimes the presence of a substance may indicate a great excess in the body, so that the kidney can’t reabsorb it all. If the kidney is diseased, substances that should not be the urine may be present. Depending on what and how much of various substances are present, a physician may be able to determine what’s wrong with a patient. Urinalysis tests for the presence or absence of different substances, so that a physician can analyze the results to find out what’s wrong with the patient.

**PROCEDURE FOR URINE ANALYSIS**

For convenience (so the lab proceeds in a timely manner), the schedule will be very different. As stated in the syllabus, you need to come prepared to micturate. Go to the rest room and fill the sample cup.

Next, we will take a reading with the [urinalysis](#) strip

Here's how:
Obtain a Multistix from the front lab bench. Dip the reagent part of the Multistix into the urine so that all reagents are completely covered. Dip quickly for not more than one second! Wipe off the excess urine by dragging the chemstrip across the lip of the urinalysis jar. For the specified number of seconds read the strip by comparing it to the colored scale on the Multistix pages provided.
Questions:
1. Considering that filtration is a passive process, what provides the force that pushes fluid/solutes into the nephron?
2. Where does the majority of glucose reabsorption take place? Should there be some glucose remaining in the urine, what might this signify?
3. What are ketones? If you have Ketosis or Ketonuria, what potential problems could you have?
4. Explain how/why Diabetes, if untreated, could lead to kidney failure?
5. Why is urine not used to test for the presence of alcohol?
6. Why does chronic high blood pressure often lead to renal failure or kidney disease?
7. A patient comes to see you with a variety of problems for which you tell him to pee on this stick, handing him a Multistix. The results show Hemolytic Anemia. You know this because ________ is shown to be elevated in the urine. Explain why this occurs as well.
8. Why is it more common for women to have Hematuria?
9. Your patient comes to the hospital unconscious. You believe one underlying cause is a urinary tract infection that has moved up into the kidney. A urinalysis can verify this. Explain how.
10. Why are Rhubarb leaves considered toxic? Explain. What affect would they have on your urine?
11. Next time your parents tell you to eat your spinach, you will tell them no because dark green leafy vegetables can cause this problem in your excretory system.
12. How does ADH work to increase urine volume?
13. Why does alcohol make an individual micturate more often?
14. Describe the process of urine formation, referring specifically to the nephron. Be specific.
15. For each of the 10 categories, write down your results and explain what each category means.