Water and Electrolytes

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

1. Cog/II Discuss the characteristics of water that make it a good biological solvent.

2. Cog/II Explain the following mechanisms that control water balance in the body.

   a. thirst center
   b. antidiuretic hormone
   c. renin-angiotensin-aldosterone axis
   d. retention of sodium and water by the kidney
   e. natriuretic peptides

3. Cog/I List causes for dehydration and overhydration.

4. Cog/II Differentiate electrolytes, minerals, and trace metals. List three important roles of electrolytes.

5. Cog/II Explain the concept of electroneutrality and note the principle extracellular and intracellular cations and anions. Explain what would happen to these extracellular/intracellular relationships over time while blood is setting in a test tube.

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.

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.

6. Cog/II Explain/discuss/list the following for each electrolyte.

   a. normal serum/plasma and urine levels
   b. movement through the kidney
   c. causes for increases in blood levels of each electrolyte
   d. causes for decreases in blood levels of each electrolyte
   e. panic or critical values and problems associated with these values.
   f. any special specimen considerations.

7. Cog/ II Describe the importance of potassium in normal cardiac function and explain potential cardiac problems in hypo- and hyperkalemia. Explain the importance of potassium levels in digitalis therapy.

8. Cog/II Describe the use of direct and indirect methods for the measurement of sodium and explain factors (such as hyperlipidemia and hyperproteinemia) that would affect the results obtained by these two methods.

9. Cog/II Describe factors that would influence the analysis of serum/plasma potassium levels.

10. Cog/II Describe specific characteristics of chloride in CSF and sweat.

11. Cog/II Describe the following methods of chloride measurement.

    a. coulometric-amperometric titration
    b. ion-selective electrode methods
    c. mecurimetric titration
    d. mecuric thiocyanate spectrophotometric method

12. Cog/II Explain the use of total CO_2 when referring to bicarbonate.

13. Cog/I Illustrate and explain the formation of bicarbonate in the tissue capillary from CO_2.

14. Cog/II Describe methods for measuring bicarbonate/total CO_2.

15. Cog/II Describe and calculate the anion gap.

B. Maintaining Water balance - Water makes up 60 - 70 percent of the total body weight. This percentage varies with many different factors. For example, as a person ages, the body carries less water. Also, the more body fat a person contains the less water their body contains since fat does not contain water.

1. An adult drinks and takes in approximately 2,500 mL of water daily. The body maintains a certain water balance by excreting about the same amount each day. The average daily loss of water is as follows:

Site	Vol/Day (mL)
Skin	500
Expired Air	350
Urine	1,500
Feces	150

C. **Mechanisms to control Water balance** - There are five primary mechanisms for the control of water balance. These include:

1. **The thirst center -** This is located in the hypothalamus and is stimulated by either an increased osmotic pressure in the interstitial fluid, or a decreased extracellular fluid volume. Intake of water triggered by thirst is considered the most important factor in the maintenance of normal water balance.

2. The effect of antidiuretic hormones on the late distal convoluted tubules and the collecting ducts.

3. **The renin-angiotensin-aldosterone system.**

4. **Retention of sodium and water by the kidney.**

5. **Atrial Natriuretic Peptide (ANP)** - ANP is released by the atria of the heart in response to too much fluid in the blood stream. This increased amount of fluid will cause the atria to stretch, releasing ANP. ANP provides negative feedback for ADH and the renin-angiotensin system to decrease the amount of water reabsorption.

a) More on Natriuretic Peptides (Moved to cardiac testing for next year)
The natriuretic function of the heart was demonstrated more than 20 years ago by the discovery of atrial natriuretic peptide (also known as A-type natriuretic peptide, atrial natriuretic factor - ANF). This led to the description of a family of structurally similar but genetically distinct peptides, constituting the natriuretic peptide (NP) family, which contribute to the maintenance of cardiovascular homeostasis. These natriuretic peptides are the naturally occurring antagonists of

the renin-angiotensin-aldosterone system and of the sympathetic nervous system. They promote natriuresis and diuresis, and act as vasodilators.

A second important NP is brain natriuretic peptide (B-type natriuretic peptide, BNP). BNP is also secreted by the hemodynamically stressed heart mainly in response to myocardial stretch induced by volume load. BNP was first identified in porcine brain, resulting in its name - brain natriuretic peptide. However, the main source of BNP in humans is the heart. ANP and BNP appear to form a dual, integrated NP system with ANP acting as a rapid-response hormone and BNP as a backup hormone activated only after prolonged ventricular overload. The NP system is activated to its highest degree in ventricular dysfunction and has an important role in maintaining the compensated state of congestive heart failure (CHF) and delaying disease progression. The increased plasma ANP and BNP seen in CHF patients is not unique: **NPs are increased in all patients with edematous disorders, such as renal failure or ascitic liver cirrhosis, that lead to increased atrial tension or central blood volume.**

The NPs are synthesized as preprohormones. The endocrinologically active C-terminal peptides (ANP and BNP) and their N-terminal prohormone fragments are found in plasma. One significant fragment that appears to be useful clinically is the N-terminal-proBNP fragment (NT-proBNP).

Questions remain regarding which NP should be measured in CHF. According to preliminary data from side-by-side comparison studies, BNP and NT-proBNP appear to be equivalent diagnostic and prognostic markers, and both appear to be superior markers over ANP and the N–terminal proANP (NT-proANP) for diagnosis and risk stratification in CHF patients.
BNP is an active hormone and is cleared from the body primarily via neutral endopeptidase enzymes and by binding to natriuretic peptide receptor sites. Since clearance is not dependent on kidney function, studies have confirmed that BNP is an effective marker for CHF even in patients with renal insufficiency. As an inactive protein, NT-proBNP is cleared from the bloodstream as a by-product and thus requires adequate kidney function. Therefore the use of NT-proBNP as a marker for CHF in patients who may have co-existing renal insufficiency may be unacceptable.

b) **Natriuretic Peptides** in normal kidney function - On the other hand, with normal kidney function, NT-proBNP has been shown to take longer to clear from the body suggesting that NT-proBNP may be more stable. Also, administration of the drug nesiritide, which is a recombinant form of BNP, does not interfere with the NT-proBNP test results. Thus, BNP and NT-proBNP both serve as useful laboratory tests in the evaluation of CHF, each having particular advantages over the other.

D. **Water loss exceeds intake** - If losses of water exceed water replacement, **dehydration** develops. The most common causes of dehydration are:

1. Vomiting
2. Diarrhea
3. Some types of renal disease
4. Diuretic administration
5. Addison's disease (decreased renal function; therefore, decreased aldosterone)

Addison's →

E. **Water intake exceeds water loss/ Increase of water in body** - May occur by two mechanisms

1. Increased Intake - This situation occurs in inadvertent overhydration (excessive intake or IV administration)
2. Bodies inability to eliminate water when intake is normal

a) congestive heart failure

b)conditions associated with hypoalbuminemia.
- i) cirrhosis
- ii) nephrotic syndrome.

No laboratory tests can measure the **exact** amount of fluid loss or gain. All tests are relative. No way to measure every ounce that goes in and out because fluids are also utilized within the body.

II. Electrolytes

A. **Medical electrolytes** - Electrolytes are free ions that exist in body fluids. In medical usage, the term electrolyte is applied to four main ions in plasma.

1. Sodium
2. Potassium
3. Chloride
4. Bicarbonate ions.

B. **Medical minerals** - Other ions that are important in clinical chemistry are usually referred to as minerals or trace metals/elements. Minerals include ions such as

1. Calcium
2. Magnesium

C. **Trace metals/elements** - include ions such as

1. iron
2. zinc.

Note: Electrolytes are the subject of this lecture. Minerals and trace metals will be discussed in later lectures.

D. **Maintaining electroneutrality** - It is important to note at the outset that the body always maintains electroneutrality, i.e., everywhere there is a positive charge there will also be a negative charge. This concept is illustrated in the table below which shows the distribution of ions in extracellular and intracellular water.

		EXTRACELLULAR		INTRACELLULAR	
	ION	mEq/L	% of total	mEq/L	% of total
CATIONS	sodium	154	92	15	8
	potassium	5	3	150	77
	calcium	5.4	2	27	14
	magnesium	2.6	2	2	1
	total	167	100	194	100
ANIONS	bicarbonate	29	17	10	5
	chloride	111	67	1	1
	phosphate	2	1	100	51
	sulfate	1	1	20	10
	organic acid	7	4	-	-
	protein	17	10	63	33
	total	167	100	194	100

E. **Ionic concentration** - In reviewing this table, one can see striking differences in composition between the intracellular and extracellular fluids.

1. Sodium - is the principal cation of the extracellular fluid while it has a low intracellular concentration.

2. Potassium - is the principal cation intracellularly while it has a low extracellular concentration.

3. Maintenance of ionic concentration - maintained by active transport mechanisms which require metabolic energy.

a) Example - the sodium-postassium pump removes sodium that diffuses into the cells and replaces this sodium with potassium that has diffused out of the cell. Notice that electroneutrality is always maintained.

b) Extracellular and intracellular ion concentrations are not numerically equal. The concentration of ions inside and outside of the cell depend upon cellular function. The important point is that electroneutrality is maintained, i.e., cations and anions balance inside the cell and cations and anions balance outside of the cell.

From Tietz:

"Maintenance of water homeostasis is paramount to life for all organisms. In mammals the maintenance of osmotic pressure and water distribution in the various body fluid compartments is primarily a function of the four major electrolytes, Na^+, K^+, Cl^-, and HCO_3^-. In addition to water homeostasis, these electrolytes play an important role in the maintenance of pH, regulation of proper heart and muscle function, involvement in oxidation-reduction (electron transfer) reactions, and participation in catalysis as cofactors for enzymes. Indeed, there are almost no metabolic processes that are not dependent on or affected by electrolytes."

"Either serum or plasma is appropriate for assay, and with direct ion-selective electrodes, whole blood may be used." "Hemolysis causes erroneously high results in K^+ assays. This problem often is undetected when analyzing whole blood. In addition, unhemolyzed specimens that are not promptly processed may have increased K^+ leakage which is exaggerated when whole blood is stored at 4°C."

This from Tietz very well illustrates the importance of the four ions known as electrolytes. We will talk in detail about each.

III. Sodium

A. Important facts about Sodium

1. **It** is the most abundant cation in extracellular fluid.

2. The major contributor to the osmolality of extracellular fluid. Sodium concentration in extracellular fluid is approximately 10- times higher than inside cells.

a) Maintenance - To maintain this gradient, an active transport system involving the sodium-potassium pump moves three sodium ions to the extracellular fluid in exchange for moving two potassium ions into cells.

B. Step-by-step process following sodium intake -

1. An increase in the extracellular concentration of sodium.
2. This excess sodium then moves into the blood stream.
3. In the kidney
   - **a)** sodium is filtered by the glomerulus
   - **b)** approximately 70 % of that filtered will be reabsorbed from the proximal convoluted tubule (PCT),
   - **c)** 25 % reabsorbed from the Loop of Henle,
   - **d)** 5-15 % reabsorbed from the distal convoluted tubule (DCT) and collecting duct (CD).
   - **e)** under normal conditions, less than 1 % of that sodium filtered at the glomerulus is actually excreted from the body. This is due to the fact that the DCT and CD are under the control of aldosterone.

C. Normal Sodium Reference range

1. **Serum - 136 - 146 mEq/L.**

2. **Urine - 40- 220 mEq/day.** A lot of controversy exists over what a normal urine sodium level should be. When the body has an excess intake of sodium over what is required, this excess will show up in the urine. **Therefore, the urine level is dependent to some extent on the dietary intake of sodium.**

D. Hyponatremia

Hyponatremia - a low serum sodium level. Hyponatremia can be found in a variety of unrelated conditions. It can occur with a sodium (solute) loss or with a water (solvent) excess. Hyponatremia may be found in association with the following situations:

1. **Diarrhea**, - or any large loss of gastrointestinal secretions.

2. **Renal disease with a malfunction of the tubular ion-exchange system of sodium for potassium,** - particularly in the late DCT and CD. This type of problem is generally referred to as **salt- losing nephritis**.
3. **Addison's Disease** - A decrease in adrenal cortical function results in a decrease in aldosterone production.
4. **In polyuric states,** - such as with diabetes insipidus the body stimulates a compensatory intake of water to offset the loss. Therefore, upon increased water intake, hyponatremia may occur.
5. **Metabolic acidosis** - in which both sodium and potassium ions are excreted into the urine as salts of keto-acids.

6. **In syndrome of inappropriate antidiuretic hormone secretion (SIADH)** - hyponatremia is seen due to increased water reabsorption from the DCT and diminished sodium reabsorption. In this condition which is secondary to head trauma, seizures, other CNS diseases, and neoplastic conditions, especially lung, breast, and ovarian cancers, that secrete ADH-like hormones, the serum sodium is depressed due to the excess retention of water in the collecting ducts. This results in depletion of water in the renal tubules, thereby concentrating the urine. Therefore, while the serum sodium is low (hyponatremia), the urine sodium concentration is elevated. This unusual pattern is diagnostic of SIADH.

7. **Use/abuse of diuretics.** - Most diuretics work by blocking renal reabsorption of sodium resulting in an increased excretion of sodium. This will be an accompanying increased excretion of water.

8. **Pseudohyponatremia,** - an analytical artifact, may be seen with lipemic specimens as a result of fluid displacement. Actually, sodium levels are not low. Whether or not pseudohyponatremia is seen depends on the method of analysis. This will be discussed below.

E. **Hypernatremia -** Increased serum sodium. Hypernatremia may be found in association with the following situations:

1.Cushings Syndrome - hyperfunction of the adrenal cortex - results in the over production of aldosterone.

2. **Severe dehydration** due to inadequate fluid intake or excessive fluid loss.

3. **Following treatment of comatose diabetes.** With the removal of glucose, more sodium than usual is pulled out of the cells in order to maintain proper osmotic gradients.

4. **Brain damage** - such as to the hypothalamic area where the thirst center is located.

5. **Diabetes Insipidus (decreased ADH)** - this occurs without sufficient fluid intake to replace the fluid loss.

F. Panic Values for Sodium - Many clinical facilities do not have panic values for sodium. Once it has been determined that the sodium concentration is abnormal, the primary goal of the physician is to determine the cause and to correct it. However, it has been noted that once sodium gets down around 120 mEq/L, the individual may suffer from mental confusion, headaches, fatigue, etc. It has also been noted that if the sodium level drops to approximately 110 mEq/L the individual is susceptible to convulsions, semicoma and comatose states.

IV. Potassium

A. Important facts about Potassium

1. **It is the major intracellular cation** - with a 30-fold greater concentration inside cells than in extracellular fluid.
2. **Only about 3% of the total potassium in the body circulates in the plasma.** As seen with sodium, the sodium-potassium pump is largely responsible for maintaining the potassium gradient necessary for proper cellular function.

B. The major physiologic functions of potassium:

1. Regulation of neuromuscular excitability
2. Contraction of the heart and cardiac rhythm
3. Affecting acid-base status

C. Process following potassium absorption from the diet

1. **Absorbed through the intestinal wall**

2. **Potassium is sent to parts of body where it is need** - once bodies needs are met the remainder from absorption is excreted by kidney.

3. **In the kidney**

   a) This removal process involves partial removal of potassium by glomerular filtration.

   b) Once in the PCT, almost all of the potassium is reabsorbed.

   c) Potassium will then re-enter the tubules in the late DCT and CD by tubular secretion under the control of aldosterone.

   d) Potassium is excreted - An important physiologic difference between sodium and potassium in the kidney is that the kidney does not have the ability to reduce potassium excretion to nearly zero as it does for sodium.

D. Normal reference range for potassium

1. **Serum - 3.5 - 5.0 mEq/L.** - This may run a little higher in newborns, but it will adjust to adult levels soon after birth.

2. **Urine - 25 - 125 mEq/day** - but this level varies greatly with diet.

E. **Specimen** -

1. **Must be free of hemolysis** - , since the concentration of potassium inside the cell is much greater than that outside the cell. It has been suggested that if 0.5 percent of the red blood cells in the body were hemolyzed, the serum concentration of potassium would be increased by 0.5 mEq/L. Even though this small degree of hemolysis may be difficult to see, considering the normal reference range given above, it would not take much hemolysis to significantly influence potassium results. Therefore, electrolytes (especially potassium) are not run on a hemolyzed specimen. A hemolyzed sample must be recollected and **all** electrolytes should be analyzed at one time.

2. **Analyzed at the same time as other electrolytes** - The importance of analyzing all electrolytes on the same sample is based on the fact that the balance between electrolytes is constantly changing as conditions in the body change.

3. **Should be performed on serum** - it has been noted that potassium is slightly higher in serum than in plasma because potassium is released from platelets during clotting.

4. **Avoid excessive centrifugation** - may falsely elevate potassium levels.

F. **Hypokalemia - Low serum potassium.**

1. **A low intake of potassium over a period of time**

2. **An increased loss of potassium through vomiting, diarrhea, etc.** - The fluids of the GI tract usually contain high levels of potassium. Therefore, any great loss of these fluids can produce serious deficits.

3. **The increased secretion of adrenal steroids** - such as aldosterone release associated with Cushing's Syndrome and Aldosteronism, will result in a high loss of potassium depleting the body's supply.

4. **Lower Panic values - 2.5,Eq/L.** 2.5 - 3.0 mEq/L - very dangerous. These low levels can result in an increased muscle irritability, specifically cardiac muscles, which can greatly affect the heart. A low serum potassium may result in cardiac dysrhythmias, or irregular heartbeat. We will talk more about this

H. **Hyperkalemia** - Increased serum potassium - may be caused by any of the following:

1. **decreased output of urine with a normal potassium intake** - Since the concentration of potassium is so high within the cell, its concentration in the plasma rises when it leaves the cell at a greater rate than can be excreted by the kidney. This type of overload may occur when there is a(i.e. shock - a disorder resulting from the ineffective circulation of the blood, characterized by a decreased blood pressure, rapid pulse, and decreased kidney function). This type of overload may also occur when there is an intracellular to extracellular shift of potassium such as in acidosis, hypoxia, insulin deficiency, and digitalis overdose.

2. **a high potassium intake** - such as with a high potassium diet, oral (or IV) potassium supplementation, or transfusion of old blood.

3. **Adrenal cortical insufficiency (referring to aldosterone)** - such as in Addison's Disease, may also lead to a hyperkalemic state.

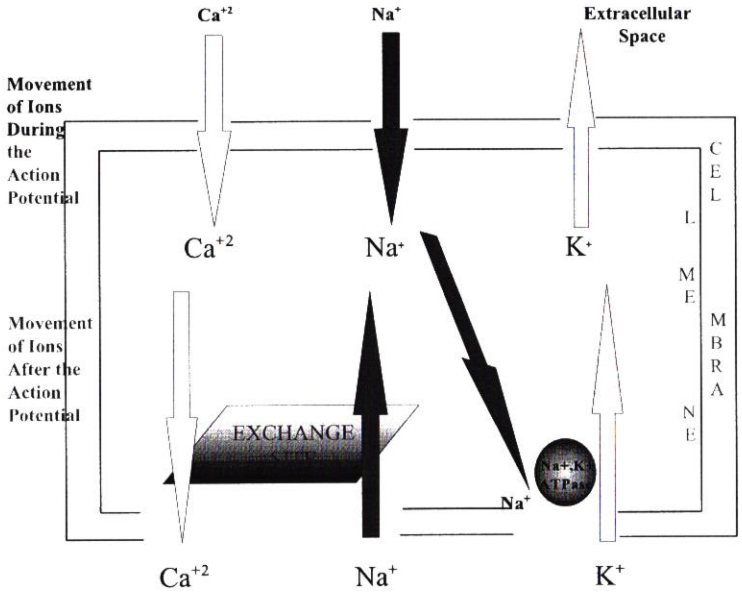
4. **Upper Panic value - 7.5 mEq/L** - High potassium levels in the body can also be very severe. At this level, potassium may seriously inhibit muscle irritability resulting in paralysis of the heart.

   a)Treatment of hyperkalemia - May use glucose infusion as treatment for hyperkalemia - This will stimulate the production of insulin causing the movement of glucose into the cell. To maintain a proper osmotic gradient, sodium moves out of the cell. To maintain a proper electrical balance, potassium moves into the cell.

I. **Normal potassium process - During the depolarization phase of the cardiac action potential, the cardiac muscle cell membrane will undergo a change in permeability allowing the rapid influx of sodium and calcium. This influx of positive charge will cause potassium to leave the cell as illustrated below. The calcium that has just entered the cell will combine with calcium released from the sarcoplasmic reticulum during depolarization, bind with troponin, and the calcium-troponin complex will inhibit tropomyosin. Inhibition of tropomyosin will allow actin and myosin filaments to interact causing the heart to contract (or beat).**

Soon after depolarization occurs, the cardiac cell will begin to repolarize. Na^+-K^+ATPase is the enzyme that controls the Na^+-K^+-pump, which exchanges sodium for potassium (sodium is pumped out of the cardiac cell, potassium is pumped into the cardiac cell). Next, sodium will move back into the cardiac cell as it exchanges for calcium that is not taken up by the sarcoplasmic reticulum. As calcium levels decrease, the cardiac muscle cell relaxes.

In the diagram on the next page, please note that the ion movement along the top of the cardiac cell illustrates ion movement during the cardiac action potential, i.e., depolarization. Also, ion movement along the bottom of the cardiac cell illustrates ion movement after the cardiac action potential, i.e., repolarization.



Movement of sodium, potassium, and calcium ions across the cardiac cell membrane

J. **Effects of Abnormal Potassium levels** - Typically the greatest concern associated with potassium (both increased and decreased levels) is in association with the heart. The diagram on the following page illustrates ion movement during cardiac function and focuses on the importance of potassium

1. **Process of hypokalemia** - there is a decreased amount of potassium to exchange with sodium.

   a). Therefore, sodium will remain in the cell during repolarization preventing the sodium-for-calcium exchange.

   b). Calcium not taken up into the sarcoplasmic reticulum will remain free in the cell creating an environment where cardiac muscle may, at any time, initiate its own contraction.

   c). As a result, cardiac muscle is said to be more irritable resulting in possible contractions which are out of sync with the rest of the heart, hence cardiac dysrhythmias.

   d). Also, Na^+-K^+ATPase activity is dependent on magnesium levels.

   1) Magnesium serves as an activator for Na^+-K^+ATPase.
   2) When magnesium levels are low, such as in acute pancreatitis, chronic alcoholism, chronic diarrhea, or chronic diuretic therapy, Na^+-K^+ATPase is inhibited possibly leading to dysrhythmias.

2. **Process of hyperkalemia** -

   a) excessive potassium leads to excessive sodium removal during repolarization.

   b) This will be followed by the excessive exchange of sodium for calcium which leads to a decreased amount of calcium being available in the cardiac cell.

   c) during the next action potential, there will not be enough calcium to adequately bind with troponin.

   d) Thus, tropomyosin will not be inhibited resulting in muscle paralysis.

   e) Therefore, potassium levels are always important and need to remain within very narrow limits. Potassium levels outside the normal reference range on either side may have significant effects, especially on the heart.

4) Increased White Cell count - White cells metabolize glucose much more rapidly than red cells; in patients with extreme leukocytosis, potassium may be released in vitro within 30 - 60 minutes.

5) Serum left exposed to clot for prolonged time - Allowing the blood to sit at room temperature for a long time, or in a refrigerator for a short time before centrifugation of the blood allows potassium to leak out of the red cells and makes the serum concentration appear high. Of all laboratory tests, this is the one which is most subject to erroneously high results. Since treatment of high potassium is a medical emergency, it is important to check for the possibility of such contamination before reporting results.

VI. Chloride

A. Chloride is the major extracellular anion in serum. It plays an important role in the maintenance of:

1. **Electrolyte balance**
2. **Hydration**
3. **Osmotic pressure**

B. Normal reference range

1. **Serum chloride - 98 - 108 mEq/L.**

2. **Urinary chloride - 110-250mEq/day**
   levels vary greatly with the amount of dietary intake. In individuals on a low salt diet, very little urinary chloride will be seen.

3. **CSF chloride - 118 - 132 mEq/L**
   Chloride concentration in normal CSF is a bit unusual in that CSF chloride levels are higher than in serum. The reason for this is that very little protein can filter from the plasma into the CSF, therefore the protein, or proteinate anion concentration is low. Since chloride can pass the blood brain barrier without much difficulty, a higher concentration moves into the CNS to balance out the electrical charges.

   a) Bacteria in CSF - If bacteria is present in the CSF, such as with bacterial meningitis, the CSF chloride levels usually fall to approximately that of the serum (98 - 108 mEq/L). This is because bacteria are protein in nature. Therefore, as the protein content of CSF increases, the chloride level decreases.

4. **Sweat chloride - 5 - 40 mEq/L.**

   a) In Cystic Fibrosis - the concentration of these ions is significantly elevated. In this disease state, the sweat chloride levels are usually greater than 70 mEq/L.

C. Absorption/Excretion of chloride - Dietary chloride is readily absorbed in the intestine. Once the body supplies of chloride have been met, chloride is removed from the body by two primary routes: sweat and urine.

1. **In the kidney** - chloride is filtered by the glomerulus and passively reabsorbed by the PCT, Loop of Henle, DCT, and the collecting duct, resulting in less than one percent of that being filtered actually being excreted.

D. Hyperchloremia - an excess of chloride

1. **Associated with a sodium excess.**

2. **Exception - metabolic acidosis** -

   Chloride is in excess without a sodium excess. In this acidosis,the bicarbonate levels will be depleted leaving an electrical gradient. Therefore, chloride will move out of the cell to try to balance the lost of bicarbonate, thereby increasing the chloride concentration, with sodium remaining essentially normal.

E. Hypochloremia, - decreased levels of serum chloride

1. **Basically caused by the same situations with a decreased sodium level**.

2. **Exception - Metabolic alkalosis.**

   This is associated with a bicarbonate excess. As a result, in order to maintain electroneutrality, chloride is lost from the extracellular fluid, both into the cell and in urine.

3. **There are no panic values for chloride.**

F. Methods of Chloride Determination

1. **Chloridometer**

2. **Ion selective electrodes**

3. **A mercurimetric method** - known as the **Modified Schales and Schales Titration**
   In this assay, chloride ions of the sample combine with added mercury to form a soluble complex - mercuric chloride. Once all the chloride has been bound in this complex, the excess mercury combines with the diphenylcarbazone indicator. When mercury binds to diphenylcarbazone, a blue color is formed and this is the endpoint of the titration.

4. A colorimetric method using mercuric thiocyanate [$Hg(SCN)_2$]:

$$Hg(SCN)_2 \xrightarrow{\text{dissociation}} Hg^{+2} + 2SCN^- + 2Cl^- \longrightarrow HgCl_2 + 2(SCN)^-$$

$$3(SCN)^- + Fe^{+3} \longrightarrow Fe(SCN)_3$$

It should be noted that the ferric ion (Fe^{+3}) in this reaction comes from the dissociation of Ferric Nitrate, as the following equation demonstrates.

$$Fe(NO3)_3 \xrightarrow{\text{dissociation}} 3NO^3 + Fe^{+3}$$

The resulting ferric thiocyanate [$Fe(SCN)_3$] is a highly colored reddish material that has λmax of 480nm.

VI **Bicarbonate (HCO_3^-) -** Bicarbonate is the second most abundant anion in the extracellular fluid.

A. **Bicarb (HCO_3^-) and CO_2 used interchangeably - Since bicarbonate ions make up 89 - 90% of the total CO_2, many in the clinical laboratory field use HCO_3^- and total CO_2 interchangeably.** Total carbon dioxide (CO_2) is the sum of [HCO_3^-] + carbonic acid [H_2CO_3]. Given that total CO_2 measurements are much easier and quicker to perform, not only do most use these two terms interchangeably, when we measure electrolytes in the clinical laboratory, we actually measure total CO_2 and report this as the fourth electrolyte. Obviously CO_2 is not an electrolyte, it is a gas. **Total CO_2 is simply used to represent HCO_3^- because of speed and ease of measurement and clinicians know that total CO_2 is used to represent HCO_3^-. The diagram on the next page attempts to illustrate this relationship.**

B. **The Chloride Shift** - Considering the diagram, CO_2 at the tissue level is a result of cellular metabolism. CO_2 will diffuse from tissues into the blood stream at the capillary level. Once in the capillary, some CO_2 will dissolve in the blood, some will combine with plasma proteins, but most will enter the erythrocyte. Once in the erythrocyte, CO_2 will combine with H_2O in the presence of the enzyme carbonic anhydrase (CA) and form carbonic acid, H_2CO_3. H_2CO_3 will dissociate into HCO_3^- and a proton (H^+). As the HCO_3^- concentration builds up inside the cell, HCO_3^- will diffuse out into the plasma. If the HCO_3^- were to remain in the cell, the cell would swell and burst. As HCO_3^- moves out of the cell, chloride moves in to maintain the electrical balance. This is known as the chloride shift.

C. **Erythrocyte function and CO_2** - The function of the RBC is to take O2 through the arterial system to the capillaries supplying the tissues with oxygen. The RBC's release the O_2 as the oxygen tension changes in the capillaries and in turn picks up CO_2 which will be transported by the erythrocytes through the venous system to the lungs to be blown off.. Also note that electrolytes are most commonly run on venous samples. If electrolytes were to be run on an arterial sample, a comment should be included on the patient's report because the HCO_3^- level would be different in arterial blood, as would chloride.

D. **Measurement of CO2 -** HCO_3^- levels can be measured directly by acid-base titration. However, in clinical laboratories, it is most commonly measured with other combined forms of CO_2 and dissolved CO_2 as total CO_2. As noted above, the value that is obtained for the total CO_2 approximates the actual bicarbonate level very closely. This is because 89 to 90 percent of all the CO_2 in the serum is in the form of HCO_3^-. Thus, it is actually total CO_2 that is being measured to obtain a good estimate of HCO_3^-, the electrolyte of real interest.

Many different techniques are available to measure total CO_2 levels. Most of these methods have several steps in common. Primarily, they all use some type of acid, such as lactic acid or sulfuric acid, as a liberating agent to give off CO_2. Methods differ in the way CO_2 is trapped for measurement.

E. **Normal reference ranges for total CO_2**

1. **Venous serum or plasma: 22 - 29 mEq/L**
2. **Arterial serum or plasma: 19 - 24 mEq/L**

F. **Electrolyte Units** - Notice that the units for electrolytes are in terms of mEq/L. Since the valence for each of these four ions is 1, mEq/L = mmol/L (which is also an acceptable unit). It is important when working with electrolytes to use mEq/L or mmol/L because electrical balance (i.e. a balance between positive and negative charges, or, electroneutrality) is the primary interest. If electrolytes were to be reported in terms of mg/dL, the charge nor the molecular weight would be taken into account. Therefore, electrical balance would not be able to be seen.

Thus, with the production of lactic acid, at physiologic pH, there will be the formation of lactate and an acidic proton. The acidic proton will be buffered by HCO_3^- to maintain normal pH. As a result, HCO_3^- levels will drop. For every HCO_3^- molecule that is lost to buffering action, there will be the formation of one lactate (also negatively charged). Thus, in this buffering reaction, electroneutrality is maintained.

Considering the anion gap equation,

$$\text{Anion Gap} = (Na + K) - (Cl + HCO_3)$$

as HCO_3^- drop, the ion (in this case the lactate ion) that maintains electroneutrality is not considered. Thus, the anion gap will increase. This change in anion gap is very indicative of metabolic acidosis.

E. **Low anion gap** - The above discussion of anion gap is the main emphasis at this time. However, while on the topic of anion gap, it would be prudent to mention that consistently low anion gaps, typically in the range of 1 to 3 mEq/L, signify the presence of high levels of basic protein, often a myeloma protein. Basic protein contains ammonium ions. The counter-ions for the ammonium ions are chloride ions. Thus, chloride levels will increase but the anion gap equation will not account for the ammonium ions. As such, the anion gap will drop. Persistently low anion gaps are a serious sign of possible malignancy (i.e., myeloma).

Consistent low ag ap = myeloma

VIII. Aldosterone - A hormone that acts in the late DCT that exerts an effect on ions and therefore H_2O excretion.

A. Released by the zona glomerulosa of the adrenal cortex

B. Function - 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)

C. Factor that 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.

IX. Regulation of Blood Pressure

A. Mechanisms The body has two major mechanisms for regulating the arterial blood pressure;

1. a central nervous system response

2. 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 <u>angiotensin II</u>.

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.**

3. Potassium levels and drug therapy - If a patient is on digitalis therapy, potassium levels are even more important. The most common drug in the digitalis group is digoxin, which is used extensively. Potassium levels are important during digitalis therapy because potassium ions and digitalis (i.e., digoxin) **compete** for binding sites on Na^+-K^+ATPase.

Patients are placed on digoxin while they are in the hospital under very close medical supervision. **The level of digoxin is adjusted based on that particular patient's potassium level**. Significant changes in that patient's potassium level can result in severe effects.

a) Drop in potassium levels in patient on digoxin - any drop in potassium from where they were when the patient was placed on digoxin, then digoxin would exert greater influence on Na^+-K^+ATPase because there is no longer enough potassium. As a result, in this situation, there will be a decreased amount of sodium pumped out of the cell eventually resulting in too much calcium in the cell creating an environment conducive to cardiac dysrhythmias.

b) Increase in potassium levels in patient on digoxin - Considering the other situation, if potassium levels were to increase from where they were when the patient was placed on digoxin, then the effect of digoxin on Na^+-K^+ATPase would be diminished because of the high amount of potassium present. Thus, the drug would be inactive and create an environment conducive to cardiac paralysis, the reason the drug was given to begin with.

V. **Measurement of Serum Sodium and Potassium** - Measurement of the concentration of electrolytes in the blood is **the** most common clinical chemistry test in most hospital laboratories in this country.

A. **Measurement by Ion Selective Electrodes (ISE)** - The use of ion selective electrodes is the most common technique used to measure sodium and potassium

1. **Serum sodium** measurement - this test measures the concentration of sodium in plasma or serum in most assay systems, including direct and indirect methods.

a) Direct Measurement - The direct measurement is made by ISEs on samples which are not diluted.

b) Indirect Measurement - In the indirect measurement, the sample is diluted prior to analysis by ISEs or flame photometry.

Normally, only 93 percent of plasma is water; the remainder is solid, such as lipid and protein, which is suspended in and displaces water. Thus, sodium concentration in plasma is about seven percent lower than sodium concentration in water. In patients who have an increase in solids, either lipid (e.g., triglycerides) or protein (e.g., in patients with monoclonal or polyclonal abnormalities), the amount of plasma that is water decreases. When performing sodium on a severely lipemic or on a specimen with increased proteins a dilution should be made prior to performing the assay. The increased displacement of water by these solids will result in a false decrease in the sodium or **pseudohyponatremia**. **If sodium is measured using a method in which the concentration of sodium is measured in water (direct ISEs measure sodium activity, which is roughly equivalent to sodium concentration in water), then the sodium concentration will not be affected by the amount of protein or lipid.**

Summarizing... ***"Pseudohyponatremia is usually caused by the presence of excess lipids in serum. No sodium ions are dissolved in lipids, which can take up considerable volume of serum. If the absolute amount of sodium in a given volume of serum is determined, as is performed when using such methods of sodium determination as flame photometry, this value is divided by the sample volume to get the concentration. But part of this volume is lipid that has no sodium. So a falsely low value of sodium can be obtained. This artifact is eliminated by the use of ISEs that directly determine the concentration of sodium and do not depend on knowledge of the volume of serum."***

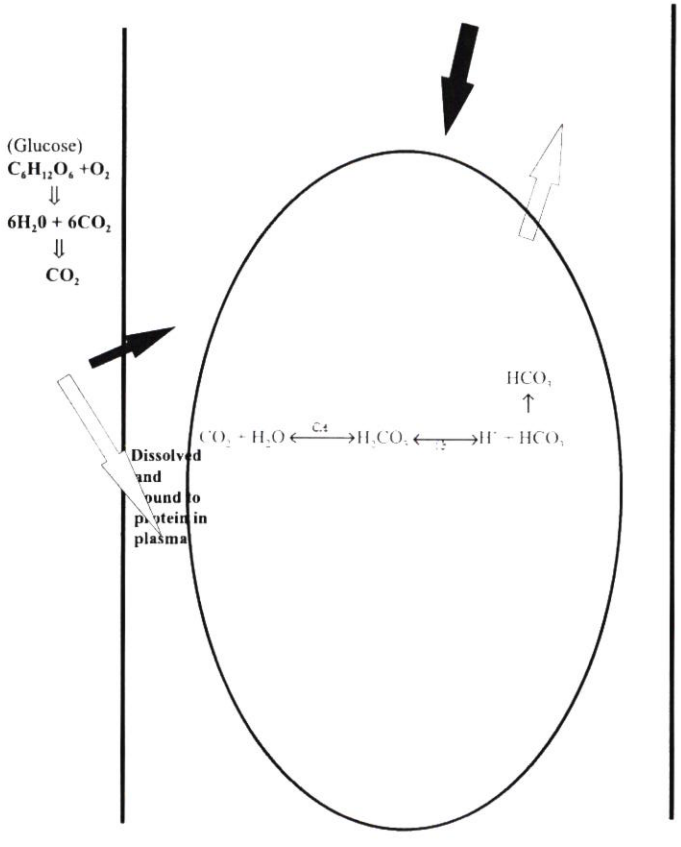
2. Serum potassium -

a) Preanalytical variables

1)Hemolysis - Hemolyzed specimens will give erroneously high potassium since intracellular concentration is approximately 30 - 40 times higher than plasma.

2) Specimen exposed to Potassium EDTA - It is common to see "serum" specimens contaminated with EDTA when blood is drawn with a syringe and injected into vacutainer tubes, frequently giving potassium concentrations over 10 mmol/L. If potassium levels this high are seen, calcium should always be measured; since this is bound by EDTA, an undetectable calcium confirms contamination. Just another reason not to use a syringe.

3) Increased Platelet count - Platelets release potassium when forming a clot; in patients with extreme thrombocytosis, serum potassium is approximately 0.1 mmol/L higher than plasma for each 100,000 mm^3 increase in platelet count.



VII. **Anion Gap** - the anion gap is a mathematical approximation of the difference between the unmeasured cations and anions in serum. This difference is what would be made up by the unmeasured ions.

A. **Anion Gap Equations** - There are two equations that may be used to calculate the anion gap.

1. **Anion Gap = Na - (Cl + HCO_3/CO2)** - This equation omits potassium because of its relatively low concentration when compared to the other electrolytes.

***2. Anion Gap = (Na + K) - (Cl + HCO_3/CO2)**

B. **Normal anion gap: 10 - 20 mEq/L** (using the equation that considers potassium). (i.e. under normal conditions, the major cations in the blood stream exceed the major anions in the blood stream by 10 - 20 mEq/L.)

C. **Anion gap and electroneutrality.** - recall that the most common ions measured in a clinical laboratory are Na, K, Cl, HCO_3 (as total CO_2).

1. If Na and K are measured as the only cations, and Cl and HCO_3 as the only anions, we would find that the electrolytes do not balance electrically. The obvious difference is the other ions that exist in the body. As illustrated earlier, if all the cations and anions in the body are considered, there will be electrical balance (electroneutrality). Considering typical normal levels of the electrolytes:

AG = (137 + 4) - (100 + 25)
AG = 16 mEq/L

This anion gap indicates that the sum of the principle cations exceeds the sum of the principle anions by approximately 16 mEq/L.

D. **High Anion gap** - The greatest utility of calculating the anion gap is in the early detection of certain types of **metabolic acidosis**. For example, consider a situation where there is the increased production of lactic acid.

At physiologic pH, lactic acid will dissociate into lactate and a proton, H^+. The acid (H^+) is buffered by HCO_3^- as illustrated in the following reaction.