Kidney Function

1. Cog/I Relate the importance of kidney function to cellular metabolism.
2. Cog/I Provide examples that support the theory that the kidney is the major homeostatic organ in the body.
3. Cog/I List the vascular components associated with blood supply to the kidney.
4. Cog/I List the tubular components that make up the functional unit of the kidney - the nephron.
5. Cog/II Describe the glomerular filtration process and the influence of blood pressure (BP), plasma colloid osmotic pressure (PCOP) and capsular pressure (CP) on glomerular filtration.
6. Cog/II Explain tubular secretion and tubular reabsorption and describe how these processes effect urine formation.
7. Cog/II Discuss how maintaining electroneutrality serves as a driving force for tubular reabsorption.
8. Cog/I Give a step-by-step description of the reclamation of filtered bicarbonate by the carbonic anhydrase system.
9. Cog/II Describe water and electrolyte movement in each segment of the nephron.
10. Cog/II Describe the effects of antidiuretic hormone, aldosterone, ethanol and caffeine on the late distal convoluted tubule and collecting duct.
11. Cog/II Differentiate diabetes mellitus and diabetes insipidus and explain the cause of each.
12. Cog/II Describe of the renin-angiotensin system and give a step-by-step response of this system to a sudden drop in blood pressure.
13. Cog/II Describe glomerular filtration, tubular secretion and tubular reabsorption characteristics of urea nitrogen, uric acid, and creatinine.

Introduction

As an introduction to kidney function, recall intracellular metabolism (e.g., carbohydrate metabolism). In order for intracellular metabolism to occur optimally, the internal environment of the cell must remain constant (i.e. constant supply of nutrients and constant removal of waste). The internal environment of the cell is a direct reflection of the composition of extracellular fluid that surrounds the cell. This extracellular fluid is, in turn, regulated by two organs: the kidneys and the lungs.

The kidneys are responsible for the removal of non-volatile waste materials by the formation of urine. The lungs function to control the volatile components of extracellular fluids. At this point, the focus will be on the kidneys. The lungs will be considered later this semester.

Excretion is a very important function of the kidney. Even though this is true, the major function of the kidney is homeostasis. The kidneys are believed to be the major homeostatic organ in the body. (Homeostasis is defined as the maintenance of constant conditions within the body; status-quo.)

For example, ions such as sodium, potassium, chloride, and bicarbonate are materials that are regulated by the kidney. It is important to note that these four ions are the four major ions in the body; and, it is the responsibility of the kidney to regulate the interstitial fluid concentration of these ions.

In addition, the kidney is the major organ in the body which regulates the concentration of organic materials such as glucose, urea, and amino acids.

Another way the kidney serves to maintain the status-quo in the body is by osmo-regulation (i.e. maintaining a water balance).

Yet another function of the kidney in regards to homeostasis is with acid/base balance. The kidney is the most powerful organ the body has to regulate acid/base balance. The kidney regulates acid/base balance primarily by regulating two ions: H^+ and HCO_3^-.

Furthermore, the kidney exerts homeostatic control in the body by the release of renin to maintain blood pressure. As a matter of fact, the kidney produces three significant products that play important roles elsewhere in the body. These include:

1. Renin - hypertension
2. 1,25 dihydroxyvitamin D - metabolic bone disease
3. Erythropoietin - anemia

Metabolic bone disease and anemia are problems that are typically seen in late stages of renal disease.

These are just a few of the ways the kidney serves to keep everything in the body at a status-quo.

Kidney Function

ADH
Aldosterone
Angiotensin

Review of Renal Physiology

Vascular Elements

It is said that theoretically 5,000 mL of blood per minute is pumped by the heart. Of this, approximately 25% or ≈ 1,250

Each kidney receives blood off of the abdominal aorta via the renal artery. The renal artery divides down into the arcuate arteries, and from here there is division into the interlobular arteries. These divide into afferent arterioles which carry blood into the glomerular capillary bed. Blood passes from the glomerulus into the efferent arterioles, and then into the peritubular capillaries. The peritubular capillaries surround the proximal convoluted tubule and the distal convoluted tubule, and the vasa recta (a specialized peritubular capillary) extends down surrounding the loop of Henle. From here, blood passes into venules into the interlobular veins, then arcuate veins, renal vein, vena cava and into the heart.

Tubular Elements

At the glomerulus, 125 mL/min of fluid is filtered out of the blood stream. This equates to 180 liters/day or 45 gallons/day. Most of the fluid that is filtered is reabsorbed (approximately 99%) back into the body. This results in 1 mL/min of urine being formed which leads to a total urine output of 1500 mL/day.

Normal - approximately 600 - 2000 mL/day
Oligouria - <500 mL/day
Polyuria - >2000 mL/day
Anuria - virtually complete suppression of urine output

From the glomerular capillary bed, fluid will first pass into Bowman's Capsule, then the proximal convoluted tubule (PCT), then the loop of Henle, then the distal convoluted tubule (DCT) and into the collecting duct. These tubules make up what is commonly referred to as the functional unit of the kidney - the nephron. It is important to note that the peritubular capillaries, including the vasa recta, completely surround the nephron. This is where materials that are reabsorbed from the nephron re-enter the circulation as well as where materials being secreted into the nephron exit the circulation.

Formation of Urine

Urine is a modified ultrafiltrate of the plasma. The amount of plasma that passes from the glomeruli into Bowman's Capsule is known as the glomerular filtrate. This glomerular filtrate must pass three layers of cells. The layers of cells are as follows:

1. endothelial cells of the capillaries - the fenestrae (pores) are approximately 160Å
2. basement membrane - this has channels approximately 110 Å wide
3. epithelium of Bowman's Capsule - fenestrae are approximately 70 Å

Compounds with a molecular weight of greater than 70,000 cannot filter from the glomerulus into Bowman's Capsule.

There are two main differences between blood and glomerular filtrate in normal individuals. These are:

1. No RBC, WBC, platelets in filtrate (i.e. no formed elements).
2. Filtrate protein is approximately 0.03% of plasma protein.

The formation of urine by the nephron involves three basic processes: 1) glomerular filtration, 2) tubular secretion, 3) tubular reabsorption.

Glomerular Filtration

There are three basic pressures that affect glomerular filtration.

1. Blood Pressure (BP) - this is a positive force which promotes filtration
2. Plasma Colloid Osmotic Pressure (PCOP) - this pressure results from the presence of protein in the blood stream. Protein is osmotically active meaning that it tends to hold onto water. Therefore, this pressure tends to retard filtration.
3. Capsular Pressure (CP) - this pressure results from the presence of fluid already present in Bowman's Capsule. Since there is already fluid present, incoming fluid meets with resistance as it tries to move into the capsule. Therefore, this pressure also works against filtration.

Consider the following equation.

Net filtration pressure = BP - (PCOP + CP)

Now plugging in some numbers that have been measured by physiologists

Net filtration pressure = 70 mmHg - (32 mmHg + 18 mmHg)

Net filtration pressure = +20 mmHg

It is this net filtration pressure of +20 mmHg that leads to the 125 mL of fluid filtering into Bowman's Capsule per minute. Therefore, anything that would increase this pressure (e.g. increased blood pressure) would lead to an increased urinary output. Likewise, anything that would decrease this pressure (e.g. increased blood protein levels such as that seen in dehydration) would lead to a decreased urinary output.

Tubular Secretion

This is an active transport process meaning that it requires the use of metabolic energy in the form of ATP. This process involves the movement of materials from the blood stream, in particular the peritubular capillaries, into the renal interstitial fluid (RIF) and from the RIF into the renal tubules. The materials that enter the nephron by this route are those, which for some reason, are unable to pass from the glomerulus into Bowman's Capsule. Examples of materials that are actively secreted into the PCT include penicillin and salicylates.

Tubular Reabsorption

As previously discussed, 99% of the fluid that is filtered returns to the circulation as a result of tubular reabsorption. This simply involves the movement of materials from the tubules into the RIF and theninto the peritubular capillaries. There are also many endogenous and exogenous materials that exhibit tubular reabsorption. These include glucose, amino acids, vitamins, and uric acid.

Handling of Ions and Water by the Kidney

First, throughout the nephron, there are pumps that will either pump sodium or chloride from the renal tubule back into the RIF and therefore the blood stream. Considering the sodium pump, when sodium (Na^+) is pumped out of the nephron it leaves an electrical imbalance inside the nephron. Therefore, to regain electroneutrality which is an absolute requirement inside the body, a chloride ion will passively follow. Therefore, it can be said that there is tubular reabsorption of Na^+Cl^-. Sodium is very osmotically active meaning that sodium will pull an osmotic equivalent amount of water with it wherever it goes. Therefore, as sodium chloride is reabsorbed, so is an osmotic equivalent amount of water.

Considering each segment of the nephron, the PCT will be considered first. Almost 100% of filtered glucose, amino acids and potassium are reabsorbed in the PCT. Also, approximately 80% of the filtered bicarbonate and approximately 70% of the filtered sodium is reabsorbed. There is no reabsorption of waste products like urea or creatinine in the PCT. Water is passively reabsorbed here.

In the PCT there is an important physiologic process occurring that serves to reclaim filtered HCO_3^-. HCO_3^- is freely filtered at the glomerulus. The body, however, needs this HCO_3^- to maintain proper pH. This physiologic process involves the carbonic anhydrase system and H^+ - Na^+ exchange. Beginning in the RIF, there are protons (H^+) that result from normal metabolic processes in the body. These protons are acidic in nature and are therefore buffered with a basic bicarbonate ion (HCO_3^-). This combination produces carbonic acid (H_2CO_3) which will dissociate into carbon dioxide (CO_2) and water. These reactions are as follows:

$$H^+ + HCO_3^- \quad H_2CO_3 \quad H_2O + CO_2$$

Inside the cell the enzyme carbonic anhydrase (CA) promotes the formation of H_2CO_3. H_2CO_3 then dissociates to form H^+ and HCO_3^-. These renal tubular cells have a pump which then pumps H^+ out of the cell into the PCT. To balance the loss of this positive charge, in order to maintain electroneutrality, Na^+ is absorbed from the tubule, hence H^+ - Na^+ exchange. Na^+, being very osmotically active, will pull an osmotic equivalent amount of water into the tubule cell as it enters the cell. For this reason, Na^+ cannot stay in the tubule cell; if it were to stay, the cell would swell and burst. Therefore, sodium is pumped from the tubule cell into the RIF, which again creates an electrical imbalance. Thus, to maintain electroneutrality, HCO_3^- passively moves from within the cell into the RIF. In addition to sodium being present in the PCT from glomerular filtration, HCO_3^- is present as well. Unmodified glomerular filtrate has the same concentration of HCO_3^- as does plasma. As noted, when H^+ is pumped out of the tubular cell into the glomerular filtrate, Na^+ moves into the cell to maintain electroneutrality. This H^+ then reacts with HCO_3^- from glomerular filtration forming H_2CO_3 which then dissociates into H_2O and CO_2. CO_2 and H_2O may then diffuse intothe PCT cell and follow the same reaction sequence as before producing HCO_3^- which then enters the RIF. Thus, HCO_3^- which was in the glomerular filtrate for possible excretion has now been conserved.

In the descending limb of the loop of Henle there is H_2O leaving the tubule but not much Na^+ or Cl^-. Actually, Na^+ and Cl^- levels appear to increase with the loss of H_2O. In the ascending limb of the loop of Henle, it is found that the walls of the tubule become impermeable to H_2O. Therefore, only ions move out of the tubule into the RIF while H_2O remains in the tubule. This process is what is responsible for urine being very hypotonic (i.e., dilute). By the end of the loop of Henle, approximately 90% of the filtered H_2O has been reabsorbed and approximately 95% of the filtered Na^+ has been reabsorbed.

In the early portion of the DCT, Cl^- is actively pumped out of the tubule into the RIF followed by Na^+ and an osmotic equivalent amount of H_2O. (This will be discussed below.) The later portion of the DCT is where the body can exert its greatest control over the amount of H_2O or fluid that leaves the body. (All other processes considered thus far are going to occur.) This control is primarily exerted by antidiuretic hormone (ADH). An older name for this hormone is vasopressin. ADH is released by the posterior pituitary as a result of stimulation of so-called ¢sodium receptors¢ that are stimulated by higher than acceptable Na^+ levels in the body. This stimulation leads to H_2O retention in order to dilute the Na^+ levels back to normal. This H_2O retention results from action of ADH on the cells of the tubule wall in the late DCT and the collecting duct (CD). ADH increases the permeability of the tubule wall cells in the late DCT and CD to H_2O. Therefore with the same osmotic gradient, now there is greater H_2O reabsorption. ADH is one of these hormones that is always released at a basal level and the body merely modifies how much is released depending on the amount of ¢sodium receptor¢ stimulation. In the absence of ADH, the late DCT and CD are essentially impermeable to H_2O.

Previously diabetes mellitus has been discussed. Now that ADH has been introduced, diabetes insipidus can be discussed. Diabetes insipidus is a disorder resulting from a decreased activity of the posterior lobe of the pituitary gland, in particular the neurohypophysis. This decreased activity results in a deficiency of ADH which in turn results in large amounts of urine being excreted. The relationship between diabetes mellitus and diabetes insipidus is the increased volume of urine excreted in both conditions. In fact, the word diabetes refers to the fact that there is an increased urine output. These two conditions differ in the cause for the increased urine output - a deficiency of ADH in diabetes insipidus and an excess amount of glucose spilling over into the urine pulling an osmotic equivalent amount of water along with it in diabetes mellitus.

While considering these two causes for increased urine output, there are two chemicals to consider. The first chemical is ethanol. Ethanol appears to block the release of ADH, and by doing so, results in an increased urine output. Caffeine is another chemical that increases urine output. Caffeine acts by selectively dilating the afferent arteriole which leads to increased glomerular filtration and increased urine output.

There is a second hormone that acts in the late DCT that exerts an effect on ions and therefore H_2O excretion. This hormone is aldosterone. Aldosterone is released by the zona glomerulosa of the adrenal cortex and functions to increase the amount of Na^+ reabsorption in this area. Here, in order to maintain electroneutrality, there is the secretion of either a K^+ or H^+. Again, with the movement of Na^+ into the RIF there is an accompanying movement of an osmotic equivalent amount of H_2O there also. Consider some the of the following effects of aldosterone.

aldosterone sodium reabsorption potassium secretion hypokalemia cardiac muscle hyperactivity - arrhythmias

aldosterone sodium reabsorption proton secretion alkalosis

aldosterone sodium reabsorption hypernatremia polydipsia blood volume cardiac output peripheral resistance and arterial pressure (polyuria)

aldosterone sodium reabsorption water reabsorption blood volume cardiac output peripheral resistance and arterial pressure (polyuria)

There are several factors that are known to stimulate an increased aldosterone production. These include:

1) low body Na^+
2) low blood volume
3) low cardiac output
4) high K^+
5) stress - physical or emotional

The mechanism of aldosterone production appears to initiate with these factors stimulating the hypothalamus to secrete glomerulotropin into the blood stream. Glomerulotropin then stimulates the zona glomerulosa of the adrenal cortex to release aldosterone which stimulates Na^+ reabsorption. Some physiologists believe that the zona glomerulosa itself may be sensitive to the factors listed above and respond directly to them.

Regulation of Blood Pressure

The body has two major mechanisms for regulating the arterial blood pressure; a central nervous system response and the renin-angiotensin system. The renin-angiotensin system is initiated in the kidney by the juxtaglomerular apparatus and therefore this will be considered here.

The origin of the DCT lies in close proximity to the afferent and efferent arterioles. Here, the DCT is made up of special epithelial cells known as macula densa cells. Adjacent to the macula densa cells are modified afferent arteriole cells called juxtaglomerular cells. Within these cells are granules that contain the proteolytic enzyme renin. Collectively, the juxtaglomerular cells and the macula densa cells make up the juxtaglomerular apparatus.

There are several factors that can initiate this apparatus. These include a drop in blood pressure, decreased plasma Na^+ and Cl^- levels, and a drop in glomerular filtration rate. Also, sympathetic stimulation of beta-1 receptors located on the juxtaglomerular apparatus can directly activate this system.

Consider the following sequence of events involving the juxtaglomerular apparatus.

Suppose an individual had a sudden drop in blood pressure. This would lead to a decreased glomerular filtration rate. The decreased glomerular filtration rate would be detected by the macula densa cells (which detect a decreased amount of Cl^- being pumped out of the tubule). The macula densa cells would in turn stimulate the juxtaglomerular cells to release renin into the blood stream. Renin, an enzyme, will then act on the protein angiotensinogen (synthesized in the liver but found in the circulation) and produce angiotensin I. Angiotensin I will then circulate through the pulmonary circulation where angiotensin converting enzymes (A.C.E.) will convert it into angiotensin II. Angiotensin II is a very potent vasoconstrictor leading to an increase in blood pressure by many different mechanisms considered below. As a result of the effects of angiotensin II, blood pressure would increase leading to an increased glomerular filtration rate which would act to shut off the release of renin.

The following are some of the effects of angiotensin II.

1. Direct vasoconstriction leading to an increased blood pressure.
2. Stimulation of release of norepinephrine from sympathetic nerves leading to vasoconstriction and an increased blood pressure.
3. Stimulation of increased synthesis and release of aldosterone which in turn leads to an increased Na^+ and H_2O reabsorption and an increased blood volume.
4. Stimulation of release of antidiuretic hormone which leads to an increased H_2O reabsorption and an increased blood volume.
5. Stimulation of an increased thirst which leads to an increased H_2O intake and an increased blood volume.

Reabsorption and Secretion of Organic Materials by the Nephron

Reabsorption/secretion patterns for organic materials very much depend on the specific organic compound that is being observed. Under normal conditions, all glucose, amino acids, and small proteins that are filtered through the glomerulus are reabsorbed in the proximal convoluted tubule. However, in situations where the transport system may become saturated, these materials may spill over into the urine.

Urea is freely filtered at the glomerulus and 40 - 80% is reabsorbed in the proximal convoluted tubule by simple diffusion. Although most cells and capillaries are freely permeable to urea, the kidney is able to concentrate urea with a gradient up to 50-fold greater than plasma.

Creatinine is freely filtered at the glomerulus and not reabsorbed significantly under normal conditions. However, tubular reabsorption has been seen with certain conditions such as severe congestive heart failure and uncontrolled diabetes mellitus. A small amount of creatinine secretion may be seen. The amount of creatinine secretion appears to increase as the serum or plasma levels increase.

Uric Acid follows a very deviated path through the nephron. Essentially 100% of the uric acid in the glomerulus undergoes glomerular filtration. Once in PCT, 98-100% of filtered uric acid is reabsorbed in the early PCT. This is followed by the secretion of uric acid into the distal portion of the PCT. Uric acid once again undergoes reabsorption in the DCT. Net urine excretion is 6-12% of filtered uric acid that was initially filtered at the glomerulus.

Laboratory Evaluation of Renal Function

Lecture Objectives: Upon completion of this class material each student will be able to do the following:

1. Cog/II Differentiate glomerular and tubular dysfunction on a basis of serum and urine laboratory results.

2. Cog/II Describe indicators of pre/post-renal azotemia and indicators of renal azotemia.

3. Cog/II Describe the cause of the following glomerular diseases and correlate laboratory findings with these glomerular diseases.

   a. arteriolar nephrosclerosis
   b. mild glomerular injury
   c. glomerulonephritis
   d. nephrotic syndrome
   e. pyelonephritis
   f. acute tubular necrosis
   g. changes in urine output

4. Cog/I Briefly describe the historic concentration and dilution tests as they were used to evaluate kidney function.

5. Cog/II Describe the rationale behind the use of the following tests in the evaluation of renal function and correlate the results of these tests with the presence or absence of disease. Note which tests are measures of glomerular filtration and which are measures of tubular function.

   a. PSP dye test
   b. urea nitrogen
   c. uric acid
   d. creatinine
   e. creatinine clearance
   f. inulin clearance
   g. p-aminohippurate clearance
   h. osmolality
   i. osmolal gap
   j. free water clearance

6. Cog/I Describe the procedure for performing a creatinine clearance or other types of clearances.

7. Cog/II Calculate a creatinine clearance and correlate the result with serum creatinine levels and with disease.

8. Cog/II Compare and contrast creatinine, unulin, and p-aminohippurate clearances.

9. Cog/I Define osmolality and note the correct units for reporting osmolality results.

10. Cog/I List the four colligative properties of solutions and relate these properties to solute concentration. Note the most commonly evaluated colligative properties in the measurement of osmolality.

11. Cog/II Explain the theory behind freezing point depression osmometry and relate freezing point depression to solute concentration.

12. Cog/II Explain the theory behind vapor pressure pressure depression osmometry and relate vapor pressure depression to solute concentration. Describe the limitation of measuring volatile solutes by vapor pressure depression osmometry.

13. Cog/II List the major contributors to serum osmolality and calculate the estimated serum osmolality using these contributors. Manipulate the equation for estimating osmolality based on the units for the major contributors.

14. Cog/II Explain why protein does not make a major contribution to serum osmolality.

15. Cog/II Give normal urine and serum osmolality ranges and relate the utility of individual serum and urine osmolality measurements to the utility of the urine:serum osmolality ratio in the evaluation of kidney function. Include the normal ratio.

16. Cog/II Explain the utility of serum osmolality in the following clinical situations.

   a. in the prognosis of trauma
   b. in monitoring fluid therapy
   c. in HHNC
   d. in poisoning

17. Cog/II Describe the rationale behind the calculation of an osmolal gap and why measurements for this calculation must be made by freezing point depression and not vapor pressure depression osmometry.

18. Cog/II Explain the calculation of the free water clearance, the rationale for the calculation, expected normal results, and the meaning of the calculations. Describe changes that will occur in active tubular necrosis.

I. Introduction

Laboratory tests play an important role in the diagnosis and assessment of renal disease because the clinical signs and symptoms of renal disease may be quite vague or absent. Some of the renal function tests reveal primarily disturbances in glomerular filtration, and others reflect dysfunction of the tubules. The table below lists the abnormal laboratory findings in glomerular and tubular dysfunction. Even though this is true, damage is seldom confined solely to a particular portion of the nephron. The anatomic portions of the nephron are closely related and have a common blood supply so that damage to one portion of the nephron gradually involves the nephron as a whole. Thus, with chronicity, both glomerular and tubular portions of the nephrons become involved irrespective of the site of the original lesion.

TYPE OF DYSFUNCTION	SERUM		URINE
Glomerular **↓ GFR***	**↑ creatinine** **↑ phosphate** **↑ potassium** **↓ bicarbonate** **↓ pH**	**↑ urea** **↑ urate** **↓ calcium** **↓ creatinine clearance**	**Urinalysis** **↓ volume** **↑ specific gravity** **↑ osmolality** **↑ urea conc., ↓ amount** **↓ sodium concentration**
Tubular	**↓ potassium** **↓ bicarbonate** **When glomeruli also involved:** **↑ creatinine**	**↓ phosphate** **↓ pH** **↑ urea**	**Urinalysis** **↑ volume, ↑ pH** **↓ specific gravity** **↓ osmolality** **↑ sodium concentration** **↓ urine concentration test**

* Findings do not apply to the nephrotic syndrome because the lesion is in the glomerular membrane; serum proteins are lost but the GFR is normal.

A. The formation of a normal urine at a normal rate requires:

1. the presence of properly functioning kidneys
2. adequate blood supply
3. sufficiently high blood pressure
4. no obstruction to urine outflow.

B. Thus, a malfunction in the formation and/or elimination of urine may be:

1. **Renal**
   - **a)** renal tubular epithelial cells
   - **b)** course granular casts
   - **c)** BUN and Creatinine abnormalities
2. **pre-renal and post-renal: can usually be identifies by**
   - **a)** serum urea nitrogen levels, serum creatinine levels, and the serum urea **nitrogen/creatinine ratio.**
   - **b)** urine sediment is typically unremarkable.

II. Renal Diseases

A. Arteriolar nephrosclerosis

is characterized by a thickening of the inner lining of the renal arterioles, resulting in a decreased lumen and increased blood pressure. As the blood vessels become necrotic, there is a progressive loss in both glomerular and tubular function. Scar tissue replaces the damaged cells and the kidney becomes contracted. Proteinuria is common. In some patients, high blood pressure cannot be controlled (malignant hypertension), and impairment of renal function becomes progressive and rapid; the outcome is fatal. In the benign form, the blood pressure can be reduced to reasonable levels by appropriate drugs, so the loss of kidney function is relatively mild and slow in its progression.

B. Mild glomerular injury

1. **proteinuria.** Because the glomerulus retards the passage of protein into the urine
2. **increased urine loss of albumin**
   is common with mild injury to the glomerulus.
3. **Transient dysfunction of the glomerulus**
   is common with febrile illness and after exercise, so that transient proteinuria is a common but insignificant finding. With ongoing glomerular injury, as occurs in diabetes mellitus and hypertension, the first indication of injury is the persistent presence of proteinuria. The earliest way to demonstrate this is to determine "microalbuminuria" on a first morning or 24-hour urine specimen.

C. Glomerulonephritis - diffuse, inflammatory disease that affects the glomeruli first but rapidly produces degeneration of the tubules.

1. **acute form,**
   a) Hematuria and proteinuria-the disease is manifested suddenly by the appearance of hematuria and proteinuria,

   b) varying degrees of hypertension, renal insufficiency, and edema.

2. **causative agent** - for the disease is usually a prior infection with Group A, β-hemolytic streptococcus. It is believed that an autoimmune reaction to the antibody produced against the streptococcus is responsible for the damage to the glomerular capillaries. Most patients recover completely from the acute phase. Those that do not recover typically undergo progressive loss of renal function and finally die in renal failure.

3. **Chronic form** - There is also a chronic form of glomerulonephritis that varies greatly in its degree of severity. In some patients, the disease is progressive, with continuing loss of renal function; in others, there are remissions and relapses that may go on for many years.

D. **Nephrotic syndrome**

1. is characterized by a heavy proteinuria, hypoalbuminemia, edema, and hyperlipidemia.

2. Lesions are present in the glomerular membrane which allows proteins to escape.

Laboratory test reveal little or no impairment of renal function. The syndrome is frequently associated with systemic lupus erythematosus, proliferative glomerulonephritis, amyloidosis, or syphilis, but frequently the causative factor is not known. Many cases respond to treatment with adrenocortical steroids.

E. **Pyelonephritis,** - a type of interstitial nephritis, is an inflammatory disease caused by infectious organisms that have ascended the urinary tract and invaded kidney tissues.

1. **Chronic or repeated infections -** may lead to replacement of renal cells by scar tissue with some loss of renal function, which typically causes damage to the tubules.
2. **Drugs are another common cause of interstitial nephritis or proximal tubule damage -** The actual manifestations are dependent on the severity of the damage and the part of the tubule damaged. The proximal part of the tubule, which is typically damaged by drugs, is involved in retention of important substances filtered at the glomerulus such as bicarbonate, sodium, potassium, amino acids, glucose, and proteins.

   a) patients with proximal tubular damage often show proteinuria, glucosuria, hypokalemia, increased fractional excretion of sodium, and acidosis.

3. **Infectious organisms with distal tubular damage.**
   The distal tubule is involved in regulation of acid-base status and potassium excretion. With damage to the distal tubule, acidosis and hyperkalemia are often present.

F. **Acute tubular necrosis (ATN) -** is one of the most common causes of acute renal failure. Severe tubular damage can cause death of tubular cells, with subsequent loss of tubular function. In severe cases, urine production falls to nearly zero, apparently because of functional obstruction to urine flow through the tubules caused by the injury. Evidence of tubular dysfunction is typically manifested by an increase in BUN and creatinine in the serum with a high urine sodium excretion and free water clearance of approximately zero.

G. **Change in urine output** - In many cases, a change in urine output is an initial mode of presentation of renal disease; however, many of these patients actually have disorders of fluid metabolism rather than renal disease. In most patients, increased urine output is a result of one of three major causes.

1. **Increased ingestion of water** - Most commonly, it is due to an increased ingestion of water (polydipsia). This may be seen in diabetes mellitus where an increased serum osmolality, due to increased plasma glucose, stimulates thirst receptors.

2. **increased urine excretion** - may occur due to an absence of antidiuretic hormone (ADH), i.e., diabetes insipidus.

3. **The inability of the kidney to respond to ADH,** i.e., nephrogenic diabetes insipidus.
   a) acute decrease in urine output - may be due to decreased renal blood flow (pre-renal azotemia), acute tubular damage, acute glomerulonephritis, or obstruction of the ureters or urethra.
   b) decreased output due to decreased renal blood flow
      1) BUN typically rises faster than creatinine producing a high BUN:creatinine ratio.
   c) tubular damage
      1) BUN and creatinine rise in parallel resulting in a normal BUN:creatinine ratio. Urine osmolality (considered below) approaches that of serum and the free water clearance (considered below) approaches zero. Acute glomerular damage usually shows hematuria, red cell casts, and proteinuria.
   d) Post-renal obstruction - may be associated with an elevated creatinine and a high BUN:creatinine ratio. This pattern also may be seen with pre-renal azotemia superimposed on renal disease. Thus, obstruction produces no diagnostic laboratory results making imaging studies necessary.

II. Laboratory Tests

A. **Concentration Test**

The principle of the concentration test is to test the ability of the kidney (specifically the DCT) to concentrate the urine (i.e. this tests the ability of the kidney to conserve liquid when necessary). This test is used as an evaluation of kidney function.

1. **Procedure -** This test is performed when the patient is dehydrated by restricting the patients fluid intake for 15 hours. The lab measures the volume of urine output over three hours and the specific gravity of the urine that is formed during that time.
2. **Under normal conditions** the kidney should be able to concentrate the urine to a specific gravity of 1.025 and there should be a low urine volume. (Normal

specific gravity is 1.005 - 1.030) The significance is that this concentrating ability of the kidney is lost early in tubular necrosis. This test is not used much anymore, however a modification of this test is still used and will be considered below.

Specific gravity is the ratio of density of a substance to the density of a standard substance which is usually water. Put another way, specific gravity is the ratio of the mass of a solution compared with the mass of an equal volume of water. Therefore, specific gravity is actually a comparison of weight. It does not measure the exact amount of solute particles.

B. Dilution Test

This test is designed to evaluate the ability of the kidney to rid the body of excess fluid. This is done by loading the patient with large amounts of fluid, generally around 1200 mL of water in a 30 minute period. The lab measures the urine volume and the specific gravity of the urine formed.

Under normal conditions, the kidney should be able to dilute urine to a specific gravity of 1.005. Approximately 1,200 mL of urine should be excreted during a four hour period. This dilution ability of the kidney is usually lost in late kidney disease.
This test is only run on rare occasions because once a patient reaches a late stage of kidney disease, it is in many cases to late to do anything for the patient. Also, most cases of kidney disease should be detected at an earlier stage with today's medical advancements.

C. PSP-Dye Test (Phenolsulfonphthalein)

The principal of this test involves dye being injected through an IV and removed from the blood by the kidney tubules. The amount of dye that ends up in the urine is a function of the kidney tubules. Only a very small amount of the dye is filtered at the glomerulus with most of it being removed by tubular secretion.

The following lists the typical steps of the PSP dye test.

1. A fixed dose of PSP dye is injected IV
2. Urine is collected at 15, 30, 60, and 120 minutes following injection
3. Once the urine is in the lab, NaOH is added to each specimen and the intensity of the pink color that is formed is measured spectrophotometrically. The color intensity is directly proportional to the concentration of PSP in the urine.

Normal values (you are not responsible for these values):
15 minutes - 25 - 30% of the total PSP dose should be excreted
30 minutes - 40 - 75% of the total PSP dose should be excreted by this time (Note: this is the total amount, including what was excreted in the 15 minute specimen)
60 minutes - 50 - 90% of the total PSP dose should be excreted
120 minutes - 60 - 90% of the total PSP dose should be excreted

The remainder of the PSP dose is removed by the liver. This is important because the PSP removed by the liver may interfere with the BSP-dye test used for the evaluation of liver function (BSP stands for bromsulfonphthalein).

The clinical significance of this test is that, on average, approximately 75% of the I njected dye should be excreted in the urine within the first hour. This test is abnormal in renal tubular dysfunction. An abnormal result would mean that only a small amount of dye is excreted.

D. Urea Nitrogen

BUN is a very common screening test for renal function. At one time it was considered the best because of the ease of measurement. Now, since other more specific waste products can be measured with the same ease (i.e., creatinine), BUN is no longer considered the best indicator of kidney function.
However, many clinicians continue to use urea nitrogen measurements to correlate with creatinine measurements. The importance of BUN has been shown with the BUN:creatinine ratio.

Urine urea nitrogen levels are not run very often. This is due to the variability previously discussed from diet, hydration state, and hormones.

The normal value for a urine urea nitrogen level is approximately 12-20 g of urea nitrogen/24 hours.

E. Uric Acid

The measurement of serum uric acid is not used as a primary test for the evaluation of kidney function. This is because urea nitrogen and creatinine serve this function much better. Uric acid, when included, is typically used as a confirmatory test. If uric acid is ordered on a routine screen, such as a diagnostic profile, it should correlate with BUN and creatinine levels.

As discussed previously, the main value of serum uric acid assays is in the diagnosis of gout. Monitoring uric acid levels is also very useful for following the treatment of patients with gout.

Serum acid levels may also be an indicator of a large-scale breakdown of nucleic acid. This is commonly seen in three cases:

1. with toxemia of pregnancy
2. after massive irradiation for tumors
3. following the administration of cytotoxic agents in the treatment of malignancies

F. **Creatinine** - Serum creatinine concentration is elevated in the following situations:

1. a significant reduction in the glomerular filtration rate occurs
2. urine elimination is obstructed

It is estimated that approximately 50% of the kidney function is lost before a significant rise in serum creatinine levels will occur. A serum and/or urine creatinine is very useful, but, perhaps a better evaluation of kidney function is the creatinine clearance test.

3. **creatinine clearance is a sensitive method of assessing renal function.**
   This gives a very good estimate of the glomerular filtration rate.
   a) Natural product of metabolism
   b) analyzed easily and inexpensively
   c) produced at a constant rate by each individual
   d) eliminated by renal action
   e) cleared only by glomerular filtration (almost none reabsorbed or secreted by the tubules)

4. **Creatinine clearance test** - The basis of this test is that the amount of any substance passing from the blood into the urine in a given length of time is a function of two factors. These include:

   a) the concentration of that substance in the blood
   b) the kidney's ability to excrete substance

5. **Glomerular Filtration Rate** -

   a) Normal rate - ~125 mL/min - The glomerulus filters about 125 mLs of filtrate out of the 1200 mLs of blood delivered to the kidney each minute. Creatinine clearance is said to be approximately equal to the normal GFR. (Tubules should reabsorb into the bloodstream all but about 1mL of the original 125 mL of filtrate they receive per/min.)

6. **Procedure** - A creatinine clearance can essentially be run for any length of time. The most frequent times are two hours, four hours, and 24 hours; 24-hours is the most common. A typical procedure is as follows.

   a) A 24-hour urine is collected by the individual. Sometime during this 24-hour period, a blood specimen is collected. The serum and urine creatinine levels are measured, as is the 24-hour total urine volume.

b) Calculations - Once all of the laboratory tests have been performed, the creatinine clearance is calculated by the following equation.

$$\textbf{Creatinine Clearance in mL/min} = \frac{\textbf{Urine Creat mg/dL}}{\textbf{Serum Creat mgdL}} \times \frac{\textbf{TV in mLs}}{\textbf{Time in mins}}$$

Example: UrCr = 226 mg/mL 24hr TV = 1000mL
Sr Cr = 1.8 mg/mL 24hrs in min = 1440

$$= \frac{226 \text{ mg/dL}}{1.8 \text{ mg/dL}} \times \frac{1000 \text{ mL/24hrs}}{1440 \text{ min/24hrs}} = 87 \text{ mL/min}$$

Creatinine excretion = Urine Creat mg/dL X TV mLs/24hrs X IdL/100mLs = mg/24hrs

Example: 226 mg/dL X $\frac{1000 \text{ mL/24hrs}}{100 \text{ mL/dL}}$ = 2260 mg/24hr

Since the creatinine clearance can be run for any number of hours, it is conventional to convert milliliters excreted, say per 24 hours, to mL/minute. This standardizes the results of this calculation by the results being report in mL/min, no matter what length of time was used for the urine collection.

c) Body surface area - The clearance is roughly proportional to size of kidney and body surface area of individual which should be taken into account when calculating the Cr/Cl. The body surface area can be directly related to the amount of creatinine that can be cleared from the blood stream under normal conditions. 1.73 m^2 is considered the standard body surface area of the average adult.

The actual body surface area of the patient can be determined from a nomogram. All nomograms are the result of a mathematical equation. The mathematical equation for this nomogram is as follows:

$$\textbf{log A = 0.425 log W + 0.725 log H - 2.144}$$

where:
A = body surface area (m^2)
W = weight (kg)
H = height (cm)

Please do not memorize this equation. The purpose for giving this equation is to illustrate that all nomograms can be programmed into hospital computers removing the need for the actual use of the nomogram such as the one illustrated in Teitz.

$$\text{Creatinine Clearance in mL/min} = \frac{\text{Urine Creat mg/dL}}{\text{Serum Creat mgdL}} \times \frac{\text{TV in mLs}}{\text{Time in mins}} \times \frac{1.73}{\text{Area}}$$

Area = body surface of patient (must know ht and wt to read nomogram)
1.73 m^2 = avg body surface

7. **Units** - The units for the creatinine clearance are: **mL of blood cleared of creatinine per minute**. Typically these units are simply reported as "mL/min." It is important to understand the complete units for the creatinine clearance in order to understand the meaning of the results.

8. **Reference Range** - The normal reference range for creatinine clearance is **80 - 120 mL of blood cleared of creatinine per minute.**
An increased creatinine clearance result has no medical significance. If a value is greatly increased, an error might be suspected in the collection and/or the timing of the clearance. Another consideration is that the bladder may not have been completely empty when the timing was started.

The table below provides the typical correlation of serum creatinine concentration with the creatinine clearance and patient status.

Serum Creatinine (mg/dL)	Creatinine Clearance (mL/min)	Patient's Status
0.6 - 1.3	100 ± 20	Normal
1.4 - 2.4	61 - 99	Some patients with proteinuria
2.5 - 4.9	24 - 60	difficulty in performing strenuous physical activity
5.0 - 7.9	12 - 23	Unable to perform all of daily physical activities except on part-time basis Acidosis, sodium loss, serum Ca ↓
8.0 - 12	7 - 12	Severe limitation of physical activity Serum K ↑, Ca ↓, Na ↓
> 12	6 or less	May be disoriented or in coma

NOTE: When there is retention of creatinine in serum, there is always retention (elevation) of serum phosphate, urate, and urea. The degree of rise in urea concentration depends to a large extent upon the amount of protein in the diet.

9. **Cr/Cl at low GFR's** - The creatinine clearance usually parallels true glomerular filtration rate. However, at low glomerular filtration rates where serum creatinine levels become significantly elevated, creatinine clearance becomes increasingly inaccurate because tubular secretion begins to occur at these elevated serum levels. At this point, creatinine clearance no longer parallels glomerular filtration because more creatinine is being excreted than was filtered at the glomerulus. This deviation is also seen in severe diabetes mellitus and severe congestive heart failure. These two situations have been found associated with tubular reabsorption of creatinine. Once again, the creatinine clearance no longer parallels glomerular filtration because in these cases, some of the creatinine that was filtered is now being reabsorbed.

10. **Alternate methods to measure GFR** -
Measurement of creatinine clearance by collection of a timed 24-hour urine specimen is burdensome to the patient and frequently difficult to perform. Inaccurate results caused by incomplete bladder emptying, failure to collect the entire specimen, and wide intraindividual variation impair the usefulness of this procedure. Numerous formulas and nomograms have been developed for estimating creatinine clearance from serum concentration, thereby bypassing the need for urine collection. The simplest, and perhaps most widely used is the formula by Cockcroft and Gault.

$$\text{Creatinine Clearance (males)} = \frac{[140 - \text{Age(years)} \cdot \text{Weight (kg)}]}{[72 \cdot \text{Serum creatinine (mg} \cdot \text{L)}]}$$

$$\text{Creatinine Clearance (females)} = \text{above equation result} \cdot 0.85$$

The creatinine clearance is lower in women and the elderly. The calculation for females is based on a 15% lower muscle mass in females than in males.

Tests similar to the creatinine clearance test may be performed with other endogenous materials such as urea. These tests, however, are not believed to be as useful as the creatinine clearance test. Similar types of clearances may also be performed on exogenous materials. One useful exogenous clearance is the inulin clearance.

F. Inulin Clearance - Inulin is a polysaccharide with a molecular weight of approximately 5100. This is a substance that is freely filtered by the glomerulus and it is neither reabsorbed nor secreted by the tubules. Therefore, inulin can provide a very accurate estimate of the glomerular filtration rate.

Based on the fact that there is no tubular reabsorption or tubular secretion of inulin, an inulin clearance is the most accurate measure of glomerular filtration. However, the Inulin Clearance Test is rarely performed due to the fact that it requires an intravenous infusion of inulin, which is not present in plasma under normal conditions. It is also very costly, takes a long time to perform, and is discomforting to the patient.

Except in cases where the glomerular filtration rate is very slow, or serum creatinine levels are very high, the creatinine clearance parallels the inulin clearance very closely. Therefore, the creatinine clearance is typically considered the test of choice in evaluating glomerular filtration, except in the situations noted above.

G. **p-Aminohippurate Clearance -** The p-aminohippurate clearance test is another exogenous clearance test. In this test, p-aminohippurate is used to assess tubular function. This test is performed essentially like the inulin clearance test. Again, the primary difference is this test assesses tubular function.

H **Osmolality -** Serum and urine osmolality are very helpful because these tests serve as good indicators of renal water regulation. Osmolality is a measure of the number of moles of solute per kilogram of H_2O; i.e., it reflects the total concentration of solutes.

1. Osmotic activity - In normal urine, over 80% of osmotic activity is due to urea and creatinine. Electrolytes only make a minor contribution. Protein and glucose do not normally contribute. Urine osmolality is considered the ultimate standard for the evaluation of urine concentration.

2. colligative properties - Briefly considering some of the theoretical background of osmolality, if a solute is dissolved in a solvent (such as water since we are dealing with a biological system) there are four phenomena that occur.

a) The osmotic pressure of the solution is increased.
b) At a given temperature, the vapor pressure of the solution is lowered below that of the pure solvent.
c) The boiling point of the solution is raised above that of the pure solvent.
d) The freezing point of the solution is lowered below that of the pure solvent.

These four properties of solutions are known as the **colligative properties** because they are very closely related and can be interconverted mathematically. These properties depend on the molar concentration of solute present and not on the nature of the solute. Stated another way, these properties do not change in proportion to the weight, size, or shape of the solute particles. These properties depend only on the collection of solutes, or, only to the number of solute molecules present.

Any one of these four colligative properties may be used to determine the osmolality of a sample. The most common colligative property utilized in the determination of osmolality is freezing point depression. The other commonly used property is vapor pressure depression.

3. Freezing point depression - is the most common method used clinically to estimate total solute concentration in body fluids. First, consider what would happen with pure water, i.e., no solute, pure solvent. After placing the sample in a vial, the vial is cooled by either placing it in a bath containing ethylene glycol at - 5°C to -7°C or by a thermoelectric cooler. Therefore, the cooling of this sample is rapid; so rapid that ice will not form even though the temperature of the sample is below its freezing point because ice crystals are slow to form and this cooling is again rapid.

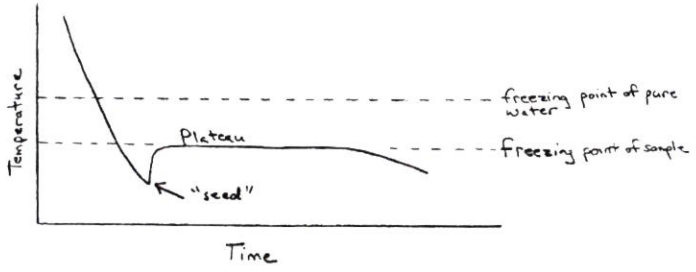
a) Supercooled liquid - An unfrozen liquid at a temperature below its freezing point is known as **supercooled** liquid.

b) Seeding - After the sample is supercooled, it is stirred vigorously which will initiate the crystallization process. This vigorous stirring is known as "**seeding**."

c) Crystallization process - Once ice crystals begin to form, additional water molecules are rapidly added to the ice crystals. During this crystallization process, heat (known as heat of fusion) is released in the freezing process just as it is absorbed in the melting process. This heat that is released is trapped in the vial because there is no good heat conductor around. Therefore, the temperature of the sample increases until the freezing or crystallization stops and an equilibrium temperature is established at the true freezing point of the sample. In the case of pure water, this would be 0°C.

d) Relationship of FPD and osm - This measurement of body fluids by freezing point depression is based on the fact that the freezing point of water is decreased 1.86°C per mole of solute. Therefore, the difference between the plateau of water at 0°C and the plateau of the body fluid under analysis is the depression of the freezing point of the solvent by the solute. There is an inverse relationship between freezing point depression and osmolality. The plateau's temperature is measured electronically by a thermistor, and the temperature reading is converted to milliosmoles per kilogram of water and displayed. The graph below illustrates this process.

4. Vapor pressure depression - involves evaporation of the solvent (water in the case of biological fluids). The theory behind this technique is based on the fact that as solute concentration increases in a solution, there is a greater chance that the solute molecules in the solution will be on the surface of the solution being tested. Solute molecules are typically not volatile. The increased number of solute molecules on the surface of the solution will reduce the amount of evaporation of the solvent. Thus, the vapor pressure from the solvent evaporating will be reduced resulting in vapor pressure depression. Vapor pressure depression is not the most commonly used technique for measuring total solute concentration because it will not detect the presence of all solutes. Ethanol (a solute), for example, is volatile.



Therefore, when ethanol is on the surface of the solution, it will evaporate just as the solvent and its presence will not be detected.

5. The major contributors to serum osmolality are:

a). Sodium or NaCl

b) Urea

c) Glucose

6. **Estimation equation** - An equation is used which allows for the **estimation** of serum osmolality utilizing these three primary materials. The units for osmolality are: mOsmo/kg H_2O (H_2O is the biological solvent)

$$\text{mOsmo/kg } H_2O = 1.86\,[Na] + \frac{[\text{Blood Glucose}]}{18} + \frac{[\text{Urea Nitrogen}]}{2.8} + 9$$

a) Variations in equation - Considering the above equation, the 1.86 (in front of sodium) takes into account the osmotic coefficient of sodium (0.93) times the number of atoms that dissociate in solution: $NaCl \rightarrow Na^{+} Cl \rightarrow 2$.

i)Thus, we have 0.93 x 2, which equals 1.86. You will find some that will round 1.86 to 2 for use in this equation.

ii) Also in the above equation, the 18 (under glucose) and the 2.8 (under BUN) is used to convert the units from mg/dL to mmol/L. If glucose and BUN are already in mmol/L, it is not necessary to divide by these numbers. You will find some that round the 18 to 20 and round the 2.8 to 3 for case of calculation.

iii) Finally, most that use this equation will include the addition of 9 mOsmo/kg H_2O to this equation. This value represents the contribution of other osmotically active substances in serum/plasma such as potassium, calcium and proteins.

7. **Normal Osmolality:**
   - **a)** Serum: 275-300 mOsmo/kg H_2O
   - **b)** Urine: 50-1400 mOsmo/kg H_2O

Notice in the above equation that estimates serum/plasma osmolality that proteins are not onsidered a major contributor. Initially this might seem to be in error. However, recalling that osmolality depends only on the number of dissolved particles in solution, because of the large molecular weight of proteins, the actual number of protein molecules is relatively small. For example, a normal serum albumin of 4 g/dL may seem like a lot, but , because of the molecular weight of albumin, the actual number of moles of albumin is relatively small (approximately 50 µmoles). This can be compared to sodium, which is present in serum/plasma at a level of approximately 150 mmoles. Thus, protein is not a major contributor to serum/plasma osmolality.

Returning to renal function, if serum or urine osmolality alone are measured, it would not be very useful in evaluating renal function. It would only tell something about the state of hydration, but nothing about renal function. However, if the ratio of urine to serum osmolality is used, this would express to what degree the kidney has concentrated the glomerular filtrate. More specifically, this measures the effective tubular concentrating function. Normal individuals who consume an average fluid intake, will have a urine to serum osmolality ratio between one and three. In patients with renal tubular deficiency, the ratio will be less than normal. Therefore, this has taken the place of the concentration test because it is a much better measure of kidney concentration or diluting ability.

8. **Other uses of osmolality in the clinical setting include:**

a) The prognosis of trauma - Osmolality serves as a very good indicator of the survival of patients in traumatic shock. In one study, three patients were admitted to the trauma unit. All showed significant elevations in serum osmolality. (An increased osmolality due to an increased lactic acid primarily). After therapy, the osmolality returned to normal in those who survived. The osmolality continued to increase in those who eventually died.

b) Monitoring fluid therapy - Osmolality levels can be very useful in monitoring fluid therapy.

c)In Hyperglycemic Hyperosmolar Non-Ketotic Coma (HHNC)

d) In certain types of poisoning - When the measured serum/plasma osmolality is significantly higher than that calculated by the estimation equation, severe liver failure or poisoning should be suspected. Typically this is because only unknown and possibly toxic substances can produce high osmolalities which are not accounted for by the equation.

9. **Osmolal gap** - To evaluate the difference between the measured and calculated osmolality, the **osmolal gap** can be calculated.

a) Osmolal gap = Measured serum osmolality - calculated serum osmolality

b) Normal - The normal is -10 mOsmo/kg H_2O to +10 mOsmo/kg H_2O . Poisons such as ethanol, ethylene glycol, and methanol increase the osmolal gap. Notice that these poisons are volatile and, if present, would increase total solute concentration, therefore increasing the measured osmolality. These poisons are not accounted for the in calculation. It is important to note that the osmolal gap can only be calculated from data collected using freezing point depression osmometry.

I. Free Water Clearance

The final evaluation of kidney function is calculating the free water clearance. This calculation is very useful in detecting acute tubular necrosis. Early in the course of acute tubular necrosis, the kidney loses the ability to concentrate the urine. As a result, with this disease process, an increase in the clearance of water from the body occurs.

Before the free water clearance can be calculated, the following measurements must be made:

1. Serum osmolality
2. Urine osmolality
3. Urine output in mL per hour

First, in determining the free water clearance, the osmolal clearance must be calculated. This is a measure of the amount of water cleared from the plasma resulting in urine that has the same osmolality as plasma.

$$C_{osm}\ (mL/hr) = \frac{\text{Urine osmol x Volume of urine (mL/hr)}}{\text{Serum osmol}}$$

The free water clearance is then calculated by the following equation:

$$C_{H2O}\ (mL/hr) = V(mL/hr) - C_{osm}\ (mL/hr)$$

where,

C_{H2O} = free water clearance
V = volume of urine excreted per hour
C_{osm} = osmolal clearance of water

With normally functioning renal tubules, this calculation will result in a negative value for the free water clearance. Normally, the osmolal clearance (C_{osm}), which is essentially the amount of water filtered from the glomerular capillary bed into the nephron is greater than the volume excreted (V) because there is tubular reabsorption occurring. With a loss of concentrating ability, a change from a negative value to a value that is very close to zero will occur indicating the amount of water filtered equals the amount of water excreted (i.e., no reabsorption is occurring because of acute tubular necrosis).

Nonprotein Nitrogenous Compounds

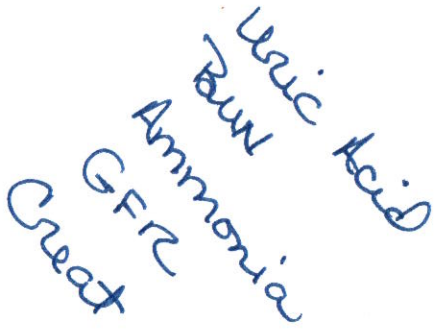
Unit 3 - Lecture #1

Lecture Goal(s): To introduce NPN compounds, their production, causes for increased production and methods of measurement. The utility of various NPN compounds in the evaluation of kidney function will be considered.

Lecture Objectives: Upon completion of this class material each student will be able to do the following:

1. Cog/I Indicate the source of NPN compounds and list the two factors that determine NPN compound levels in the body.
2. Cog/II Explain the utility of NPN compounds in evaluating kidney function.
3. Cog/II Describe the source of urea and its movement throughout the body, especially the kidney.
4. Cog/I Note the relationship between BUN and urea and convert between the two.
5. Cog/I List the normal values for BUN and urine urea nitrogen and explain factors that may cause urea nitrogen levels to fluctuate.
6. Cog/II Provide a general definition of azotemia and make the following associations:
   a. significantly decreased BUN/creatinine ratio suggests acute tubular necrosis
   b. high BUN/creatinine ratio with normal serum creatinine suggests pre-renal azotemia
   c. high BUN/creatinine ratio with high serum creatinine suggests post-renal azotemia
7. Cog/I List situations other than azotemia that will cause increased BUN levels and conditions that will lead to decreased BUN levels.
8. Cog/II Describe the following methods of urea nitrogen/urea measurement.
   a. Nessler reaction
   b. Berthelot reaction
   c. enzymatic method
   d. Fearon reaction
9. Cog/II Describe the relationship between creatine and creatinine.
10. Cog/II Compare the steady production of creatinine with the variable production of BUN and relate this to creatinine being considered a better measure of kidney function.
11. Cog/II Describe the renal diffusion characteristics of creatine and creatinine and give normal serum and urine values of each.

12. Cog/II Discuss the utility and possible arguments against the use of a 24-hour creatinine measure as an indicator of completeness of the 24-hour collection.
13. Cog/I List possible causes for increased serum levels of creatine and creatinine.
14. Cog/II Describe the Jaffe and enzymatic methods for measuring creatinine in urine and serum. Note concerns with the Jaffe method.
15. Cog/II Describe the source for uric acid and the chemical characteristics of this NPN compound.
16. Cog/II Explain the complex movement/diffusion characteristics of uric acid in the kidney.
17. Cog/I List normal urine and serum uric acid levels.
18. Cog/II List and explain possible causes for increased uric acid levels.
19. Cog/II Describe the characteristics of gout and associate this with serum uric acid levels.
20. Cog/II Describe the Kern-Stransky (phosphotungstic acid) and they enzymatic methods for uric acid measurement.
21. Cog/II Describe the production of ammonia and relate this to urea production. Explain the causes for increased serum ammonia levels and the associated concerns.



NON-PROTEIN NITROGENOUS SUBSTANCES (NPN)

I. NPN - Nitrogen exists in the body as proteins and nucleic acids. When proteins and nucleic acids are broken down, the nitrogen released is not stored in the body in large amounts except during periods of tissue growth, such as with pregnancy or in childhood. The nitrogen that is not stored is broken down into compounds known as on-protein nitrogenous (NPN) compounds. The kidneys are primarily responsible for the excretion of these compounds. Because of this, these NPN compounds can be used to evaluate kidney function

More than 15 different NPN compounds are found in the plasma. The total NPN content of plasma is normally 25 - 40 mg/dL. However, NPN compounds are not measured as a total. Of these 15, there are six major NPN compounds that are of interest in the clinical laboratory.

A. Components in decreasing order of nitrogen contribution

1. Urea - 45%
2. Amino Acids - 20%
3. Uric Acid - 20%
4. Creatinine - 5%
5. Creatine - 1-2%
6. Ammonia - 0-2% (primarily used to monitor liver function.)

B. Concentration of each depends on two factors

1. The rate of protein catabolism (i.e. destructive metabolism)
2. The rate of their excretion

II. Urea - Blood Urea Nitrogen (BUN)

Urea is the major end product of protein and amino acid catabolism. It is generated in the liver through what is known as the urea cycle. The goal of this cycle is to remove ammonia (or ammonium ions) which are very toxic to the central nervous system.

A. Metabolism -

Proteins ➤ Amino Acids ➤ Ammonia ➤ Urea

From the liver, urea enters the blood to be distributed to all intracellular and extracellular fluids. It freely diffuses across most cell membranes. .

B. Excretion- Urea is transported via plasma to the kidneys where most of it is ultimately excreted in the urine. Minimal amounts are excreted in sweat and degraded by bacteria in the intestines. 40-50% passively diffuses through renal tubules and back into renal interstitium and eventually diffuses back into plasma. The back diffusion amount of urea depends on urine flow rate (Glomerular Filtration Rate, GFR). More urea diffuses back when GFR is slow otherwise urea is freely filtered by the glomeruli and removed from the body by urinary excretion.

C. Factors Affecting Normal Values In Health - Urea accounts for 80-90% of total urinary nitrogen. Urea in the blood is expressed as Blood Urea Nitrogen (BUN) in the US. European countries tend to express the results in amounts of urea where in the US we use BUN in mg/dL.

Reference Range - 6-20 mg/dL

BUN results may be influenced by:

1. Dietary intake of protein

2. State of hydration (Decreased hydration- increased BUN; Increased hydration- decreased BUN

3. GFR - a measurement of renal function; GFR decreases by 50% before an increase is seen in BUN.

4. Hormones - This is due to hormonal effects on protein anabolism and catabolism.

   a. Glucocorticoids & Thyroid hormones -
      - decrease protein anabolism
      - increase protein catabolism
      - increase BUN
   b. Androgens & Growth hormones
      - increase protein anabolism
      - decrease BUN

D. Pathological Increases in BUN - Two terms used to describe increases.

Azotemia - significant elevations in nitrogen-containing end products, including urea, creatinine and uric acid

Uremia refers to increased urea in the blood stream.

Since urea is by far the major NPN compound, many people use the words uremia and azotemia interchangeably with azotemia being preferred. Azotemia is frequently classified as pre-renal, renal, and post-renal.

1. Prerenal azotemia - (Before Kidney) the result of inadequate blood perfusion of the kidneys. Therefore, there is a decreased glomerular filtration. Otherwise, renal function is normal. Some etiologies for this include the following:

   a. Shock - loss of fluid to extravascular tissues with the result of a decrease plasma volume. ↓BP; ↓GFR; ↑BUN

   b. GI Bleed - Reabsorption of blood proteins account for more amino acids back in pool and thus and increased BUN

   c. Mild Dehydration

   d. High protein diet - more protein to break down

   e. Increase protein catabolism - seen in fever, stress, burns

   f. muscle wasting - starvation

   g. treatment with cortisol - or its' synthetic analogue (hormonal effect)

   h. Congestive heart failure (CHF) - decrease flow of blood supply to kidneys; #1 cause of increased BUN

2. Renal Azotemia - Within the kidney; Results from acute or chronic renal disease. ↓GFR; ↑urea; ↓urine output.

   a. Glomerulonephritis - The loss of protein thru the kidneys results in edema

   b. Polycystic kidney; c.Tubular necrosis; d.Chronic nephritis;e. Nephrosclerosis
   A group of kidney diseases which result from a decrease in blood supply to the renal tubules (ischenia) or from toxic exposure . Also involves destruction of RTE's.
   ↓↓GFR; ↓↓Urine output; Leads to expansion of extracellular fluid (edema), hypertension, and congestive heart failure (CHF)

3. Post Renal Azotemia - result of some obstruction blocking the flow of urine out of the body. As a result there is back-diffusion of urea from renal tubules into the blood stream.
   a. Renal Calculi
   b. Enlarged prostate gland
   c. Tumors of the bladder

4. Uremia - May be know as a clinical syndrome occurring with severe azotemia. BUN usually >100 mg/dL. Includes acidosis, H2O and electrolyte imbalance, nausea, vomiting, anemia, neuropsychiatric changes and sometimes coma. Patient's exhibit a "Uremic frost" where their skin has a grayish appearance. Urea has actually crystallized on the skin after being excreted as sweat. BUN increases progressively faster and higher than other kidney analytes, i.e. Creatinine & Uric acid. Seen as chronic renal failure. Relatively rare today because of hemodialysis.

All forms of azotemia are associated with an increased BUN.

E. Pathological Decreases in BUN - Only occurs in a few situations

1. Poor nutrition - decreased protein intake
2. High fluid intake
3. Excessive administration of IV fluids
4. Pregnancy (change in glomerular filtration rate and protein synthesis)
5. Severe liver disease (decreased urea syntheses due to a decreased action of the urea cycle) .
6. Familial hyperammonemia - synthesis of BUN decreased due to enzyme deficiency in urea cycle.

II. BUN Assay Methods

At the outset it should be noted that urea is susceptible to bacterial decomposition. Therefore, especially with a urine specimen, urea levels should be determined shortly after the specimen has been collected. If this cannot be done, the specimen should be refrigerated.

A. Indirect Methods for the Measurement of Urea Nitrogen

1. **Nessler Reaction of Blair-Taylor**

1st - The protein has to be removed by preparing a protein free filtrate (PFF) using acid.
2nd - The enzyme urease is added.

$$\text{Urea} \xrightarrow{\text{Urease}} NH_3 + CO_2$$

3rd - Nessler's reagent is added

$$NH_3 + 2\ HgI \bullet 2KI \xrightarrow{\text{Alkaline}} \underset{\text{Yellow}}{NH_2 Hg_2 I_2} + KI + NH_4 I$$

The ammonia from the first step reacts with Nessler's reagent (2 HgI • 2KI) to produce a yellow compound whose absorbance is directly proportional to the concentration of urea.

Major problems with this reaction:
1. long reaction times
2. turbidity in the product solution may interfere with spectrophotometric readings
3. this technique is nonspecific in that it measures NH_3. There is the potential for contamination from NH_3 in the atmosphere.

2. **Berthelot Color Reaction**
The first two steps are the same as before. First protein is removed followed by the addition of urease producing ammonia from urea.

In the third step, the following reaction occurs.

$$NH_3 + NaOCl + \underset{\text{(excess)}}{\text{phenol}} \xrightarrow{\text{nitroprusside}} \text{indophenol} \xrightarrow[\text{alkaline}]{\text{make}} \underset{\text{(560 nm)}}{\text{phenol blue}}$$

Again, the absorbance of the blue color formed is directly proportional to the concentration of urea.

The primary problem with this reaction is that it is nonspecific and typically has a long run time.

B. Direct Method for the Measurement of Urea

1. Fearon Reaction or Diacetyl Monoxime - this is the only method that measures urea as a total molecule. The reaction is as follows:

$$\text{Diacetyl Monoxime} \xrightarrow{\text{ferric alum, heat}} \text{diacetyl} - \text{Hydroxylamine}$$

$$\text{Diacetyl} - \text{urea} \xrightarrow{\text{acid, heat}} \text{Diazine Derivative}$$

In this reaction ferric alum acts as the acid catalyst in the presence of heat to start the reaction. The first step in the reaction is to form the active ingredent, diacetyl from the primary reagent - diacetyl monoxime. Diacetyl is very reactive and must be prepared as the reaction is being run. The end product is a pink material whose absorbance is directly proportional to the concentration of urea, which is read at 540 nm.

One advantage of this test over the others is that there is not a step that forms ammonia. Therefore, the interference does not occur. Also, with this procedure, there is no protein free filtrate needed. It is run directly on urine or serum. The biggest disadvantage is the toxic reagents.

C. Enzymatic Measurement of Urea Nitrogen
This enzymatic reaction is also considered an indirect method.

$$\text{Urea} \xrightarrow{\text{Urease}} NH_3 + CO_2$$

$$NH_3 + \text{ketoglutarate} + NADH \xrightarrow[\text{dehydrogenase}]{\text{glutamate}} \text{Glutamate} + NAD$$

In this reaction, the ammonia that is released as a result of urease is tied to an enzymatic reaction involving glutamate dehydrogenase and the coenzyme NADH.

In this reaction sequence, the conversion of NADH to NAD is monitored spectrophotometrically at a wavelength of 340 nm where NADH absorbs light at 340 nm and NAD does not. Therefore, in this particular reaction, a decrease in absorbance will occur as the concentration of urea nitrogen increases (i.e. the absorbance is inversely proportional to the concentration). Currently, this enzymatic technique is the most commonly used method to measure BUN.

CREATININE/CREATINE

I. Metabolism -CREATINE is synthesized in the liver, pancreas, and kidney from three amino acids; 1. arginine, 2. Glycine, 3. Methionine.

- Transported via vascular system to certain tissues (especially nmuscles)
- In muscle cells it becomes phosphorylated to form creatine phosphate.
- Creatine phosphate serves as a high energy source in muscle tissue for contraction. (Creatine concentration proportional to muscle mass)
- During this use of phosphocreatine by the muscles some of the free creatine spontaneously converts to creatinine (irreversible).
- Creatine is found in very low concentrations in the blood stream and there is typically no clinical significance associated with blood creatine levels. Thus, there are no normal reference values for serum creatine.
- Creatine is filtered by the glomeruli but it is almost completely reabsorbed by the proximal convoluted tubule.
- Free creatinine is not reutilized in the body's metabolism and is considered as a waste product of creatine.

II. Excretion - Since creatinine is considered only a waste product its' excretion is what makes it of primary interest in clinical medicine. Creatinine,like creatine is filtered by the glomerulus, but unlike creatine almost none of the creatinine is reabsorbed. It is excreted. This makes it an good measure of GFR.

2% Creatine →Creatinine 24°

A. Creatinines role as a measurement of GFR - A constant waste product

- The formation of creatinine is fairly constant - Approximately two percent of the creatine is transformed into creatinine every 24 hours.
- Creatinine formation is directly related to muscle mass thus creatinine levels in males are typically higher than in females.
- Creatinine is freely filtered by the glomeruli, and its removal from the blood stream can serve as a measure of glomerular filtration rate.
- A small quantity of creatinine is reabsorbed by the tubules and a small quantity of creatinine is secreted into the tubules under normal conditions.
- The result of this reabsorption and secretion causes final urine creatinine to exceed creatinine filtered at the glomerulus by a factor of approximately 1.1 to 1.2 under normal conditions.
- This small factor is not considered to be significant in most cases and urine creatinine is considered a good measure of glomerular filtration.
- However, significant tubular reabsorption of creatinine has been observed in severe congestive heart failure and in uncontrolled diabetes mellitus.

III. Normal Values

A. Serum Reference Ranges
Males - 0.6 - 1.2 mg/dL
Females - 0.5 - 1.0 mg/dL
Most hospitals have a single normal reference range of 0.5 - 1.5 mg/dL.

As previously noted, creatinine formation is relatively constant. This consistency makes creatinine measurement a useful index of renal function, primarily glomerular filtration.

B. Urine Reference Ranges
Males - 1.0 - 2.0 g/24 hours
Females - 0.6 - 1.5 g/24 hours
Most hospitals just use one normal of 1.0 - 2.0 g/24 hours.

Constant waste product = Constant excretion - This relationship allows urine creatinine to be used to test for completeness of a 24hr urine collection in the absence of renal disease.

C. Factors affecting urine creatinine levels which would negate it as a measure of a complete 24hr urine collection..

1. Severe excercise - ↑Creatinine excretion
2. Excessively High meat intake - ↑Creatinine excretion.

Creatinines constant formation and excretion as well as its independence of diet, hydration, and protein metabolism made it more reliable screening of renal function than BUN. Much less sensitive to these things than BUN.

IV Creatinine Assay Methods

A. Jaffe Reaction
Step one - remove proteins

Step two - Creatinine + NaOH + Picric Acid ⟶ Creatinine Picrate

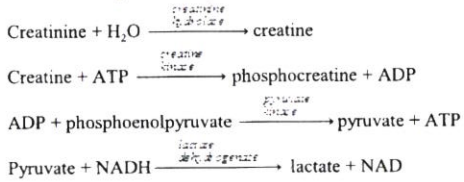
520 nm

NaOH and Picric Acid are typically combined forming alkaline picrate. Creatinine Picrate absorbs at 520 nm, and its absorbance is directly proportional to the concentration of creatinine in the sample being measured.

This reaction is run at a constant temperature of less than 30°C. At temperatures above 30°C, reducing substances such as glucose, uric acid, and ascorbic acid will interfere and falsely elevate results. A constant temperature is also important because absorbance of the colorimetric product will increase with increasing temperature.

Some techniques used to measure creatinine by the Jaffe' method attempt to remove possible interferences by the addition of Fuller's earth (Floridin) or Lloyd's reagent to the protein free filtrate. This selectively adsorbs creatinine away from the other reducing substances. The Jaffe' reaction is then run on this adsorbed creatinine. This gives better specificity, but it is very time consuming.

B. 2. Enzymatic assay



Creatinine + H_2O → creatine

Creatine + ATP → phosphocreatine + ADP

ADP + phosphoenolpyruvate → pyruvate + ATP

Pyruvate + NADH → lactate + NAD

This is a very involved reaction with many places for things to go wrong. For this reason, the enzymatic assay for creatinine was slow to be accepted. It is now, however, the most commonly used method for measuring creatinine. Perhaps, a couple of pertinent questions at this point would be:

Considering the enzymatic reaction above, what components of the reaction sequence would be provided by the reagent? What would you expect to see with absorbance if you were measuring this enzymatic reaction using a manual spectrophotometer? Answers are at the end of this lecture.

BUN:CREATININE RATIO

The BUN and creatinine are routinely requested together because of the relationship of the two levels helps in the differential diagnosis of azotemia.

Keep in mind that the main source of BUN is metabolic catabolism of dietary protein. The level depends on the GFR, dietary intake, and hydration state. It is filtered by the glomerulus and ~50% is reabsorbed by the tubules.

The creatinine on the other hand is freely filtered by the glomerulus and almost all is excreted in the urine. Creatinine levels depend on muscle mass and GFR. It is unaffected by ordinary variations in the diet or activity.

I. Normal Ratio = 10-15:1

A. Variations seen in azotemia

1. Pre-renal Azotemia - Usually and increased ratio due to augmented tubular reabsorption of urea in the presence of a decreased GFR

2. Renal Azotemia - Increased to normal. Both BUN and creatinine are increased. The exception would be in Nephrosis in which you would see a decreased ratio because of the excessive loss of protein in the urine.

3. Post-Renal Azotemia - Increased ratio. Urea will be reabsorbed to a greater degree than creatinine.

II. BUN:CR > 15:1 - Main Causes

A. Decreased glomerular filtration

1. Heart Failure - The urea increases due to the decreased GFR, increased diffusion of urea, and increased protein catabolism which occurs in response to the stress.

2. Underperfused Kidneys - less volume being presented to the kidneys results in decreased GFR. May result from
   a) dehydration
   b) shock
   c) hemorrhage
   d) glomerular disease

B. Increased nitrogen load

1. Excessive protein intake
2. Gastrointestinal bleeding - Large amounts of blood proteins are absorbed. The ammonia produced from the blood breakdown products are also absorbed and converted to urea by the liver.

↑ Prot Catab =

3. Increased protein catabolism - The is known to occur in
   a) fever
   b) burns
   c) heart failure
   d) tetracycline therapy

↑ tub reabsorp =

4. Increased tubular reabsorption of urea - Occurs in
   a) heart faliure
   b) Urinary tract obstruction
   c) familial azotemia (rare disorder associated with active distal tubular transport of urea.

III. BUN:CR < 10:1 - Primary causes

A. Decreased protein intake - seen inpatients who are starving and also in patients with renal failure, i.e. Nephrosis increases protein loss

B. Overhydration - Causes each nephron to filter more urea resulting in increased urea clearance. Chronic renal failure mimics the condition of overhydration because nephron death increases the urea load of the remaining nephrons.

C. Severe liver disease - Severe hepatocellular dysfunction results in decreased urea synthesis

D. Dialysis - Urea rather than creatinine tends to diffuse out of the extracellular fluid, so urea level drops more rapidly than creatinine.

E. Familial hyperammonemia syndromes - These individuals have a deficiency of one the urea cycle enzymes and are characterized by an absence of urea in the blood.

F. Inappropriate secretion of ADH - Excessive amounts of antidiuretic hormone cause increased amounts of water and urea to be reabsorbed by the collecting tubule. The urea accumulates in the medullary interstitium; the high concentration causes some of the urea to be secreted into the descending limb of the Loop of Henle to be recirculated, resulting in increased excretion.

G. Rhabdomyolysis - Dissolution of muscle results in increased release of muscle creatinine.

URIC ACID

I. Metabolism and Excretion

A. Major product of purine metabolism

Purines are derived from ingested nucleic acids or from the destruction of tissue cells. Two major purines are adenosine and guanosine.
Urate is the end product for humans and apes but it is oxidized to allatoin in most other mammals.
It is a week acid and at body fluid pH exists almost entirely as the urate anion.

B. Excretion - Urate is freely filtered by the glomerulus, reabsorbed by the tubules and reabsorbed a second time. Any disease affecting tubular secretion or absorption will have an effect on renal clearance of Uric Acid. The remainder of the uric acid is excreted into the GI trat and degraded by bacterial **enzymes. The steps of excretion are as follows:**

1. Glomerular filtration of virtually all uric acid from the glomerular capillary bed into the nephron.
2. Reabsorption of 98%-100% of the filtered uric acid from the proximal convoluted tubule.
3. Subsequent secretion of uric acid from the renal interstitial fluid into the distal portion of the proximal convoluted tubule.
4. Reabsorption of some uric acid in the distal convoluted tubule.

Following all of this movement, approximately 6-12% of the uric acid initially filtered at the glomerulus is excreted

II. Reference Ranges - Diets high in purines increase the levels of Uric Acid in normal individuals, i.e. visceral meats (kidneys, liver) leguminous vegetables.

Serum/plasma Uric Acid = 2.5 - 7.0 mg/dL

Urine Uric Acid = 400-800 mg/24hrs

A. Hyperuricemia

1. Renal failure - The UA is not being cleared so levels are increased. Levels typically parallel urea nitrogen and creatinine in kidney disease.

2. Lactate excess - competitive inhibitor for renal excretion of urate. Seen in alcohol intoxication, severe toxemia in pregnancy and ethylene glycol intoxication.

3. Diuretic use - not sure of action, but may increase reabsorption and/or decrease secretion.

4. Nucloprotein catabolism (cell turnover) - proliferation of tumor cells as seen in leukemia or lymphoma or in a destruction of tumor cells as seen with chemo.
5. Ketoacidosis - Both lactate and ketones will competitively inhibit the renal excretion of UA. They compete for active tubular secretion.

6. Gout - A rise in UA is typically associated with gout. Disorder of purine metabolism or renal excretion. Precipitation of the Uric Acid forms crystals in the tissues and joints. Primary danger is disposition of urates in the kidney.

   a) Increased UA
   b) Precipitation of monosodium urates as tophi - have a special predilection for joints, bones, bursae, and subcutaneous tissue. Results in pain and swelling in affected area. Most frequently in big toe.
   c) Recurrent arthritis resulting in permenant damage and deformation of joints.
   d) Nephropathy - the uric acid deposits in the kidneys
      1) Nephrolithiasis - results from formation of renal calculi in this case produced by uric acid....10-30% of gout patients exhibit renal calculi
   e) ~95% of cases are in men and first attack rarely occurs before the age of 30
7. Lyesch Nyan syndrome - enzyme deficiency in purine salvage pathway. Increase breakdown in purines with a large increase uric acid. Results in abnormal muscle movement, sel-mutilation and mental retardation.
8. Alcohol consumption may trigger gout attack by increasing UA

B. Hypouricemia

1. Renal tubular reabsorption defects - congenital such as Fanconi's syndrome, and Wilson's disease.

2. Severe liver disease

3. Drugs - Allupurinal (drug for gout)

III. Uric Acid Assay Methods

_____A. Kern-Stransky Method (*Tietz* calls this the phosphotungstic acid method.)
As with most of the other laboratory methods for measuring NPN compounds, the first step involves preparing a protein free filtrate. This is followed by:

$$\text{Uric Acid} + \text{Phosphotungstic Acid} \xrightarrow[650\text{ nm}]{\text{alkaline}} \text{Tungsten Blue} + \text{Allantoin}$$

The absorbance of the blue color that is produced is directly proportional to the concentration of uric acid.

B. Enzymatic Method

$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow[\text{Alkaline condition}]{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

Uric acid absorbs at 290 nm. In this reaction, uric acid reacts with the enzyme uricase to produce allantoin. Allantoin does not absorb at 290 nm. Therefore, it can be said that the measured absorbance at 290 nm is inversely proportional to the concentration of uric acid.

There are many variations available for this enzymatic reaction. The following reaction sequence would allow us to monitor an increased absorbance at 340 nm as concentration of uric acid increases.

$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{ethanol} \xrightarrow{\text{catalase}} \text{acetaldehyde} + H_2O$$

$$\text{Acetaldehyde} + \text{NAD} \xrightarrow[\text{dehydrogenase}]{\text{aldehyde}} \text{acetate} + \text{NADH}$$

AMMONIA

I. **Metabolism** - Ammonia is a product of amino acid metabolism. As noted with the production of urea, most of the ammonia in the body is removed as urea. In addition to the ammonia produced as a result of protein breakdown, a considerable amount is absorbed from the intestinal tract. Ammonia from the GI tract comes from bacterial breakdown of proteins and urea in the GI tract. It is also released from metabolic reactions that occur in skeletal muscle during exercise.

At physiologic pH, ammonia typically exist as the ammonium ion (NH_4^+).

II. **Excretion - Carried by the portal vein to the liver where it is converted to urea. This conversion is a very important step as NH3 is toxic** to the CNS and the urea produced is not only non-toxic but is also readily excreted.

Ammonia may be stored as glutamine which is formed in the liver, brain, and skeletal muscle. The **kidneys take up the glutamine from the circulation and produce NH3 through the following reaction**.

Glutamine —(Glutaminase)→ **Glutamate** —(Glutamate Dehydrogenase)→ Ketoglutarate

$NH_4^+ \rightleftharpoons NH_3^+ + H^+$

NH_4^+ = Ammonium ion dissociates into NH3 (Ammonia) + H+ to be released in the urine as NH4+. NH3 is an improtant buffer against H+ build up. In chronic renal failure the kidneys are uanble to generate enough NH3 to buffer the H+ produced and this contributes to acidosis.

III. **Normal Values** - <120 mg/dL -Low amounts of ammonia are usually excreted by the kidney and low amounts are typically found in the blood stream (approximately ≤ 120 ug/dL).

IV. **Clinical Significance of Abnormal Values**

a. Severe Liver Disease - hepatic coma, Urea cycle slows down NH3 increase.
The most common cause for an increase in ammonia levels is liver disease. In this case, the liver is unable to incorporate ammonia into urea. The build up of ammonia in the body is of concern because increased ammonia is toxic, particularly in the brain.

1. Impaired parachymal function
2. Shunting past liver - Seen in cirrhosis patients. Nh3 not removed

B. Reye's Syndrome - Encephalohepatitis - occurs after some type of viral infection i.e. chicken pox, flu and after ingestion of salicylates. Associated with damage to mitochondria in cells in liver, brain, muscle. Patient usually dies from intracranial pressure. NH3 markedly elevated. May cause hepatic coma. (Medical mystery)

C. Urea cycle enzyme deficience - Rare - increase in NH3

D. Portocaval shunt- Protal vein from intestine to lever bypasses the liver completely or partially empties into vena cava. Results from congenial malfunction or results as a surgical procedure on cirrhotic patients.

V. **Ammonia Assay Methods**

Berthelot - Old method
reaction between ammonia and an alkaline solution of phenol hypochlorite.

VI. **Specimens - Very picky - Not following reccomendations may lead to preanalytic sources of error**
A. Smoking - Patient should not smoke past midnight for a fasting collection

Laboratory Tests in Metabolic Acidosis

TESTS	RESULT	POSSIBLE INTERPRETATION
Total CO_2/HCO_3	Low	Acidosis
Serum chloride	Normal (with low HCO_3 and increased anion gap)	See causes of normochloremic metabolic acidosis.
	Elevated (with low HCO_3 and normal anion gap)	Acidosis due to HCO_3 loss or early renal disease. See causes of hyperchloremic metabolic acidosis.
Anion gap	Normal	See causes of hyperchloremic metabolic acidosis.
	Increased	See causes of normochloremic metabolic acidosis.
Potassium	Increased	As hydrogen ion concentration increases in the plasma, hydrogen ions will begin to move into the cells where Hb buffer is found. To maintain electroneutrality, potassium will leave the cell.
Glucose	Increased	Diabetes mellitus
BUN/Creatinine	Increased	Renal Disease
Blood gases	pH decreased	Acidemia present
	pCO_2 decreased	Pulmonary compensation
	pO_2 decreased	May indicate maximal pulmonary compensatory mechanisms, hypoxia present and lactic acidosis likely.
Urinalysis	pH decreased	Kidney excreting acid effectively.
	pH increased (with low blood pH)	Renal tubular acidosis likely due to excessive excretion of bicarbonate.
	Ketones	Check for increased blood ketones. This is likely due to acid overproduction or caloric deficit.
Blood lactate	Increased	Lactic Acidosis
Blood salicylates	Increased	Poisoning with aspirin.
Serum osmolality	Increased	BUN, glucose, methanol, ethylene glycol
WBC count	Increased	Leukocytosis may be seen as a result of acidosis stimulating nerve endings to release catecholamines which in turn stimulate WBC production.